UNIVERSITÁ POLITECNICA DELLE MARCHE

FACOLTA' DI MEDICINA E CHIRURGIA

DOTTORATO DI RICERCA IN SCIENZE BIOEMDICHE

Coordinator: Prof. Carlo Catassi

Evaluation of the Anti proliferative effect of phenolic compounds from Garlic on human breast cancer cells through the modulation of different molecular mechanisms involved in their growth and proliferation

Ph. D. dissertation by | Supervisor

Johura Ansary | Prof. Maurizio Battino

Co-supervisor

Dr. Francesca Giampieri

XXXIV Triennial Academic Cycle 2018-2021

To my beloved parents, daughter

&

my PhD supervisor

TABLE OF CONTENTS

ITALIAN SUMMARY **14**

CHAPTER 1. STATE OF ART **17**

1.1. An overview on breast cancer 18

1.1.1. Epidemiology of breast cancer 18

1.1.1.2. Breast cancer incidence and mortality rate 18

1.2. Breast tumour progression 22

1.3.1. Type of breast cancer 23

1.3.1.1. Ductal Carcinoma and Lobular Carcinoma breast cancer 23

1.3.1.2. Luminal A/B and Hormone Receptor Positive Breast Cancer 24

1.3.1.3. HER2+ Breast Cancer 25

1.3.1.4. Triple Negative Breast Cancer 26

1.3.1.5. Breast cancer and metastasis 27

1.3.1.6. Inflammatory breast cancers 29

1.3.1.6.1. Inflammation in breast tumoural microenvironment 30

1.3.1.6.2. Pathway-mediated transmission 32

1.3.1.6.3. Breast cancer and NFκB signalling 32

1.3.1.6.4. Breast cancer and EGFR pathway 33

1.3.1.6.5. Breast cancer and PTGS2 pathway 35

1.4. Risk factors 36

1.4.1. Endogenous hormonal factors 36

1.4.2. Exogenous hormonal factors 36

1.4.3. Reproductive factors 37

1.4.4. Family history and genetic mutations 38

1.4.5. Age and Sex 38

1.4.6. Lifestyle 38

1.5. Breast cancer and Reactive Oxygen Spices 40

1.6. Breast cancer and Apoptosis 40

1.7. Breast cancer and Angiogenesis 42

1.8. Breast cancer and autophagy 47

1.8.1. Autophagy 47

1.8.2. Autophagy induction in breast cancer 50

1.8.3. Autophagy inhibition in breast cancer 51

1.9. Garlic 51

1.9.1. Garlic Origin and Geographical discrimination 52

1.9.2. Bioactive profile of Garlic 54

1.9.3. Chemical composition of garlic 55

1.9.3.1. Proximate composition 55

1.9.3.2. Mineral composition 56

1.9.3.3. Organosulfur profile 57

1.9.3.4. Lipid profile 58
1.9.3.5. Phenolic profile 60
1.9.3.5.1. Antioxidant properties of garlic 62
1.9.4. Bioavailability and Metabolism 63
1.9.5. The potential impact of garlic on human health 65
1.9.5.1. Garlic and Cancer 65
1.9.5.1.1. Therapeutic anticancer effect of garlic on human studies 65
1.9.5.1.2. Garlic treatment and management of breast cancer 68
OBJECTIVE 70
CHAPTER 2. MATERIALS AND METHODS 72
2.1. Plant materials 73
2.2.1. Chemicals and Reagents 73
2.2.2. Antibodies 74
2.3. Preparation of Garlic extract 74
2.3.1. Ethanolic extract of garlic powder 74
2.4. Evaluation of total phenol and flavonoid contents 74
2.5. Evaluation of phenolic compounds by HPLC-MS 75
2.6. Evaluation of organosulfur compounds in garlic extract 76
2.7. Evaluation of mineral content in garlic extract 77
2.8. Evaluationof total antioxidant capacity 77

2.9. Evaluation of *in vitro* digestion of garlic 79

2.9.1. Gastric digestion 79

2.9.2. Intestinal digestion 80

2.10. Cell culture 81

2.11. Evaluation of cytotoxic activity by MTT assay 81

2.12. Evaluation of intracellular ROS levels by Tali® Image-Based Cytometer 82

2.13. Cellular lysates preparation 83

2.14. Evaluation of antioxidant enzyme activity 83

2.14.1. Evaluation of glutathione reductase 83

2.14.2. Evaluation of glutathione peroxidase 84

2.14.3. Evaluation of glutathione transferase 84

2.14.4. Evaluation of reduced glutathione (GSH) determination 85

2.14.5. Evluation of superoxide dismutase 85

2.14.6. Evaluation of catalase 86

2.15. Evaluation of oxidative stress markers 87

2.15.1. Evaluation of lipid peroxidation 87

2.15.2. Evaluation of protein carbonyl content 87

2.16. Evaluation of apoptosis by Tali® Image- Based Cytometer 88

2.17. Seahorse phenotype 89

2.17.1. Evaluation of the mitochondria respiration and glycolysis by XF24 Extracellular Flux Analyzer 89

2.18. Evaluation of wound healing assay 90

2.19. Evaluation of Colony formation 90

2.20. Cell cycle phase control analysis 91

2.21. RNA isolation and quantitative real-time PCR analysis 92

20.22. Protein extraction and western blotting 94

20.23. Human protein array analysis 94

2.24. Statistical analysis 95

CHAPTER 3. RESULTS AND DISCUSSION 96

REULTS AND DISCUSSION: PART I 97

3.1. Phytochemical analysis of garlic extract 97

3.1.1.TPC and TFC 97

3.1.2. Identification and quantification of phenolic compounds 98

3.1.3. Organosulfur compounds 99

3.1.4. Mineral profile 101

3.1.5. Total Antioxidant Capacity (TAC) 102

RESULTS AND DISCUSSION: PART II 104

3.2. Phenolic composition after *in vitro* digestion 104

3.2.1. Determination of potential bioavailability and bioaccessibility of poly phenols in the gastric and intestinal fraction by UV-VS spectrophotometry 104

3.2.1.1. Efficiency of Extraction Conditions 107

3.2.2. Antioxidant capacity after *in vitro* digestion 109

3.2.3. Relationship between phenolic compounds and antioxidant capacity of undigested and digest garlic extract 111

RESULTS AND DISCUSSION: PART III 114

3.3. Anti-proliferative effect of garlic on human breast cancer MCF_7 cells 114

3.3.1. Cytotoxic activity of garlic extracts on breast cancer MCF_7 cell lines 114

3.3.2. Cytotoxic effect of bioaccessible fractions of garlic on breast cancer MCF_7 cells 116

3.3.3. Cytotoxic activity of garlic extracts on MCF10A cell lines 118

3.3.4. Garlic extract by modulating Oxidative stress in MCF_7 cells 119

3.3.4.1. Effect garlic extract induces intracellular ROS production on MCF_7 cells 119

3.3.4.2. Effect of garlic extract on antioxidant system on MCF_7 cells 121

3.3.4.3. Effect of garlic extract on the biomarker of oxidative stress in MCF_7 cells .. 127

3.3.5. Effect of garlic extract on apoptosis on MCF_7 cell 129

3.3.5.1. Intrinsic Pathway 132

3.3.5.1.1. P^{53} dependent intrinsic apoptosis 132

3.3.5.1.2. Apoptosis induction with Bax/Bcl2 family 133

3.3.5.1.3. Caspase dependent intrinsic apoptosis 134

3.3.5.1.4. SMAC mediated apoptosis 135

3.3.5.2. Extrinsic pathway 137

3.3.5.3. ER induced apoptosis 139

3.3.6. Effect of garlic Seahorse phenotype on MCF_7 cells 140

3.3.6.1. Garlic extract targets on OXPHOS inhibition and impairment of mitochondrial respiration 142

3.3.6.2. Garlic extract targets on glycols-deficient and imparement of metabolic profiles. 144

3.3.7. Garlic extract suppressing AMPK induced cell survival in metabolic stress conditions 146

RESULTS AND DISCUSSION: PART IV 149

4.1. Anti-metastatic activity of garlic extract in breast cancer MCF_7 cells 149

4.1.1. Wound healing assay revealed garlic extract induced inhibition of MCF_7 cell migration 150

4.1.2. Garlic extract down regulated the migration capacity of MCF_7 cells by decreasing migration marker expression. 151

4.1.3. Garlic extract decreased mesenchymal and increased epithelial markers expression in MCF_7 cells by β-Catenin and TGF-β pathway 152

4.1.4. Garlic extract inhibits metastasis by blocking Angiogenesis in MCF_7 cells 153

4.2. Effect of garlic extract on the colony growth of MCF_7 cells 163

4.3. Garlic extract suppressing inflammation and prevent tumour growth by down regulating NFκβ/EGFR pathway 164

4.4. Garlic extract on cell cycle progression in MCF_7 cells 165

4.5. Garlic extract stimulated Autophagy induction in MCF_7 cells 171

4.5.1. Garlic extract induced autophagy in non-canonical pathway 171

4.5.1.1. Effect of garlic extract on mTOR signalling 172

4.5.1.2. Effect garlic extract on ATG family protein 172

4.5.1.3. Effect of BNIP3-mediated mitochondrial autophagy 175

4.5.2 Garlic extract induced autophagy in canonical pathway 175

CONCLUSIONS, FUTURE PERSPECTIVES AND LIMITATIONS 180

REFERENCES .... Error! Bookmark not defined.

ACKNOWLEDGMENTS 232

ABSTRACT

Cancer treatment and prevention are consistent challenges for clinicians and scientific community. Recent research has focused on the study of various bioactive compounds, including natural compounds, to ascertain whether they could be used to prevent cancer rather than to treat it. Epidemiological evidence has indicated an inverse association between garlic and various types of cancer, including breast cancer. In the last two decades, garlic has received considerable attention for its potential role in cancer prevention. The main objective of the present work was to evaluate the anti-proliferative potential of garlic extract on *in vitro* breast cancer models, targeting different molecular mechanisms.

Garlic extract (BARI RASHUN-1) showed high amounts of phenolic compounds, such as dihydroxybenzoic acid and ferulic acid via HPLC analysis. Organosulfur compounds (Diallyl disulfide, Dipropyl disulfide and Dipropyl sulfide) were evaluated by Gas chromatography. In addition, six mineral elements (Ca, Cu, Fe, K, Se, and Zn) were identified and quantified by using coupled plasma mass spectrometry. After that, we evaluated the gastric and intestinal bioavailability of garlic phenols for the first time. In this work, spectrophotometric were used to analyse the total phenolic compounds, flavonoids and the antioxidant capacity of garlic extract before and after *in vitro* digestion. Our results illustrated that there was a significant decrease in the number of phenolic compounds and flavonoids after gastrointestinal digestion within the bio accessible fraction; the decrease was lower for the antioxidant activity of the samples but had antiproliferative effect on human breast adenocarcinoma MCF_7 cells .

The anticancer effects of garlic extract on human breast adenocarcinoma MCF_7 cells were observed by cell viability, intracellular reactive oxygen species (ROS) production, apoptosis assay, oxidative stress via the determination of protein carbonyl

content, lipid peroxidation, and DNA damage, as well as the antioxidant enzyme activities. Furthermore, garlic extract suppressed Nrf2/Keap1/NOQ dependent pathway, activity of antioxidant enzyme, expression of superoxide dismutase (SOD), catalase and hemeoxygenase 1(HO-1). Additionally, decreased the activity of glutathione, glutathione peroxidase, reductase, and transferase enzymes in MCF_7 cells that were confirmed by decreased gene expression of SOD, catalase, GSTP1, Nrf2, NOQ and increased Keap1 that were evaluated by RT-PCR and reduced protein expression of HO-1 was determined by western blot. An increase in intracellular ROS levels, an increased number of apoptotic cells, mainly due to a strong down-regulation of various apoptosis inhibitors such as BIRC-2, BIRC-5, BCL-2, MCL-1 gene, was found after garlic treatment. The apoptosis event was associated with the increased expression of p53, Bax, Bid cleaved-PARP and caspase-3 and with the activation of both intrinsic (caspase-9) and extrinsic (FADD, caspase-8) apoptotic and ER induced (XBP1, ATF6α, EIF2α and CHOP) pathways. Bioenergetic characterization was determined by observing various parameters of mitochondrial respiration, glycolysis and lactate balance and metabolism (by reducing LDH-A gene expression) in MCF_7 cell lines. Garlic extract treatment decreased AMPK activation through suppression of PGC1α protein expression, which prevented MCF_7 cell survival by repressing stress adjustment.

Anti-metastasis effects of garlic extract were observed through migration and colony formation assay. The expression of invasion (MMP-2 and MMP-9), epithelial mesenchymal transition (EMT) and metastasis markers (E-cadherin, N-cadherin, and β-catenin) were observed by RT-PCR analysis. Anti-inflammatory effects were observed through the suppression of NFκB, IL-1β, IL-6 p-IκB-α protein expression, while the suppression of EGFR expression indicated the growth inhibitory effects. Garlic extracts also showed anti-angiogenesis effect though it significantly regulated

the levels of some pro-angiogenic factors, increasing FN-γ, TIMP-1 and TIMP-2 expression and decreasing VEGF, VEGF-D, b-FGF, ENA-78, EGF, PlGF, TGFβ1, MCP-1, IL-8. Our work demonstrated that garlic extract might induce autophagy in breast cancer MCF_7 cells through the modulation of autophagy related protein expression by increasing ATG3, ATG4A, ATG5, ATG7, ATG 12, ATG13, Becline, BNIP3, GABARAP, LC3A, LC3B, and decreasing P62, MSK1, NSB1, LMP1, Rheb that was previously unknown. Garlic extracts inhibited cell growth in breast cancer MCF_7 cells by arresting cells at the G1/G0 phase, along with the decreased gene expression of pRb, cyclin D1, cyclin E, cdk2 and cdk4 and increased gene expression of p21Cip, p27Kip and P53.

In summary, this study demonstrated, for the first time, that garlic could be a promising candidate for anticancer effect that inhibits breast cancer growth and metastasis by modulating the expression and activities of several targeting molecular pathways. Our preliminary results suggest a potential Chemopreventive action of garlic, and that it should be investigated in further detail to identify novel compounds for the treatment of breast cancer.

ITALIAN SUMMARY

La prevenzione e la cura del cancro rappresentano una sfida costante per i clinici e per l'intera comunità scientifica. Negli ultimi anni la ricerca si è concentrata sullo studio di vari composti bioattivi naturali dai potenziali effetti antitumorali. Negli ultimi due decenni, l'aglio ha ricevuto notevole attenzione per il suo potenziale ruolo nella prevenzione del cancro, incluso il tumore al seno. L'obiettivo principale del presente lavoro è stato quello di determinare in vitro il potenziale effetto antiproliferativo di un estratto di aglio su un modello di cancro al seno, valutando i principali meccanismi molecolari.

L'estratto di aglio è stato analizzato per determinare il contenuto di polifenoli fitochimica, mediante analisi di HPLC, di composti organo solforati mediante gas cromatografia e di minerali utilizzando la spettrometria di massa. Gli effetti antitumorali sono stati valutati nella linea cellulare di adenocarcinoma mammario MCF-7, attraverso la determinazione della vitalità cellulare, del tasso di apoptosi e dei livelli intracellulari di specie reattive dell'ossigeno (ROS). Lo stress ossidativo è stato misurato mediante la determinazione della perossidazione lipidica, del contenuto di gruppi carbonili e del danno del DNA, nonché mediante la valutazione delle attività degli enzimi antiossidanti nei lisati cellulari. I livelli di espressione delle proteine correlate all'infiammazione, all'apoptosi, alla via molecolare dell'EGFR sono stati determinati attraverso saggi di Western blot. La caratterizzazione bioenergetica è stata analizzata valutando diversi parametri di respirazione mitocondriale (tasso di consumo di ossigeno) e glicolisi (velocità di acidificazione extracellulare). Gli effetti anti-metastatici sono stati osservati attraverso il saggio di migrazione cellulare e di formazione delle colonie; l'espressione di marcatori di invasione, transizione

mesenchimale epiteliale (EMT) e metastatizzazione è stata osservata mediante Western blot.

I risultati hanno dimostrato che l'estratto di aglio (BARI RASHUN-1) ha elevate quantità di composti fenolici, come l'acido diidrossibenzoico e l'acido ferulico, di composti organosolforati quali diallil disolfuro, dipropil disolfuro e dipropil solfuro, e di minerali, come Ca, Cu, Fe, K, Se e Zn.

L'estratto di aglio ha dimostrato profondi effetti inibitori sulla crescita delle cellule tumorali, senza mostrare, al tempo stesso, alcun effetto tossico in cellule sane non cancerose. Nello specifico, dopo il trattamento con l'estratto di aglio, è stato evidenziato un forte aumento dei livelli di ROS intracellulari, del numero di cellule apoptotiche, principalmente a causa della downregolazione di vari inibitori dell'apoptosi, come il gene BIRC-2, BIRC-5, BCL-2, MCL-1. L' apoptosi è stata associata anche con un aumento dell'espressione di p53, Bax, Bid cleaved-PARP e caspase-3 e con l'attivazione della via intrinseca (caspase-9), della estrinseca (FADD, caspase-8) e di quella indotta dallo stress del reticolo endoplasmatico (XBP1, ATF6α, EIF2α e CHOP). Inoltre, l'estratto di aglio ha soppresso la via molecolare dipendente da Nrf2/Keap1/NOQ, l'espressione degli enzimi antiossidanti, sia a livello genico che proteico, e la loro attività.

È stata evidenziata anche una riduzione della funzionalità mitocondriale, correlata con il potenziale di sopravvivenza cellulare, e, contemporaneamente, una diminuzione del tasso di glicolisi. Inoltre, il trattamento con l'estratto di aglio ha ridotto i livelli di espressione di p-AMPK/AMPK, PGC1α e SIRT1 coinvolte nella sopravvivenza delle cellule MCF7 in condizioni di stress metabolico. Inoltre, è stato evidenziato un arresto del ciclo cellulare nella fase G1/G0, insieme alla modulazione dell'espressione di pRb, ciclina D1, ciclina E, cdk2, cdk4, p21Cip, p27Kip e P53, ed ad una diminuzione della proliferazione (con una diminuzione dell'espressione dell'EGFR); abbiamo

riscontrato anche una diminuzione delle abilità di migrazione cellulare e di formazione delle colonie, così come delle capacità di invasione e metastatizzazione, come dimostrato dalla riduzione dell'espressione delle metallo-proteinasi, delle E-, N-caderine, delle β-catenine e di NF-κB, nelle cellule trattate con l'estratto di aglio.

L'estratto di aglio ha mostrato anche un effetto anti-angiogenico, aumentando l'espressione di FN-γ, TIMP-1 e TIMP-2 e diminuendo quella di VEGF, VEGF-D, b-FGF, ENA-78, EGF, PlGF, TGFβ1, MCP-1, IL-8. Infine, l'estratto di aglio è stato in grado di promuovere l'autofagia nelle cellule MCF7 del cancro al seno attraverso la modulazione dell'espressione proteica di ATG3, ATG4A, ATG5, ATG7, ATG 12, ATG13, Becline, BNIP3, GABARAP, LC3A, LC3B, P62, MSK1, NSB1, LMP1, Rheb.

In conclusione, questo studio ha dimostrato per la prima volta che l'aglio potrebbe essere un candidato promettente per contrastare la crescita e la metastasi del cancro al seno modulando l'espressione e le attività di diverse vie molecolari. I nostri risultati, sebbene preliminari, suggeriscono una potenziale azione chemiopreventiva, che andrebbe ulteriormente analizzata per identificare nuovi composti per la terapia del cancro al seno.

CHAPTER 1. STATE OF ART

1.1. An overview on breast cancer

1.1.1. Epidemiology of breast cancer

Breast cancer is the most common malignancy in women around the world and epidemiology of breast cancer is the study of the factors affecting cancer, as a way to infer possible trends and causes. The study of breast cancer epidemiology is essential for planning health measures.

1.1.1.2. Breast cancer incidence and mortality rate

According to International Agency for Research on Cancer (IARC) and World Health Organization (WHO) on-line database, female breast cancer has now surpassed lung cancer as the leading cause of global cancer incidence in 2020 (Figure 1). It is the fifth leading cause of cancer mortality worldwide.

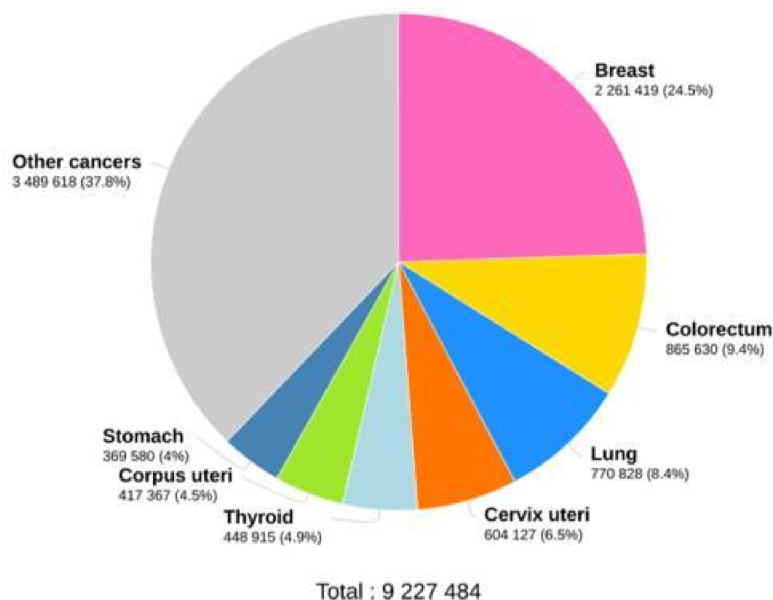


Figure 1.1: Estimated number of new cases in 2020, worldwide, female, all ages. The data was obtained from the GLOBOCAN database in 2020.

However, breast cancer incidence and mortality data in 2020 were obtained from the GLOBOCAN online database. In 2020, new breast cancer cases 24.5% of all cancer cases (Figure 1). Among women, breast cancer accounts for 1 in 4 cancer cases and for 1 in 6 cancer deaths, ranking first for incidence in the vast majority of countries (159 of 185 countries) (Fig 2. A).

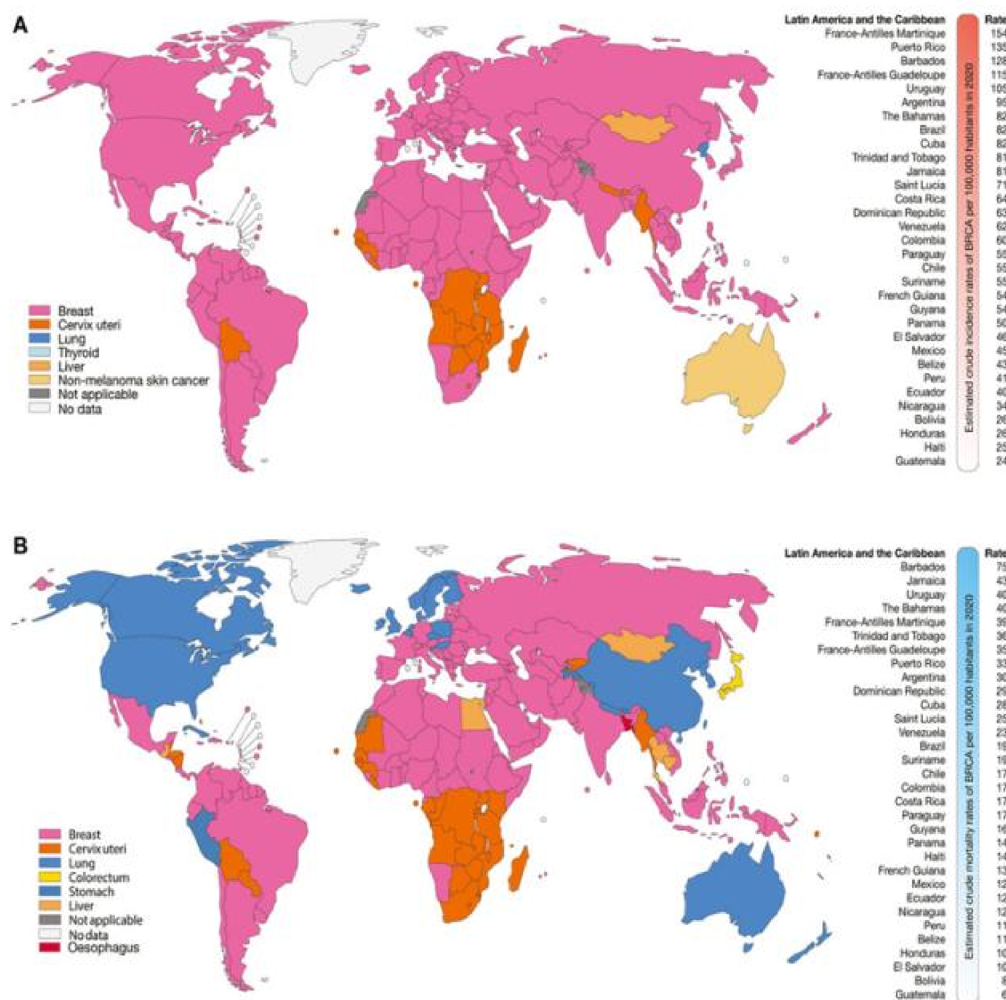


Figure 1.2: Distribution of Standardized Breast Cancer A) Incidence Rate and B) Mortality Rates in World in 2020 (Extracted from Globocan 2020)

Epidemiology of breast cancer from (figure 2A) top cancer crude incidence per country in females of all ages (left) and ranking of estimated crude incidence rates of

breast cancer per 100,000 inhabitants in Latin American and the Caribbean countries (right). From figure 3 and 2B top cancer mortality per country in females of all ages, and ranking of mortality crude rates of breast cancer per 100,000 inhabitants in Latin American and the Caribbean countries (Figure 2B) (Varela et al. 2021)

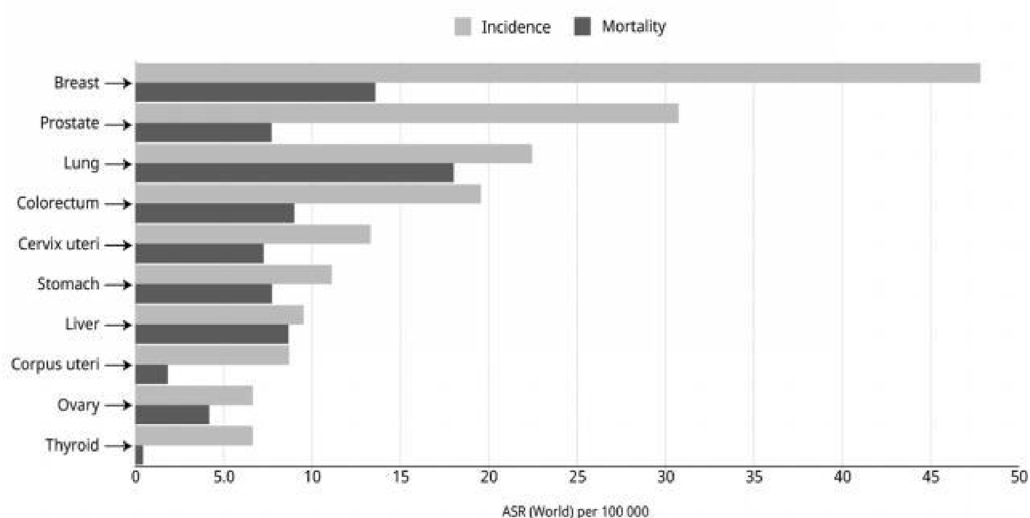


Figure 1.3: Age-standardized (world) incidence (ASR) and mortality rates per 100 000 population for the 10 most common cancer types, worldwide for both sexes and all ages in 2020. The data was obtained from the GLOBOCAN database in 2020.

Approximately, incidence 2.3 million and deaths 685,000 in worldwide (Figure 1 and Figure 2) female breast cancer were diagnosed in 2020. The crude and age-standardized incidence rates of breast cancer were 58.5 and 47.8 per 100,000 population in the different countries (Figure 3). Developing countries (Iran, China, Mexico, Cameroon, and Costa Rica) have lower incidence rates than the world age-standardized incidence rates, whereas highly developed countries (Belgium, Demark, Australia, USA, United Kingdom, and Italy) have much higher incidence rates than the world age-standardized incidence rates (47.8 per 100,000 population) (Figure 4).

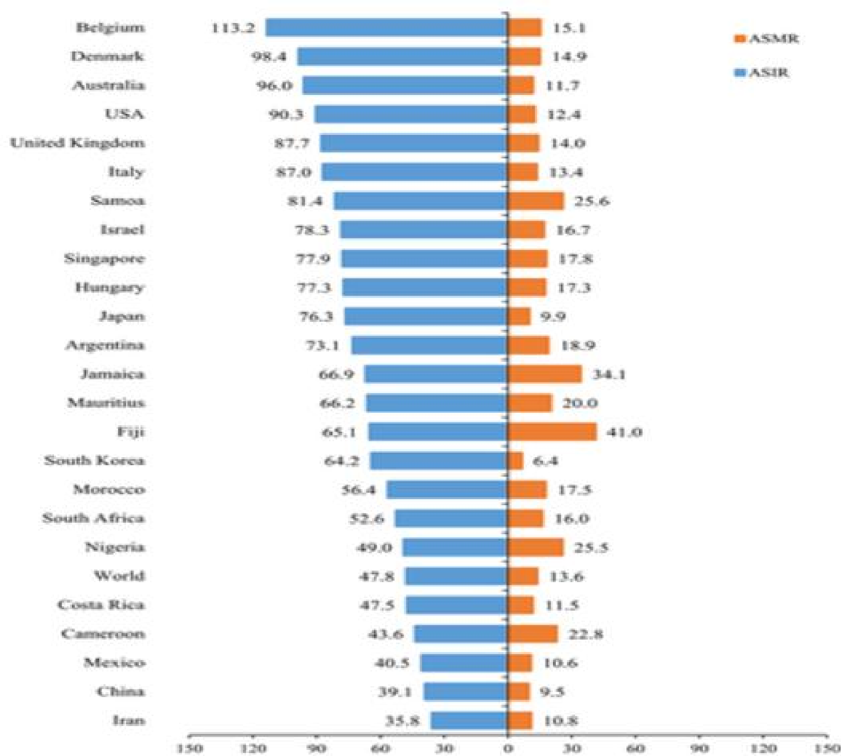


Figure 1.4: Estimated age-standardized (world) incidence (ASIR) and mortality rates (ASMR) per 100 000 persons for female in different countries in 2020. The data was obtained from the GLOBOCAN database in 2020 Abbreviations

In case of age-specific incidence rates of breast cancer, relatively low for female <25 years old in all countries (Figure). Although, the peak age of breast cancer varied across the world regions such as South Korea and Cameroon had the youngest onset peak age of 40 years old. China, Japan, Iran, Fiji, Morocco peaked among female aged 55-60 years old. The summit onset age of breast cancer in the USA, Belgium, Australia, and the United Kingdom were latest with age of 70 years. The age-standardized incidence rates among countries varied over 3-fold, from 113.2 per 100,000 population in Belgium to 35.8 per 100,000 population in Iran (Figure 4). China had the largest number of breast cancer cases, accounting for approximately 18.4% of global breast cancer cases, followed by the USA, with 11.8% breast cancer

cases in the world (Lei et al. 2021). Furthermore, IARC speculates that by 2040 the incidence of breast cancer will increase by more than one third, to more than 3 million new cases per year, and breast cancer mortality will increase by more than one half, to more than 1 million deaths per year.

1.2. Breast tumour progression

There're two theoretical speculations for breast cancer start and progression: the cancer stem cell hypothesis and the stochastic theory (Polyak 2007; Sgroi 2010). The cancer stem cell hypothesis proposes that all tumour subtypes are determined from the same stem cells or transit-amplifying cells (progenitor cells). Obtained hereditary and epigenetic changes in stem cells or progenitor cells will lead to distinctive tumour phenotypes (Figure 5A).

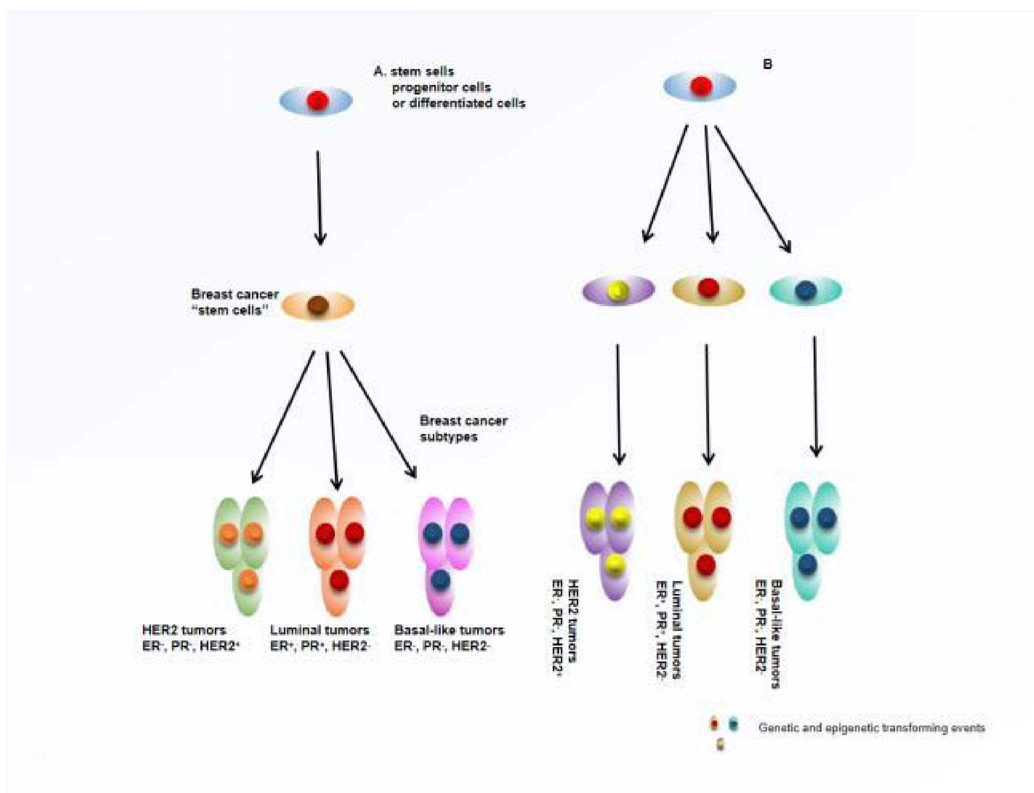


Figure 1.5: Two speculative hypotheses of breast cancer start and movement. (A) All subtypes of tumour are determined from the same stem cells or progenitor cells. Different tumour phenotypes are at that point determined by subtype-specific changing events. (B) Each tumour subtype is started from a single cell type (stem cell, begetter cell, or separated cell). Irregular transformations can slowly amass in any breast cells, driving to their change into tumour cells when a satisfactory number of transformations have gathered. Figures are partially modified from (Polyak 2007; Sgroi 2010).

The stochastic hypothesis is that each tumour subtype is started from a single cell type (stem cell, progenitor cell, or separated cell) (Figure 5B). Irregular transformations can slowly gather in any breast cells, driving to their change into tumour cells when satisfactory transformations have gathered. In spite of the fact that both speculations are upheld by plenty of information, neither can completely clarify the root of human breast cancer(Polyak 2007; Sgroi 2010).

1.3.1. Type of breast cancer

Breast cancer heterogeneity has to lead to the requirement of a common classification of these tumours, mainly based on four different parameters: histopathology, grade, stage, and receptor status.

1.3.1.1. Ductal Carcinoma and Lobular Carcinoma breast cancer

The clinically approved histological subtypes of breast cancer are classified based on the microanatomy of the tissue and the organization of the cellular structure as ductal carcinoma in situ (DCIS), infiltrating ductal carcinoma (IDC), lobular carcinoma in situ (LCIS) and invasive lobular carcinoma (ILC) (Figure 6). Whereas DCIS is defined by the proliferative malignant cells that gather within the lumen of the

membrane of mammary ducts without invading the surrounding tissue (Burstein et al. 2004; Schnitt et al. 2010). Usually, DCIS recognizes as a precursor of IDC type, the most frequent subtype of breast cancers. However, IDC is characterized by the infiltration of cancer cells in the stromal tissue and in the surrounding tissues. The first step of metastatic propagation, LCIS, and ILC represents a small percentage of breast cancers, and they localize to epithelial lobules in situ (LCIS) or lobules and stroma (ILC) (Gump et al. 1987; Weigelt et al. 2005; Hanby and Hughes 2008; Siziopikou 2013).

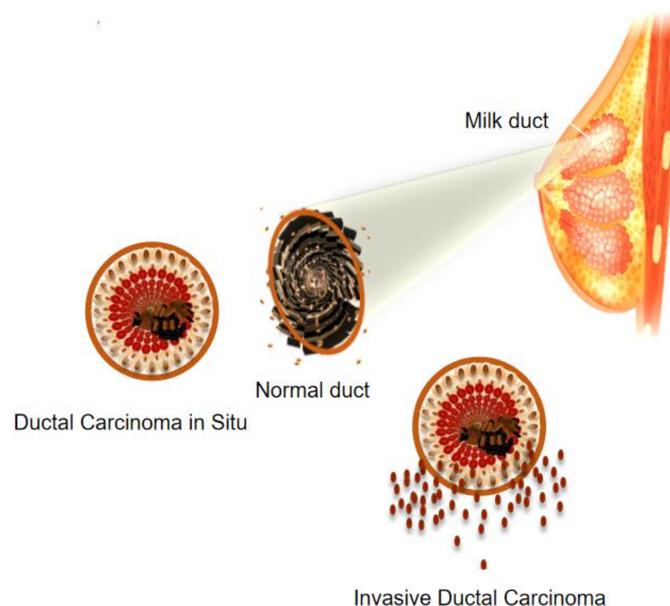


Figure 1.6: The histological types of breast cancer. Adapted from Schnitt et al. 1988.

1.3.1.2. Luminal A/B and Hormone Receptor Positive Breast Cancer

Further, highly indicative of the aggressiveness of the tumour of breast cancer defined as a grade type. Generally, grade-type tumour cells are differentiated by grade - I indicate low, intermediate grade - II indicates moderately differentiated, and high grade- III indicates poorly differentiated. In addition, luminal and ductal epithelial cells lose their morphological architecture by progressing reduction of differentiation

grade and the uniformity of cell nuclei and they divide without control (Elston and Ellis 1991).

There are most common types of breast cancers that comprise greater than 70% of patient diagnoses are estrogen receptor (ER) and progesterone (PR) positive breast cancers. Where, estrogens act as a key factor that involves both initiation and proliferation of breast cancer (Sommer and Fuqua 2001; Faria et al. 2010). In ER+ breast cancer, estrogen receptor activated by estradiol binding results in translocation of the estrogenic receptor to the nucleus where it binds to DNA and regulates cell proliferation and cell survival (Sommer and Fuqua 2001). However, ER/PR+ breast cancers are characterized into Luminal A or Luminal B classification based on expression levels of the proliferative marker Ki-67. Thus, Luminal A cancers have low levels of Ki-67, are slowly proliferating, and have a better prognosis as compared to the high levels of Ki-67 observed with the quickly proliferating Luminal B cancer that normally have a worse prognosis (Perou et al. 2000). Hormone therapies such as tamoxifen, fulvestrant, and aromatase inhibitors (letrozole, anastrozole, exemestane) block estrogen receptor activity and subsequently decrease cancer burden and are common treatments for ER and/or PR+ cancers in patients whose molecular profiling tests indicate potential benefit with these therapies. Moreover, the presence of all three receptors (ER/PR/Her2) are considered collectively when making these treatment decisions (Markopoulos 2013).

1.3.1.3. HER2+ Breast Cancer

HER2+ is another key player in breast cancer malignancy approximately 75% and a part of the epidermal growth factor receptor family; includes four major proteins named EGFR (also known as HER1 or ErbB1), HER2 (p185 neu/ErbB2), HER3

(ErbB3) and HER4 (ErbB4) (figure 7). Which breast cancers have amplification of the HER2 gene that classifies as HER2+ breast cancers (Perou et al. 2000; Davies and Hiscox 2011). Additionally, HER2+-breast cancers shows more resistant to the cancer therapy than without HER2 expression breast cancer (Yarden 2001). Moreover, over expression in both primary tumours and metastatic sites, makes HER2 a primary target for the development of novel strategies for cancer prevention or treatment (Davies and Hiscox 2011). Therefore, down regulation of HER2 expression can be validated therapeutic targets for breast tumour.

1.3.1.4. Triple Negative Breast Cancer

When lack expression of the estrogen receptor, progesterone receptor, and HER2, is often, but not always, a basal-like breast cancer that indicate Triple-negative breast cancer (TNBC), the most aggressive histological subtype of breast cancer. TNBC could be presented 15% among all type of breast cancer patients (Rakha et al. 2007; Telli and Carlson 2009) (Figure 7).

Another parameter of breast cancer is characterized by a staging system that is established based on tumour, node, metastasis (TNM) classification. It also considers the tumour size (T1 to T4), involvement of lymph nodes (N0 to N3), and presence of metastasis (M0 or M1). therefore, Breast cancer staging provides useful information about the current status of cancer detection and management, and the success of implementing new strategies (Singletary and Connolly 2006).

Accordingly, on the basis of receptor status: 1) human epidermal growth factor-2 (HER2)- amplified, 2) hormone-receptor-positive: estrogen receptor (ER) and progesterone receptor (PR); subdivided into Luminal A, Luminal B and normal-like

breast cancer 3) Triple-negative breast cancer: HER2, ER, and PR negative breast cancer which is a more aggressive cancer with a poor prognosis (Golub et al. 1999; Perou et al. 2000; Reis-Filho and Tutt 2008).

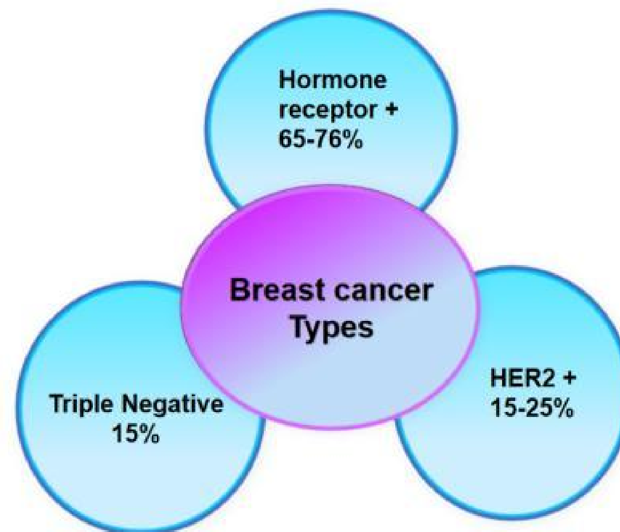


Figure 1.7: The classification of clinical breast cancer types and their percentage level in each. Adapted from (Vivek 2018) with modification.

While approximately 75% of breast cancer patients carrying the BRCA1 (Breast Cancer 1) mutations have a Triple negative phenotype, indicating that BRCA1 is an important vulnerable gene for Triple negative tumours (Singletary and Connolly 2006).

1.3.1.5. Breast cancer and metastasis

Last decades, breast cancer metastasis responsible for most deaths from breast cancer patients. However, exhaustive efforts have established that breast cancer shows metastatic heterogeneity with exclusive metastatic develop to various organs such as bone, lung, liver, and brain (Steeg 2006; Scully et al. 2012; Liang et al. 2020) (Figure 8). Where, bone is the most common location for breast cancer metastasis, approximately 75 % metastasis cases (Tulotta and Ottewell 2018).

The development of metastasis of breast cancer is a series of sequential process. Metastasis begains with the local invasion of neighbouring host tissue by cells activating from the primary tumour and continues as far as the tumour cells invade and intrastate into blood or lymphatic vessels, survival in the circulation as circulating tumour cells (CTCs), Therefore, the tumour cells undergo cell cycle arrest and adhere to capillary beds within the target organ, before extravasating into the organ parenchyma, growth and promoting angiogenesis within the organ (Nguyen et al. 2009; Chaffer and Weinberg 2011; Pantel and Speicher 2016; Mohme et al. 2017).

However, various mechanisms are responsible to develops the early steps of metastasis (Figure 8), including local invasion, intravasation, and survival in circulation. The EMT is a critical pathway in the mesenchymal movement of single migratory cells. Here, the cells will undergo changes from an epithelial phenotype to a mesenchymal-like phenotype (Lou et al. 2008; Thiery et al. 2009). Further, EMT begains with the disintegration of cell-cell adhesion via loss of EMT-related transcriptional factors, such as E-cadherin, vimentin, slug, snail, ZEB1, ZEB2 and Twist, various microRNAs and epigenetic changes are crucial in mediating the EMT of cancer cells (Batlle et al. 2000; Kessenbrock et al. 2010). For invasion tumour cell need to be migrate and EMT helps invasion and intravasion to the blood stream. In addition, EMT and MMPs family involves degradation of extracellular matrix (ECM) which is a component of a complex network of solid tumours and build up a microenvironment which influences tumour progression (Kessenbrock et al. 2010; Bonnomet et al. 2010). Where, malignant tumour cells continually interact with non-malignant cells in microenvironment at both the primary and metastatic sites (Joyce and Pollard 2009). Thus, these interactions give stimulation for the progression of ‘in

situ' breast cancer to metastatic breast cancer (Coghlin and Murray 2010) (figure 8). Moreover, Angiogenesis plays an essential role in breeding metastasis and subsequent metastasis growth (de Castro Junior et al. 2006).

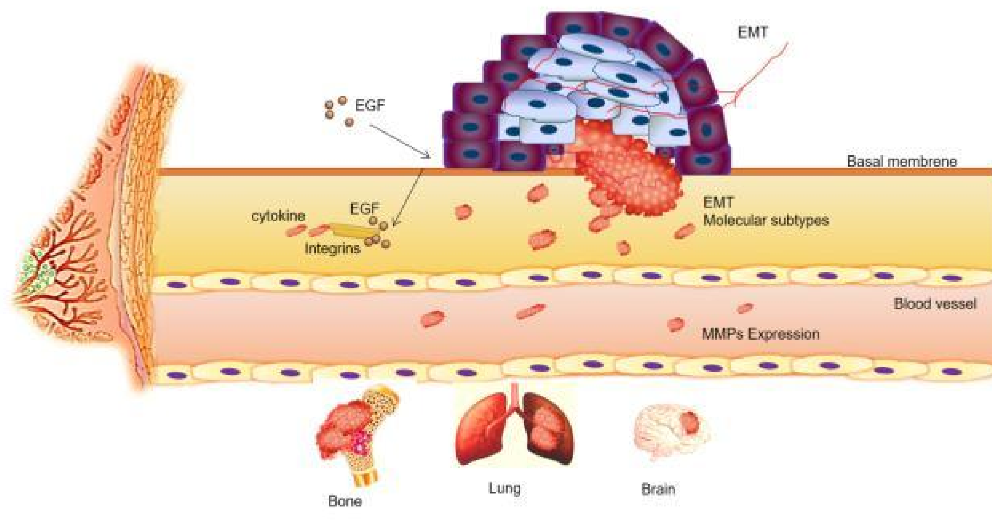


Figure 1.8: The flow diagram of early-stage breast cancer metastasis formation. Breast cancer metastasis may be a multi-step process, including invasion through the basement layer, intravasation into the vascular framework, circularization, extravasation to the target organ, and colonization. In addition to the beginning driving oncogenic mutations, stromal cells in the microenvironment help tumour cells obtain the capacity to invade and pre-select cells with diverse preferences to far-off organs.

1.3.1.6. Inflammatory breast cancers

Inflammatory breast cancer (IBC) is an uncommon and aggressive type of invasive breast cancer due to its rapid proliferation that comprise 1% – 6% of all breast cancers (Hance et al. 2005). IBC has different symptoms, prognosis, and treatment from other type of breast cancer. Where, often a lump absents in breast and may not identified by mammograms. It's located in where the breast cancer cells grow into the skin at

advanced stages of cancer and makes more difficulty to treat successfully (Hulka 1996; Hance et al. 2005; Polyak 2007; Colditz et al. 2012).

1.3.1.6.1. Inflammation in breast tumoural microenvironment

IBC is the diffusion of tumour cells in the form of tumour emboli into the dermal–lymphatic system. The development of tumour emboli involves various processes. Early, migrates cumulative cells as clusters and direct exposure to the immune cells within the vessels that stimulates invasion into the ductal system (for example, mediated by the RHOC GTPase). After, the ample formation of blood and lymphatic vessels, that bind grouped cells to allow them for survive and metastasis. Then, a cell–cell contact between cancer cells or between cancer cells and tumour microenvironment (TME) type of cells which requires for the formation of the unique emboli structure. Therefore, tumour emboli are an end product of a well-arranged and unique ability of both IBC cells and the aberrant immune and/or angiogenic microenvironment (Vermeulen et al. 2009; Sauer et al. 2017).

However, IBC have more interest to Tumour-associated macrophages (TAMs) (Fig. 9) and fibroblast, that responsible to secrete cytokines, chemokines, enzymes and alternatively activates M2 macrophages and contribute to cell grow, tumour progression and invasion in breast cancer (Schmidt et al. 2012; Su et al. 2014; Noy and Pollard 2014). Notably, Cancer-associated fibroblasts (CAFs), the major stromal cells that contribute to the TME in IBC and help to promote cancer metastasis (Figure 9).

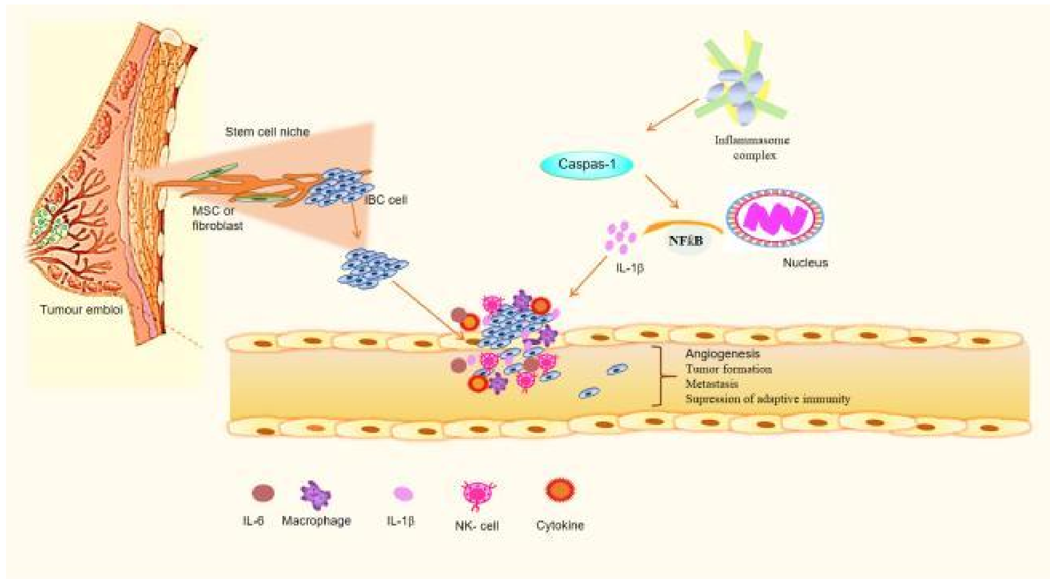


Figure 1.9: Hypothetical role of inflammation in breast cancer. Different members inside the tumour microenvironment connected with inflammatory breast cancer (IBC) cells specifically and/or indirectly, advancing stemness, and metastatic and invasion potential. NLRP3 inflammasome leads to activation of caspase-1 (CASP1), which at that point cleaves the pro-forms of interleukin-1beta (IL-1β) and interleukin- 6 (IL6), coming about within the emission of biologically active cytokines, coming about in pyroptosis through the arrangement of pores at the plasma layer. Inflammasomes are activated through different components and discharge IL-1β and IL-6 to start inflammation, tumour formation, angiogenesis.

Recently, *in vivo* study showed that the CAF-derived cytokine chitinase 3-like protein 1 (CHI3L1) increases in the stroma of metastatic loci and several exosome miRNAs (for example, miR-9) secretes from breast cancer cells that contribute to the differentiation of fibroblasts into CAFs (Baroni et al. 2016; Zhang et al. 2017; Cohen et al. 2017). What's more, the interleukin IL-1 family plays vital roles in tumoral immunity including seven ligands with pro-inflammatory activity (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, IL-36γ). Whereas IL-1α and IL-1β are bind to the same receptor (IL-1R) and recruit the IL-1R accessory protein that activates a cascade of

immune and inflammatory genes. Furthermore, chronic inflammation occurs through the activation of NLRP3 inflammasome; a multiple-protein complex also help to activate caspase 1, that responsible to cleaves pro-IL-1β and pro-IL-18 and generates the mature forms of these inflammatory cytokines, IL-1β and IL-18 as well as triggering the release of pro-inflammatory cytokines (Latz et al. 2013; Mantovani et al. 2018) and helps breast cancer progression (Figure 9). Accordingly, lymphatic endothelial cells within IBC tumours that supports the active participation of endothelial cells in the TME of IBC and the angiogenesis-mediating factors VEGF and platelet-derived growth factor (PDGF) are secreted from endothelial cells and promote the formation of vessels around IBC cell (Colpaert et al. 2003).

1.3.1.6.2. Pathway-mediated transmission

Intrinsic and extrinsic components of IBC can also communicate via the activation of pathways that have critical biological effects on both the tumour and the TME. These pathways can be activated intrinsically in tumour cells or extrinsically in the TME. Also, intrinsically activated pathways in tumour cells can restructure the composition of surrounding cell types within the TME.

1.3.1.6.3. Breast cancer and NFκB signalling

The transcription factor, nuclear NFκB pathway a major inflammation-mediated pathways by modulating several genes responsible for the generation of pro-inflammatory mediators and cytokines. The NF-κB pathway mediates the crosstalk between inflammatory pathways within the TME in IBC, that might lead to the emergence of resistance to chemotherapy and endocrine therapy via evasion of appropriate apoptosis (Drygin et al. 2011).

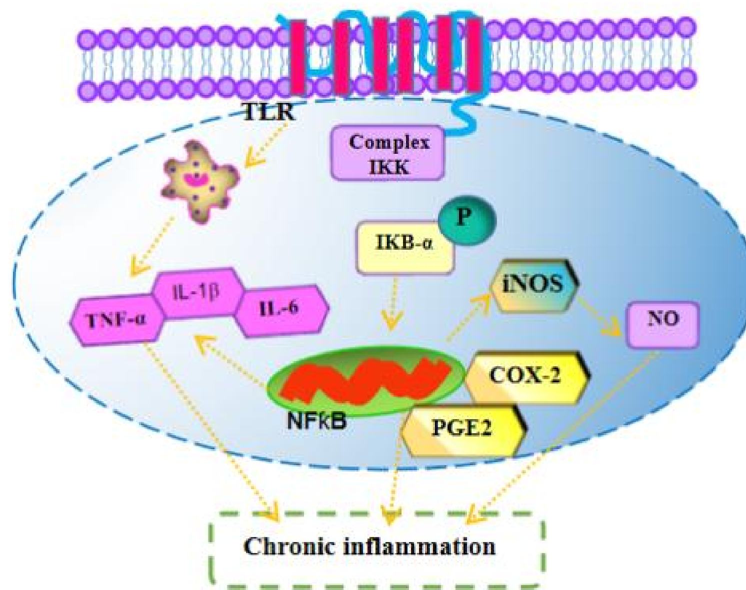


Figure 1.10: Inflammation development in NFκB signalling pathway in breast cancer.

However, in respose of multiple pro-inflammatory stimuli, inhibitor of kappa B (IκB) is degrading by the ubiquitin-proteasome pathway and inducing the translocation of NFκB subunits into the nucleus. Where they bind to the promoter regions of target genes, including COX-2, iNOS, IL-1β, and TNF-α and stimulate transcription genes that encode cytokines, chemokines, growth factors, and anti-apoptotic factors. In addition, activated NFκB pathway also boost cell proliferation, anti-apoptosis, genomic instability, glycolysis, resistance to chemotherapy, radiotherapy and develop invasive breast cancers (Wang et al. 2015)(Figure 10).

1.3.1.6.4. Breast cancer and EGFR pathway

Epidermal growth factor receptor (EGFR) is over expressed among all subtypes of breast cancer, specially in IBC (Cabioglu et al. 2007). For example, up regulating EGFR signalling from the TME increases of skin invasion and metastasis in the SUM149 xenograft model with IBC breast cancer and establishes a strongly

correlated between the stromal and tumour cells in IBC tumour samples (Lacerda et al. 2015). Moreover, upregulation of EGFR observes in 30% of IBC patients through immunohistochemical staining. In addition, EGFR-expressing IBC associates with a truly poor 5-year overall survival rate than EGFR-negative IBC. Therefore, over expression of EGFR indicates increase the risk of IBC (Cabioglu et al. 2007).

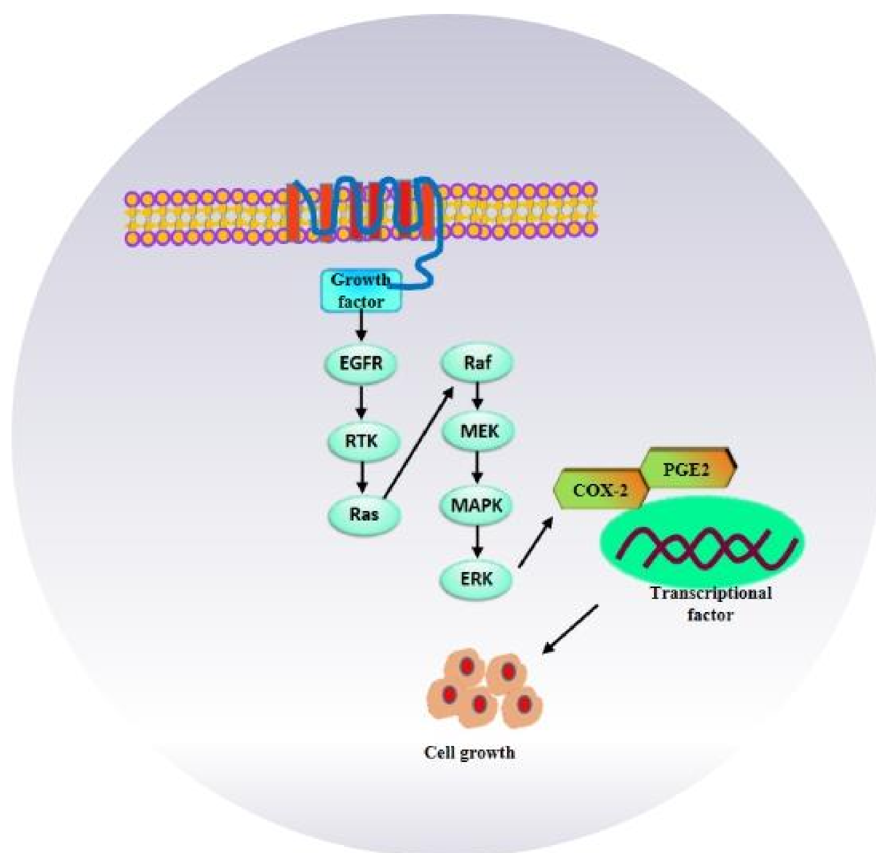


Figure 1.11: Cell growth in EGFR signalling pathway in inflammatory breast cancer (IBC).

Surprisingly, there is a positive correlation between CSC regulation in IBC and EGFR/COX-2 signalling axis (Figure 11). Furthermore, Nodal (a member of the TGFβ superfamily), is a promising driver of EGFR/COX2 pathway that regulate regulated invasive capacity and the CSC phenotype of IBC cells (Wang et al. 2017). EGFR may represent a potential therapeutic target in IBC and EGFR tyrosine kinase inhibition can be changed the phenotype of IBC cells from mesenchymal to epithelial

and inhibited IBC tumour growth and metastasis. Thus, clinical samples from an ongoing clinical trial evaluating EGFR-targeted agents in IBC will provide further evidence of the biological effects of the EGFR pathway (Zhang et al. 2009).

1.3.1.6.5. Breast cancer and PTGS2 pathway

Prostaglandin-Endoperoxide Synthase 2 (PTGS2) pathway also known as cyclooxygenase 2 (COX2) pathways which has prominent contributes to both intrinsic and extrinsic pathways in IBC, that which mediates inflammation. Over expression of COX2 are found in IBC tumour than non-IBC tumour (Kim et al. 2012). Also, COX2 responsible to cancer stemness characteristics, including invasion, migration, EMT and metastasis in breast cancers. The increase expression of COX2 and prostaglandin E2 (PGE2) assists to formation of chronic inflammation and promotes the invasion and metastasis of breast cancer cells (Ristimäki et al. 2002; Wang et al. 2017) (Figure 10).

Besides, COX activity in tumour cells is reported as a key suppressor of type I interferon-mediated and T cell-mediated tumour suppression, and COX-dependent immune evasion is showed to be critical for tumour growth in colorectal cancer, melanoma, and breast cancer models (Zelenay et al. 2015). Whereas activates of the COX2 pathway promotes activation of the protein kinase A signalling pathway and transcription of the aromatase gene CYP19 that up regulates expression of aromatase and the progesterone receptor in the breast tissues in overweight or obese women. Down regulation of the COX2 pathway, including the PGE2 receptors may be a potential target for breast cancer prevention (Lee et al. 2013).

1.4. Risk factors

Recent evidence demonstrate that some established factors are associated with breast cancer risk such as age, early age at menarche, late menopause, height, post-menopausal obesity, family history of breast cancer, oral contraceptives, hormonal replacement therapy, mammographic density, some gene mutations, and clinical conditions are associated with an increased risk of breast cancer.

1.4.1. Endogenous hormonal factors

Several studies demonstrated that the onset of the first period before the age of 12 raise the risk of breast cancer (Clinton et al. 2020) (Figure 12). Based on biological, this association corresponds to the early and prolonged exposure to the hormonal impregnation that exists during the period of activity of the ovaries. Thus, this exposure is appreciable when menstrual cycles are regular. Usually, women who have high level of estrogen levels they show early period (Key et al. 2001).

Further, late menopause of women can be increased risk of breast cancer, compared with early menopause of women. Due to prolong production of ovarian hormones causes of late menopause within women. The risk of breast cancer raised approximately 3% per year from the presumed age of menopause. This association between age and breast cancer risk is similar when menopause occurs naturally (Beral, V. et al. 2004).

1.4.2. Exogenous hormonal factors

Using oral contraceptives increases approximately 25% risk of breast cancer in women. However, the risk of breast cancer does not change significantly with duration of use and is independent of the type of estrogen or the combination of

preparations used. Oppositely, contraceptives show late effects in reproductive life and develops the risk of breast cancer when the natural risk becomes appreciable. Thus, the garter birth control pills are used, the greater the number of resulting breast cancer cases (Beral, V. et al. 2004).

According to Women's Health Initiative Investigators 2002, Hormone replacement therapy (HRT) increases risk of breast cancer in women approximately 26% to 35%, compared to women who have never used it and the risk of breast cancer increases with duration of use. Furthermore, long term uses also a factor that increases the risk is respectively 2, 6 and 12 cases per 1,000. Moreover, women, who using an estrogen-progestogen combination, risk increased by 30% than who receiving estrogen therapy alone (Key et al. 2001).

1.4.3. Reproductive factors

However, first pregnancy after the age of 30 have, on average, a 25% higher risk of breast cancer with respect to nulliparous women. Thus, different ways can be influenced the risk of breast cancer are known or suspected. Furthermore, the reproductive period seems to have a dual effect; the risk is raised immediately after childbirth, after gently reducing. Pregnancy causes accelerated differentiation of breast tissue and rapid proliferation of epithelium. The changes initiated during the first pregnancy, particularly if it occurred early, are accentuated by each subsequent pregnancy, and the development of breast cancer is linked to the rate of proliferation of mammary epithelial cells and inversely to the degree of differentiation (Layde et al. 1989; Russo et al. 2000; Hinkula et al. 2001)

Further, in case of natural breastfeeding 33% reduces breast cancer risk, compared to women who never breastfed. Where, lactation helps reduction of estrogen hormone

and increases pro-lactin production in women and suppress the appearance and development of breast cancer (Key and Pike 1988; Beral, V. et al. 2004).

1.4.4. Family history and genetic mutations

Another risk factor is family history, is consistently associated with an increased risk of breast cancer (Figure 12). Women, who have previously breast cancer history in their family (for example, mother, sister, and grandmother) who have more the risk to develop breast cancer. Furthermore, genetic mutations of BRCA1 and BCRA2 gene increases the risk of breast cancer in women. The fact of having the same environment, the same lifestyle and a common genetic heritage, added to the genomic instability related to mutations, would partly explain the increased risk of breast cancer associated with family aggregation and genetic mutations (Pharoah et al. 1997).

1.4.5. Age and Sex

Besides sex, aging is one of the most important risk factors of breast cancer (Figure 12), because the incidence of breast cancer is highly related to the increasing age between 50 and 75 years. In 2016, approximately 99.3% and 71.2% of all breast cancer-associated deaths in America were reported in women over the age of 40 and 60, respectively. Therefore, it is necessary to have a mammography screening in women after aged 40 or older (Boyd et al. 1998; Siegel et al. 2018).

1.4.6. Lifestyle

Modern lifestyles for example too much dietary fat intake and alcohol consumption can be increased the risk of breast cancer (Figure 12). Alcohol consumption can increase the level of estrogen-related hormones in the blood and trigger the estrogen

receptor pathways. A meta-analysis-based study shows that excess intake of alcohol per day can increase the risk of breast cancer by 32%. The modern western diet contains too much fat and excess intake of fat, mainly saturated fat, is associated with mortality (RR=1.3) and poor prognosis in breast cancer patients. Even though the relationship between breast cancer risk and smoking remains controversial, mutagens from cigarette smoke have been detected in the breast fluid from non-lactating women. The risk of breast cancer increases in women who both smoke and drink (RR=1.54). Previous evidence explains that smoking, especially at an early age, has a higher risk of breast cancer occurrence (Makarem et al. 2013; Jung et al. 2016; Knight et al. 2017; Kispert and McHowat 2017). Avoidance behaviour of modifiable risk factors depends on lifestyle and socio- economic background and has been associated with the knowledge of women about breast cancer.

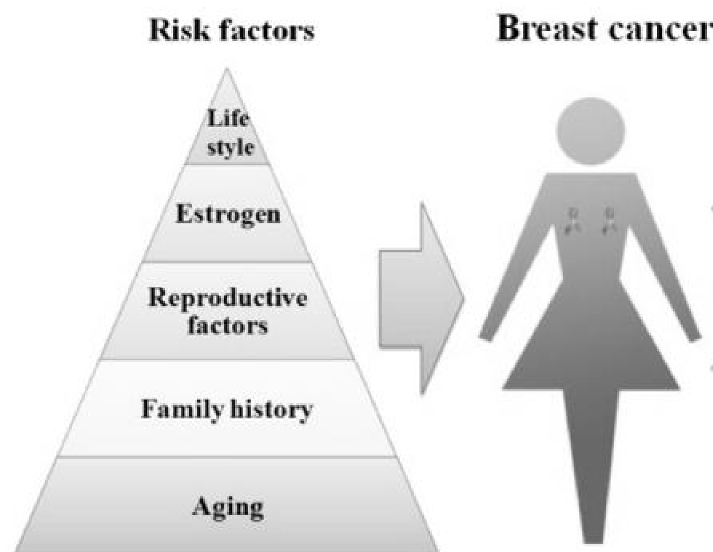


Figure 1.12: Schematic diagram of risk factors of breast cancer. Age, family history, reproductive factors, estrogen and lifestyle are five important risk factors of breast cancer, represented in the pyramid chart.

1.5. Breast cancer and Reactive Oxygen Spices

The part of receptive oxygen species (ROS) and oxidative stress in tumorigeneses is questionable, as they have been appeared to have both tumour-promoting and tumour-inhibiting properties.

Although low to moderate ROS levels can be advantageous to cells by inducing DNA changes and pro-oncogenic signalling pathways where, initiation and development of breast cancer is supported by increasing ROS, that attack DNA and may be induced DNA damage that in turn can either hinder or induce transcription, replication errors, genomic instability such as BRCA1 gene down regulate cellular levels of ROS and signal transduction pathways. These conflicting impacts have vital implications for potential anticancer strategies that point to modulating levels of ROS (Valko et al. 2006; Saha et al. 2009; Gorrini et al. 2013). Control of redox homeostasis is exceptionally important because expanded oxidative stress takes part in all stages of carcinogenesis (initiation, promotion, and progression). Numerous signalling pathways that are connected to tumorigenesis can also control the metabolism of receptive oxygen species (ROS) through direct or indirect mechanisms.

1.6. Breast cancer and Apoptosis

Evasion of apoptosis could be a trademark of cancer. Recently, considerable attention has been focused on the role of apoptosis process in mediating the lethal effects of anti-neoplastic agents in breast cancer cells, and in particular on the sequence of events referred to this process. Apoptosis is characterized by a series of cellular events that are typical of this process, such as membrane blebbing, cell shrinkage, chromatin

condensation and formation of a DNA ladder with multiple fragments caused by inter nucleosomal DNA cleavage (Steller 1995; Rozeboom et al. 2019).

However, pro-apoptotic and anti-apoptotic signals are strongly directed in typical breast epithelial cells. Where, dysregulation of this balance is required for breast tumorigeneses and increments obtained resistance to treatments, counting molecularly focused on treatments, radiation, and chemotherapies. The pro-apoptotic or anti-apoptotic Bcl-2 family individuals connected with each other to preserve mitochondrial judgement and direct cellular commitment to apoptosis. Among the anti-apoptotic Bcl-2 family individuals, Mcl-1 is extraordinarily directed by various oncogenic signalling pathways (Williams and Cook 2015).

A novel combination treatment that can initiate tumour cell apoptosis, a basic cellular mechanism by delaying tumour movement (Rozeboom et al. 2019). Conceivable apoptotic pathways actuated in breast cancer where, apoptosis may be executed through the intrinsic and extrinsic pathways. Within the inherent apoptotic pathway, the pro-apoptotic signals such as Bax and Bak translocate to the mitochondria, coming about in mitochondrial membrane permeabilization. This in turn gives a route for the discharge of intermembrane space proteins such as Cytochrome c (Cyt c), and Smac/DIABLO into the cytosol. Once within the cytosol, Cyt c advances the arrangement of the apoptosome for the actuation of caspase 9. In turn, dynamic caspase-9 catalyzes the proteolytic activation of caspase-6 and/or-7 which leads to actuation of caspase-8 and consequent passing signals and chromatin condensation. Within the extrinsic pathway, pro-caspase-8 is cleaved and actuates downstream executioner caspases-7, and-6 which leads to actuation of passing substrates, chromatin condensation, and cell passing. Loss of mitochondrial transmembrane

potential and discharge of Cyt c has been appear but is not entirely essential for this apoptotic pathway to initiate cell passing. Crosstalk between the extrinsic and intrinsic pathways happens when activation of caspase-8 includes mitochondria-dependent signalling and results in cleavage of the pro-apoptotic Bcl-2 family protein Bid to t-Bid. Translocation of t-Bid to the mitochondria is accepted to be one of the signals for mitochondrial occasions during apoptosis. This results in translocation of Cyt c, arrangement of the apoptosome, and enactment of caspase 9. After, successful enactment of caspase-9, cells undergo apoptosis and the downstream inhibits caspases-7, and -6 which leads to activation of death substrates, chromatin condensation, and cell death. Smac/DIABLO advance apoptosis indirectly by binding to and antagonizing individuals of the inhibitor of apoptosis protein (IAP) family. (Simstein et al. 2003; Hu and Kavanagh 2003; Bayir and Kagan 2008).

Oppositely, activated Akt represses apoptosis by avoiding cytochrome c discharge, phosphorylating B-cell lymphoma 2 related passing promoters (Bad) and expanding NFκB. Furthermore, Akt stimulates cell expansion through blocking kinase movement by diminishing GSK3β which diminishes Cyclin D1 degradation and raises β-catenin collection. Moreover, AKT-mediated phosphorylation of tuberous sclerosis (TSC) discharges TSC-inhibition of the GTPase, which actuates the mammalian target of rapamycin complex 1 (mTORC1) kinase. Actuated mTORC1 phosphorylates eukaryotic interpretation start figure 4E-binding protein 1(4E-BP1) and ribosomal protein S6 kinase beta-1 (P70S6K) which permits protein interpretation start and prolongation (Huang and Chen 2009; Zhang et al. 2013)

1.7. Breast cancer and Angiogenesis

An angiogenic infection is depicted as either an excessive or lacking development of micro vessels. At first, angiogenesis involves in cancer, arthritis, and psoriasis. Be

that as it may, the impact it has on several other diseases has been described (Tímár et al. 2001) The nature and composition of tumours make them inherently prime for compelling angiogenic development. An active vascular system is made up of adipose tissue, enveloped by stromal cells which gives it a supporting system for the tumour's vascular system to develop. In *in vivo* studies, white fat tissue supports the growth of the new vasculature and bolsters of the development and movement of breast cancer in mouse models, (Bertolini et al. 2015) and the brown fat tissue (made up of cells with various mitochondria) supports for the tumour development by giving a constant supply of oxygen and supplements (Christiaens and Lijnen 2010). Further, both types of adipose tissues can produce angiogenic factors, most commonly VEGF A, B, and C, basic fibroblast development factor (bFGF)/FGF-2; matrix metalloproteinases (MMPs); and IL-8, factors associated with breast cancer (Yoshida et al. 1997; Christiaens and Lijnen 2010). This aberrant growth of blood vessels presently involves in many life-threatening and impairing diseases conditions, such as cardiovascular illness, cancer and diabetics (Lu et al.,1992; Qi et al., 2003; Voelkelet al., 2002; Joukov et al., 1996).

However, anomalous angiogenesis is responsible for developing breast cancer metastasis. (Lu et al. 2020) (Figure 12). Angiogenesis includes a coordinated regulation of a few vascular growth factors, such as fundamental fibroblast development calculate (bFGF), transforming growth factor beta-1 (TGFβ-1), platelet-derived EC growth factor, placenta growth factor, and a few other development components,(Voelkel et al. 2002; Qi et al. 2003) and clinical studies have appeared shown appeared that it plays a basic role in breast cancer movement and metastasis (Joukov et al. 1996). These growth factors are communicated and/or upregulated in aggressive human breast cancers, and among these growth factors, the expression of

VEGF and its diverse isoforms has been characterized as the most significant in breast cancer, (Relf et al. 1997) although low-level protein expression has been identified in a healthy human mammary organ (Berse et al. 1992).

VEGF and interleukin-8 (IL-8) highly express in breast cancer cell lines, recommending that they plays very pivotal parts within the advancement of angiogenesis in breast cancer angiogenesis (Marjon et al. 2004; Chelouche-Lev et al. 2004). A high level of VEGFR-3 expression recognizes in invasive breast cancer that up regulates in the endothelium of angiogenic blood vessels (Valtola et al. 1999). The interaction between VEGF-A and VEGFR-1 or 2 is intricately included in breast cancer development, progression, and metastasis (Guo et al. 2010; Lushnikova et al. 2010; Linardou et al. 2012; Madu et al. 2020).

One of the prognostic markers for survival is the level of angiogenesis in breast cancer. (Weidner et al. 1991). A high level of angiogenic growth factors within the breast cancer cells relates to the aggressiveness and chance of invasive breast cancer,(Hanahan and Folkman 1996; Linderholm et al. 1999; George et al. 2001) and it's connected to p53 genes inactivation. Besides, the number of micro vessels in invasive breast cancer from surgical tests may be an indicator of metastasis or relapse (Weidner et al. 1991). Considers that, for tumour advancement and metastasis occur by angiogenesis within the tumours must include an transaction of a few or all these growth factors VEGF, IL-8, essential basic fundamental essential fibroblast growth factor (bFGF/FGF-2), and matrix metalloproteinases (MMPs) (Liekens et al. 2001; LI et al.). Interleukins are a bunch of proteins and signal particles, for the most part called cytokines, and to begin in leukocytes (Brocker et al. 2010). They are discharged by cells as an immune response to different pathological stimuli. IL-8 can

be a part of the IL family that's delivered by macrophages, aviation route smooth muscle cells, tumour cells, and other cell types (Koch et al. 1992; Norgauer et al. 1996; Wolff et al. 1998; Hedges et al. 2000) and has been reported to excite the generation of VEGF in ECs by binding with its receptor, and in this manner activating VEGF receptors (Martin et al. 2009) IL-8 incorporates a coordinate impact on angiogenesis by improving the proliferation and survival of EC, upregulating lattice metalloproteinases in certain EC lines, (LI et al.) (and stimulating the arrangement of capillary tubes *in vitro*. All these are basic features of breast cancer progression and metastasis (Bar-Eli 1999; Marjon et al. 2004; Lin et al. 2004; Razmkhah et al. 2010). Furthermore, breast tumours with upregulated IL-8 levels have been observed to be more forceful and invasive, making IL-8 levels an appealing target for anti-angiogenic medicines, (Liekens et al. 2001; Chelouche-Lev et al. 2004) and a potential prognostic biomarker for different cancers, including breast cancer (Liekens et al. 2001). Fibroblast growth factors (bFGF/FGF-2) are collectively a family of. effective angiogenic stimulators connectes to breast cancer risk (Vlodavsky et al. 1990; Fang et al. 2003; Sørensen et al. 2006; Slattery et al. 2013). Substances can modulate the interactions between FGF-2 and its receptor within the extracellular environment driving the direction of angiogenesis, consequent tumour movement, and metastasis. (Yiangou et al. 1997; Fang et al. 2003; Brady et al. 2013) (Figure 9). MMPs have a place in a bigger family of proteases (Brady et al. 2013) included in angiogenesis due to their ability to degrade extracellular matrix proteins, and thereby redesign the extracellular network. They are primarily included in the destabilization of the existing blood vessel wall, degradation of network proteins, and migration of ECs- stages that have been depicted as the start process of angiogenesis.(WOESSNER 1993; Heppner et al. 1996). Therefore, antiangiogenic treatments that have been approved for clinical utilize target these pro-angiogenic growth factors, and/or their receptors,

cytokines, and proteases related to them. (Gasparini and Harris). During these tightly directed forms, a complex signal adjustment, between pro- and anti-angiogenic components, is forcefully maintained within the microenvironment, to create and stabilize the recently formed blood vessels. Various studies, hence, have confirmed that these angiogenic activators play a basic part in the improvement of tumours (Liekens et al. 2001; Papetti and Herman 2002) (Figure 13).

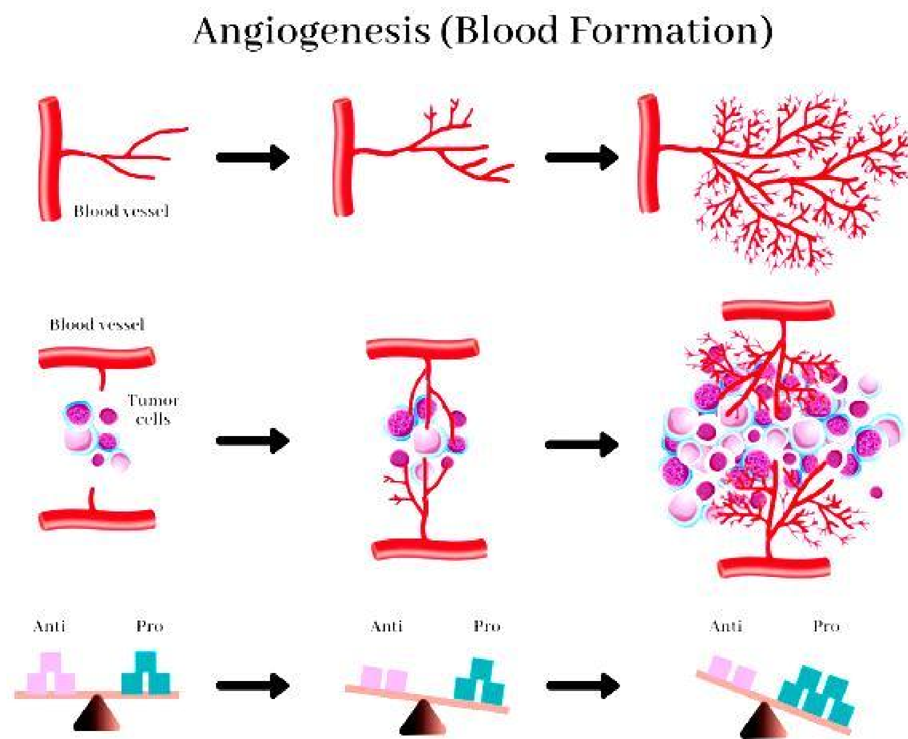


Figure 1.13: Angiogenesis, the physiological prepare by which improvement of new blood vessels from pre-existing vessels. New blood vessels form out of pre-existing capillaries. The new blood vessels, close and within the tumour, give it with basic nutrients for development. Angiogenesis in solid tissues is controlled by the adjust between anti- and pro-angiogenic factors (bottom), and this adjust is destroyed by the predominance of angiogenic components in tumours, resulting in anomalous structure and function of blood vessels and driving to hypoxia. This returns the adjustment and normalizes the vasculature. Figure adapted from (Singh et al. 1995).

1.8. Breast cancer and autophagy

1.8.1. Autophagy

Autophagy is a highly kept catabolic control mechanism accountable for the bulk degradation of cytoplasmic organelles and intracellular molecules in a lysosome-dependent manner to keep cellular homeostasis. Three common types of autophagy; microautophagy, macroautophagy, and chaperone-mediated autophagy are identified until now (He and Klionsky 2009; Lee et al. 2012) .

Shortly, autophagy is frequently activating as an adaptive survival response to diverse stress stimuli for example, nutrient deprivation is sensed by AMP-activated protein kinase (AMPK), dephosphorylate the serine-threonine protein kinase ULK1, that is the main part of the initiation of multi-protein complex comprising involving ATG13, ATG101 and FAK Family Kinase Interacting Protein of 200 kDa (FIP200). Afterwards, during the initiation of autophagy, ULK1 dissociates from the mammalian target of rapamycin complex 1 (mTORC1), leading to ULK1 autophosphorylation and at this moment enzymatically active and phosphorylates ATG13 (He and Klionsky 2009; Kim et al. 2011; Yu et al. 2018) (Figure 14A). Later, the autophagic process begins with formation of double membrene containing phagophors that further involves elongation and forms autophagosomes.

Afterwards, the ULK1 complex also phosphorylates Beclin1, leading to the recruitment of class III phosphatidylinositol 3-kinase (PI3K) Vacuolar Protein Sorting 34 (VPS34) and its activating kinase Vacuolar Protein Sorting 15 (VPS15), ATG14L and Beclin-1; forming a complex is involved in the nucleation of the phagophore (Hamacher-Brady 2012; Parzych and Klionsky 2014).

The elongation of autophagosomes needs two ubiquitin-like conjugation systems, ATG12-ATG5-ATG16L (Figure 14B) and LC3-II (LC3-I/phosphatidylethanolamine), that contribute to the continuation of the phagophore. Therefore, VPS34 starts phosphatidylinositol 3-phosphate (PI3P), that turns to bound by the WD-repeat protein Interacting with Phosphoinositide 1 and 2 (WIPI1 and 2) proteins that serve as adaptors for the ATG7-mediated formation of the ubiquitin-like conjugation ATG5–12-16L complex (Figure 14B). After that, the microtubule-associated light chain 3 (LC3) proteins (LC3A, LC3B, LC3C) and other ATG8 homologs, GABA Type A Receptor Associated Protein (GABARAP, GABARAPL1, GABARAPL2) are processed by ATG4 proteases into LC3-I (GABARAP-I). LC3-I is conjugated to phosphatidylethanolamine (PE) by the ATG3-7 and the ATG5-12-16L complex generating LC3-II (GABARAP-II), that incorporates into the phagophore membrane. This permits the elongation and closure of the phagophore that is giving rise to an autophagosome (Figure 14B,C) (Hamacher-Brady 2012).

At the same time as elongation, autophagosomes engulf intracellular constituents for example, organelles and protein aggregates and finally fuse with lysosomes to form autolysosomes where the content is degraded via hydrolysis. Then, the intracellular yields products are amino acids, glucose, and free fatty acids, after autophagic degradation which acts as energy substrates for macromolecule synthesis (He and Klionsky 2009; Mizushima and Komatsu 2011) (figure 14D, E).

In breast cancer, autophagy acts as a tumour-suppressor and tumour promoter, playing double roles that could be contributed to distinct context and stages of tumorigeneses.

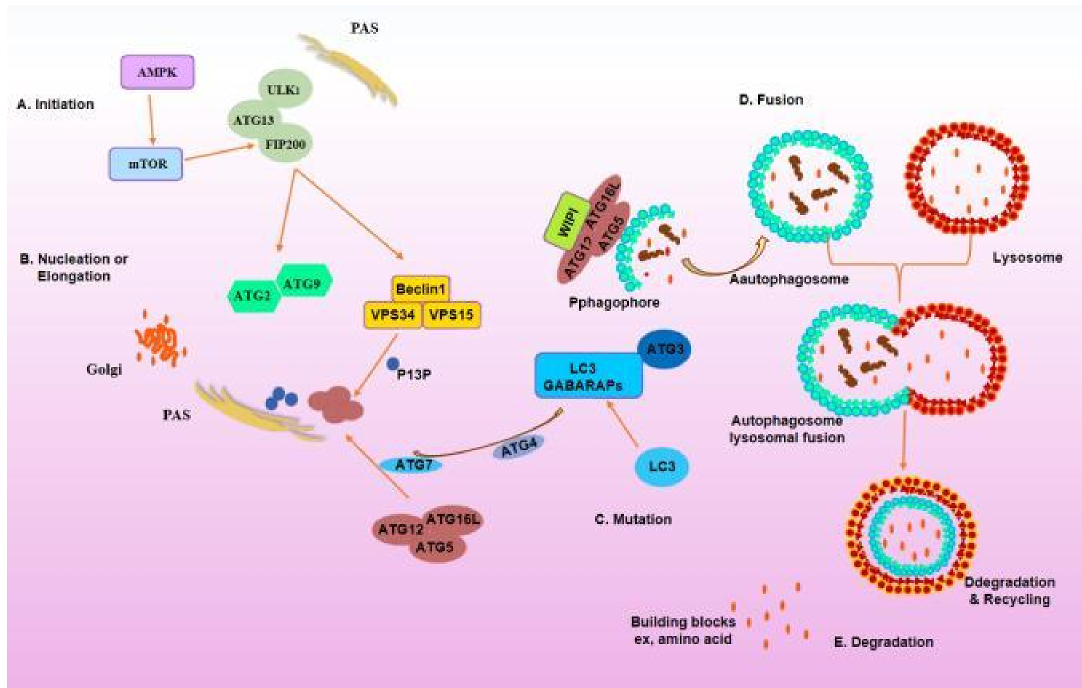


Figure 1.14: Autophagic pathway. The autophagic pathway can be separated into five primary steps: Start, Stretching, Development, Fusion, and Degradation. (A) During the start, push components to make ubiquitin-like complex by repressing mTOR. (B) The ubiquitin-like complex initiates and actuates the ATG9–ATG2 complex and the Beclin1 complex that start phagophore arrangement at the PAS. The Beclin1 complex further encourages layer elongation by selecting the primary ubiquitin-like conjugation system composed of ATG5, -12, and -16L. (C) During maturation, the moment ubiquitin-like conjugation system that incorporates LC3 and GABARAP-I by the ATG4 protease taken after by the conjugation and attached with AGT3 with LC3-II, GABARAP-II and joining into the confinement layer (IM) through ATG5/12 /16L and ATG3/7complexes, that leads to the Fusion and degradation of the autophagosome, subsequently consolidating cytoplasmic substance. (D, E) When in near vicinity, autophagosomes and lysosomes meld, and the cargo is corrupted into cellular building blocks (i.e., amino acids)

1.8.2. Autophagy induction in breast cancer

Thus, during tumour initiation, autophagy is thought to serve a beneficial tumour suppressing role via instance, binding inflammation, tissue damage, and genome instability, and via inhibition of oncogene-induced senescence, thereby restricting the invasion and dissemination of cancer cells from the primary site (Dong et al. 2013). In addition, while cancer cells overcome cytotoxic effect in response of anticancer agents due to dysfunction of apoptotic signals, autophagy induction shows therapeutic benefit such as, autophagy efficient drug-induced cell death in caspase-3 deficient in breast cancer by inducing cell cycle arrest through autophagy mediated down regulation of cyclin D1expression (Chen et al. 2019; Li et al. 2019). Moreover, HDAC inhibitors deals a potential candidate to induces autophagy in LC3B-dependent autophagy by activating cathepsin B and inhibits the survival of breast cancer MDA-MB-231 cells (Han et al. 2017). Several evidence demonstrates that natural bioactive compounds play an effective autophagy inducer. For example, grapes polyphenol resveratrol, curcumin, aaptamine, demethyloxyaaptamine, and isoaaptamine promotes autophagy by the down regulating of Wnt/β-catenin signalling pathway. Additionally, resveratrol significantly reduces the percentage of BCSCs in SUM159 and MCF_7 cell lines and inhibits mammospheres (Alayev et al. 2014; Fu et al. 2014; Guan et al. 2016; Wu et al. 2018). Similarly, flavonoids show anti proliferative effects via induction of autophagy by inhibiting Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit γ (PI3Kγ), that provokes the PI3K/AKT/mTOR/ Ribosomal Protein S6 Kinase (p70S6K)/ULK pathway in breast cancer cells (Park et al., 2016).

1.8.3. Autophagy inhibition in breast cancer

Inhibition of autophagy involves in different types of cancer including breast cancer where its play as a tumour-suppressive and tumour-promoting functions. For inhibiting autophagy, targeting PI3K/AKT/mTOR pathway is more potential in cancer. For instance, activates PI3K/AKT/mTOR pathway by down regulation of ETS transcription Factor ELK3 (ELK3), activates PI3K/AKT/mTOR pathway via inhibition of autophagy in MDA-MB-231 cells that increase chemosensitivity of anticancer drugs (Kong et al. 2016; Park et al. 2016a). Furthermore, reduction of cell viability by inhibiting autophagy via down regulation of genes ATG5, BECN1, or ATG7 and LC3genes in MCF_7 and T47D breast cancer cell lines (Qadir et al. 2008; Vazquez-Martin et al. 2009). In *in vitro* studies shows that up regulating autophagy inhibitor and ATG12 silencing reduces tumour proliferation and reduces cell viability, colony formation and migration in ER2+ SUM-190 basal breast cancer cell lines (Dziri et al. 2012; Vega-Rubín-de-Celis et al. 2018; D'Archivio et al. 2019). Interestingly, autoghagy potentially inhibits while autophegic gene Beclin1 bind with over expressive HER2 in breast cancer (Vega-Rubín-de-Celis et al. 2018). Finally, autophagy suppression leads to significant drug re-sensitization in different settings, indicating that autophagy acts as a survival mechanism to overcome the cytotoxic effect of chemotherapy or targeted cancer drugs or molecules, thereby supporting the translational use of autophagy-modulating components.

1.9. Garlic

Recent consideration paid to agricultural and nourishment crops especially those that combine nutritional and health benefits, so-called ''functional foods''. Among them, members of the sort of Allium, primarily garlic have gotten specific consideration as a

solidified source of functional ingredients (Kamanna and Chandrasekhara 1980; Dziri et al. 2012; Ritota et al. 2012; D'Archivio et al. 2019).

Table 1.1. Examples of different forms of garlic and their active compounds

Garlic form	Active compounds in garlic product
Raw garlic	Allicin and γ-glutamyl-S-allylcysteine
Garlic powder	Alliin and a small amount of oil-soluble sulfur
Garlic essential oil	Oil-soluble sulfur compounds (DAS, DADS, etc.)
Aged garlic extract	Mainly water-soluble compounds (e.g., SAC,
Garlicin®	Alliin, allicin, and other allyl thiosulfinates
Kyolic® supplement	Mainly water-soluble compounds, γ-glutamyl-S-

(Adapted from Farhat et al., 2021)

1.9.1. Garlic Origin and Geographical discrimination

Garlic (*Allium sativum L.*) is developed since ancient times and has been utilized for centuries as a nourishment condiment and both preventive and healing specialist in traditional medication. However, it should be noted that the content of these bioactive compounds can vary depending on the genotype, agronomic conditions, environmental factors, maturity, and post-harvest conditions (Hrbek, et al., 2018). Also, the elemental composition of plants reflects the soil from where these are grown. Classification of garlic according to the cultivars and the topographical origin was endeavoured to utilize compositional information given by 1H high resolution magic angle spinning-nuclear magnetic resonance (HRMAS-NMR) spectroscopy (Ritota et al. 2012), high resolution mass spectrometry (Hrbek et al. 2018), fourier transform

infrared spectroscopy (Montaño et al. 2011), high performance liquid chromatography (Montaño et al. 2011). The over studies demonstrated the unique mark capacity of the organo-sulfur compounds and other metabolome components, counting natural acids, sugars, fatty acids and amino acids.

Garlic samples cultivated in various countries that differentiates with good accuracy by comparing the trace metal profile of the sample with authentic reference samples. The mineral composition of garlic similar where it's cultivates rather than the origin of the bulb, that supports the close connection between garlic components and that of the growing soil. Although trace metal data are useful to establish the country where garlic is cultivated to enforce existing importation laws and regulations, the discriminatory power of mineral composition for tracing local garlic varieties cultivated in specific regions. Regarding this, they found the great potentiality of the mineral composition for the geographical discrimination of garlic such as the discriminant chemical species (Ba, Ca, Fe, Mg, Mn, Na and Sr) and also garlic cultivated in SU and CL or PR and PR sites, they reflect the greater similarity in the soil characteristics of these territories (D'Archivio et al. 2019) (Figure 15).

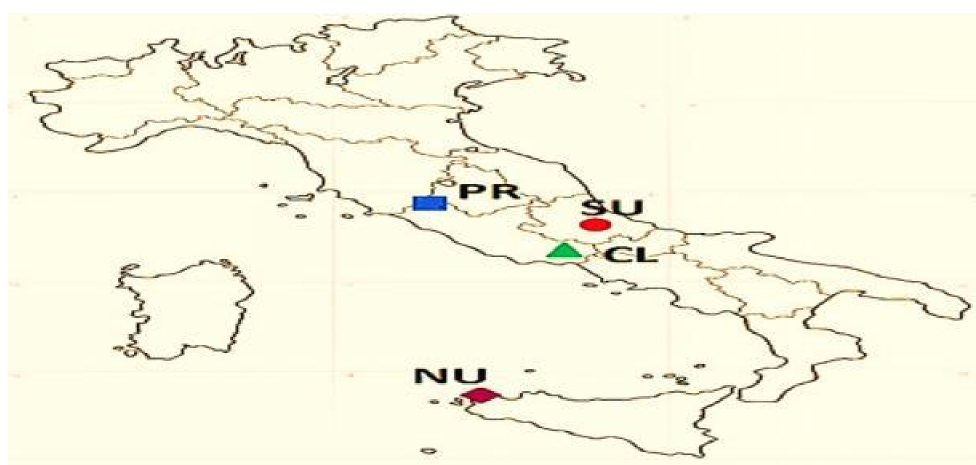


Figure. 1.15. Geographical origin of the Italian red garlic samples: Sulmona (SU), Proceno (PR), Castelliri (CL) and Nubia (NU). Adapted from D'Archivio et al., 2019.

In addition, this geographical variation can be seen in also for polyphenol in garlic. The high quantity of polyphenols obtained from the the Australian grown garlic cultivars which exhibits a potent antioxidant capacity and stronger antimicrobial activity than the commercial non-Australian grown garlic (Phan et al. 2019). further, total phenolic content differs from the different garlic cultivars grown at four locations in Andalusia and Spain (Agarwal, 1996).

1.9.2. Bioactive profile of Garlic

Garlic is covered as a functional flavor because of its differing cluster of wholesome constituents, phytochemicals, and fiber. It contains high levels of potassium, phosphorus zinc, and sulfur, moderate levels of selenium, calcium, magnesium, manganese, press, and low levels of sodium, vitamin A and C, and B-complex (Agarwal 1996). Within the last long time, impressive consideration has been given to its fundamental bioactive compounds, especially polyphenols, flavonoids, flavonols, tannins (Gorinstein et al. 2008), saponins (Diretto et al. 2017), polysaccharides (Wang et al. 2018), sulfur-containing compounds (counting alliin, allicin, ajoene, allylpropyl disulfide, DATS, S-allylcysteine, vinyldithiins, SAMC), proteins (like allinase, peroxidase, myrosinase), and other compounds, such as β-phellandrene, phellandrene, citral, linalool, and geraniol (Setiawan et al. 2005). There are more than twenty well-known polyphenolic compounds in garlic, counting kaempferol 3,7-di-O-rhamnoside, kaempferol-3 glucuronide, kaempferol-3-O-glucoside, kaempferol-3-O-beta-D-glucoside-7-O-alpha-L-rhamnoside, luteoline, and apigenine (Dziri et al. 2012). In addition, it contains 17 amino acids with eight fundamental amino acids (Fenwick et

al. 1985). For the most part, bioactive compounds are shown in intact garlic, but, after chopping or smashing, a better number of compounds, such as allicin, DAS, Fathers, dithiins, and ajoene have been found after distinctive sorts of chemical responses (Londhe et al. 2011; Rana et al. 2011).

1.9.3. Chemical composition of garlic

1.9.3.1. Proximate composition

Nutritional significance based on the content of protein, carbohydrates, fats, and minerals ordinarily evaluated by proximate compositional analysis. According to previous investigations, proximate composition of garlic contains moisture contents 64.58 %, crude protein 7.87 %, crude fat 0.52 %, crude fiber 2.3 %, ash 2.46 % and NFE 22.27 %, based on dry matter (Sajid et al. 2014) (Figure 2). Also, comparative studies between Multi-clove and Single clove garlic in Bangladeshi Indigenous shows that Imported Large Multi clove garlic cultivator have moderately high in protein, fat and ash substance, while indigenous multi clove garlic is composed of more prominent carbohydrate and total energy value (Lina et al. 2020).

Table 1.2. Proximate composition of garlic

Parameter	Quantity (%)
Moisture	64.58± 2.06
Crude protein	7.87±0.32
Crude fat	0.52±0.01
Crude fiber	2.3±0.08
Ash	2.46±0.09
NFE	22.27±0.95

Data are collected from Sajid et al., 2014

1.9.3.2. Mineral composition

Minerals play a critical part in human biological system through holding typical metabolism, ensuring the intemperate generation of free radicals, controlling the circulatory system and generation and catalyst distinctive biochemical responses. Hence it is vital they are provided by diet (da Silva et al. 2016). Extensive research has been carried out to estimate the number of mineral elements present in garlic. According to the previous data, mineral profile of garlic (Table 3) mention that it composed of potassium as a crucial mineral in a maximum quantity followed by phosphorous, iron, calcium, sodium and magnesium respectively. Furthermore, other minerals like zinc, manganese and copper were present in lowest quantities respectively (Sajid et al. 2014) which make garlic a potential controller of blood pressure, liquid balance, anti-cardiac arrhythmias, anti-sudden cardiac death, anti-hypertension, anti-atherogenesis, anti-ischemic heart malady, anti-diabetic and fundamental in teeth and bone formation (Karppanen 1991). Therefore, low amount than normal dietary, intake of Mg has been recognized as a strong risk factor for hypertension, ischemic heart illness, atherogenesis, cardiac arrhythmias, and sudden cardiac death (Altura and Altura 1991). Chromium (Cr) and Zinc (Zn) acts as a co-factor for insulin that makes garlic pertinent to the treatment of diabetes (Kimura 1996) whereas, P, Ca and Mg are basic for bone and teeth arrangement (Okwu and Omodamiro 2005). The non-detection of Pb and Co is of extraordinary advantage to consumers of garlic as these components can be profoundly toxic even at low concentrations (Oloyede 2005)

Table 1.3. Mineral composition of garlic.

Mineral	Concentration mg/100g
Potassium	54.65±1.74
Calcium	19.83±0.83
Phosphorus	9.54±0.34
Iron	4.21±0.15
Sodium	4.1±0.18
Magnesium	3.97±0.13
Zinc	0.34±0.01
Manganese	0.016±0.00
Copper	0.012±0.00

Data are collected from Sajid et al., 2014

1.9.3.3. Organosulfur profile

The most important intial organosulfur compounds presents in intact garlic clove is alliin (S-allylcysteine sulfox-ide) that greatly differ from that present in garlic juice obtained after crushing garlic. However, the whole bulbs consist of non-volatile γ-glutamyl-S-alk(en)yl-l-cysteines, namely, γ-glutamyl-S-trans-1-propenyl-l-cysteine, γ-glutamyl-S-allyl-l-cysteine, and S-alk(en)yl-l-cysteine sulfoxides such as S-(trans-1-propenyl)- l-cysteine sulfoxide (isoalliin), S-allyl-l-cysteine sulfoxide (alliin), and S-methyl-l- cysteine sulfoxide (methiin) (Block 1992; Amagase 2006). Besides, cutting or crushing garlic cloves discharges allinase enzyme sequestered in the vacuoles, which encounters cytosolic alliin to transform it into thoisulfinates of which the most prominent is allicin. Therefore, the highly unstable, reactive, and volatile allicin

decomposes to yield a large number of sulfides such as diallyl disulfide (DADS), diallyl trisulfide (DATS), diallyl sulfide (DAS), methyl allyl sulfide, methyl allyl disulfide (MADS), vinyl dithiins (2-vinyl-1,3-dithiin, 3-vinyl-1,2-dithiin) ajoen (Brodnitz et al., 1971). Particularly, when allicin react with -SH groups and produce water soluble compounds S-allyl cysteine (SAC) or S-allylmercaptocysteine (SAMC). Water soluble organosulfur have less flavour than oil soluble compounds. The initial compound GSAC is converts into SAC and this reaction is catalyzed by γ-glutamyltranspeptidase (γGT) (Rabinkov et al. 2000).

Whole garlic contains γ-glutamyl-S-allyl-L-cysteines. When whole garlic is cut/crushed, alliinase, an enzyme found in the cell wall, converts alliin into allicin and other thiosulfinates diallyl disulfide (DADS), diallyl sulfide (DAS), diallyl trisulfide (DATS)). When garlic is extracted in an aqueous solvent γ-Glutamyl-S-allyl-L-cysteines are converted into water-soluble compounds including S-allyl-cysteines (SAC) (Farhat et al. 2021).

1.9.3.4. Lipid profile

In several studies demonstrate oleic, linoleic and linolenic are the major unsaturated fatty acids that have significance pharmacological properties in garlic. However, chloroform-methanol extricated lipids of garlic (*Allium sativum Linnaeus*) measured to 0.6% compared to 0.5% extricated by petroleum ether on a dry weight basis. Fractionation by silicic acid column chromatography appeared that garlic lipids include 62.6% neutral lipids 14.0% glycolipids and 23.4% phospholipids. Garlic lipids contain a considerably high rate of polar lipids (Table 4).

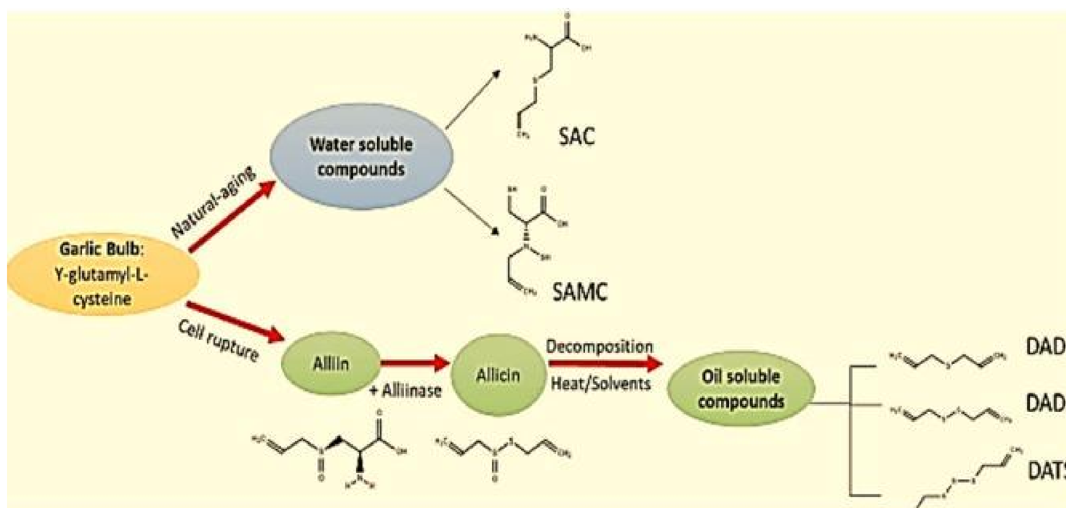


Figure 1.16: Conversion of oil and water-soluble compound to their metabolite of garlic organosulfur. Adapted by partial modification from (Farhat et al. 2021).

The fatty acid composition of the total lipids (TL) and component divisions appeared that palmitic, oleic, linoleic and linolenic acids constituted the major fattyacids; capric, laurie, myristic and stearic acids produced about 6% in total lipid. The unsaturated fatty acids together produced to 72-80% and among these, linoleate was transcendent in add up to lipids as well as in the neutral (NL) and phospholipid (PL) divisions. In contrast the glycolipid division was wealthier in linolenate (37.5%) compared to 4-9.5% in TL, NL and PL. consequently, in the neutral lipids, triglycerides were predominant (80.5~83.6 mg/g) with smaller amounts of free sterols, free fatty acids, and sterol esters being present. 1, 2-Diacylglycerol acetates, 1, 3-diacylglycerol acetates and cholesterol acetates were too probably distinguished. aditionally, esterified steryl glycosides and steryl glycosides were main sugar-containing lipids, but monogalactosyl diglycerides, cerebrosides, digalactosyl diglycerides and sulfo lipids were relatively minor components and the phospholipids, phosphatidyl cholines and serines, and phosphatidyl ethanolamines were the main components, comprising over 85% of this class. A Phosphatidic acid and phosphatidyl inositols were also

present. The major fatty acids in the total and three lipid classes were palmitic, linoleic, linolenic acid and oleic (Kamanna et al., 1980; Kennedy and Wightman, 2011; Jang et al., 2008).

Table 1.4. Lipid composition of garlic

Fatty Acid	Total amount (%)			
	Total lipid	Neutral	Glycolipid	Phospholipi
CIO:0	0.5	1.0	0.7	TR b
C12:0	0.5	0.5	2.8	1.0
C14:0	TR	TR	1.7	TR
C16:0	24.6 ± 0.6	13.8 ± 0.4	21.0 ± 1.1	26.6 ± 0.8
C18:0	TR	TR	0.9 ± 0.2	0.5 ± 0.1
C18:1	3.1 ± 0.7	6.6 ± 0.4	6.0 ± 0.3	3.5 ± 0.2
C18:2	64.8 ±0.8	64.3 ± 1.0	28.5 ± 2.0	64.1 ± 0.8
C18:3	5.7 ± 0.5	9.5 ± 0.3	37.5 ± 2.6	4.0 ± 0.4
Total unsaturated fatty acids	72.6	80.4	72.2	71.6

1.9.3.5. Phenolic profile

Polyphenols are naturally occurring compounds found largely in fruits and vegetables and that can be separated into flavonoids (flavonols, flavones, flavanols, flavanones, anthocyanidin, chalcones and isoflavones) and non-flavonoids (phenolic acids). All these compounds are frequently found as a secondary plant digestion system and are characterized by the presence of numerous phenolic groups related to more or less complex structures (Kennedy and Wightman 2011). The antioxidant properties of these poly phenols including total phenolic content (TPC), total flavonoid content (TFC), tannin content, in fruits and vegetables.

Interestingly, garlic contains diverse range of phenolic compound, for example, tannin where, tannins are mostly composed of proanthocyanidins, which are polymers of oligomeric flavonoids with mostly catechin and epicatechin. Other phenolic compouns such as, kaempferol-3-Glucuronide, kaempferol-3-O-glucoside, kaempferol-O-beta-D-glucoside-7-O-alpha-L-rhamnoside, 3,7-di-O-rhamnoside, luteoline and apigenine in different varieties (Gorinstein et al., 2008; Dziri et al., 2012). However, black garlic whole bulbs contain 2.5-fold higher polyphenol compare with raw garlic (Jang et al. 2008).

In addition, garlic extract also contains various type of phenolic acid such as vanillic acid, caffeic acid, p-Coumaric acid, ferulic acid, sinaptic acid and flavonoids (quercetin, isoquercetin, and kaempferol) and Anthocyanins (Cyanidin-3-(6'-malonyl)- Glucoside, Cyanidin-based compound, Pelargonidin-based compound) are found in garlic and garlic skin that indicate garlic by-product also a potential source for poly phenols(Phan et al. 2019; Maharani et al. 2020).

The presence of phenolic compound has been recognized as the most capable for the antioxidant activity of garlic, that's primarily related with the capacity of free radical scavenger, through the arrangement of more steady and less poisonous atoms. Phenolic compounds stabilize free radicals when they grant off hydrogen from one of their hydroxyls gather; the degree of movement is related to the number of their hydroxyl groups (Chen et al. 2013). Moreover, poly phenols may be able to balance supplement accessibility through the inhibition of digestive enzymes involved in lipid and starch breakdown, which might lead to advantageous impacts on calorie intake,

weight (McDougall et al. 2009), antioxidant, anti-microbial and anticancer (Dziri et al. 2012).

However, the chemical structure of flavonoid like estrogen that have unique advantages in the prevention and treatment of breast cancer. Flavonoids can hinder the occurrence and advancement of breast cancer from different viewpoints. Most instruments incorporate the restraint of high impact glycolysis, the advancement of apoptosis, the impediment of the cell cycle, the suppression of intrusion and relocation, the acceptance of DNA damage, and the restraint of aromatase and microtubule generation (Vaya and Tamir 2004).

1.9.3.5.1. Antioxidant properties of garlic

Natural antioxidant, especially cancer prevention agents from natural sources such as natural products, vegetables, and herbs have gained popularity due to their defensive properties to contribute the protection against oxidative stress in humans. Garlic possesses potential health-promoting effects due to its high phenolic and flavonoid contents and shows potential antioxidant activity (Nuutila et al. 2003a). Antioxidant activity determines mainly by free radical scavenging against 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) and 2,2′-azino-bis-(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS+•), Fe (III) reducing ability (FRAP) in garlic and its components. Garlic applies antioxidant activity by scavenging ROS, improving the cellular antioxidant proteins superoxide dismutase, catalase, and glutathione peroxidase, and expanding glutathione within the cells and reducing the radiation sensitivity of normal tissues that are adjacent to tumours. When comparing the evolution between crude garlic and finished dark garlic, an eminent increase is

observed within the total polyphenol substance, antioxidant capacity and total polyphenol index (Kang et al. 1996).

1.9.4. Bioavailability and Metabolism

Based on several *in vitro* studies for cancer treatment, an expansive number of *in vivo* and clinical thinks have been conducted on crude garlic and/or its definition, although results are clashing. In fact, the most sulfur containing groups show diverse bioavailability between crude garlic and particular garlic supplement formulations. For illustration, the bioavailability of allicin from nine garlic-based nourishment and garlic supplements was tried on 13 subjects measuring the concentration bend of breath allyl methyl sulfide, the foremost imperative garlic metabolite, highlighting the next bioavailability of allicin from garlic supplements than that of pulverized crude garlic (Lawson and Hunsaker 2018).

In crushed raw garlic cloves, allicin is liable for most of the pharmacological activity and it is metabolized immediately under enzyme-inhibiting gastrointestinal conditions (half-life <1 min) to allyl-mercaptan. After consuming a large amount (25 g) of crushed raw garlic, allicin and its metabolites are available in the blood, urine, and stool (Figure 17) (Lawson and Hunsaker 2018). Similarly, after intravenous injection, allicin rapidly disappears from circulation and is transformed into secondary metabolites, including E-ajoene, 2-ethenyl-4H-1, 3-dithiin, and DADS (Freeman and Koder 1995).

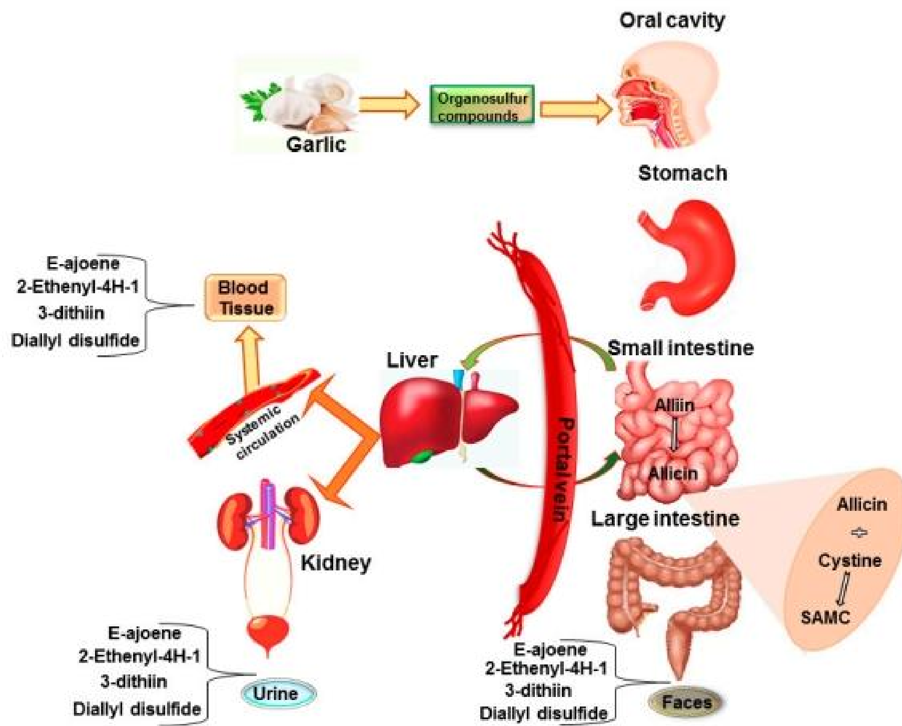


Figure 1.17: Schematic illustration of absorption, metabolism, and distribution of garlic organosulfur compounds in the gastrointestinal (GI) tract. The absorption of garlic occurs in the GI tract where allicin is released from alliin and contacts with cystine that is released from protein diet, forming S-allylmercaptocysteine (SAMC). After metabolism, the secondary metabolites of allicin including E-ajoene, 2-ethenyl-4H-1, 3-dithiin, and diallyl disulfide (DADS) are available in blood, urine, and faces.

On the other hand, allicin bioavailability of enteric tablets varies from 36% to 104% at ≥0.5 h after garlic product consumption, which was decreased from 22% to 57% in breath, when eating with a high protein meal. Independent of meal type, garlic capsules gave 26–109% lower bioavailability, while non-enteric tablets showed 80–111% higher bioavailability (Lawson and Hunsaker 2018).

Additionally, protein derivative cystine interacts with allicin quantitatively at body temperature to form two equivalents of SAMC. This probably happens when cysteine is released from digested meal protein and comes in contact with allicin released from garlic products in the gastrointestinal tract. In addition to this, after an oral administration of 200 mg/kg of DADS in rats, the main metabolites, such as allyl methyl sulfoxide and allyl methyl sulfone, were found in plasma, stomach, liver, and urine (Germain et al. 2002). Aged garlic extract (AGE) contains primarily water-soluble organosulfur compounds, for example, S-allyl cysteine (SAC) and SAMC that have different pharmacokinetic behaviours than oil-soluble organosulfur compounds (Nagae et al. 1994). After garlic oral administration, SAC is absorbed immediately from the gastrointestinal tract (GI) tract, its half-life is more than 10 h and the renal clearance is more than 30 h in humans. The result after the evaluation of the safety and efficacy of SAC illustrated that it seems to play a key role in the biological effects of garlic (Berginc et al. 2010). Finally, extraction may improve the bioavailability of the whole garlic as well as of different crude ingredients and reduce toxicities. For example, in AGE, during the extraction process, the odorous, harsh, and irritating compounds of garlic are naturally transformed into stable and safe sulfur compounds; moreover, different toxicological studies have confirmed the safety of aged garlic (Miraghajani et al. 2018).

1.9.5. The potential impact of garlic on human health

1.9.5.1. Garlic and Cancer

1.9.5.1.1. Therapeutic anticancer effect of garlic on human studies

Cancer is one of the main causes of deaths worldwide. Based on National Cancer Database and the Surveillance, Epidemiology, and End Results, about 16.9 million

people were identified with cancer in 2019 and this number will probably rise to more than 22.1 million in 2030 (Miller et al. 2019). The Food and Drug Administration's evidence-based review system for the scientific evaluation of health showed no reliable evidence for the relation between garlic and a reduced risk of gastric, breast, and lung cancer (Rivlin 2009). However, credible evidence for an association between garlic intake and colon, prostate, oesophageal, larynx, oral, ovary, and renal cell cancers has been reported, even if all studies were observational and the number of such trials that are scientifically considered valid in this analysis is remarkably few and the number of subjects involved generally small. As a result, relations between garlic and reduction of risk of cancers are still uncertain (Kim and Kwon 2009; Zuniga et al. 2019). Interestingly, garlic can provide symptomatic relief of various cancer conditions, including breast, colorectal, colon, gastric, lung, and pancreatic cancers. It may be a therapeutic potential for specific cancer treatment that has been reported in human-based clinical studies. In this context, personalized diets with supplemented functional elements, including functional phytochemicals, such as allyl sulfur compounds or allicin, have provided high amounts of antioxidants to patients in chemotherapy and in remission (Lee et al. 2015). A randomized controlled trial showed that dietary intervention for six months among breast cancer survivors increased adherence to a Mediterranean style diet and consequently raised the consumption of anti-inflammatory spices, such as garlic (Zuniga et al. 2019). Another randomized double-blind factorial trial on garlic highlighted a decreased appearance of precancerous gastric lesions or gastric cancer (You et al. 2006), while consumption of 200 mg of synthetic allitridum (diallyl trisulfide) with 100 mg of selenium reduced gastric cancer risk (Li et al., 2004); similar results were found with 7.3 years consuming garlic supplementation at a dose of 200 mg caps or steam-distilled garlic oil 1 mg two times per day that reduced advanced gastric lesions (You et al. 2006). In

addition, long-term consumption of garlic, garlic supplements or garlic with vitamins reduced gastric cancer (Ma et al. 2012), precancerous gastric lesions (Gail and You 2006), and mortality rate (Li et al. 2019). Garlic supplementation at a dose of two capsules two times a day for 7.3 years increased serum folate and improved moderate folate deficiency in patients with gastric lesions in rural Chinese populations(Wang et al. 2009). In addition, the intake of garlic supplements <0.60 to >3.65 kg per year for two years was significantly associated with decreased risk of colorectal adenoma, which is a precursor of colorectal cancer (CRC) (Dreher 2018; Wu et al. 2019). Epidemiological studies of randomized controlled trials explained that the administration of GE decreased colon adenomas and CRC in patients with CRC (Ngo et al. 2007) via increased NK cell activity (Ishikawa et al. 2006). Correspondingly, AGE at a dose of four caps per day for six months prevented the reduction of NK cell number in patients with liver and pancreatic cancer (Ishikawa et al. 2006).

Few epidemiological studies conducted in the Chinese population found a significant inverse relation between consumption of raw garlic or garlic components 8.4 g or 33.4 g per week for seven years and lung cancer (Jin et al. 2013). Finally, GE showed preventive effect on febrile neuropathy after receiving chemotherapy in patients with hematological malignancies, potentially reducing the risk of chemotherapy related febrile neutropenia after receiving GE at 900 mg per day for three weeks compared with placebo (Gatt et al. 2015).

Numerous mechanisms have been recommended to explain the chemo-preventive effects of garlic, including the inhibition of DNA adduct formation, the inhibition of mutagenesis by blocking metabolism, through its free-radical scavenging, or by decreasing cell proliferation and tumour growth (Jin et al. 2013). In this context,

(Charron et al. 2015)performed a clinical trial on gene expression related to immunity, apoptosis, and xenobiotic metabolism in humans, after consumption of 5 g raw, crushed garlic daily for 10 days. A single meal containing raw crushed garlic activated the expression of seven genes, such as activating protein with immunoreceptor tyrosine-based activation motif 1, aryl hydrocarbon receptor nuclear translocator, aryl hydrocarbon receptor, hypoxia-inducible factor 1α, c-Jun, nuclear factor of activated T cells, oncostatin M and V-rel avian reticuloendotheliosis viral oncogene homolog in blood of healthy volunteers, thus inhibiting tumourigenesis.

This section adapted from the paper already published: Ansary, J., Forbes-Hernández, T. Y., Gil, E., Cianciosi, D., Zhang, J., Elexpuru-Zabaleta, M., ... & Battino, M. (2020). Potential health benefit of garlic based on human intervention studies: A brief overview. Antioxidants, 9(7), 619. doi: https://doi.org/10.3390/antiox9070619

1.9.5.1.2. Garlic treatment and management of breast cancer

Garlic, an Allium vegetable, contains rich vitamins, fatty acids esters, polyphenols/phenolics, flavonoids sulfur containing compounds that have strong anticancer properties. Epidemiological considers appeared compelling prove that garlic utilization is related with diminished chance of breast cancer due to its effects on scavenging free radicals, modulating the immune system and ROS (Farhat et al; Malki et al., 2009; LI, 1995).

Garlic shows anti proliferative effect when treatment with garlic components DATS reduces cell proliferation of breast cancer MCF17 and MCF12A cell lines (Malki et al. 2009). Similarly, aged-garlic extract and two water-soluble compounds, SAMC and SAC exhibits in breast cancer MCF_7 and MCF_7ras cells (Li et al. 1995). Cell

proliferation inhibits by the accumulation of cells within the G0/G1 or G2/M phase of the cell cycle, depending on the concentration of allicin, and not by a noteworthy increment in cell passing. Moreover, SAC up regulates E-cadherin expression and downregulates MMP-2 expression in MDAMB-231 cells and prevents migration, adhesion, and invasion as well cell proliferation (Gapter et al., 2008).

In this regard, it might be concerning that alliin, a precursor of allicin, did not successfully prevent the expansion of MCF-7 cells. Allicin is metabolized exceptionally quickly, and a few metabolites have been tried for anticarcinogenic action against human and canine breast cancer cell lines (Sigounas et al. 1997). It might be concern that, oil-soluble compound such as DAS, DADS, DATS has greater antiproliferative effects compare with twater-soluble compound such as SAC and SAMC (Sundaram and Milner 1996).

OBJECTIVE

The main objective of the present work was to evaluate the anticancer potential of garlic extract on *in vitro* breast cancer models targeting different molecular pathways.

For such purpose human adenocarcinoma breast cancer cells MCF_7 cell model was chosen and treated with garlic extract. The following specific objectives were set as:

- To characterize the phytochemical contents of garlic extract and their antioxidant capacity.
- To evaluate the *in vitro* digestion of garlic by characterization the phytochemical contents of digested garlic extract and their antioxidant capacity.
- To evaluate the anti-proliferative effects of garlic extract on human breast cancer cells by performing cell viability, intracellular ROS production assay, apoptosis and cell cycle and evaluate their effects on some autophagy related protein.
- To evaluate the cytotoxic effects of garlic extract on human non-tumourigenic epithelial MCF10A cell line.
- To determine the energy metabolism by observing several parameters of mitochondrial respiration or oxygen consumption rate (OCR) and glycolysis or extracellular acidification rate (ECAR) in breast cancer cell line.
- To determine the anti-metastasis effects of garlic, extract on human breast cancer cells by migration and colony formation assay, as well as by observing the expression of invasion, EMT, metastasis marker and evaluate their effects on some pro angiogenic factors.

- To evaluate effect of garlic extract on the expression of proteins and gene related to, inflammation, apoptosis, cell cycle, ER stress, migration, Nrf2/Keap1, EGFR and NFκβ signalling in breast cancer cell lines.

CHAPTER 2. MATERIALS AND METHODS

2.1. Plant materials

Garlic (*Allium sativam*), the best time of planting is between the mid October to mid-November and harvesting time takes 135 to 145 days after planting. BARI Rashun-1 variety were cultured and provided by Spices Research Centre, Bangladesh Agricultural Research Institute, Bogra, Bangladesh. Garlic cloves were washed and dried at 40 ^{o} C in a microwave oven, and then ground into powder using a manual grinder. The powdered mass obtained was stored in airtight Polyvinyl chloride (PVC) package at 4 ^{0} C for further analysis.

2.2.1. Chemicals and Reagents

Sodium carbonate (Na2CO3), 2,4,6-tripyridyl-S-triazine (TPTZ), ferric chloride (FeCl3), gallic acid, sodium nitrite (NaNO2), sodium carbonate (Na2CO3), aluminum chloride (AlCl3), sodium hydroxide (NaOH), (+)-catechin, glacial acetic acid (CH3CO2H), methanol (CH3OH), phosphoric acid (H3PO4), L-Leucine, sodium chloride (NaCl), ninhydrin, acetic acid, potassium persulfate (K2SO4), potassium sulfate (K2SO4), cadmium chloride hemi (pentahydrate), ammonium ferrous sulfate and α, α-diphenyl-β-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Pepsin, bile extract porcine, pancreatic, ethanol (CH3CH2OH), Folin–Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,20-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, diammonium salt) were purchased from Fluka Chemie (Buchs, Switzerland). Reagents for cell culture were purchased from Sigma Aldrich (Milan, Italy). The Tali® Cell Cycle Kit, Tali™ Apoptosis Assay Kit-Annexin V Alexa Fluor® 488 and Tali™ CellROX® Orange Reagents were purchased from Invitrogen TM, Life Technologies (Milan, Italy). Purified Millipore

water was used throughout this works. All chemicals and solvents were of analytical grade. The other chemicals or kits the not mentioned in this section will be directly mentioned in the relative descriptive sections of the specific methods.

2.2.2. Antibodies

The primary antibodies hemeoxygenase 1 (HO-1), 8-Oxoguanine DNA glycosylase (OGG1), ATF6, XBP1, EIF2α, AMPK, PGC1α, NFκB, IL-1β, IL-6, phosphorylated (p)-IκBα, EGFR and glyceraldehyde-3-phosphate dehydrogenase (GADPH) were purchased from Santa Cruz Biotechnology (Dallas, TX, USA), while goat anti-rabbit IgG peroxidase secondary antibody was purchased from Sigma-Aldrich (Milan, Italy).

2.3. Preparation of Garlic extract

2.3.1. Ethanolic extract of garlic powder

Garlic samples were extracted according to procedures previously described by (Abdul Qadir et al. 2017), with minor modifications. Dried samples were grounded to pass 80 mesh sieves. Each sample (80g) was extracted with 800 ml of 80% ethanol and shaken for 1 day at room temperature followed filtration by using a Whatman grade 1 qualitative filter paper. The obtained ethanolic extract was stored in brown bottles at 4 ° C for further analysis.

2.4. Evaluation of total phenol and flavonoid contents

The total phenolic content (TPC) was evaluated through the Folin-Ciocalteu method, as already described by (Singleton et al. 1999) with a few alterations. Gallic acid (GA) was utilized for the calibration curve (0 - 6 mg/ml). 250 μL of standards or extract, appropriately diluted, were included to 1.25 ml of Folin-Ciocalteau reagent (10 %),

after 5 min of incubation at room temperature 1 ml of 7M Sodium carbonate (Na_2CO_3) was included. Then, the mixed solution was incubated at room temperature within the dark for two hours, and thus, the absorbance was measured at 760 nm by utilizing an UV-Vis spectrophotometer (show DU®6400 Spectrophotometer, Beckman, Fullerton, CA, USA). The total phenolic content was expressed in mg GA eq/g DW of garlic.

Total flavonoid content (TFC) was measured by the $AlCl_3$ method portrayed by (Ariza et al. 2016). Each test was appropriately diluted to be inside the calibration curve run. Catechin (Cat) was utilized to develop the calibration bend (0.0008-0.05 mg/ml). About 125 µl of standards or extract were mixed with 625 µl of Milli-Q water and 37.5 µl of Sodium Nitrite ($NaNO_2$) (5 %); after 6 minutes, 75 µl of $AlCl_3$ x $6H_2O$ solution (10 %) were added to the mixture. After 5 minutes, 250 µl of sodium hydroxide (4 %) were added and a final volume of 1,250 ml was reached with Milli-Q water. The absorbance was measured by a UV-Vis spectrophotometer (model DU®6400 Spectrophotometer, Beckman, Fullerton, CA, USA) at 510 nm. The TFC was expressed in mg Ceq/g DW of garlic.

2.5. Evaluation of phenolic compounds by HPLC-MS

The determination of phenolic compounds in the extracts was performed by a Liquid Chromatography system (Agilent model 1260 Infinity, Palo Alto, CA, USA) coupled with triple quadrupole tandem mass spectrometry MS/MS AB SCIEX Triple Quad 3500 (AB Sciex, Foster City, CA, USA), equipped with an electrospray ionization source (ESI)(Seraglio et al. 2016).

The chromatographic separation was done with a Phenomenex Luna C18 column (150 × 2 mm; 3-µm particle diameter). The flow rate was 300 µl/min, the injection

volume was 5 µl, and the mobile phase was composed of Solvent A (water with 0.1% formic acid) and Solvent B (acetonitrile with 0.1% formic acid). The mobile phase gradient was programmed as follows: 98% A (v/v) from 0 to 4.0 min, 80–98% A (v/v) from 4.0–7.0 min, 10–80% A (v/v) from 7.0–14.0 min, 10% A (v/v) from 14.0–15.0 min, 10–98% A (v/v) from 15.0–17.0 min. For the mass spectrometric analysis, a turbo V™ source, operating both in positive and negative ionization modes, was set as follows: ion spray (IS) voltage: 4500 V; curtain gas: 25 psi; nebulizer gas (GS1) and auxiliary gas (GS2): 55 psi; source temperature: 400 ° C. Nitrogen was used as the nebulizer and collision gas. The acquisition was performed in multiple reaction monitoring (MRM) mode, and the Analyst 1.6.2 software (AB Sciex, Foster City, CA, USA) was used for data acquisition and processing. The phenolic compounds were characterized according to their UV–Vis, mass spectra and their retention times, compared with authentic standards and data provided by the literature. Calibration curves were obtained by injection of known concentrations of standards for quantification.

2.6. Evaluation of organosulfur compounds in garlic extract

Garlic extract was evaporated at 40 ° C under reduced pressure, using a rotary evaporator and dry extract was stored in at -20 ° C for further analysis. Approximately 50 mg of garlic-dried extract were added, and 1 ml of dichloromethane was added. Then, the compounds were extracted by vortexing for 3 minutes and assisting the extraction with sonication for 10 minutes. The mix was centrifuged at 14000 rpm for 10 minutes and the supernatant was filtered. After filtration, 1 µl of the extract was injected in split-less mode with Helium (99.999% purity) at 250 ºC and a flow rate of 1 ml/min through a capillary column RTX-5 (Restek, PA, USA) of 5 m length, 0.25 mm internal diameter, and 0.25 µm film. GC oven was programmed as follows: 50º C

for 1 min, 5º C/min to 150º C, 150º C for 1 min, 15º C/min to 300º C, kept for 20 min. MS detector was operated at 250 ºC, ionization energy 70 eV. Scan range was from 35 to 350 m/z and scan rate 0.9 scan/s. Organosulfur compounds were identified by NIST and Wiley mass spectral libraries, and quantified with calibration standards of the identified compounds .

2.7. Evaluation of mineral content in garlic extract

Ethanolic garlic extracts were evaporated at 40 ° C under reduced pressure, using a rotary evaporator and dry extract was stored in at -20 ° C for further analysis. Minerals in the dried extracts were analyzed in triplicate using an inductively coupled THERMO X7-model mass spectrometer (ICP-MS) quadrupole. The sample (1 mg) was acidified with 0.1 mL nitric acid (1/1, v/v) and filtered through a 0.25 µm filter. The instrumental measurement parameters in the ICP-MS plasma were radio frequency power (W): 1300; sample flow (l/min): 0.6; Argon flow (l/min): refrigerant at 13.0, auxiliary at 0.7, and nebulizer at 0.85. The standard multi-element reagent VI (Merck), consisting of 30 elements, was used to develop the standards in the same way at different concentrations, together with a blank control. It was used as an internal 103Rh standard, adding a well-known concentration to both samples, standards, and control. To verify that spectral interferences were effectively removed or corrected, a study was carried out with the standards in synthetic solutions and known concentrations of the elements that make up as interfering compounds.

2.8. Evaluationof total antioxidant capacity

Total antioxidant capacity (TAC) of samples was quantified by Diphenyl-1 picrylhydrazyl (DPPH), Trolox equivalent antioxidant capacity (TEAC) and Ferric reducing antioxidant power (FRAP) tests. The DPPH assay is based on the method of

(Kumaran and Joel Karunakaran 2007). The standard Trolox was used to prepare the calibration curve (50–1000 μM). 50 μl of extract dilute with 450 μL miliQ water then 50 μl of standards or diluted extract were added to a solution of 550 μl of ethanol (70 %) and 400 μl of DPPH in methanol. The mixture was incubated within the dark at room temperature for 15 min and then the absorbance was measured by the UV–Vis spectrophotometer (model DU®6400 Spectrophotometer) at 517 nm. The percentage of radical-scavenging activity (RSA) was calculated according to the following equation:

$$\% \text{ RSA} = (\text{Abs control} - \text{Abs sample}/\text{Abs control}) \times 100$$

Where, Abs is the absorbance. Trolox was used for the calibration curve (50–1000 μM), and the DPPH results were expressed in μmol Teq/g DW of garlic.

The TEAC assay was performed following the method of (Re et al. 1999). This method is based on the ability of antioxidant compounds to quench the 2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation (ABTS+) and reduced the radical to the colorless neutral form. Trolox equivalent was used to construct the calibration curve (50 –1000 μM). The radical solution (ABTS solution) was prepared 16 h before the analysis by reacting ABTS (7 mM) dissolved in Milli-Q water with K2S2O8 (2.45 mM) and maintained overnight in the dark at 4 ° C. The following day, the radical solution was diluted with ethanol (115 μL of ABTS solution in 10 ml of 100% ethanol). About 10 μl of blank Milli-Q water (for control), standards, or extract were added to 1000 μl of ABTS + EtOH working solution into 1.5 ml eppendrof. The mixture was vortex for 20 and 90s and then the absorbance was measured by the UV–Vis spectrophotometer (model DU®6400 Spectrophotometer) at 734 nm. TEAC was expressed in μmol Teq/g DW of garlic. The percentage of inhibition was calculated with the following equation:

% inhibition = (Abs control - Abs sample/Abs control) × 100

Where, the control consisted of a mixture of Milli-Q water, ABTS working solution, and ethanol.

The FRAP assay was performed according to a modified method as described by (Benzie and Strain 1996). The principle of this method is based on the reduction of a ferric 2,4,6-tripyridyl-S-triazine complex ($Fe3+$-TPTZ) to its ferrous coloured form ($Fe2+$-TPTZ) in the presence of antioxidants. 50 µl of extract sample was dissolved in 450 µl of distilled water and then about 150 µl standards or diluted extract were added to 1350 µl of FRAP solution. The fresh FRAP reagent contained 2.5 ml of a 10 mM TPTZ solution in 40 mM HCl, 2.5 ml of 20 mM FeCl3 and 25 ml of 0.3 M acetate buffer, pH 3.6 and kept in the dark at 37 0 C. The reaction mixture was incubated at 37 0 C for 5 min and the absorbance was measured at 593 nm using a Beckman Du 640 spectrophotometer (Instruments Inc., Fullerton, CA, USA).

Trolox (50 – 500 µM) was used as the standard to calculate the calibration curves. The FRAP results were expressed as µmol of Trolox equivalents (Teq) per g DW of garlic.

2.9. Evaluation of *in vitro* digestion of garlic

2.9.1. Gastric digestion

Garlic was processed *in vitro* by using the strategy of (Gil-Izquierdo et al. 2002) with a few adjustments. Briefly, dried garlic powder (10 g) was dissolved with distilled water (100 ml), adjust pH up to 2 by expansion of 6M HCl then, included in pepsin from porcine gastric mucosa (315 U/ml; 0.734 mg/g of garlic powder) and this arrangement was incubated mixing within the dim at 37 ° C for 2 hours to get the

gastric fraction. After 2h, the gastric phase containing breaker is put in ice to stop the enzymatic response. After, a cellulose dialysis layer of molecular weight cut-off 12000 Da holding ultra-pure 25ml water was put in a beaker containing gastric fraction to get gastric In and OUT fraction. The fraction which was the inside of layer (that containing antioxidants able of crossing the membrane for absorption) called Gastric fraction OUT (GF-OUT). Another fraction which was the outside of membrane (that containing compounds capable of reaching the small intestine) called Gastric fraction IN (GF-IN). Samples were collected and centrifuged at 10000 rpm at 4 ° C for 10 min and stored at −80 ° C for further analysis.

2.9.2. Intestinal digestion

After 2 hours of gastric digestion, 20ml of aliquot with mixture of 4.5 ml pancreatin from porcine pancreas (4 mg/ml) and porcine bile salts (25 mg/ml) was taken to determine the titratable acidity. The remaining 80 ml of the gastric fraction obtained were divided into 4 aliquots of 20 ml and transferred to corresponding 4 beakers. For each 20 ml of gastric fraction, added 4.5 ml of solution of porcine pancreatin (4 mg/ml) and bile salts (25mg/ml) were dissolved. After calibrated amount of sodium bicarbonate (NaHCO3) required to reach a pH of 7.8, similar with intestinal tract, was added in the 25 ml of distilled water and mix and fill in segments (20 to 25 cm) of cellulose dialysis membranes (molecular weight cut-off 12000 Da). These membranes were closed with nodes and placed in beaker that containing gastric fraction, pancreatin and bile salts and incubated at 37 ° C in the water bath for another 2 hours. At the end of the incubation, the dialysis membranes were recovered from the beakers; the fraction contained inside is called intestinal fraction “IN”, which represents the “bioaccessible” fraction that contained the absorbable antioxidant, while the fraction that is outside the dialysis membrane, in the beaker, is called “OUT” fraction which

consisted of compounds capable of reaching the “colon”. The above-described fractions were purified by centrifugation at 10 000 rpm at 4 ° C for 10 min and stored at −80 ° C. The percent bioaccessibility intestinal digestion fractions were estimated as follows

% of Bioaccessibility = (Polyphenol release from each digested fraction / undigested extract) ×100

Potential bioavailability (%) = (GF-OUT×100)/undigest extract

2.10. Cell culture

MCF_7 breast cancer cell line was compassionate given by Prof. MircoFanelli of the Division of Biomolecular Sciences (Università Degli Studi di Urbino Carlo Bo, Urbino, Italy) and was grown in Dulbecco’s Adjusted Eagle’s Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum as well as 0.1% penicillin and 0.1% streptomycin. Cells were kept up in a CO2 incubator at 37 °C under a humidified climate (95% discuss, 5% CO2). MCF10A cell line was grown in Gibco Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) supplemented with 5% heat-inactivated fetal bovine serum as well as 20ng/ml Epidermal growth factor (EGF), 0.5µg/ml Hydro cortisone, 10 µg/ml insulin and 0.1 % penicillin/streptomycin.

2.11. Evaluation of cytotoxic activity by MTT assay

Cells were seeded at a thickness of $5×10^3$ cells/well into 96-well plates utilizing the particular complete growth medium. To permit cell attachment, they were incubated overnight. After overnight incubation, the MCF_7 and MCF10A cells were treated with 0-10 mg/ml of concentrate garlic extract with or without digestion for 24, 48, and 72 h. After the treatment time, 30 µL of RPMI medium containing 2 mg/mL of the 3-(4,5-

Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were included, and cells were incubated for other 2h. The produced formazan crystals were dissolved by including 100 µL of dimethyl sulfoxidein each well and measured by a microplate reader (Thermo Logical Multiskan EX, Thermo Fisher Logical, Waltham, MA, USA) at 590 nm. The proportion of viable cells was computed as absorbance of treated cells/ absorbance of control cells x100. The ultimate results were expressed as the concentration that inhibits cell growth by 50% (IC50 values) versus control cells (untreated cells) (Reboredo-Rodríguez et al. 2018)

2.12. Evaluation of intracellular ROS levels by Tali® Image-Based Cytometer

Intracellular ROS aggregation was decided by the CellROX® Oxidative Stress kit taking after the manufacturer's instructions. Cells were seeded at a density of 1.5×10^5 cells/well into 6 well plates and incubated for 48 h with the concentrated garlic extract (0, 1.85, 4.97 and 8.1 mg/ml) for MCF_7 cells. These concentrations were chosen according to the MTT viability test ensuring 90 to 20% cells were viable at 48 h. Cells were collected 48 h post-treatment and centrifuged for 10 min at 1500 rpm. Another, CellROX® Orange reagent (2 µl/ ml) was included, and cells were incubated at 37°C for 30 min. Once more, cells were centrifuged for 10 min at 1500 rpm to take out excess dye and medium and after that resuspended with 100 µl PBS. For each test, 25 µl of cell suspension was loaded into one Tali™ Cellular Analysis Slide's chamber and analyzed within the Tali™ Image-Based Cytometer. In this test, "RFP fluorescence" (530 nm EX/580 nm EM) represented the fluorescence signal from CellROX® Orange Reagent. Untreated cells, which were moreover labeled with CellROX® Orange Reagent, were utilized to determine baseline levels of oxidative movement. Cells are fluorescent in an oxidation state and non-fluorescent 62in a

decreased state. The results were expressed as the fold alter and all data were reported as the mean value of three free analyses ± SD (Giampieri et al. 2017).

2.13. Cellular lysates preparation

Cells were seeded in T75 flasks at a density of 2 x 10^6 cells/flask and treated with the concentrate garlic extract (0, 1.85, 4.97 and 8.1 mg/ml) for 48h in MCF_7. These concentrations were chosen according to the MTT viability assay ensuring 90 to 20% cells were viable at 48 h. After 48h, the medium was removed, and cells were washing two times with PBS. The cellular lysates were prepared by using the RIPA buffer (Sigma-Aldrich, Milan, Italy) for the oxidative marker (lipid and protein) and antioxidant enzyme activity measured. 1 ml RIPA buffer (Sigma-Aldrich, Milan, Italy) was added into each flask after the desire treatments and the cells were incubated at 4°C for 5 to 8 minutes. After the incubation time cells were scraped and collected in a micro-centrifuge tube and stored at - 80°C until analysis. Before analysis, the cells lysates were thawed at 4°C and centrifuged at 8000 x g for 10 minutes at 4°C for the precipitation of cells debris.

2.14. Evaluation of antioxidant enzyme activity

2.14.1. Evaluation of glutathione reductase

The action of glutathione reductase (GR) was measured by the alteration method was already depicted by (Carlberg and Mannervik 1985). This measure is based on the capacity of GR to diminish oxidized glutathione turn around to decreased glutathione. Briefly, 50 µL of cellular lysate or 50 µl of PBS (for the blank) was mixed with 800 µl of PBS (100 mM, pH 7.6), 50 µl of EDTA (0.5 mM), 50 µl of NADPH (2 mM) and 50 µl of glutathione disulfide (20 mM). The action of GR was decided following the

NADPH oxidation within the samples. The concentration was measured at 340 nm against clear at 10 s interims for 3 min, in a Beckman DU-640 spectrophotometer and the comes about were communicated as µmole of NADPH oxidized per min per mg protein. All information was reported as a mean value of three free investigations ± SD.

2.14.2. Evaluation of glutathione peroxidase

The action of glutathione peroxidase (GPx) was measured by the alteration strategy that was already depicted by (Sies et al. 1979). The assay is based on the capacity of GPx to evacuate H2O2 by coupling its reduction to H2O with oxidation of decreased glutathione. Briefly, 50 µL of cellular lysate or 50 µL of PBS-EDTA buffer (for the clear) was mixed with 795 µL of PBS-EDTA buffer (50 mM to 0.40 mM, pH 7.4), 25 µL of glutathione (40 mM), 25 µL of glutathione reductase (5 UI/mL), 50 µL of sodium azide (1 mM) and 50 µL NADPH (2 mM). Finally, the reaction was begun by adding 5 µL of H2O2 (0.25 mM), and the GPx activity was determined by the disappearance of NADPH within the tests. The intensity was measured at 340 nm against blank at 10 s intervals for 3 min, in a Beckman DU-640 spectrophotometer. The results were expressed as µmole of NADPH oxidizes per 64min per mg of protein. All information was detailed as the mean value of three free analyses ± SD.

2.14.3. Evaluation of glutathione transferase

The action of glutathione transferase (GST) was measured by the alteration method that was already described by(Habig et al. 1974). This method is based on the capacity of GST to conjugate 1-chloro-2,4-dinitro benzene (CDNB) with decreased glutathione and create a dinitrophenyl thioether which can be effectively measured. Briefly, 300 µL of cellular lysate or 300 µL of PBS (for the blank) was mixed with

1.475 mL of PBS (100 mM, pH 6.8), 200 µL of diminished glutathione (1 mM), and 25 µL of CDNB (1 mM). The advancement of CDNB- glutathione conjugate was measured instantly and after 1 min against clear at 340 nm in a Beckman DU-640 spectrophotometer. The comes about were expressed as the amount of protein producing 1 µmole of CDNB- glutathione conjugate per min per mg protein. All data were detailed as a mean value of three free examinations ± SD.

2.14.4. Evaluation of reduced glutathione (GSH) determination

The method is based on the decrease of 5,5 dithiobis (2-nitrobenzoic corrosive) (DTNB) with decreased glutathione (GSH) to create a yellow compound (Ellman 1958; Griffith 1980). Briefly, 100 µl of the cell RIPA preparation, or 100 µl of buffer, within the case of blank, were added at 1.375 ml of phosphate buffer (5.3% of NaH2PO4 0.023 mM + 94.7% of Na2HPO4 2 mM; pH 8.0) and 25 µl of DTNB (0.1 mM). The solution was mixed for roughly 15 seconds and the absorbance was measured at 412 nm in a Beckman DU-640 spectrophotometer, against blank. A calibration curve was arranged using GSH as a standard (working extend: 10-100 µg/ml). The results were expressed as µmol GSH/mg of protein. Each sample was analyzed in three replicates and the final data was generally reported as a mean value ± SD.

2.14.5. Evluation of superoxide dismutase

The action of superoxide dismutase (Sod) was measured by the method described by (Kakkar et al. 1984) with a slight alteration. In this strategy, the Sod activity was determined by employing a superoxide radical generator (chemical, photochemical or enzymatic) and a detector (blue tetrazolium, NBT). The NBT is decreased to formazan, a blue chromogen within the presence of superoxide. The concentration of

blue chromagen can be measured by 540 to 560 nm in a Beckman DU-640 spectrophotometer. Briefly, 100 µL of cellular lysate or 100 µL PBS (for the blank) was blended with 600 µL of sodium pyrophosphate buffer (0.025 M, pH 8.3), 50 µL of phenazine methosulphate (186 µM), 150 µL of NBT (300 µM) and 500 µL de-ionized water. At last, the response was begun by including 100 µL of NADH and the mixture was incubated at 30°C for 90 seconds. The response was blocked by the addition of 500 µL of glacial acidic acid and the mixture was shaken Then the tube permitted standing for 10 min at room temperature and centrifuge at 1500 rpm for 1 min. The intensity of the chromogen within the butanol layer was measured at 540 nm in a microplate reader (Thermo Logical Microplate Peruser, Multiskan® EX, USA) coupled to an Ascent software (Thermo LabSystems Oy, Adaptation 2.6). To realize a standard curve, a serial standard dilution of Grass (extending from 25 to 500 IU/mL) was treated and read along with the samples. All data were reported as a mean value of three independent analyses ± SD.

2.14.6. Evaluation of catalase

The action of catalase was measured by the alteration strategy was already described by (Aebi 1984). This test is based on the decomposition of hydrogen peroxide by the action of the enzyme. Briefly, 10 µL of cellular lysate or 10 µL of PBS (for the blank) was mixed with 990 µL of sodium phosphate buffer (50 mM; pH 7.0) and 600 µL of H2O2 (30%). The absorbance was diminished due to H2O2 degradations, and it was measured at 240 nm against blank for 10 to 70 seconds of reaction, in a Beckman DU-640 spectrophotometer. One unit of catalase was defined as the sum of chemicals that decayed 1 µmol of H2O2 per min per mg protein.All data were reported as a mean value of three independent analyses ± SD.

2.15. Evaluation of oxidative stress markers

2.15.1. Evaluation of lipid peroxidation

Lipid peroxidation was measured by the thiobarbituric acid-reactive substances (TBARS) test according to an altered method depicted by (Ohkawa et al. 1979). Briefly, 300 μL of cellular lysate was mixed with thiobarbituric acid reagent (TBA, 0.37% in 0.2 M HCl) and 15 % trichloroacetic acid (TCA) and warmed at 95 °C for 20 min. At that point, the mixture was cooled, centrifuged at 1200 x g for 15 min at 4°C and the absorbance of the supernatant was measured at 532 nm in a Beckman DU-640 spectrophotometer. The amount of the formed MDA was calculated considering the MDA extinction coefficient as 1.56 x 105 M-1 cm-1. The MDA values were calculated utilizing 1,1,3,3-tetra ethoxy propane as standard and expressed as nmoles of MDA per mg of proteins. All information was detailed as a mean value of three independent analyses ± SD.

2.15.2. Evaluation of protein carbonyl content

Protein carbonyl substance was decided by the dinitrophenylhydrazine (DNPH) method as already described by (Liang et al. 2020) with minor alterations. This method is based on the reaction between DNPH and protein carbonyl, the appearance of a Schiff base to produce the corresponding hydrazone that can be identified spectrophotometrically. Briefly, 25 µl of cellular lysate was taken in two microcentrifuge tubes with 25 µl of TCA (20%), then centrifugation at 2800 x g for 10 min at 4°C. One micro-centrifuge tube represented the test and another one the control. Then included 750 µl of DNPH (10 mM) within the treatment tubes and 250 µl of HCl (2.5 M) into the control tubes and incubated 1 h within the dark at room temperature with mixing at each 15 min interval. After the incubation, 750 µl of TCA

(10%) was added into both the solutions (treatment and control) and once more incubated at room temperature for another 15 min. The mixture was centrifuged for 10 min at 3400 x g at 4°C and the sample precipitation was washed two times with 500 µl of an ethanol-ethylacetate (1:1) solution to remove abundance DNPH and the final precipitations were dissolved in 625 µl of guanidine hydrochloride (6 M). Finally, for removing the leftover debris, the samples were centrifuged for 10 min at 3400 x g at 4°C and the absorbance of the supernatant was measured at 370 nm in a microplate reader (Thermo Logical Microplate Peruser, Multiskan® EX, USA), against the control. The protein carbonyl substance was communicated as nmole per mg protein. All data were reported as a mean value of three independent analyses ± SD.

2.16. Evaluation of apoptosis by Tali® Image- Based Cytometer

Apoptotic cells were recognized by the Tali™ Apoptosis Assay Kit–Annexin V Alexa Fluor® 488 (Invitrogen) according to the manufacturer's information. Cells were seeded at a density of 1.5×10^5 cells/well into 6 well and treated for 48 h with the MCF_7 cells (0, 1.85, 4.97 and 8.1 mg/ml). These concentrations were chosen according to the MTT viability measure ensuring 90 to 20% cells were viable at 48 h. The IC50 values of garlic extract treatments were utilized for treating the refined cells in 6-well plates for 48 h. Cells were collected after 48 h treatment and centrifuged for 15 min at 1500 rpm at 4°C. After removing the supernatant and re-suspending the cells with 100 µl of Annexin binding buffer (ABB, Component C), 5 µL of Annexin V Alexa Fluor® 488 (Component A) was added to each 100 µl of re-suspended.

The mixture of cell and Annexin V Alexa Fluor® 488 was incubated at room temperature into the dark for 20 minutes and after that once more centrifuged. After

removing the excess mixture and re-suspending with 100 µl of AB, samples were incubated at room temperature into the dark for 1 to 5 minutes after including 1 µl of Tali™ PI (component B). For each sample, 25 µl of cell suspension was loaded into one Tali™ Cellular Analysis Slide's chamber and analyzed within the Tali™ Image-Based Cytometer. The instrument works at different excitation/emission wavelengths: 530/580 nm and 458/495 nm for the PI and Annexin V, separately. The Tali™ Image-Based Cytometer was assessed the live, apoptotic and dead cells. The annexin-V-negative/PI negative cells were recognized as viable cells by the cytometer program while the annexin-V positive/PI negative cells were recognized as apoptotic cells. Additionally, the annexin V positive/PI positive cells were recognized as dead cells. All data were reported as a mean value of three independent analyses ± SD

2.17. Seahorse phenotype

2.17.1. Evaluation of the mitochondria respiration and glycolysis by XF24 Extracellular Flux Analyzer

XF24 Extracellular Flux Analyzer from Seahorse Bioscience, Inc. (North Billerica, MA, USA) was utilized to ECAR and OCR, representing glycolysis and oxidative phosphorylation (OXPHOS), separately. MCF_7 cells were seeded in 24-well XF cell culture microplates at 2.0×10^5 cells/well for 24 h and after that treated with various concentrations of the garlic extract (0, 1.85, 4.97 and 8.1 mg/ml) for 48 h. At the end of the treatment, the medium was replaced with 500 µl/well of XF-24 running media. The plates were pre-incubated at 37 °C for 20 min within the XF Prep Station hatchery (Seahorse Bioscience, Billerica MA, USA) within the absence of CO2 and after that run on the XF24 analyzer to get ECAR and OCR. Injections of different compounds that modulate glycolysis or mitochondrial respiration already optimized

were injected successively in each well: for ECAR, rotenone (1 µM), glucose (30 mM) and 2-Deoxy-D-glucose (100 mM), whereas for OCR, oligomycin A (3 µg/mL), 2,4-DNP (300 µM) and rotenone/antimycin A (1 µM/10 µM). ECAR and OCR were recorded during specified programmed time periods (three readings each) as the average numbers between the injections of inhibitors specified above. The ultimate data calculation was performed after the readings had been normalized by counting the cell number in each well. ECAR and OCR are expressed as mpH unit change/min per 2.5×10^5 cells and pMoles/min per 2.5×10^5 cells, individually.

2.18. Evaluation of wound healing assay

The wounding measure was performed as an already depicted method by Liang et al., (2007). Briefly, a straight wound was made in MCF_7 cells intersecting the cellular populace by scratching the base of the plate with a sterile pipette (200 µL) tip. After the scratch, cells were washed two times with PBS and incubated with particular media of each cell containing 2% serum. The wounds were at that point captured at zero time and incubated for 48 h after the treatment. The media contained different treatments of garlic extract (0, 1.85, 4.97 and 8.1 mg/ml) for MCF_7 cells. These concentrations were chosen to agree to the MTT viability measure ensuring 90 to 20% cells were reasonable at 48 h. The IC50 values of garlic extract were too utilized for treating the cultured cells in 6 well plates for 48 h. After 48h, cells were settled with methanol and stained with 0.2% methylene blue stain. The photos were taken by the light microscope and the wound region was calculated by the picture J software for calculating the percent of wound closure after the treatments.

2.19. Evaluation of Colony formation

The colony formation test was performed on the marginally altered method depicted by (Waghela et al. 2015). MCF_7 cells were seeded at a density of $5×10^5$ cells/well into 6 wells and permitted to follow for 24 h. After that, the cells were treated with garlic extract (0, 1.85, 4.97, and 8.1 mg/ml) for MCF_7 cells. These concentrations were chosen to agree to the MTT viability measure ensuring 90 to 20% of cells were viable at 48 h. The IC50 values of garlic extract were moreover utilized for treating the cultured cells in 6-well plates for 48 h. After the treatment time, cells were trypsinized and 1000 cells were seeded in a 6 well plate. Cells were permitted to grow for 10 to 12 days until little colonies were obvious. The colonies were settled with methanol and recolored with 0.2% methylene blue recolor. The plating effectiveness (PE) was considered by the capacity of a single cell to outlive and to develop in the shape of a colony. The PE was characterized by the taking after equation: Rate PE = (Number of colonies formed/Number of cells.

2.20. Cell cycle phase control analysis

Cell cycle investigation was determined by the Tali® Cell Cycle Kit containing propidium iodide (PI), RNase A, and Triton® X-100 to label cells for cell cycle analysis using the Tali® Image-Based Cytometer (ThermoFisher Logical, Milan, Italy). Cells were cultured in 6-well plates, at a density of $5×10^5$ cells/ well, and incubated with different concentrations of garlic extract (0, 1.85, 4.97, and 8.1 mg/ml) for MCF_7 cells for 48 h. These concentrations were chosen concurring to the MTT viability measure ensuring 90 to 20% cells were viable at 48 h. The IC50 values of garlic extract were too utilized for treating the cultured cells in 6-well plates for 48 h. At that point, cells were trypsinized and centrifuged for 5 min at 500 x g after removing the excess media, re-suspending with PBS, centrifuging for 5 min at 500 x g, and transferring the cells into ice. Briefly, the cells were gathered and settled with

70% cold ethanol at -20°C overnight. The settled cells were washed twice with PBS, re-suspended in 100 µL PBS. The fixed cells were washed twice with PBS, re-suspended in 100 µL PBS based PI solution containing 0.1% Triton® X-100, 0.2 mg/ml RNase A (Invitrogen), and 20 µg/ml PI (Invitrogen), and incubated for 30 min at room temperature ensured from the light. For each sample, 25 µL of cell suspension was loaded into one Tali™ Cellular Analysis Slide's chamber (Invitrogen) and analyzed within the Tali™ Image-Based Cytometer. The results were expressed as the rate of cells in each stage and all data were reported as cruel value of three independent analyses ± standard deviation (SD).

2.21. RNA isolation and quantitative real-time PCR analysis

Cellular lysates preparation was decscribed in paragraph 2.13. MCF_7 cells were treated with different concentrations of garlic extract (0, 1.85, 4.97, and 8.1 mg/ml) for 48 h. The total RNA content of the cells was isolated using a PureLink® RNA Mini Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocol. RNA concentrations and purity were checked by using the microplate spectrophotometer system (BioTek Synergy HT, Winooski, USA). Reverse transcription was used to obtain 100 ng of cDNA with 5X All-In-One RT Mastermix Kit (Applied Biological Materials Inc., Canada). EvaGreen 2X qPCR Mastermix (Applied Biological Material Inc., Canada) was used for performing Real-Time PCR using the following forward (F) and reverse (R) primers (Sigma-Aldrich, Milan, Italy) (Table 2.1). GAPDH was used, applying the same PCR condition, for normalizing the quantitative data, expressed as a relative mRNA level compared to the control. The 2-ΔΔCt was used for calculating the fold-change value.

Table 2.1. Sequences and list of primer used for quantitative Real-Time PCR analysis

Primer	Forward	Reversed
P53	5′-TTGCCGTCCCAAGCAATGGAT-3'	5′-AGTCACAGACTTGGCTGTCCCAGA-3'
MCL1	5'-GCCAAGGACACAAAGCCAAT-3'	5'-AACTCCACAAACCCATCCCA-3'
BCL2	5'-ACCACTAATTGCCAAGCACC-3'	5'-ATTTTCCATCCGTCTGCTCTT-3'
Bid	5'-CCTTGCTCCGTGATGTCTTTC-3'	5'-TCCGTTCAGTCCATCCCATTT-3'
BAX	5'-CCCTTTTGCTTCAGGGTTTC-3'	5'-ACAAAGTAGAAAAGGGCGACAA-3'
BIRC2	5′-CCCAAAGACTTTTCCCAGGTCCC-3′	5′-ACTGAGCTTCCCACCACAGGCA-3′
BIRC5	5′-ATTCGTCCGGTTGCGCTTTCC-3′	5′-CACGGCGCACTTTCTTCGCAG-3′
Smac	5′-CTTTGTGAGCTGTGAGTGC-3′	5′-CAGTTGCTGAACTTAAGGGC-3′
FADD	5′-ACGCTTCGGAGGTAGATG-3′	5′-CCTGGTACAAGAGGTTCA-3′
Caspas 3	5′-AAAGGATCCAAAGATCATA CATGGAAGCGAATCAAT-3′	5′-AAAGAATTCCAGTGCTTTTA TGAAAATTCTTATTAT-3′
Caspas 8	5′-AGAGTCTGTGCCCAAATCAAC-3′	5′-GCTGCTTCTCTCTTTGCTGAA-3′
Caspas 9	5′-TGTCCTACTCTACTTTCCCAGGTTTT-3′	5′-GTGAGCCCACTGCTCAAAGAT-3′
LDH-A	5'-GGTTGGTGCTGTTGGCATGG-3'	5'-TGC CCC AGC CGT GAT AAT GA-3‘
MMP2	5′-CACCTACACCAAGAACTTC-3′	5′-AACACAGCCTTCTCCTCCTG-3
MMP9	5′-TTGAGTCCGGCAGACAATCC-3′	5′-CCTTATCCACGCGAATGACG-3′
E-cadherin	5′-CTCACATTTCCCAACTCCTCT-3′	5′-GTGAATAGCCGCAGGGACA-3′
N-cadherin	5′-GCTTATCCTTGTGCTGATGTTT-3′	5′-GTCTTCTTCTCCTCCACCTTCT-3′
β-catenin	5’-CGAAATCTTGCCCTTTGTCC-3’	5’-GTTGTGAACATCCCGAGCTAG-3’
Cyclin D1	5'-GAACAAACAGATCATCCGCAA-3’	5'-TGCTCCTGGCAGGCACGGA-3’
Cyclin E1	5'-GAGCCAGCCTTGGGACAATAA-3’	5'-GCACGTTGAGTTTGGGTAAACC-3’
CDK2	5'-TTTGCTGAGATGGTGACTCGCCG-3’	5'-CCGGGCCCACTTGGGGAAAC-3’
CDK4	5′-CTTCCCGTCAGCACAGTTC-3′	5′-GGTCAGCATTTCCAGTAGC-3′
P27 Kip	5′-ATGTCAAACGTGCGAGTGTC-3′	5′-TCTCTGCAGTGCTTCTCCAA-3′
P21waf1/cip1	5′-GCGATGGAACTTCGACTTTGT-3′	5′-GGGCTTCCTCTTGGAGAAGAT-3′
Rb	5′-ATCCGAGGCAACTACAGCCTA-3′	5′-CCTTTCCAACCGTGGGAATAAT-3′
CHOP	5′-ATGGCAGCTGAGTCATTGCCTTTC-3′	5′-AGAAGCAGGGTCAAGAGTGGTGAA-3′
SOD	5′-GTTGGCCAAGGGAGATGTTAC-3′	5′-AGCAACTCCCCTTTGGGT TC-3′
Catalase	5′–GAGGCC TCCTGCAGTGTTCT–3′	5′–CATTAAGCCATGACGGTGCT–3′
NQO1	5′-ATGTATGACAAAGGACCCTTCC-3′	5′-TCCCTTGCAGAGAGTACATGG-3′
Keap1	5′-GGAGTGCAAGGCAGAGGTGA-3′	5′-GGCAGCTGGGATGTCCTCAT-3′
GSTP1	5′-TACACCAACTATGAGGCGGG-3′	5′-AGCGAAGGAGATCTGGTCTC-3′
Nrf2	5′-TTCTGTTGCTCAGGTAGCCCC-3′	5′-TCAGTTTGGCTTCTGGACTTGG-3′
c-PARP	5′-GGGAGGAGATGGAATGTCAG-3′	5′-CTGCTGGGTCCAAAAGAGAC-3′

20.22. Protein extraction and western blotting

Cell seed and treatment for protein lysate were described in passage 2.13. After 48h of treatment, the medium was expelled, and cells were washed two times with PBS. Protein lysates were arranged from cell pellets by utilizing lysis buffer (120 mmol/L NaCl, 40 mmol/L Tris (pH 8), 0.1% NP40) with protease inhibitor cocktails (Sigma) and centrifuged at 13000 x g for 15 min. Proteins from cell supernatants were alienated on 8 or 10% polyacrylamide gel and after that transferred into a nitrocellulose film, utilizing the transblot SD semidry electrophoretic transfer cell (Bio-Rad, Hercules, CA, USA). The membranes were blocked with 5% non-fat-milk with Tris HCl buffered saline with Tween 20 (TBST) for 1 h at room temperature.
HO-1, OGG1, XBP1, ATF6α, EIF2α, AMPK, PGClα, NFκB, IL-6, IL-1β, p-IκBα, EGFR and GADPH essential antibodies (1:500 dilution) were used after overnight incubation at 4°C. Membranes were washed 3 times by TBST and incubated with their specific alkaline phosphatase conjugated secondary antibodies (1:80,000) for another 1h. Immunolabeled proteins were identified by using a chemiluminescence method (C-DiGit Blot Scanner, LICOR, Bad Homburg, Germany) and bands were quantified by image studio digits software 3.1 (C-DiGit Blot Scanner, LICOR, Bad Homburg, Germany).

20.23. Human protein array analysis

Protein array analysis was conducted by using Human Autophagy Antibody Array (CODE: AAH-ATG-1-4, Ray Biotech) and Human Angiogenesis Antibody Array (ab134000) kits (Abcam®, Cambridge, UK), following the manufacturer's instructions. These two tests were carried out for MCF_7 cell lysates (cell seeded according to manufracture protocol) that treated with garlic extract 0, 1.85, 4.97 and

8.1 mg/ml for 48 h. These concentrations were chosen according to the MTT viability measure ensuring 90 to 20% cells were viable at 48 h. The membranes were detected using a chemiluminescence method (C-DiGit Blot Scanner, LICOR, Bad Homburg, Germany) and array spots were quantified using Image Studio 3 (C-DiGit Blot Scanner, LICOR, Bad Homburg, Germany), normalizing the signal with spots of positive control present in each membrane as follows:

Where:

P1= mean signal density of positive control spots on reference array

P(y)= mean signal density of positive control spots on array "y"

X(y)= mean signal density for spot "X" on array for sample "y"

X(Ny)= normalized signal intensity for spot "X" on array "y"

The results were expressed as fold increase of protein expression respect to the Crlt group. All data were reported as a mean value of the two spots present in the membrane ± SD.

2.24. Statistical analysis

The results are expressed as the mean values with SD of three experiments and the statistical analysis was performed using STATISTICA software (Statsoft Inc., Tulsa, OK, USA). The significant differences represented by letters were obtained by a one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test ($p < 0.05$). Correlations were determined by Pearson's correlation coefficient (r). Differences at $P < 0.05$ were considered to be statistically significant.

CHAPTER 3. RESULTS AND DISCUSSION

REULTS AND DISCUSSION: PART I

3.1. Phytochemical analysis of garlic extract

3.1.1.TPC and TFC

Phenolic compounds are one of the most efficient bioactive constituents of plants (Magalhães et al. 2008) and they are the principal source of antioxidants found in plants (Cai et al. 2004). Thus, the evaluation of these compounds is highly relevant when assessing the bioactive potential of any plant extract. Therefore, here we have carried out a spectrophotometric analysis of garlic extracts to determine the total phenolic and flavonoid contents. The value of TPC (5.8 ± 0.06 mg GAE/g DW) of garlic extract obtained in our study was very close to those obtained by (Beato et al. 2011) but higher than (Park et al. 2016a) at different processing conditions (Gorinstein et al. 2008). Moreover, our obtained TPC value is higher than those found in Hungarian and Polish garlic (Nuutila et al. 2003b; Gorinstein et al. 2008). In addition, we have found a higher level of total phenol and flavonoid in 80% ethanol garlic extract than studies of (Bozin et al. 2008) with 80% methanolic extract (0.98 mg GAE/g and 6.99 μg QE/g, respectively).

Considering TFC value, we observed the value obtained for TFC (0.74 ± 0.01 mgCeq/g DW) in garlic inconsistent with those obtained by (Abdul Qadir et al. 2017) with similar experimental conditions. Also, we found a higher amount of flavonoid than different types of solvent extraction done by (Jang et al. 2008), where they quantify flavonoid by using different standards than us. They claimed that the amount of TFC in aged garlic was 338.04 ±1.60 μg QE/g for water solvent, 124.08 ± 3.22 μg QE/g for ethanol solvent, and 28.07 ± 1.97 μg QE/g for chloroform solvent.

According to our observations, a significant ($p<0.05$) increase in total phenol and flavonoids was found in an 80% ethanolic solvent, suggesting that components in garlic may have been converted into these highly hydrophilic and lipophilic compounds.

3.1.2. Identification and quantification of phenolic compounds

In this context, we have analyzed the garlic extracts by HPLC-MS/MS where we identified and quantified seven poly phenolic compounds. However, the name, molecular formula, molecular weight, m/z value, as well as their retention time of identified main poly phenol and flavonoid compounds are reported in Table 3.1. Moreover, we detected phenolic acids, but flavonoids didn't detect in garlic extract that complied with previously found by Sultana and Anwar (2008). In our experiment, we identified and quantified caffeic acid, p-coumaric acid, dihydroxybenzoic acid, gallic acid, salicylic acid, sinapic acid, and ferulic acid in garlic extract (Table 3.1). In garlic extract, we found lower values of caffeic acid, ferulic acid, p-coumaric acid, as well as the higher values of sinapic acid, compared to those obtained by (Beato et al. 2011). This disagreement could be due to genetic and environmental differences and agronomic practices. Moreover, Beato et al. (2011) showed a meaningful effect of genotype/location on phenolic compounds in garlic.

Surprisingly, we found a higher amount of dihydroxybenzoic acid (0.36 ± 0.010 µg/mg) and ferulic acid (0.5 ± 0.006 µg/mg) in garlic extracts, respectively. In this view, our results are very significant ($p<0.05$) in the context of fact that many polyphenols, including dihydroxybenzoic acid, gallic acid, caffeic acid, and p-coumaric acid have been shown to be strong antioxidants and suppressors of oxidative

stress-related damage and anti-inflammatory substances (Sroka and Cisowski 2003; Kim et al. 2013).

3.1.3. Organosulfur compounds

The major volatile compounds of garlic are sulfur-containing compounds, which were identified through GC-MS analysis (Table 3.2). In our study, diallyl disulfide (DADS), dipropyl sulfide (DPS) and dipropyl disulfide (DPDS) were identified in dichloromethane extract of dried garlic extract. DADS is found after decomposition of diallyl thiosulfinate (Allicin), upon gas chromatography. At room temperature, diallyl thiosulfinate undergoes re-arrangement reactions and produces sulfur dioxide and mono, di, tri sulfide which contribute to garlic flavor (Berger 2007). Unfortunately, our analysis did not trace tri sulfide in the samples. DADS has antithrombotic, lipid-lowering, anticancer antithrombotic, lipid-lowering, anticancer activities (Agarwal 1996). We found a much higher value of DADS (1530.34 ± 13.90 mg/kg) which is consistent with that reported by (Wan et al. 2007). The value of organosulfur compound in garlic extract differed depending on ecotypes and locations. Additionally, we also found more amount of DPDS than allium cepa, which belong to the allium family (Cecchi et al. 2020).

Table 3.1. Identification and quantification of polyphenols and flavonoids in garlic ethanolic extract by HPLC-MS/MS analysis

Compound	Molecular formula	Molecular Mass	Retention Time	Experimental m/z	Fragments detected	Garlic µg/g
Caffeic acid	$C_9H_8O_4$	179	12,1	178,9	107/135	0.13 ± 0.003
p-coumaric acid	$C_9H_8O_3$	163	13,1	162,9	119/93	0.02 ± 0.001
Dihydroxybenzoic acid	$C_7H_6O_4$	154	8,3	152,9	90,9/109	0.36 ± 0.010
Gallic acid	$C_7H_6O_5$	169	3,7	168,9	124,9/79	0.21 ± 0.016
Salicylic acid	$C_7H_6O_3$	138	14,3	136,9	93,1/64,9	0.04 ± 0.001
Sinaptic acid	$C_{11}H_{12}O_5$	223	13,3	223	163,9/192,9	0.06 ± 0.001
Ferulic acid	$C_{10}H_{10}O_4$	193	13,3	195	176,9/89	0.5 ± 0.006
Quercetin	$C_{15}H_{10}O_7$	302	14,5	301	150,9/221	n.d.

* Identified with analytical standards.

n.d. not detected; Results are expressed as mean ± SD (n = 3)

Table 3.2. Content of organosulfur compounds in ethanolic garlic extract obtained by gas chromatography analysis.

Compounds	mg/Kg
Diallyl disulfide	1530.34 ± 13.90
Dipropyl disulfide	95.38 ± 2.50
Dipropyl sulfide	965.02 ± 1.54

n.d. not detected; Results are expressed as mean ± SD (n = 3)

3.1.4. Mineral profile

We obtained 6 different minerals in garlic extract, as shown in Table 3.3. The mineral contents obtained in garlic extract, including K (5761.94 mg/kg), Zn (35.22 mg/kg), and Cu (5.71 mg/kg), were higher with respect to those reported by (Bhowmik et al. 2008; Otunola et al. 2010; Khalid et al. 2014) except Ca (155.10 mg/kg) and Fe (8.14 mg/kg).

Table 3.3. Average concentration levels of minerals elements in the analysed of garlic extract by ICP-MS analysis.

Name of mineral	mg/Kg
Ca	155.1 ± 3.2
Cu	5.71 ± 0.11
Fe	8.14 ± 0.12
K	5762 ± 157
Se	< 3.64 ± 0.12
Zn	35.22 ± 0.72

n.d. not detected; Results are expressed as mean ± SD (n = 3)

This finding mainly differs from previous results because of different soil conditions including geographical origin, cultivation technique(Vadalà et al. 2016). Interestingly, dietary minerals such as Ca, Cu, Fe, K, Se, and Zn are acting as antioxidants, and they reduce the risk

of arterial hypertension, cardiovascular, coronary heart disease bone demineralization, and related metabolic disturbances (Kourounakis and Rekka 1991; Abramovič et al. 2018).

3.1.5. Total Antioxidant Capacity (TAC)

TAC of garlic extract was quantified by DPPH, TEAC and FRAP assays. The TAC of garlic extracts can be demonstrated by their ability to reduce Fe^{3+} to Fe^{2+}, as well as their capacity to scavenge DPPH radicals.TAC of garlic extract was 8.04 ± 1.31µmol Txeq/g DW (DPPH), 6.37 ±0.61 µmol Txeq/g DW (TEAC), 105.17 ± 11.51 µmol Txeq/g DW (FRAP).

We investigated the DPPH assay, the ability of the measured garlic extract containing antioxidant to perform as donors of hydrogen ions or atoms in the conversion of DPPH radical into its reduced form DPPH -H. The assessed garlic extracts were able to decrease the stable, purple-colored radical and convert it into yellow-colored when DPPH-H reached 50% of reduction. However, our results accordance with the previously reported, who stated garlic has a good reducing ability, interacting with DPPH (Kourounakis and Rekka 1991). Moreover, a positive correlation between the radical scavenging activities and the total phenolics of the extracts was observed by (Nuutila et al. 2003b).

In garlic extract, we found higher FRAP (105.167± 11.51 µmol Txeq/g DW) values and lower TEAC (6.37 ±0.61 µmol Txeq/g DW) values than the previous values (11.95 ± 0.8 uM TE/g) obtained by(Gorinstein et al. 2008). While the FRAP assay is concerned with the ion reduction process, which represents the ability of the tested compound to convert ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). However, we measured the electron transfer reaction and represent the radical scavenging activity of the tested samples by ABTS assays (Abramovič et al. 2018).

These values are different from others due to their relationship with the antiradical activity of bioactive compounds and hydrophilic and lipophilic polyphenols present in 80% ethanolic extract (Karadag et al. 2009; Rochín-Wong et al. 2013; Hayano-Kanashiro et al. 2016) or may be used of various solvents for compounds extraction where the antioxidant properties of phenolic compounds exhibit different results based on the solvent type and reaction mechanism, solubility parameters, polarity as well as structural property (Çelik et al. 2010).

According to the results of this study, garlic extract exhibited strong antioxidant properties due to its high antioxidant content. In addition, our results differ from the other published results most likely because of the differences in the experimental conditions (different reaction mechanisms) used in different assays (Bozin et al. 2008) or a significant variation in antioxidant properties between the various garlic genotypes mostly due to differences in chemical composition and bioactive compounds content (Petropoulos et al. 2018).

Even though the obtained results make it difficult to draw any conclusions about the additive or synergistic effects of individual antioxidants on the overall antioxidant activity of the extract since all antioxidant compounds are still unknown. The evidence from this study supports the hypothesis that the antioxidant activities of garlic extracts could be due to their composition of phenolic and/or other non-phenolic compounds.

RESULTS AND DISCUSSION: PART II

3.2. Phenolic composition after *in vitro* digestion

However, *in vitro* studies have many limitations by it has an effective way of evaluating the effects of physiological processes on the fate of specific food components such as phenolic compounds. Here, we have evaluated the gastric and intestinal fractions obtained from the *in vitro* studies with similar human physiological conditions, facilitating the release and absorption of phenolic compounds from the garlic food matrix. For this reason, we have evaluated gastric and intestinal bioaccessibility and potential bioavailability of garlic by UV–Vis spectrophotometry and HPLC-MS for the first time.

3.2.1. Determination of potential bioavailability and bioaccessibility of poly phenols in the gastric and intestinal fraction by UV-VS spectrophotometry

In the present study, we investigated the availability of poly phenols from garlic during simulated GI digestion, i.e., gastric phase and intestinal phase. UV–Vis spectrometry was used to determine TPC, TFC, and TAC in the gastric and intestinal digestion fractions. As reported in Table 3.4 after the simulated gastric digestion, GF-In and IN-out fraction (waste fraction) contained greater amounts of phenolic compounds than did GF-Out and IF (dialisable fraction).

Our result suggests, the digestion process could be liberated phenolic compounds from the garlic matrix where they are bound to macromolecules such as proteins and fibers. Similar evidence has been found on the biostability of Hibiscus cannabinus L. seed extract (Wong et al. 2014). As we mentioned before, we did *in vitro* digestion experiment of garlic polyphenol. We only found one paper regarding garlic phenol digestion where they differentiated between chemical and physiological extraction of garlic (Tagliazucchi et al. 2010). For this reason, we are not able to compare our results with other garlic digestion studies.

Table 3.4: Spectrophotometric determination of total phenolic and flavonoid content and antioxidant capacity of undigested and digested fractions from garlic

	TPC (mg GAeq/g DW)	TFC (mg CATeq/g DW)
Garlic extract	5.8 ± 0.06	0.74 ± 0.01
Gastric digestion		
Gastric Out	2.974 ± 0.07	0.26 ± 0.03
Gastric IN	9.54± 0.86	0.62 ± 0.03
Intestinal digestion		
Intestinal In	2.06 ± 0.02	0.044 ± 0.01
Intestinal Out	13.18 ± 1.25	0.86 ± 0.18

Amount of total phenolic content (TPC), expressed as gallic acid equivalents (GAE) and total flavonoid content (TFC), expressed as catechin equivalents (CATeq), determined from dried garlic powder (100g) using ethanolic extraction (EtOH) and after simulated gastro-intestinal digestion, i.e., phenolics remaining after the gastric phase (GF-In and GF-Out) and intestinal phase (IF and WF). Data shown represent means ± SD (n = 3 independent experiments). Significant differences ($p < 0.05$) between the different sample are indicate lower cases in superscript (in a column). Abbreviation: TAC, total antioxidant capacity; Txeq, Trolox equivalents.

However, the percentage of polyphenol that could go through the stomach during gastric digestion (i.e., the potentially bioavailable fraction) compare with undigested extraction was TPC for 51.28 % and TFC for 35.14 %. In general, the amount of polyphenol released from gastric to intestinal phase i.e., % of recovery gastric to intestine was TPC for 21.68% and TFC for 7.10%. Our results highlight that majority of polyphenols and flavonoids were released during the gastric phase which is agreed with gastrointestinal studies in grape by Tagliazucchi et al. (2010) and strawberry by (Ariza et al. 2016) and some phenolics were absorbed through the stomach during gastric environment (Fang 2014).

These results suggest that gastric digestion improves the release of phenolic compounds from the garlic matrix. This may be attributed to the acidic pH and enzymatic activity in the gastric

environment, which may induce the hydrolysis of a few phenolic compounds bound to other food components (Baublis et al. 2000; Liyana-Pathirana and Shahidi 2005)

This study indicates that not all detected poly phenols are present in intestinal digestion. TPC and TFC were decreased by 36% and 6% in the dialyzed fraction, with respect to the undigested extract respectively, which agreed with experiments where Broccoli has lost 75% phenolic compounds after intestinal digestion (Vallejo et al. 2004).

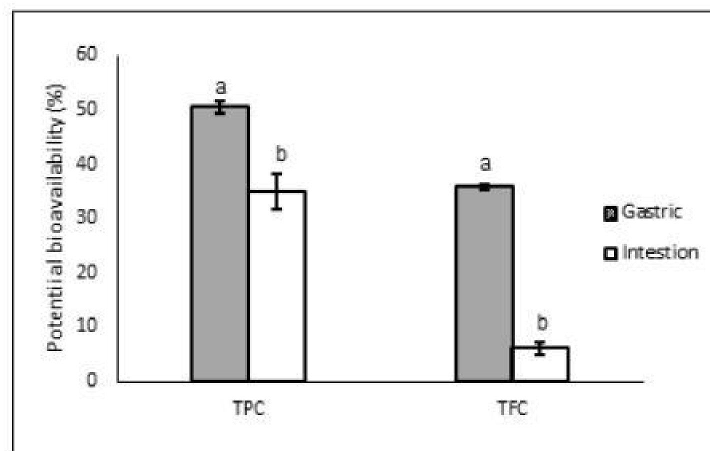


Figure 3.1: Potential bioavailability of total phenolic and flavonoid compound of garlic after gastrointestinal digestion compared to the undigested sample. Data were reported as a mean ± SD. Columns labelled with different letters are significantly different ($p<0.05$).

Therefore, the proportion of phenolics potentially going through the intestine during intestinal digestion (i.e., the potentially bioavailable fraction) was 35.52% for TPC, 5.95% for TFC. Above all, which phenolic compounds were not absorbed during gastric or intestinal digestion and reached the colon, we found for TPC and TFC values 13.18 mg GAE/g DW and 0.86 mg CATeq/g DW in the waste fraction that was 3.5-fold and 19.5-fold higher with respect to the IN fraction (dialyzed fraction).

These results are in agreement with a different type of berry digestion conducted by (Giampieri et al. 2018) and found a high amount of TPC and TFC in the intestinal out fraction (waste fraction). Consequently, after reaching the colon, metabolized by microbiota and absorption of intact polyphenols through the colonic epithelium and passage into the

bloodstream or breakdown of the original polyphenol structures into metabolites (Williamson and Manach 2005). After that the residue of the indigestible fraction, fermentation is a part of the unavailable matter excreted in faces as well as non-bioaccessible polyphenols(Guillon et al. 1995). Based on these results, garlic has potential prebiotic activity in the form of specific growth stimulation of bifid bacteria in the human gut (Laparra and Sanz 2010; Sunu et al. 2019).

3.2.1.1. Efficiency of Extraction Conditions

However, the pH of a sample solution might significantly influence extraction efficiency, specially when acidic or basic solutes are extracted from the food matrix. Taking into this consideration, extraction conditions can be affected phenolic compound release (Saura-Calixto et al. 2007).

After comparison between gastric digestion and undigested extracts, our data showed surprising result where most compounds of garlic were released to a greater extent after gastric digestion in comparison with undigested extracts. From Table 3.5, we can see the gastric conditions increase the extract ability of phenolic compounds from the food matrix which is agreed with (Ariza et al. 2016)

The values of extraction efficiency (Ee) were more than 1 (Table 3.5). It could be in chemical extraction, all antioxidants were not completely released in solvent from the food matrix, some bound polyphenols were retained in residual portion and their antioxidant capacity are ignored (Pérez-Jiménez and Saura-Calixto 2005).

Table: 3.5. Extraction efficiency of gastric conditions of garlic

	Extraction efficiency (%)
TPC	2.15
TFC	1.19
DPPH	2.59
TEAC	3.26
FRAP	1.33

Ee; Gastric fraction vs. undigested extracts

Furthermore, the bound form of phenolic compounds with proteins and other biomolecules are gently delivered during the digestive process (Hsu et al. 2004) rather than the other process such as, chemical and cooked garlic extract had lower antioxidant potential than digestive extract due to loss of phenolic compounds (Bhatt and Patel 2013). During heating, garlic has lost some polyphenols (Gorinstein et al. 2008). It could be heated processing promotes the polymerization of phenolic compounds to form brown-colored macromolecules (Ryan and Prescott 2010).

Finally, in our experiments, we observed the different amounts of TPC, TFC, and TAC between the solvent extraction and gastrointestinal digestion of garlic. Whereas the physiological environment will be preferred more antioxidants extraction by resembling the biological behaviour of antioxidants which agreed with the result of (Bhatt and Patel 2013) where they found fewer antioxidants in chemical extraction.

In this sense, the amount of antioxidants in gastric digested of garlic were quite higher than in undigested samples, indicating that *in vitro* digestion provide better conditions (i.e., low pH and pepsin) for the release of phenolic compounds from the matrix and/or for their stability (Ryan and Prescott 2010; NAVARRO GARCIA and SIMÓN 2013).

On the other hand, a drastic reduction of phenolic compounds and flavonoids was observed in the intestinal fraction. Thus, little number of phenolic compounds suggests that the intestine imposes a limitation to their availability that can result either from chemical modifications or physical barriers. Where chemical effects could arise from antioxidant degradation or from alteration of the antioxidant properties by the effects of intestinal conditions (i.e., in our study, pH 7.7, bile salts, and pancreatin). Since the action of the local microbiota is not considered in our study, it contributes significantly to the digestive process (Murkovic et al. 2000; Pérez-Vicente et al. 2002). Though, the low rate of antioxidant recovery in the intestinal fraction does not indicate a low absorption, because some phenolic compounds can be absorbed through the gastric wall. In addition, *in vivo* studies have revealed that antioxidants could be passed through the gut membrane either by passive diffusion or by specific membrane transporters after the interaction with certain glucosides (active glucose transporter: SGLT1) (Clifford and Scalbert 2000; Talavéra et al. 2003; Del Rio et al. 2013; Fang 2014).

3.2.2. Antioxidant capacity after *in vitro* digestion

During the *in vitro* digestion, antioxidant compounds might be chemically altered with consequent modification of their functions and chemical properties, which can lead to a considerable antioxidant capacity and show different results with different evaluation methods. Hence, evaluation of antioxidant capacity measurement by more than one method is recommended by many authors (Bhatt and Patel 2013; Tommonaro et al. 2017).

In this study, we have assessed antioxidant capacity with three different methods by DPPH, FRAP, and TEAC (Table 3.4 and 3.6). Antioxidant that has been absorbed by the stomach, showed antioxidant activities in the stomach (GI-Out fraction) for DPPH 43.07 %, TEAC 55.84 %, and FRAP 34.18 % with respect to undigested extract. Specifically, in all three analytical methods, a significant difference ($p<0.05$) was found between gastric digestion, intestinal digestion, and waste fraction in the FRAP test. As regards, DPPH and TEAC assay, no significant ($p<0.05$) difference was found between gastric digestion and elimination.
Most often, the stomach and gastrointestinal tract are exposed to produce ROS by host-related factors, such as by respiratory bursts of immune cells following their activation by diet-derived bacteria and toxins (Halliwell et al. 2005). Thus, antioxidants may play a curial role in the GI tract by maintaining redox balance against harmful oxidants and preventing GI tract diseases caused by ROS generation during digestion processes. Our results suggest that the antioxidant compounds released during simulated GI digestion were able to reduce free radicals either by hydrogen donation, as assessed by ABTS test, or by electron donation, as assessed by the FRAP test and ability to scavenge hydroxyl radical DPPH test, possibly favoring hydrogen atom transfer mechanisms, requiring lower energy (Leopoldini et al. 2011). In the present study, total antioxidant activities in both GI-out and IF fraction were higher than those determined by undigested extracts Table 3.6. Consequently, antioxidant activities increased in gastric digestion 2.6-fold for DPPH 3.3-fold for TEAC, and 1.3-fold for FRAP compared with the undigested extract. Apparently, due to the higher concentrations of polyphenols present in these digested fractions compared to chemical extraction. Preferentially, it can be other compounds or hydrophilic compounds present in the digest extract that acts as an antioxidant and interact with phenolic compounds or are associated with the radical cation ABTS+ and increase their antioxidant activity (Rochín-Wong et al. 2013; Helal et al. 2014; Hayano-Kanashiro et al. 2016).

Table 3.6. Total antioxidant capacity, determined by FRAP, DPPH and TEAC in garlic extracts before (undigested) and after gastric (gastric fraction) and total digestion (intestinal fraction).

	DPPH (µmol Txeq/g DW)	TEAC (µmol Txeq/g DW)	FRAP (µmol Txeq/g DW)
Undigested extract	9.24 ± 0.27 c	7.70 ± 0.61c	105.17 ± 11.51a
Gastric digestion			
Gastric OUT	3.98 ± 1.33 dns	4.30 ± 2.87 cdns	35.95 ± 2.82 b*
Gastric IN	19.91 ± 1.79 b*	20.81 ± 0.71a*	103.45 ± 1.77 ans
Intestinal digestion			
Intestinal IN	2.72 ± 0.29 dns	1.94 ± 0.02 dns	22.17 ± 0.65 b*
Intestinal OUT	25.10 ± 1.08 a*	12.68 ± 0.08 b*	107.21 ± 4.23 ans

Results are expressed as mean ± SD (n = 3). n.s. not significant; Significant differences ($p < 0.05$) between the different garlic digested extract and raw extract are indicated with differing lower-case letters in superscript (in acolumn). * Signifificant differences ($p < 0.05$) between IN and OUT fractions of gastric and intestinal digestion.

Furthermore, the scenario of the intestine was the opposite, a significant loss ($p < 0.05$) of total antioxidant activity was observed during IF digestion (bioavailable fraction) regarding undigested extract. The antioxidant activity DPPH, FRAP, and TEAC were decreased 29.44%, 21.8%, and 25.19%, respectively compared to undigested extract. When GI-Out and IF were compared, the antioxidant capacity of intestinal fraction DPPH, FRAP, and TEAC were decreased by 13.66%, 21.43% and 9.32% agreed with another lab studies those deals with honey digestion (Cianciosi et al. 2019).

The reduction of antioxidant activities in the intestinal environment could be associated with the fact that some components might be transformed into different structural forms with their other chemical properties due to their affectability to alkaline conditions (Bermúdez-Soto et

al. 2007). In addition, these compounds are capable to bind with other components and forming large complexes, that could not pass through the dialysis membrane. For instance, the total antioxidant capacity of digested apples was decreased 57% and 46% for FRAP and ABTS tests, respectively compare with fresh apple after gastrointestinal digestion (Bouayed et al. 2011). However, The IN-Out (waste fraction) showed significantly ($p<0.05$) increased antioxidant activity 9.2-fold, 6.5-fold, and 4.8-fold for DPPH, TEAC, and FRAP than IF. Indeed, waste fraction exhibited the highest value among overall antioxidant capacity. Our results showed similarities with (Giampieri et al. 2018). strawberry digestion where antioxidant activity (DPPH, FRAP, and TEAC) of different types of digested berries has been lowered in the intestinal fraction than the elimination fraction. It could be a significant amount of flavonoids is not able to pass through the dialysis membrane and found in out fraction where they are degraded by colonic microflora to phenolic acids showed high antioxidant activity (Del Rio et al. 2013). It can be concluded that an escalation in free phenolics is related to their antioxidant potential and is expected to improve their bioavailability in the large intestine (Bhanja et al. 2009). Apart from the aforementioned, antioxidant activity changes during simulated digestion, suggesting that generated bioactive polyphenol compounds might play the main role.

3.2.3. Relationship between phenolic compounds and antioxidant capacity of undigested and digest garlic extract

To assess the relationship between variables, evaluated values from undigested (ethanolic extract) and digested extracts (GF-Out, GF-In, IF and WF) from garlic. The correlation between the total phenolic content and antioxidant parameters of garlic (digested and undigested) are shown in Table 3.7.

In our studies, we found a strong correlation between the TPC and TFC (R^2 = 0. 861) of the undigested and digested fraction of extract. Similarly, a significant strong positive correlation was observed between TPC and DPPH ($R^2 = 0.994$, $p \leq 0.001$), our result matched with those found a positive correlation between TPC and antioxidant in different undigested allium spices, (Nencini et al. 2011) found $R^2 = 0.468$ for DPPH test and $R^2 = 0.669$ for FRAP test (Nencini et al. 2011); Soto et al., found for DPPH test $R = 0.51$, $p \leq 0.01$ and also for FRAP test $R = 0.80$, $p \leq 0.01$ (Soto et al. 2016). Previously, a significant positive correlation ($R = 0.95$, $P < 0.05$) was found between phenolics content and antioxidant capacity among five

well-known anticancer spices, ginger, red pepper, garlic, green onion, and leek (Brand-Williams et al., 1995). On the other side, a weak correlation with TPC of different garlic species was found (Nuutila et al. 2003b). It could be used the differences in the experimental conditions. Simultaneously, a strong positive correlation was found between TPC and TEAC (R^2 = 0.785) and TPC and FRAP (R^2 = 0.836), which was also observed previously in the undigest extract of garlic (Nencini et al. 2011). This positive correlation is due to the hydroxyl group of phenolic compounds and flavonoids that donate hydrogen and electron (Brand-Williams et al. 1995; Rice-Evans et al. 1996).

Furthermore, a high correlation was found among TFC and FRAP (R^2 = 0.969, $p < 0.006$) and DPPH (R^2 = 0.824). Our data agreed with (Prakash et al. 2007) where, the ability to scavenge hydroxyl radical in garlic was mainly due to flavonoids. This scavenges hydroxyl radical can help to prevent oxidative damage that involves different pathological processes such as cancer and different type of diseases (Guo et al. 2011). While low correlation coefficients were obtained between TFC and TEAC (R^2 = 0.678).

However, previously reported a similar correlation between phenolic compounds and antioxidant activities observes in honey (for TPC R^2 = 0.869-0.895; for TFC R^2 = 0.704-0.739) and berry for TPC R^2 = 0.835; $p \leq 0.0001$ and TFC) samples (Ariza et al. 2016; Cianciosi et al. 2019). Our garlic results also alien with these results.

Table 3.7: Regression coefficient of TPC and TFC in correlation with DPPH, FRAP and TEAC

	TFC	DPPH	TEAC	FRAP
TPC	0.861	0.988**	0.617	0.699
TFC		0.6796	0.459	0.939*
DPPH			0.837	0.821
TEAC				0.782

TPC, Total phenolic content; TFC, Total flavonoid content; FRAP, ferric reducing antioxidant power assay; TEAC, Trolox equivalent antioxidant capacity assay; DPPH,

Diphenyl-1-picrylhydrazyl assay; a 95% confidence interval, * Significant at $p \leq 0.05$ ** Significant at $p \leq 0.001$.

According to the correlation values of undigested and digested samples of garlic confirmed that polyphenols and flavonoids significantly contributed to the TAC of garlic extract.

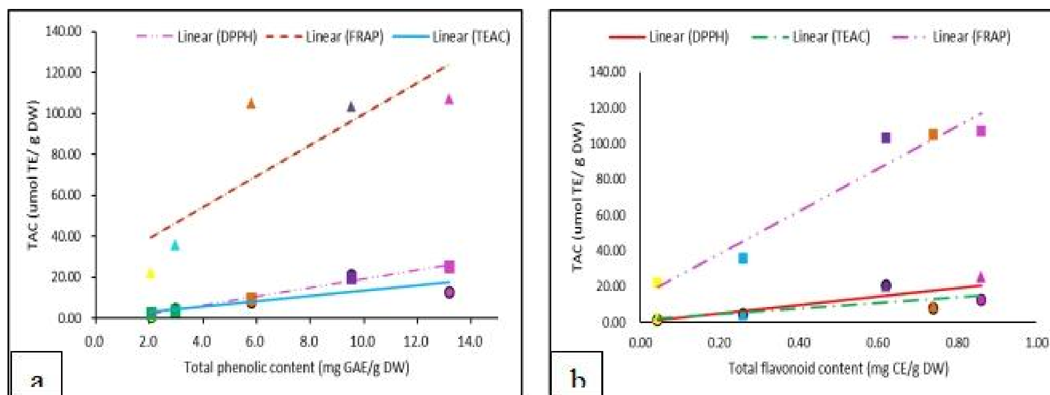


Figure 3.2: Linear relationship between total phenolic content and antioxidant activity a) Total phenolic content and TAC (DPPH, TEAC and FRAP); b) Total flavonoid content and TAC (DPPH, TEAC and FRAP). The colour of the dots in the figure represents digestion condition; orange the undigested samples, cyan, green the GI-out fraction, violate the GI- In fraction, yellow the IN fraction and pink the WF fraction after digestion.

Finally, a strong correlation was observed between DPPH and FRAP ($R^2 = 0.821$, $p>0.05$) as well as DPPH and TEAC ($R^2 = 0.837$, $p > 0.05$) including FRAP and TEAC ($R^2 = 0.782$) but value did not demonstrate significant ($p<0.05$) statistical differences.

In summary, this work highlights not only the relevance of garlic extracts antioxidant capacity but their potential healthy properties under physiological-like conditions. These results give new insights for garlic phenol and open a wide range of applications for improving human health (i.e., pharmaceutical uses).

RESULTS AND DISCUSSION: PART III

3.3. Anti-proliferative effect of garlic on human breast cancer MCF_7 cells

Focusing on the third part of my Ph. D. project was to evaluate the anticancer effects of garlic extract human breast adenocarcinoma MCF_7 cell lines by evaluating cell viability, ROS, Oxidative stress, Antioxidant enzyme activity, Apoptosis, Cell metabolism effect, and their related molecular mechanism. Although, aged garlic extract exerts its cancer-inhibitory action in different and complementary ways, due to the presence of a variety of compounds in the extract (Borek 2001). To the best of our knowledge, this is the first report to demonstrate the anticancer effects of phenol enriched garlic extract on breast cancer MCF_7 cells.

3.3.1. Cytotoxic activity of garlic extracts on breast cancer MCF_7 cell lines

In the next step of our experiment, we evaluated the anti-proliferative effect of undigested and digested garlic extract on human breast cancer MCF_7 cells. In addition, we also analyzed the cytotoxic effect of garlic extract (undigested) on non-malignant breast epithelial cells MCF10A cells to ensure the toxic effect of garlic extract on normal cells.

The cytotoxic effects of garlic extract were determined by performing MTT assay to investigate the anti-proliferative effect on breast cancer cell line MCF_7. Our results indicated the possible anti-proliferative effect of the garlic extract with relation to increasing concentration at 0-10 mg/ml and exposure time at 24 h, 48 h, and 72 h on MFC_7 cells. Our results confirm that garlic extracts significantly ($p<0.05$) reduced breast cancer MCF_7 cell proliferation at various concentrations in a time-dependent manner (Figure. 3.3). Whereas high concentrations of garlic extract decreased cell viability on MCF_7 compared to untreated cells in a dose and time-dependent manner which are consistent with previous results of (Ghazanfari et al. 2011), where they point out the effect of garlic extract on breast cancer MCF_7 cells and the IC_{50} (concentrations necessary for 50% inhibition of cell growth) value 3.087 mg/ml, 1.821 mg/ml and 1.744 mg/ml at 24h, 48h and 72h (Ghazanfari et al. 2011). Similar cytotoxic effects obtain by Isbilen and his co-workers on MCF_7 cells in a concentration and time-dependent manner at 5 mg/ml and 10 mg/ml concentrations for 24h, 48h, and 72h compared to the control (Isbilen et al. 2018). These results are validated to our results on MCF_7 cells, where we found the IC_{50} values of dried garlic extract were 3.85 mg/ml, 4.97 mg/ml, and 8.1 mg/ml at 24h, 48h, and 72h respectively.

Oppositely, (Bagul et al. 2015) shows 80–90 % ($P < 0.05$) anti-proliferative effect of crude garlic extract at high concentration 0.25, 0.5, or 1 g/ml at 72h on MCF_7 breast cancer cells (Bagul et al. 2015). This result was further strengthened with *in vivo* studies done by (Talib 2017); they demonstrated, garlic aqueous extract showed anti-proliferative effect and the IC_{50} value of 78.9 mg/ml in mouse mammary EMT6/P cells line. Comparatively, our result showed satisfactory cytotoxic effectiveness at a lower dose of garlic extract. In addition, (Ohkubo et al. 2018) claim 73 % and 65 % cytotoxic effect of aged garlic extract at dose 10 mg/ml for 72 h in wild-type and multidrug-resistant human colon cancer cells LoVo WT and LoVo DX cells, respectively. This data suggests that anti-proliferative effects of garlic extract where a robust reduction is observed at a lower concentration than higher concentrations that comply with other spices results on breast cancer cells; *Nigella sativa* shows cytotoxicity at dose 2.72 mg/ml for 48h and 72h, Cloves oils (*Syzygium aromaticum L.*) oils at dose 36.43 µg/ml and 17.6 µg/ml on MCF_7 cells, respectively (Mahmoud and Torchilin 2013; Baharetha et al. 2013; Kumar et al. 2014). Along with ethanolic extract of *Cinnamomum zeylanicum* have no anti proliferative effect at low dose of 25 µg/mL against human breast cancer MDA-MB-231 cell line (Husain et al. 2018). Likewise, some African traditional spices Fagara leprieuri, Fagara xanthoxyloïdes, Mondia whitei and Xylopia aethiopica and Goji berry are exhibited cytotoxic effects on breast cancer MCF_7 cells, respectively (Choumessi et al. 2012; Wawruszak et al. 2021).

In accordance, the current study on dried garlic extract exerted an accentuated anti-proliferative effect and reinforced the notion of the presence of the different compounds with profound anticancer potential.

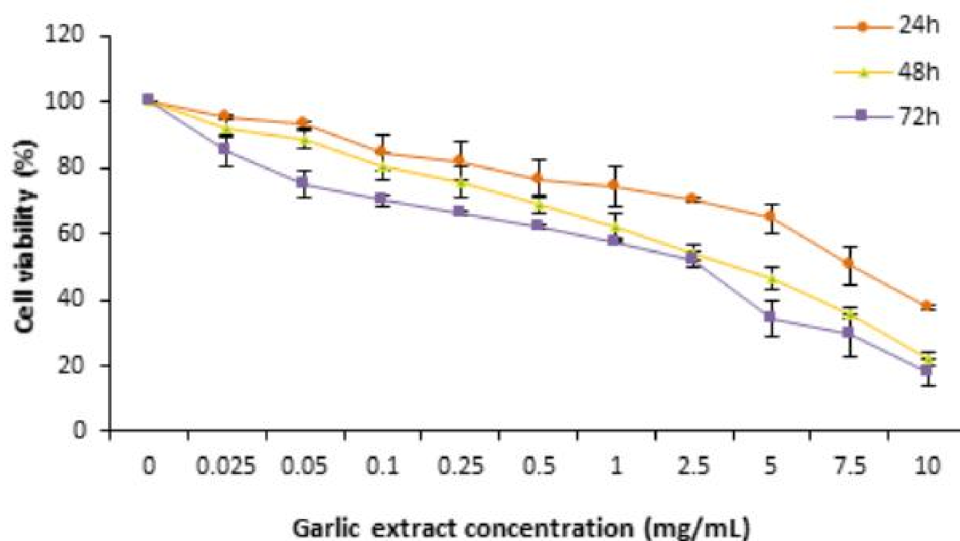


Figure 3.3: Viability of MCF_7 determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were treated with various concentrations of phenol enrich garlic extracts (0–10 mg/ml) for 24 h, 48 h and 72 h. Data are expressed as the ratio to untreated group with treated group. Data are expressed as mean ± SD for three replicas (n = 3) of three independent experiments.

3.3.2. Cytotoxic effect of bioaccessible fractions of garlic on breast cancer MCF_7 cells

Until now the cytotoxic effect has only been evaluated to undigested garlic and breast cancer cells. According to our knowledge, it is the first work, where we evaluated the cellular state and potential cytotoxic action of the bioaccessible polyphenols on breast cancer MCF_7 cells. In this part of study, we investigated the anti-proliferative effect of digested garlic extract on breast cancer MCF_7 cells. The MCF_7 cells were pre-incubated with concentrate intestinal IF (bioaccessible fraction) of digested garlic extract 0 -10 mg/ml for 24h, 48h and 72h.

Remarkably, the values of cell viability obtained after comparing with untreated cells (Figure 3.4) and this comparison will give more plausible results on the real action of the antioxidant present in the digested fraction (after the possible series of macro and micronutrient changes that influence their final bioaccessibility during the digestion procedure. Thus, the results showed a reduction of viable cells compared to untreated cells in a dose and time-dependent manner. After 24h incubation, digested garlic extract didn’t show any significant ($p<0.05$) effect on cell viability (75% - 89%).

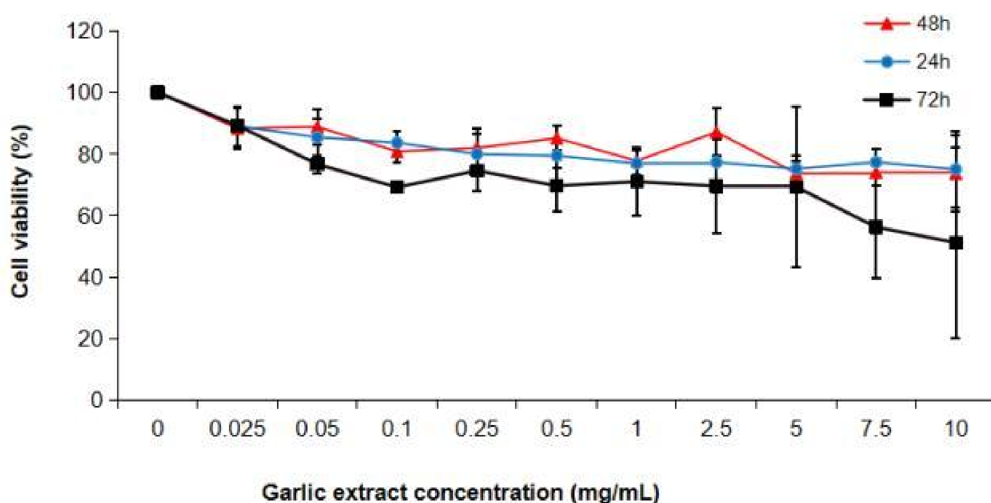


Figure 3.4: Viability of MCF7 cells determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were treated with various concentrations of polyphenol enrich digested garlic extracts (0–10 mg/ml) for 24 h, 48 h and 72 h. Data are expressed as the ratio to untreated group with treated group. Data are expressed as mean ± SD for three replicas (n = 3) of three independent experiments.

While, after 48h and 72h incubation, cell viability started to a significant ($p < 0.05$) reduction 73% - 88% for 48h and non-significant ($p<0.05$) reduction 51% - 89%, respectively. We noticed that, surprisingly, the IC_{50} value of undigested and digested garlic extract were almost similar at 24h and at 48h, and cell viability decreased for garlic digested extract, especially at higher concentrations. The marked observation from these results; the highest cytotoxic effect was obtained, a dose and time-dependent manner especially, at 48 and 72 h of incubation of cells with digested garlic extract.

In fact, a small decrease of cell proliferation compared with undigested extract at 48h after possibly due to the low bioavailability of antioxidant compound in the intestinal fraction mentioned earlier. Furthermore, there is no work on garlic digestion and cellular anti-proliferative effect. On account of this, our experiment showed a close resemblance to previously published results on the digested phenolic compound of strawberry; an inhibition of proliferation of HepG2 (human liver carcinoma cells) cells from intestinal fraction from 100% to 63% was noticed by (Ariza et al. 2018), following a dose and time depended on

manner. In addition, honey polyphenols also retained their anti-proliferative activity after gastrointestinal digestion, particularly intestinal fraction on colon cancer HCT-116 cell lines (Cianciosi et al. 2019).

Our study depicted that some factors such as the phenolic composition, the bioavailability of antioxidants such as polyphenol, and the antioxidant capacity may be influenced the anti-proliferative activity of the garlic. The higher bioaccessibility of the bioactive compounds depended on the complexes formed between polyphenols and the components of the cell wall that can be reversible or irreversible depending essentially on the affinity, polyphenols structures, proteins and polysaccharide concentrations, the action of digestive enzyme, temperature, and pH (Zhu 2018).

According to literature studies, there is no significant antiproliferative activity of garlic phenolic compounds on breast cancer cells with before and after *in vitro* digestion, as we mentioned before. The anti-proliferative effect of digestive garlic in accordance with the anti-proliferative effect of the digested phenolic compound of berries on human breast cancer cell line (Guo et al. 2017).

Overall, in both digested and undigested garlic extract, a cytotoxic effect on MCF_7 cells was observed indicating that phenolic compounds entrapped in the garlic matrix especially in the undigested extract. We hypothesized that our results would help to understand the bioavailability of garlic antioxidant compounds in the site of absorption and their anti-proliferative effects on breast cancer MCF_7 cells growth.

3.3.3. Cytotoxic activity of garlic extracts on MCF10A cell lines

Plant-derived natural compounds are important sources of anticancer agents because they induce less toxicity in non-cancer cells. (Phang et al. 2016; Bhardwaj et al. 2016). According to other studies, garlic components DAD induced the cytotoxic effects on breast cancer cells but not on normal breast cell lines (Kiesel and Stan 2017).

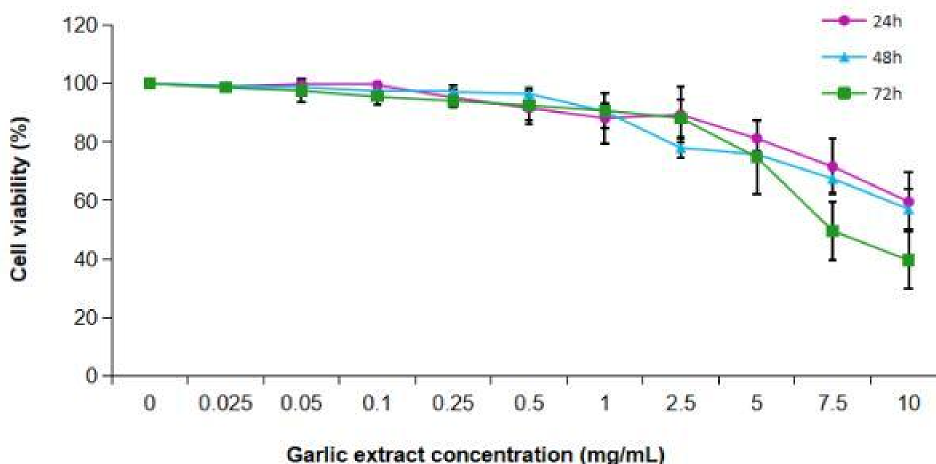


Figure 3.5: Viability of MCF10A cells determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were treated with various concentrations of polyphenol enrich garlic extracts (0–10 mg/ml) for 24 h, 48 h and 72 h. Data are expressed as the ratio to untreated group with treated group. Data are expressed as mean ± SD for three replicas (n = 3) of three independent experiments.

Regarding this present study, we evaluated, similar concentrations of garlic extract treatment (0-10 mg/ml) at 24, 48, and 72 h to determine the cytotoxic effects on non-cancerous human normal breast MCF10A cells (a non-tumorigenic cell line originally isolated from a fibrocystic breast disease and spontaneously immortalized). In particular, garlic extract exhibited no toxic effects on MCF10A cells, compared with untreated cells up to 7.5mg/ml (Figure 3.5). After, the cell viability was less decreased and showed less toxicity at the concentrations of 7.5 mg/mL for garlic extract. In these cases, non-tumorigenic MCF10A cells were significantly ($p < 0.05$) less sensitive to toxicity for garlic extract compared to breast cancer MCF_7 cells at 24h, 48h and 72h. Interestingly, garlic bioactive compound DAS has effectively recovered cell viability on non-tumorigenic MCF10A instead of showing cytotoxic effect (Chandra-Kuntal et al. 2013; McCaskill et al. 2014).

3.3.4. Garlic extract by modulating Oxidative stress in MCF_7 cells

3.3.4.1. Effect garlic extract induces intracellular ROS production on MCF_7 cells

Reactive oxygen species (ROS) are moderators of intracellular signalling cascades and can activate a sequence of pathways for programmed cell death such as apoptosis or autophagy by generating and accumulating in cancer cells. In cancer cells, there is an unbalanced ROS

level due to the defective cell metabolism and proliferative capacity, acting as a pro-tumorigenic factor (Nogueira and Hay 2013). In addition, recent evidence has revealed that natural compounds exhibit anti-tumour potential by generating ROS content in cancer cells that help to generate oxidative stress. Oxidative stress acts as a possible mediator of cell death by activating apoptosis or autophagy (Santandreu et al. 2011; Gunasekaran et al. 2015; Enayat et al. 2016; Bhardwaj et al. 2016), without cytotoxic effect against healthy cells (Ding et al. 2009). For example, the main phenolic component of turmeric; curcumin has potent anticancer activity against gastric cancer cells by inducing ROS-mediated apoptosis (Liang et al. 2014). Similarly, hexane extracts of garlic cloves has generated and accumulated ROS and the association of these effects with apoptotic cell death on using a human hepatocarcinoma cell line Hep3B *in vitro* (Kim et al. 2012). Recently, garlic component S-allyl cysteine (SAC) has also increased ROS accumulation in breast cancer MCF_7 cells (Vijayan et al. 2021). In particular, phenolic components such as p-coumaric acid, which suppressed cell growth in breast adenocarcinoma MCF_7 cell lines by generating ROS (Stefani et al. 2021). Also, caffeic and sinaptic acid which have activated AMPK pathway via ROS generated apoptosis induction in breast cancer MCF_7, MDA-MB-231 and MDA-MB-435 cells respectively and down regulated mitochondrial potential (Jia et al. 2014; Ahn et al. 2020). Likewise, salicylic as well as sodium salicylate has increased ROS production and the association of these effects with apoptotic tumour cell death (Chung et al. 2003).

In this present work, we investigated the intracellular ROS production in MCF_7 cells. In order to determine the intracellular ROS levels, MCF_7 cells were treated for 48 h with or without various concentrations of garlic extract (1.87 mg/ml, 4.97 mg/ml, and 8.1 mg /ml) for MCF_7 cells. The results were analyzed by using the CellROX® Orange assay kit by Tali™ Image-based Cytometer. We observed increased intercellular ROS accumulation in MCF_7 cells in a dose-dependent manner. After garlic extract treatment at dose 1.85 mg/ ml, 4.94 mg/ml and 8.1 mg/ml, ROS accumulated significantly ($P<0.05$) increased 1.77 fold, 5.73 fold and 6.87 fold respectively with respect to the control in a dose and time dependent manner. Thus, a high effect of garlic ROS generation was found with a concentration of 8.1 ml/mg. Our results showed that polyphenol enriched garlic extract increased intracellular ROS in a dose-dependent manner in breast cancer MCF_7 cells and fluorescence intensity showed a significant and dose-dependently increase in intracellular ROS levels in MCF_7 cells after being treated with garlic extract (Figure 3.6) for 48 h. In a word, ROS has also been

implicated in the mediation of apoptosis in cancer cells (Gupta et al. 2012; Paulsen and Carroll 2013).

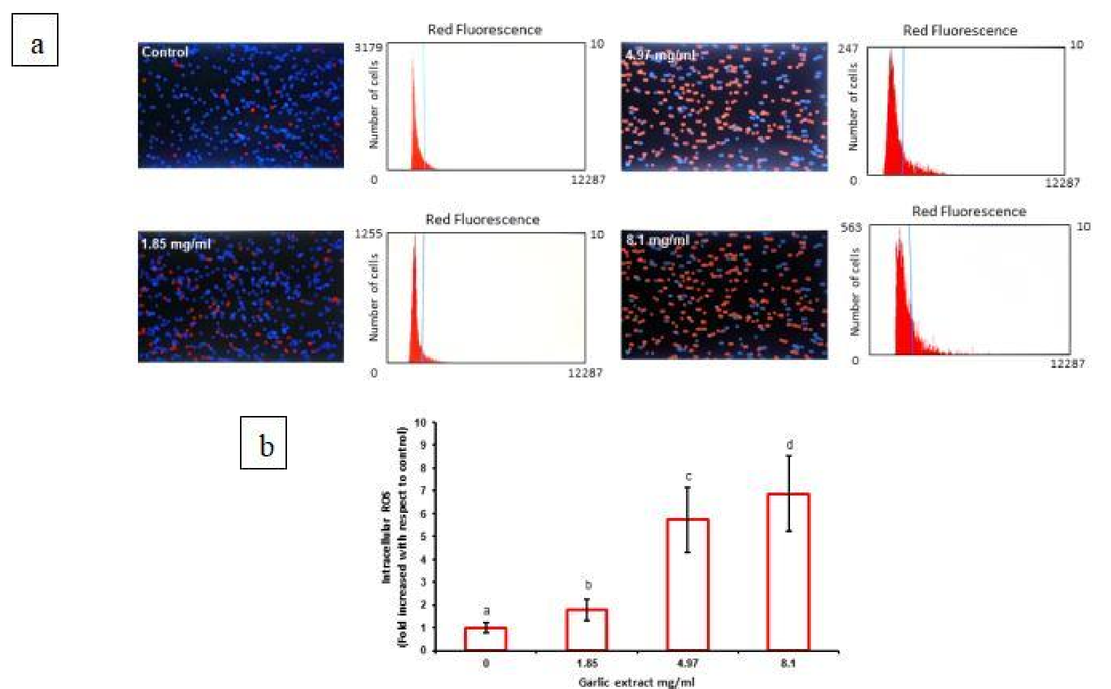


Figure 3.6: Intracellular ROS level in breast cancer MCF_7 cells treated with 1.84 mg/ml, 4.97 mg/ml and 8.1 mg/ml of garlic extract for 48 h. Intracellular ROS levels were evaluated by using CellROX® Orange assay kit and the Tali™ Image-based Cytometer. a) Representative images of intracellular ROS quantification are shown following; stressed cells appear red after 48 h garlic treatment. Scale bar = 50 µm, average cell size =13 µm). The cornflower blue line of the thumbnail histogram indicated the set threshold. b) Column's graph belongs to the same set of data compare to untreated cells with different superscript letters are significantly different ($p < 0.05$). All data shown were the mean ± SD of three independent experiments.

3.3.4.2. Effect of garlic extracts on antioxidant system on MCF_7 cells

Antioxidant defense systems in the human body maintain the balance between the generation and neutralization of ROS and antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), and glutathione (GSH). Moreover, the imbalance between ROS and antioxidant defence systems leads to oxidative stress and oxidative molecules can expose damage to all classes of biological macromolecules and eventually lead to cell death. According to previous studies, natural compounds can behave as pro-oxidants and have an

appreciable oxidative impact on cellular redox status, essential for (i) disruption of other antioxidant defence mechanism (ii) reduction of Nrf2 gene activation (Atsumi et al. 2007; Dewangan et al. 2017; Zedan et al. 2021; Sharma et al. 2021). A similar result was observed in our experiments with garlic extracts treatment in MCF_7 cells (Figure 3.7). Whereas we examined the activity and the expression of the antioxidant enzymes by using 1.85, 4.97, and 8.1 mg/ml of garlic extracts in breast cancer MCF_7 cells at 48h.

However, garlic, has ability to change the status of the GSH, GPx and GST in the liver by inducing oxidative stress (Bhuvaneswari et al. 2004). Here, we observed, garlic extracts decreased the activity of antioxidant enzymes, such as GST, GPX, GR and GSH enzymes up to 0.47-fold, 0.49 fold, 0.63 fold and 0.76 fold respectively, compare to untreated MCF_7 cells (Figure 3.7) in a dose and time depended manner.

However, GSH is an intracellular thiol antioxidant; a lower level of this GSH causes higher ROS production (Ribas et al. 2014) which changed in the intracellular redox environment, as a result, the activation of apoptotic enzymes and the progression of programmed cell death. Moreover, GSH depletion is an early hallmark observed in apoptosis (Franco et al. 2008). We evaluated the relationship between GSH depletion, the generation of reactive oxygen species (ROS), and the progression of apoptosis (Figure 3.8).

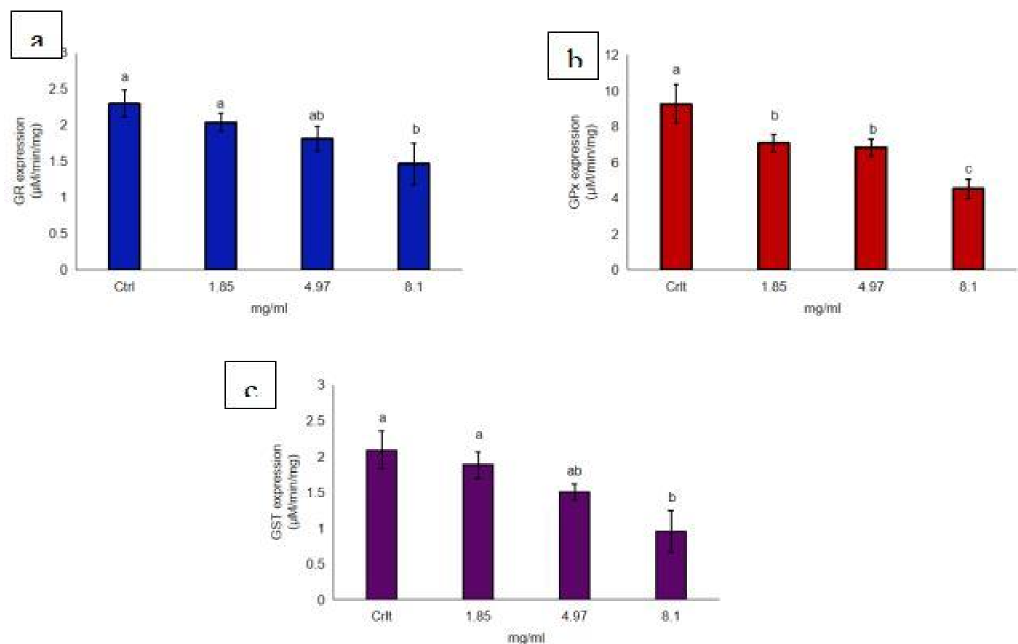


Figure 3.7: Garlic extracts reduced the antioxidant enzyme GR, GPx, and GST activity in MCF_7 cells. MCF_7 cell were incubated to different concentrations Crlt, 1.85 mg/ ml, 4.97 mg/ml and 8.1 mg/ml of garlic extracts for 48 h. a) GR, b) GPx, and c) GST activity was expressed as unit per mg protein and Column's graph belongs to the same set of data compare to untreated cells with different superscript letters are significantly different ($p < 0.05$). All data shown were the mean ± SD of three independent experiments.

After, we hypothesized that GSH efflux during death receptor-mediated apoptosis occurs via a GSH transporter. A similar cytotoxic effect has been shown in gallic acid and curcumin via ROS generation along with the glutathione depletion in breast cancer MDA-MB-231 cells line (Moghtaderi et al. 2018). Another study of garlic water preparation, allicin has showed an anti-proliferative effect in human breast and colon cancer via decreased intracellular GSH level (Hirsch et al. 2000). It has been reported that garlic extract increased cytotoxicity by compromising the cellular antioxidant enzyme system, depleted GSH, formed reactive oxygen species, collapsed the mitochondrial membrane potential, and induced lipid peroxidation (Figure 3.7- 3.11).

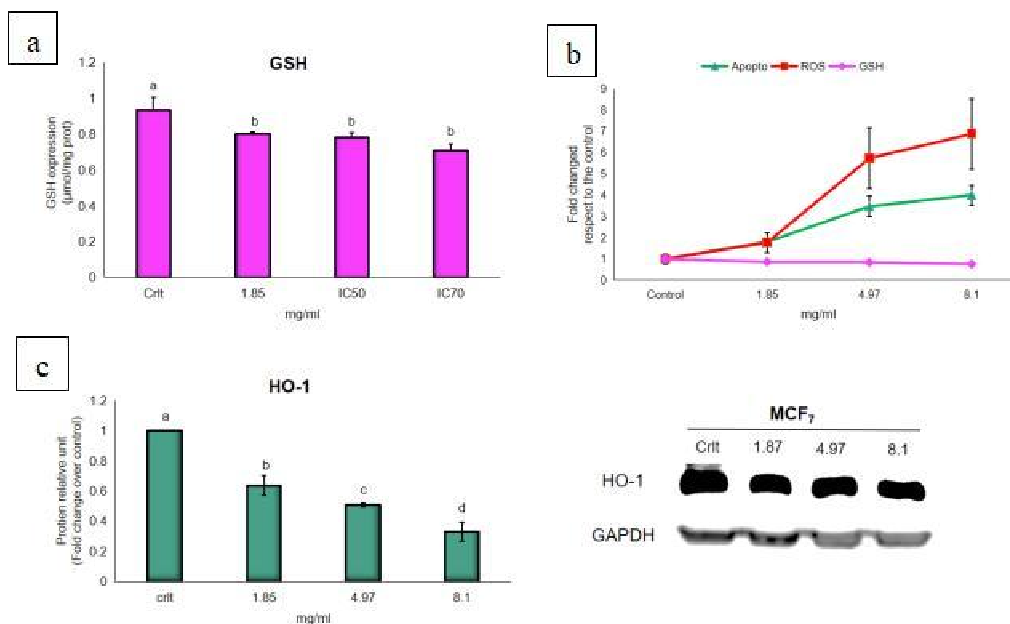


Figure 3.8: Garlic extracts reduced the antioxidant enzyme GSH activity in MCF_7 cells. MCF_7 cell were incubated to different concentrations of Crlt, 1.85 mg/ ml, 4.97 mg/ml and 8.1 mg/ml of garlic extract for 48 h. a) GSH enzyme activity was expressed as unit per mg protein and Column's graph belongs to the same set of data compare to untreated cells. b) Graphical representation expresses the relationship between level of GSH, ROS generation and apoptotic cells with the different treatment or

without treatment conditions. c)The expression of HO-1 was determined by western blotting analysis. GADPH was used as a loading control protein. All data shown were the mean ± SD of three independent experiments. Different superscripts letter for each column indicated significant differences ($p < 0.05$).

Recently, the nuclear factor erythroid-2-related factor 2 (Nrf2)-Keap1-ARE (Keap1) pathway, a major regulator of oxidative stress and a promising target for cancer therapy (Jaramillo and Zhang 2013). Nrf2 has a dual role in the case of cancer, cancer development and cancer prevention. For instance, it increase cancer survival by increasing GSTP in hepatocellular carcinoma (IKEDA et al. 2004; Lau et al. 2008).

On the other hand, Nrf2 is reclusive in the cytoplasm by an actin-binding protein, Keap1, and upon exposure of cells to inducers such as oxidative stress, Nrf2 detach from Keap1, translocate to the nucleus, binds to AREs, and transactivates phase 2 detoxifying enzymes or proteins, such as NAD(P)H: quinone reductase-1 (NQO1), heme oxygenase-1 (HO-1), and glutathione synthetase (GSS) such as GST, GSH.

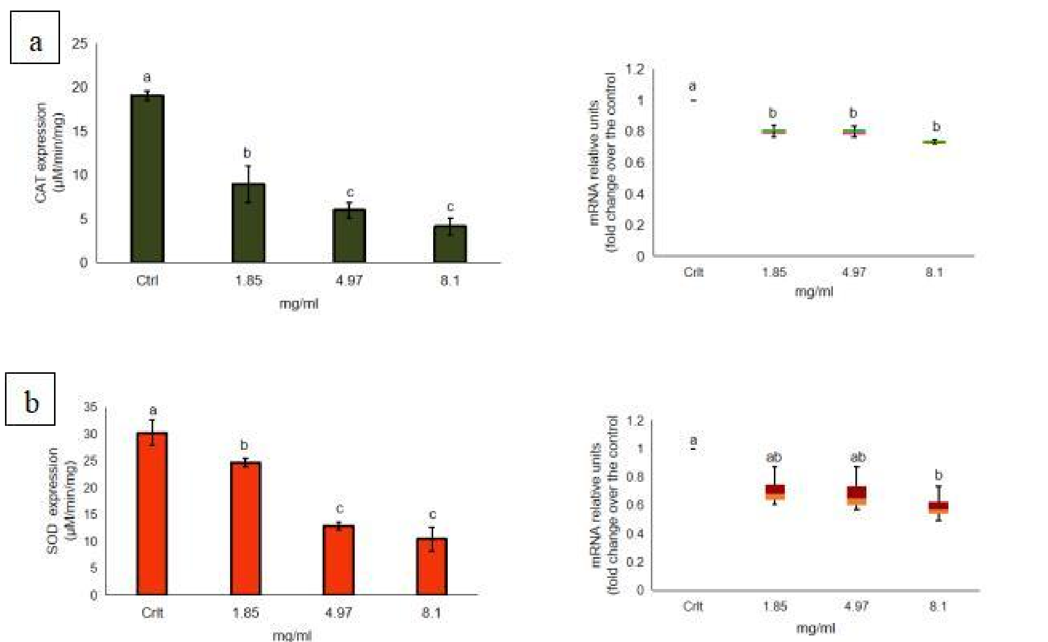


Figure 3.9: Garlic extracts reduced the antioxidant enzyme SOD and Catalase (CAT) activity in MCF_7 cells. MCF_7 cell were incubated to different concentrations Crlt, 1.85 mg/ ml, 4.97 mg/ml and 8.1 mg/ml of garlic extract for 48 h. a) CAT and b) SOD activity was expressed as unit per mg protein and Column's graph belongs to the same set of data compare to untreated cells. mRNA expression of

antioxidant marker SOD and Catalase were analysed by real-time PCR. GADPH used as housekeeping gene that was amplified under the same PCR conditions for normalized quantitative data. Results are expressed as median, first quartiles, third quartiles, minimum and maximum values. All data shown were the mean ± SD (n = 3). Different superscript letters for each column indicated significant differences ($p < 0.05$).

After then, Nrf2 showed mitochondrial protection from oxidative stress by increasing GSH protein in the mitochondrial (Jeong et al. 2006; Dinkova-Kostova and Abramov 2015; Tebay et al. 2015). Together with, loss of function of Keap1 may result in prolonged activation of Nrf2 providing cancer cells with a growth advantage by up-regulating of Nrf2 downstream genes (Wang et al. 2008). Considering this, dysfunction of Keap1-Nrf2 interaction in the breast has been able to repress Nrf2 activity and decreased antioxidant enzyme activities (Nioi and Nguyen 2007). In this part of the study, we investigated phenolic compound enrich garlic extract effect on antioxidant enzyme activities in the breast cancer MCF_7 cells; to target the interruption of Keap1-Nrf2 interaction.

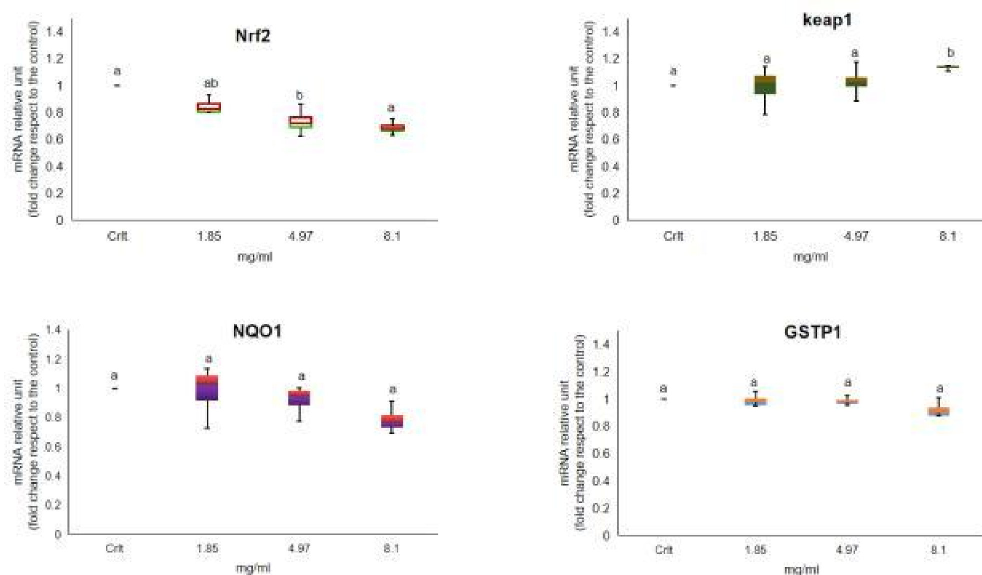


Figure 3.10: Garlic extracts provokes antioxidant enzyme activity in MCF_7 cell. MCF_7 cell were incubates to different concentrations 1.85 mg/ ml, 4.97 mg/ml and 8.1 mg/ml of garlic extracts for 48h. mRNA expression of Nrf2, keap1, NQO1 and GSTP1 were analyzed by real-time PCR. GADPH use as housekeeping gene that was amplified under the same PCR conditions for normalized quantitative data. Results are expressed as median, first quartiles, third quartiles, minimum and maximum values.

All data shown were the mean ± SD (n = 3). Different superscript letters for each column indicated significant differences ($p < 0.05$).

To demonstrate the modulation effect on the Nrf2-Keap1 pathway by the garlic extracts, the gene and protein expression of Nrf2 target genes have also been evaluated (Figure 3.10). The mRNA expression of SOD, catalase, and GST significantly ($P < 0.05$) decreased up to 0.37-fold, 0.22 fold, and 0.85 fold respectively, compared with untreated cells (Figure 3.9 and 3.10) in a dose and time depended manner. And also, the mRNA expression of Nrf2 and NQO1 were also significantly ($P<0.05$) downregulated up to 0.69-fold and 0.78 fold, respectively, compared with untreated cells in a dose and time depended manner. Moreover, garlic extracts significantly ($P<0.05$) upregulated the expression of the Keap1 gene up to 1.34-fold compared with untreated cells in a dose and time depended manner.

Henceforth, the mRNA and protein expression of Nrf2 and its downstream targets, SOD, catalase, and HO-1 (Figure 3.8 and 3.9); confirmed that effects of garlic extracts facilitated MCF_7 cell cytotoxic in response to oxidative stress in a dose and time depended manner.

As mentioned, HPLC analysis revealed the presence of several phenolic compounds; caffeic, p-coumaric acid, dihydroxy benzoic acid, galic acid in garlic extracts. It could be explained that attributed to their antioxidant activity due to presence of high phenolic contents. Our results corroborate with Sharma experiment; the phenolic extracts of *Halophila ovalis has* reduced the antioxidant defense system in MCF_7 cells by decreasing the expression of Nrf-2 and regulatory antioxidant enzymes, SOD-2 and HO-1 (Sharma et al. 2021). Likewise, garlic studies with gastric cancer showed, diallyl trisulfide (DTS) has inhibited tumour by decreasing Nrf2 protein expression in *in vitro* and *in vivo* studies (Jiang et al. 2017). In contrast, DATS has showed a cytoprotective effect via significantly up-regulated expression of NQO1 and Nrf2 in non-tumorigenic breast epithelial MCF-10A cells and in human hepatoma HepG2 cells (Chen et al. 2004; Cho et al. 2019).

Notably, *in vivo* studies, garlic could modulate oxidative stress depending on its route of administration. After oral administration of the high dose of garlic increases antioxidant enzyme activity including catalase and SOD in rat brain. Whereas intra peritoneal administration of garlic decreased the enzyme activities of catalase and SOD (Hamlaoui-Gasmi et al. 2011).

Finally, taken all together, garlic extracts caused a pronounced redox impairment in MCF_7 cells by inhibiting the antioxidant activity of SOD, catalase, GST, GPx, GR, and GSH antioxidant modulating the Nrf2/Keap pathway. These findings recommend that the anti-proliferative effect of garlic extracts is associated with its oxidative stress-induced down regulating antioxidant protection mechanism by stimulating ROS production in human breast cancer MCF_7 cells.

3.3.4.3. Effect of garlic extracts on the biomarker of oxidative stress in MCF_7 cells

To evaluate whether garlic extract inhibited cell proliferation in human breast cancer MCF_7 cells is mediated by oxidative stress, we evaluated the oxidative damage of lipid (TBARS level) and protein (carbonyl content level) and DNA (OGG1 protein expression). Because the oxidative burst resulted in protein, lipid and DNA damage as demonstrated by protein carbonyl accumulation, lipid peroxidation and damage of DNA in the cells. In the presence of polyunsaturated phospholipids, cell membranes are susceptible to oxidative damage that can be resulted in the formation of lipid peroxides. Our result obtained from TABARS and carbonyl protein assay showed a significant ($p < 0.05$) increased level of lipid and protein up to 2.20 fold and up to 3.78 fold respectively compare to the control respectively (Figure 3.11) in a dose and time dependend manner.

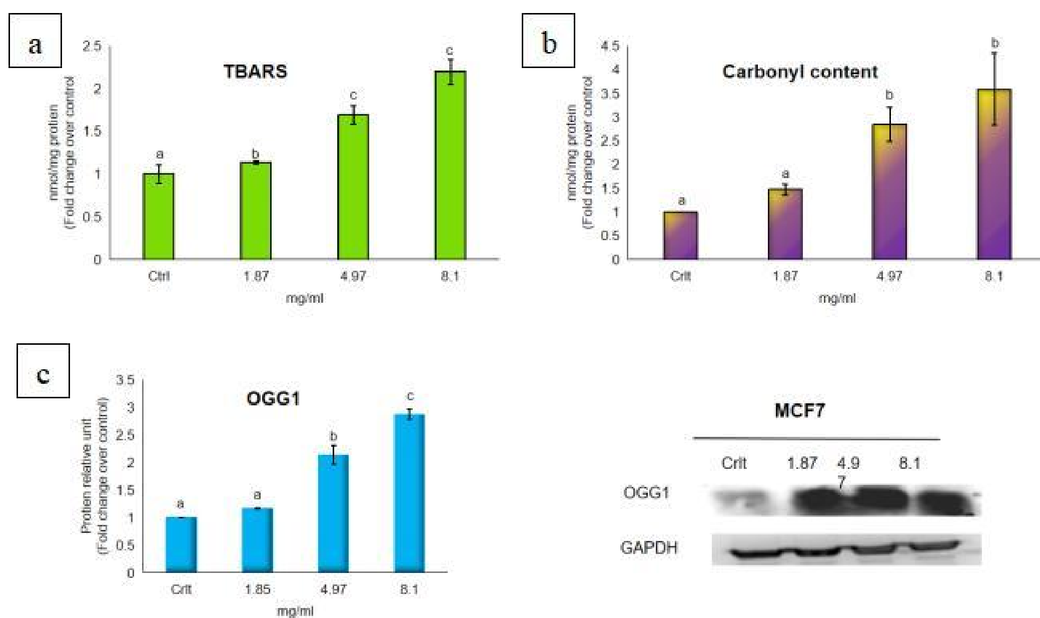


Figure 3.11: Induction of oxidative stress in breast cancer MCF_7 cell and lipid and protein oxidative damage was evaluated from the a) TBARS levels and b) protein carbonyl content. Incubation of

MCF_7 cell with garlic extracts with Crlt (untreated cell), 1.84 mg/ml, 4.97 mg/ml and 8.1 mg/ml for 48 h. The results were measured and expressed as unit per mg protein and the fold change compared to the control. c) The expression of OGG1 was determined by western blotting analysis. GADPH was used as a loading control protein. All data shown were the mean ± SD of three independent experiments. Different superscripts letter for each column indicated significant differences ($p < 0.05$).

We found a maximum level of lipid peroxidation and carbonyl content in MCF_7 cells at high conc. (8.1 mg/ml) of garlic extracts treatment compared to the untreated cells respectively. This increased amount of lipid peroxidation demonstrated that garlic extract increased peroxide production in MCF_7 in a dose-dependent manner. Similarly, garlic extracts significantly ($p<0.05$) increased malondialdehyde (a by-product of lipid peroxidation) in HL-60 cells compared to the untreated cells. This increase revealed that garlic extracts upregulate MDA production in HL-60 cells at high concentrations (6mg/ml) (Yedjou and Tchounwou 2012a). Likewise, garlic component, ajoene increase peroxide production in HL-60 cells after ROS generation in a dose and time-dependent (Dirsch et al. 1998). Also, DADS increase 2-fold oxidative mediated lipid peroxidation and carbonyl protein after 12h and 24h treatment and showed cytotoxicity (Filomeni et al. 2003). Oppositely, in the case of breast cancer, diallyl sulfide (DAS) decrease lipid peroxidation in the breast tissue of female ACI rats after being treated for 4/6 weeks (Gued et al. 2003). Recently, researchers demonstrated that phenolic compounds enrich extracts such as ferulic, vanillic, and p-hydroxybenzoic acids are increased the lipid peroxidation in breast cancer MDA-MB231 and MCF_7 cell lines (Sorice et al. 2016). In our experiments, garlic extracts also enriched with a high amount of ferulic and p-hydroxybenzoic acids that also comply with Sorice et al., (2016) result. Whereas, the oxidative damage of lipids, is associated with the down regulation of Nrf2 their corresponding signalling pathways (Orozco-Morales et al. 2021).

However, the formation of carbonyl protein by ROS generating has been used as an index of oxidation of protein (Berlett and Stadtman 1997). Our results show that incubation of cell suspensions with garlic extracts at 48h caused a marked increased of the protein carbonyl content due to oxidatively damaged protein in MCF_7 cells (Figure 3.11). These results suggested that it might be phenol molecules direct interacting with nucleic acids and proteins that are one of the major causes of garlic extracts cytotoxicity in MCF_7 cells. For example, Piper nigrum ethanolic extracts rich in piperamides causes ROS overproduction, oxidative damage that increase lipid peroxidation, and carbonyl proteins content that alien with our

results (de Souza Grinevicius et al. 2016). Correspondingly, natural polyphenols also showes these identical effects (Ivanova et al. 2019).

Consequently, for confirmation of DNA damage, we investigated the protein expression of OGG1; a bifunctional glycosylase represents a DNA damage marker that is capable to break the DNA backbone strand and splitting the glycosidic bond in the mutagenic area (Jacobs and Schär 2012). Previously reported that the absence of OGG1 is associated with aggressive breast cancer. Notably, polyphenol-containing food could oxidative DNA breakage and increased the OGG1 expression in a stressed condition in *in vitro* studies (Afrin et al. 2018). In our study, western blot analysis revealed that garlic extracts treatment significantly ($p < 0.05$) increased the protein expression of OGG1 up to 2.88-fold in MCF_7 cells compared to untreated cells. Thus, this study revealed oxidative DNA damage in the breast cancer MCF_7 cells after treatment with garlic extract (Figure 3.11). A similar effect was found in human leukemia HL-60 cells where, garlic extract has fragmented nucleosomal DNA (Yedjou and Tchounwou 2012b).

The above mentioned it could be concluded that phenolic enrich garlic extracts induced oxidative stress and increased lipid peroxidation, carbonyl protein, and damaged DNA as well as the anti-proliferative effect in breast cancer MCF_7 cells.

3.3.5. Effect of garlic extract on apoptosis on MCF_7 cell

Morphologically, apoptosis is characterized by cellular shrinkage and condensation of the nuclear contents, followed by plasma membrane ruffling and blebbing. Then, the cell is divided into membrane-bound apoptotic bodies containing a different type of the original cellular contents, that are shed from the dying cells and phagocytes by surrounding cells (Orlowski 1999).

However, apoptosis occurs mainly in mitochondrial-mediated pathways (intrinsic pathway) and death receptor-mediated pathways (extrinsic pathways), but endoplasmic stress has also some role to induce apoptosis (Choudhury et al. 2011). Previous research has focused on many spices and their bioactive components exhibited inhibitory effects of cells proliferation on breast cancer through inducing apoptosis in breast tumour cells (Mousavi et al. 2009; de

Souza Grinevicius et al. 2016). For example, curcumin-induced apoptosis in MDA-MB-231 and MCF-7 cells, respectively, and suppressed cells proliferation (Lv et al. 2014). Another study showed flavonoids, as diet-derived nutrients are recently introduced to deal with tumour cells via modulation of apoptosis pathways and inhibited cell growth (Tu et al. 2018b).

Therefore, first, we analyzed the apoptosis with apoptosis assay kit using Tali™ Image-based cytometer for quantifying the number of living, apoptosis, and dead cells to confirm garlic induced anti-proliferative effect which associated with apoptosis induction (Figure 3.12).

To determine the apoptotic effect, MCF_7 were treated for 48 h with or without various concentrations of garlic extract (1.87 mg/ml, 4.97 mg/ml and 8.1 mg/ml). Then, the number of apoptotic cells was significantly ($p < 0.05$) increased up to 4.0 fold in MCF_7 cells compared to untreated cells (Figure 3.12). The highest number of apoptotic cells was observed at 8.1 mg/ml dose for MCF_7 cells with respect to untreated cells. Along with, the dead cells significantly ($p<0.05$) increased up to 4.56-fold with respect to the untreated cells (Figure 3.12).

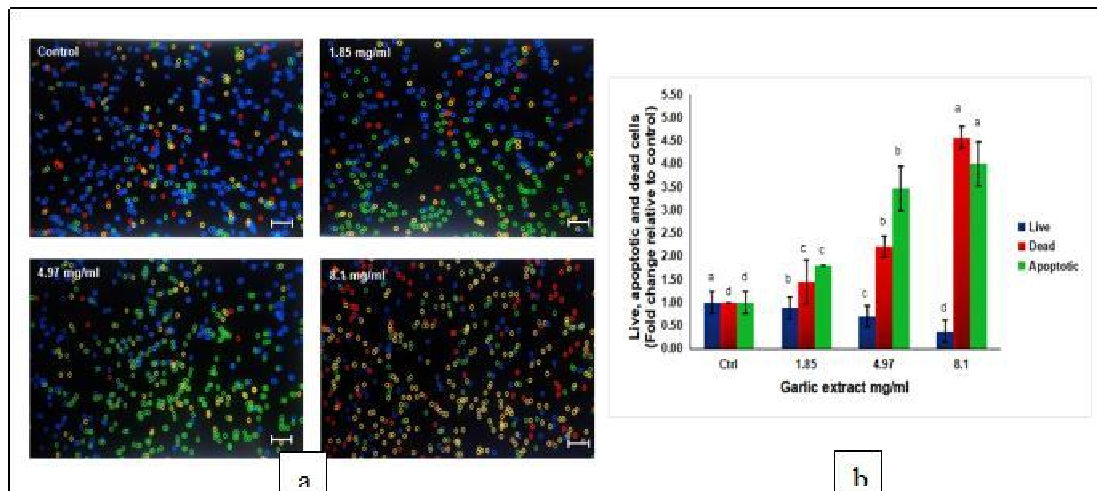


Figure 3.12: Induction of apoptosis in breast cancer MCF_7 cells treated with 1.84 mg/ml, 4.97 mg/ml and 8.1 mg/ml of garlic extract for 48 h. a) Percentage of live, dead, and apoptotic cells were evaluated by using Annexin V Alexa Fluor® 488 and PI staining by using the Tali™ Image-based Cytometer. Representative images of apoptosis quantification are shown following the picture; blue colour corresponds to live cells; green colour corresponds to apoptotic cells and red and yellow colour corresponds to dead cells after 48 h garlic extract treatment. Scale bar = 50 µm, average cell size =13 µm. b) Column's graph belongs to the same set of data compare to untreated cells with different

superscript letters are significantly different ($p < 0.05$). All data shown were the mean ± SD of three independent experiments.

In recent times, the mechanism of apoptosis induction in breast cancer is a topic of great interest to determine the underlying cause of cell death. For this, we evaluated several control points that determine the activation of apoptosis consequent to pro-apoptotic signals (Figure 3.13). Indeed, we investigated the molecular mechanism of garlic induction apoptosis; investigated by mRNA and protein expression of apoptotic marker which is involved in the intrinsic, extrinsic and ER stress apoptotic pathway via western blot and Real-time PCR analysis. For regulating these mechanisms, several groups have applied systems biology progress to study the mechanistic regulation of these apoptotic networks (Fussenegger et al. 2000; Eissing et al. 2004; Bagci et al. 2006; Legewie et al. 2006; Albeck et al. 2008; Zhang et al. 2009; Ballweg et al. 2017).

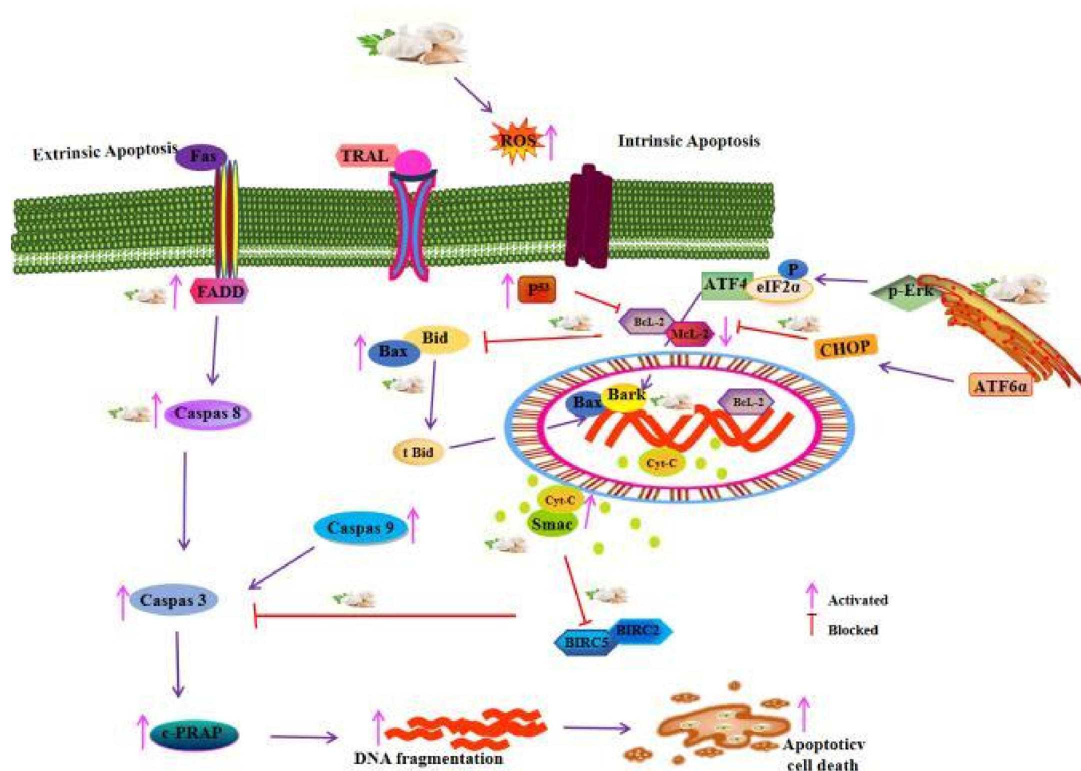


Figure 3.13: Possible anti apoptosis pathway of garlic extracts in MCF_7 breast cancer cells. Where mitochondria plays a central role in regulating the intrinsic and extrinsic apoptotic pathway responses to DNA damage. Apoptotic cell death can be induced by several triggers, resulting in the release of cytochrome c, Smac/Diablo, and apoptosis-inducing factor (AIF), from the mitochondrial outer membrane, inducing caspase-dependent and independent pathways of cell death. Garlic phenolic

compounds increased the release of these small molecules from mitochondria that decreased the over expression of Bcl-2 and McL1 and blocked BIRC-2 and BIRC-5.

3.3.5.1. Intrinsic Pathway

In intrinsic-mitochondrial apoptotic pathway, anti-apoptotic protein Bcl-2 and Mcl-1 and pro-apoptotic proteins Bax and Bid play an important role in controlling the outer mitochondrial membrane permeabilization and leakage of apoptogenic protein (Cyto C and Smac) and other apoptotic factors, that in turn activate caspase-9 and caspas-3 (Elmore 2007; Zhang et al. 2014).

3.3.5.1.1. P^{53} dependent intrinsic apoptosis

Further, we evaluate the activation of tumour suppressor P53 gene expression by RT-PCR. In our present work, after being treated with garlic extract at 1.84, 4.97 and 8.1 mg/ml for 48h, we observed garlic extract increased the gene expression of P53 up to 1.53 fold compared to untreated cells, remarkably. In the mitochondrial apoptosis pathway, Bax and Bark are the two key molecules that were interdependently activated by p53 and leading to cytochrome c release, followed by apoptosis.

We assumed that up-regulated P53 had blocked BCl-2 family and up regulated Bax gene. Notably, our observation is in line with previous experiments where, garlic components DATS, DADS and sallylmercaptocysteine (CySSA) increased P53 expression, indicating apoptosis by a mitochondrial pathway in breast cancer MCF_7 and MDA-MB-231 cells (Lei et al. 2008, p. 71; Malki et al. 2009; Zhang et al. 2014). Markedly, some spices constituents such as 6-gingerol and thymoquinone showed p53-dependent intrinsic apoptosis in breast cancer cells (Ng et al. 2011; de Souza Grinevicius et al. 2016; Sp et al. 2021).

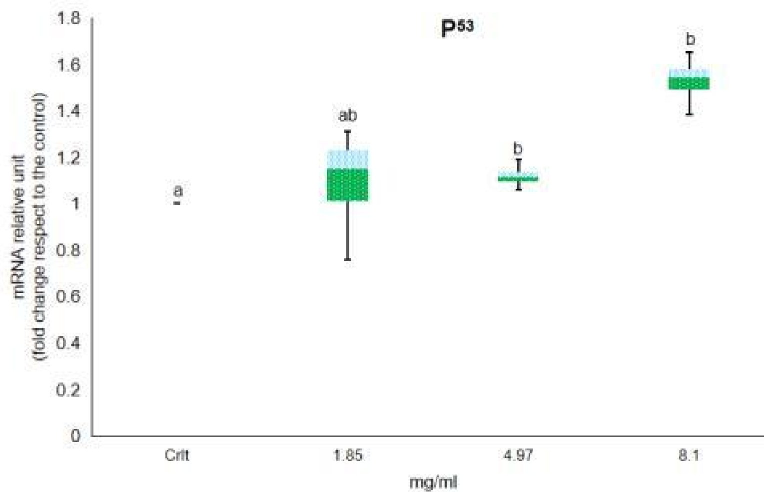


Figure 3.14: Garlic extract induces intrinsic apoptosis on MCF_7 cell. MCF_7 cell were incubates to different concentrations 1.85 mg/ ml, 4.97 mg/ml and 8.1 mg/ml of garlic extract for 48 h. mRNA expression tumour suppressor gene P53 was analysed by real-time PCR. GADPH use as housekeeping gene that was amplified under the same PCR conditions for normalized quantitative data. Results are expressed as median, first quartiles, third quartiles, minimum and maximum values. All data shown were the mean ± SD (n = 3). Different superscript letters for each column indicated significant differences ($p < 0.05$).

3.3.5.1.2. Apoptosis induction with Bax/Bcl2 family

The anti-apoptotic Bcl 2 family are also plays important role in apoptosis. Basically, Bcl-2 protein family regulates the permeability of the outer mitochondrial membrane and includes both pro-apoptotic Bad, Bax, and Bid and anti-apoptotic members such as Bcl-2 and Bcl-xl (Choumessi et al. 2012). In our experiment, we noticed, after 48h garlic treatment of MCF_7 cells significantly ($p < 0.05$) decreased anti-apoptotic genes Bcl-2 expression up to 0.79-fold and Mcl-1 gene expression up to 0.67 fold compared to the untreated cells. In particular, the expression of the pro-apoptotic gene Bax and Bid was significantly ($p < 0.05$) increased up to 1.84-fold and 1.78 fold respectively, compared to the untreated cells.

In addition, the intrinsic or mitochondrial program cell death cascade is started as a reaction to cellular stress by increasing ROS production and pro-apoptotic BH3 marker are activated in the program cell death intrinsic pathway while, Bax is directly activated through BH3 marker by making its direct interactions, further, it can also activate through indirect interaction with Bcl2 marker (Alshammari et al. 2020).

According to our results, garlic extract induced intrinsic apoptosis via up-regulation of pro-apoptotic genes (Bax and Bid) and down-regulation of anti-apoptotic genes (Bcl-2 and Mcl-1) in breast cancer MCF_7 cell line in a dose-dependent manner that consistent with previous findings; garlic oil and it's component DADS and water-soluble compounds S-allyl mercaptocysteine (SAMC) was responsible for induction apoptosis in breast cancer MCF_7 cell by modulating several mechanism actions through upregulating the protein expression level of Bax, Bark, Bid and downregulating the expression of Bcl-xL and Bcl-w in a dose-dependent manner in MCF_7 and MDA-MB-231 cells (Altonsy et al. 2012; Zhang et al. 2014). Likewise, Phenolic compound such as curcumin has potentially reduced cell growth of MCF_7 via up-regulation of Bax and downregulation of Bcl-2 (Masuelli et al. 2013)

3.3.5.1.3. Caspase dependent intrinsic apoptosis

Upon activation of bax that helps to form pores in the mitochondrial outer membrane that release cytochrome c. Increased cytosolic cytochrome c concentration activates caspas-9 and caspas-3 that indicate loss of mitochondrial membrane potentiality and causes succeeding cell death (Alshammari et al., 2020).

In our experiment, we found gene expression of caspase-9 significantly ($p < 0.05$) unregulated up to 1.55 fold and caspase-3 up to 1.61 fold. Our results have a number of similarities with previous studies of different types of spices where they induced caspase-dependent intrinsic apoptosis in breast cancer cells, without affecting non-cancerous cells (Mousavi et al. 2009; Basile et al. 2015; de Souza Grinevicius et al. 2016; Kello et al. 2020).

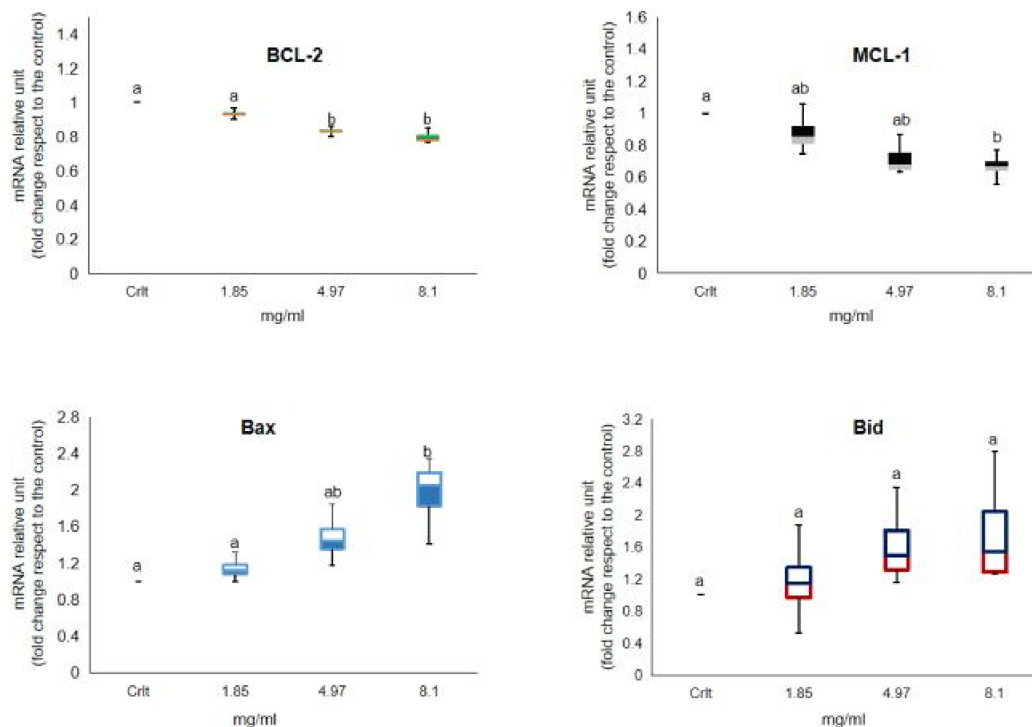


Figure 3.15: Garlic extract induces intrinsic apoptosis in breast cancer MCF_7 cell. MCF_7 cell were incubates to different concentrations 1.85 mg/ ml, 4.97 mg/ml and 8.1 mg/ml of garlic extract for 48 h. mRNA expression of intrinsic apoptotic markers Bcl-2, McL-1 Bax, Bid were analyzed by real-time PCR. GADPH use as housekeeping gene that was amplified under the same PCR conditions for normalized quantitative data. Results are expressed as median, first quartiles, third quartiles, minimum and maximum values. All data shown were the mean ± SD (n = 3). Different superscript letters for each column indicated significant differences ($p < 0.05$).

3.3.5.1.4. SMAC mediated apoptosis

In the mitochondrial pathway (the intrinsic pathway), mitochondria release many death-promoting factors such as smac (also known as DIABLO) and cytochrome c in response to death signals via the so-called sensor molecules, such as the BH3 only Bcl-2 family proteins. Recently, polyphenol enriched spices such as gingerol and curcumin induced apoptosis via Smac/DIABLO pathway (Shankar and Srivastava 2007; Ogrodzinski et al. 2017). However, Smac induced apoptosis by binding to the inhibitor of apoptosis proteins (IAPs), the whole family of these proteins is now more precisely known as BIR containing (or BIRC) proteins rather than IAPs that acts as an indirect activator of caspases by inhibition of the BIRC proteins (Du et al. 2000; Verhagen et al. 2000; Fulda et al. 2002).

While we investigated the levels of mRNA expression of pro-apoptotic gene Smac and anti-apoptotic BIRC gene (BIRC-2 and BIRC-5) of MCF_7 breast cancer cells after 48 h treatments with garlic extract. Thereafter, we found mRNA expression Smac gene significantly ($p<0.05$) increased up to 1.31 fold and BIRC-2 and BIRC-5 gene expression significantly ($p<0.05$) decreased up to 0.55 and 0.73 fold respectively, compared with untreated cells. Our result lends support to (Das et al. 2007) findings on human glioblastoma T98G and U87MG cells after treatment with garlic.

The mitochondrial release of Smac into the cytosol correlated well with downregulated BIRC-2 and BIRC-5 levels which indicate that garlic extract induced apoptosis in MCF_7 cells with a mitochondrial release of Smac into the cytosol and suppression of BIRC-2 and BIRC-5 level. Several studies showed that Smac decreased BIRC levels and induced apoptosis in different cancerous cells (Du et al. 2000; Fulda et al. 2002; Chuturgoon et al. 2015). Eventually, being after garlic extract treatment, Smac could suppress the survival effects of BIRC by down-regulating BIRC-2 and BIRC-5 gene in breast cancer MCF_7 cells for promoting apoptosis.

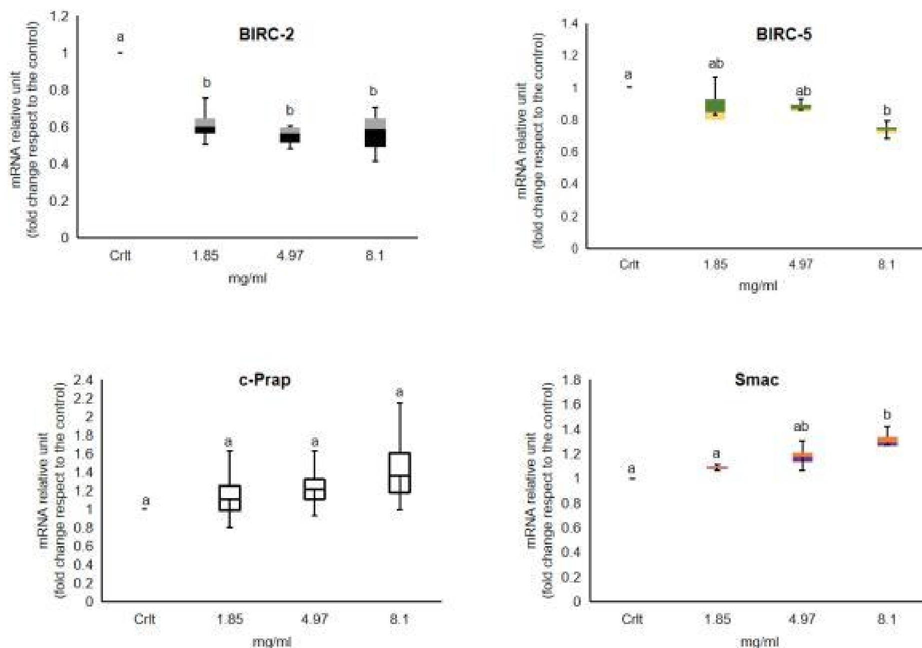


Figure 3.16: Garlic extract induces intrinsic apoptosis on MCF_7 cells. Mitochondrial release of Smac into the cytosol for inhibition of BIRC in MCF_7 cell line. MCF_7 cells were incubates to different concentrations 1.85 mg/ml, 4.97 mg/ml and 8.1 mg/ml of garlic extract for 48 hrs. mRNA expression of mitochondrial apoptotic markers Smac, BIRC-2, BIRC-5 and hallmark of apoptosis marker c-

PRAP were analysed by real-time PCR. GADPH use as housekeeping gene that was amplified under the same PCR conditions for normalized quantitative data. Results are expressed as median, first quartiles, third quartiles, minimum and maximum values. All data shown were the mean ± SD (n = 3). Different superscript letters for each column indicated significant differences ($p < 0.05$).

3.3.5.2. Extrinsic pathway

Consequently, we observed the extrinsic pathway of apoptosis by evaluating the mRNA expression levels of Fas-associated protein death domain (FADD) and caspas-8 in the breast cancer MCF_7 cell line. After, the activation of death receptor and mitochondrial pathways, garlic extract may be increased FADD protein as well as activated a series of processes that lead to the activation of pro-caspase 8 in caspase 8 via an auto catalytic reaction.

As can be seen, in Figure 3.17, the gene expression of FADD increased significantly ($p<0.05$) up to 1.40 fold and caspase-8 up to 1.58 fold respectively, compared to untreated cells in a dose-dependent manner. From this caspase, a cascade process is activated, markedly leading to the activation of the effector caspase including caspas 3 and caspase 7 (Choudhury et al. 2011), and induced apoptosis. For example, garlic water-soluble compounds SAMC potentially reduced cell growth of MCF_7 and MDA-MB-231 cells, respectively, by inducing apoptosis via activation of FADD and caspase 3 (Zhang et al. 2014). Indeed, our results were consistent with the previous report by other natural extracts such as Tribulus Terrestris and Hibiscus flower extracts on breast cancer cells (Patel et al. 2019; Nguyen et al. 2019). Likewise, traditional Chinese medicine Ganoderma lucidum spore oil induced apoptosis of breast cancer MDA-MB-231 cells via caspas activation (Jiao et al. 2020).

However, FADD is an important protein of the death receptors that bridges death receptor signalling to the caspase cascade. It could be directly involved in the death province of the Fas receptor, without the involvement of the pre-employment of the TRADD, and Fas receptor is commonly considered to just trigger apoptosis and activate the apoptosis signalling (Fantl et al. 1993; Algeciras-Schimnich et al. 2002; Gibcus et al. 2007). In our results, we recognized that the garlic extract significantly ($p<0.05$) increased the expression of FADD gene leading to apoptosis of MCF_7 cells, mediated by caspase 8. However, Caspase 8 causes the activation of pro-enzyme caspase 3 to its active form by cleaving it into subunits and their dimerization. In this study, the gene expression of caspas 9 and caspase 3 were significantly ($p<0.05$) elevated by treating with garlic extract and indicating the probable

triggering of extrinsic apoptotic pathway in accordance with (Basaiyye et al. 2018). DNA fragmentation has been to occur in later stages of apoptosis (Johnson et al. 2000) that could be confirmed by increasing expression of Poly (ADP-ribose) polymerase c-PRAP. In this case, we also found gene expression of c-PRAP increased up to 1.42-fold with respect to untreated cells by RT-PCR analysis after garlic treatment at 1.85, 4.97 and 8.1 mg/ml for 48h (Figure 3.16).

As mentioned, treatment with garlic extract on MCF_7 cells induced cleavage PARP, which is considered a hallmark of apoptosis. Similar results for induction of apoptosis have been reported for various types of spices and herbs on different types of cancer cell lines (Hu and Kavanagh 2003; Sun et al. 2012; Masuelli et al. 2013). Subsequently, DNA fragmentation in MCF_7 cells that might be responsible for activation of the cellular endonucleases. And Apoptosis-inducing factor (AIF) is a flavin adenine di nucleotide-containing, NADH-dependent oxidoreductase presents in the inter membrane space of the mitochondria. Apoptotic assault results in the proteolysis of AIF followed by its translocation to the nucleus where it causes chromatin condensation and large-scale DNA degradation in a caspase-independent manner (Sevrioukova 2011).

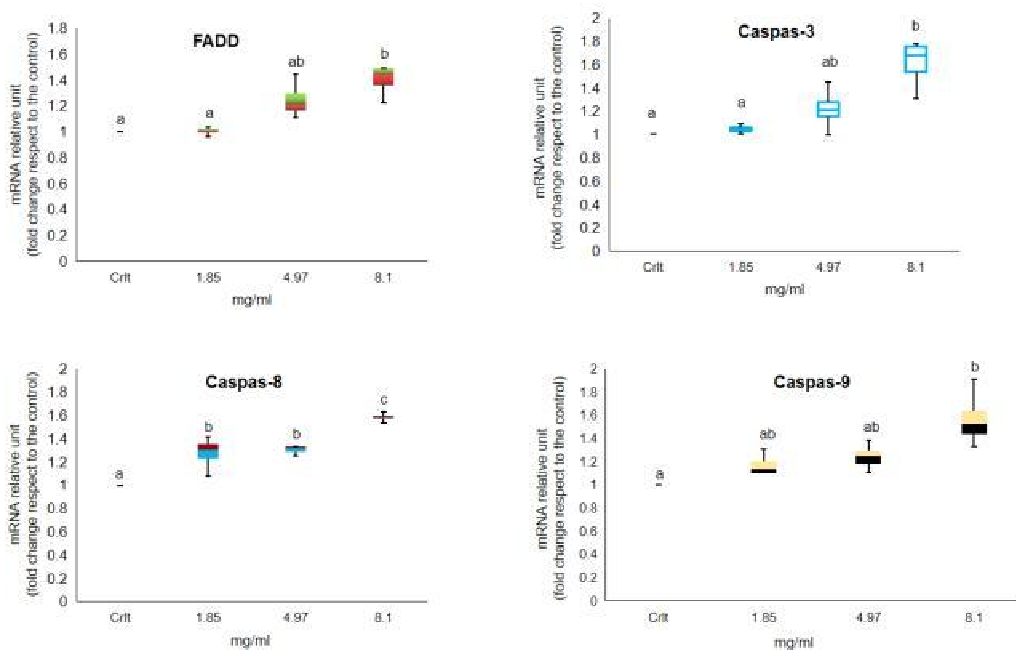


Figure 3.17: Garlic extract induces extrinsic apoptosis on MCF_7 cell. MCF_7 cell were incubates to different concentrations 1.85 mg/ ml, 4.97 mg/ml and 8.1 mg/ml of garlic extract for 48 h. mRNA

expression of extrinsic apoptotic markers FADD, caspas-9, caspas-8 and caspas-3 were analysed by real-time PCR. GADPH use as housekeeping gene that was amplified under the same PCR conditions for normalized quantitative data. Results are expressed as median, first quartiles, third quartiles, minimum and maximum values. All data shown were the mean ± SD (n = 3). Different superscript letters for each column indicated significant differences ($p < 0.05$).

In short, garlic extract can be induced apoptosis through cell surface death receptors and mitochondria-dependent pathways in breast cancer by inducing oxidative stress via ROS generation. In addition, our result complies with the observation of De et al., (2019) experiment, where Marine Sponge Hyrtios Erectus induced apoptosis of Breast cancer MCF_7 cells via ROS production through Intrinsic or Extrinsic apoptosis pathways (De et al. 2019). Moreover, ethanolic extracts of lemongrass and aqueous extracts of dandelion root were induced apoptosis via able increased oxidative stress by decreasing mitochondrial membrane potential (Ovadje et al. 2016; Philion et al. 2017).

3.3.5.3. ER induced apoptosis

To further interpret the mechanisms for apoptosis, we investigated the protein level of ER stress-associated molecules, ATF6 and XBP1 and EIF2α by western blotting and mRNA expression of CHOP gene by RT- PCR. The expression of XBP1, ATF6 and EIF2α proteins significantly ($p < 0.05$) up regulated up to 2.10 fold, 2.77 fold and 3.29 fold respectively, at concentration depended on manner of garlic extract (1.85, 4.97 and 8.1 mg/ml) in breast cancer MCF_7 cells compared to untreated cells. Besides, gene expression of CHOP significantly ($p<0.05$) upregulated up to 1.13 fold with respect to the untreated cells. However, various natural compounds are responsible for activation of ER stress, a common mechanism action for stress-induced apoptosis [84,371,378,393]

Although, ER stress response established a cellular process that is triggered by a variety of conditions that disturb the folding of proteins in the ER stress. A large volume of studies established that the induction of Grp78/Bip and CHOP is a marker for ER stress (Lee 2005; Nishitoh 2012; Sano and Reed 2013). Fresh garlic extract induced apoptosis by leading to the accumulation of unfolded proteins (also known as GRP78) and regulated ER stress and effects on the cellular redox regulation (Petrovic et al. 2018). Furthermore, it should be noted that garlic components affected protein folding, leading to ER stress, where, a compound of garlic can bind to glutathione (GSH), and to cysteine residues on proteins, and thereby

modify both the cellular redox state as well as protein folding (Biswas et al. 2006; Bhuiyan et al. 2015; Kaschula et al. 2016; Yagdi et al. 2016; Song et al. 2016). Furthermore, the ER stress sensor ATF6 (type II transmembrane protein) and its signalling molecule XBP1 (transcriptional activator of UPR genes) play a vital role in response to ER stress (Cao and Kaufman, 2014). In our present study, we observed garlic extract significantly ($p < 0.05$) unregulated protein expression of ATF6, XBP1, and EIF2α (Figure 3.18), demonstrating that garlic extract has ability to activate ER stress and induced apoptosis in breast cancer MCF_7 cell line. In addition, other natural compounds studies have also been aliened to our study (Zhang et al. 2013; Garrido-Armas et al. 2018). Recently, ER stress can lead to apoptosis through several pathways, such as activation of the JNK (c-Jun NH2-terminal kinase) pathway and the MAPK (mitogen-activated protein kinase) pathway responds to changes in the redox state of the environment (Patya et al. 2004; Mitchell et al. 2013; Hobbs et al. 2014). Indeed, up-regulation of ER stress induced by garlic extract, that elevated ROS levels and induced oxidative stress-mediated apoptosis and cell death. Apart from the aforementioned molecular mechanism, it can be concluded that polyphenol enrich garlic extract treatment in breast cancer MCF_7 cells induced apoptosis through oxidative stress in different targeting molecular pathways.

Taken all together, we hypothesized that garlic extract inhibited cell growth by generating ROS, resulting in oxidative stress and in a consequent activation of apoptosis in breast cancer MCF_7 cells (Figure 3.13).

3.3.6. Effect of garlic Seahorse phenotype on MCF_7 cells

The function of cellular metabolism is a biological process that essential for cancer metabolism and significantly improves the cancer biology acceptance to us. The diagnosis of cancer-related mutations in genes encodes important metabolic enzymes that help to establish a clear correlation between altered metabolism and cancer (Yang et al. 2013). However, reprogramming of energy metabolism is one of a hallmark of cancer (Ogrodzinski et al. 2017).

Therefore, to supply the energy essentials for anabolic reactions, where cancer cells use aerobic glycolysis in the existence of functioning mitochondria called the Warburg effect.

Although, the Warburg effect is a basic feature of metabolic reprogramming and tumour cells depend on both mitochondrial oxidative phosphorylation and glycolysis (Gao et al. 2019). Furthermore, cancer cells have more metabolic plasticity influenced by a complex tumour microenvironment which enables them to reprogram their metabolic shift between glycolysis and mitochondrial oxidative phosphorylation (OXPHOS), with both pathways being flexibly switched under different conditions (Fendt et al. 2020). For this reason, the role of cancer cell metabolism in cancer cell proliferation and their related pathways are important to understand.

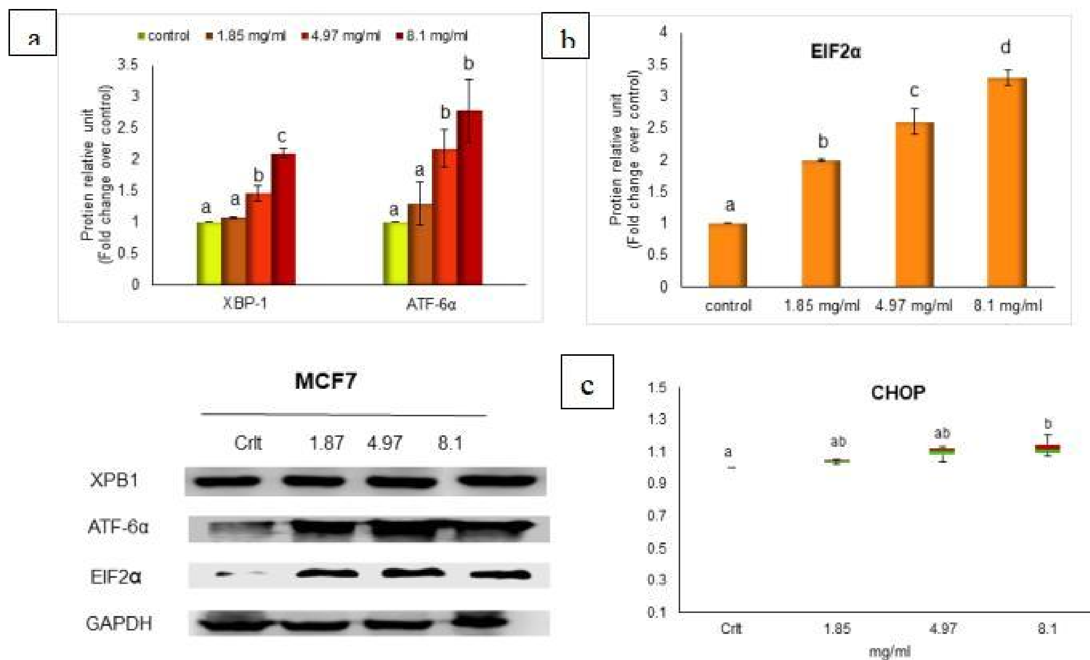


Figure 3.18: Garlic extract induces endoplasmic reticulum stress on MCF_7 cells. MCF_7 cells were incubates to different concentrations 1.85 mg/ ml, 4.97 mg/ml and 8.1 mg/ml of garlic extract for 48 h. The protein involved on endoplasmic reticulum stress (a) XBP1, b) ATF6α and c) EIF2α); that were analyzed by western blotting in MCF_7 cells. GADPH used as a loading control. mRNA expression of ER stress marker; c) CHOP was analyzed by real-time PCR. GADPH used as housekeeping gene that was amplified under the same PCR conditions for normalized quantitative data. Results are expressed as median, first quartiles, third quartiles, minimum and maximum values. All data shown were the mean ± SD (n = 3). Different superscript letters for each column indicated significant differences ($p < 0.05$).

Mitochondria are highly regulating organelles, and their complex structure is interlinked with cellular bioenergetic functions to increase energy and maintain physiological activities (Osellame et al. 2012). In the case of cancer cells survival, mitochondria play an important

role to grow cancer cells through glycolysis and generate a higher level of ATP production with the help of mitochondrial OXPHOS for adapting to hostile microenvironments (Moreno-Sánchez et al. 2007; Pastò et al. 2014; Liemburg-Apers et al. 2015). Oppositely, mitochondria can play a dysfunctional role when ROS is generated in pathological conditions (Liemburg-Apers et al. 2015). Recently, breast cancer MCF_7 cells exhibit bioenergetic and metabolic profiles by showing higher dependency on mitochondrial respiration (Lunetti et al. 2019). The aim of this study is to evaluate garlic extract as a potent inhibitor of mitochondrial metabolic function in breast cancer MCF_7 cells. Therefore, to obtain the metabolic profile of the OCR and glycolysis (ECAR) rate of MCF_7 breast cancer cells, treated with different concentrations of garlic extract (1.85mg/ml, 4.97 mg/ml, and 8.1 mg/ml) was assessed by using the Seahorse XF-96 analyzer. Here, we analyzed the effect of garlic extract in mitochondrial bioenergetics generating oxidative damage that is mediated by ROS.

3.3.6.1. Garlic extract targets on OXPHOS inhibition and impairment of mitochondrial respiration

In OCAR, untreated and treated cells were exposed sequentially to each of three well-defined small modulators of oxidative phosphorylation: oligomycin, 2,4 dinitrophenol (2,4-DNP), and rotenone (Figure 3.19). Garlic extract treatment markedly inhibited the mitochondrial respiration of MCF_7 cells, with significant ($p < 0.05$) reduction in basal respiration up to 0.29 fold (23.60 ± 5.48 pmol/min per 2.5 ×105 cells), maximal respiration up to 0.33 fold (29.46 ± 5.52 pmol/min per 2.5 ×105 cells) and Spare level up to 0.48 fold (17.0 ± 6.30 pmol/min per 2.5 ×105 cells) respectively, compared to untreated cells (Figure 3.19) in a dose-dependent manner after 48h garlic treatment. Our results showed that garlic extract impaired mitochondrial respiration by disrupting OXPHOS via blocked complex V that indicates the suppression of mitochondrial respiration rate while 2,4-DNP transports proton across the inner mitochondrial membrane instead of proton channel for bypass ATP synthesis and increase respiration. Garlic treatment demonstrates the possible effect that the bioactive compounds present in the extract may affect, directly or indirectly, mitochondrial functionality (Figure 3.19). In MCF_7 cells, OCR levels were significant ($p < 0.05$) lowered

after being treated with garlic extract, compared to untreated cells. The addition of oligomycin, a substrate that inhibits the ATP synthase activity, caused a significant ($p<0.05$) decrease of the OCR rate in a dose-dependent manner. The subsequent addition of 2,4-DNP, an ionophore that allows the electron flux without ATP production, increased OCR in MCF_7 cells, and the final addition of rotenone, a substrate that inhibits flux electrons from complex I to Ubiquinone, caused a marked decrease of OCR in MCF_7 cells (Figure 3.19). Therefore, OCR was dramatically decreased by garlic treatment in the MCF_7 cell line.

Recently, poly phenols showed impairment of mitochondrial respiration and prevent cell growth in breast cancer (Jung et al. 2016; Fuentes-Retamal et al. 2020). Finally, our results revealed that garlic extract decreased breast cancer MCF_7 cell growth by imparement of mitrochodrial repiration.

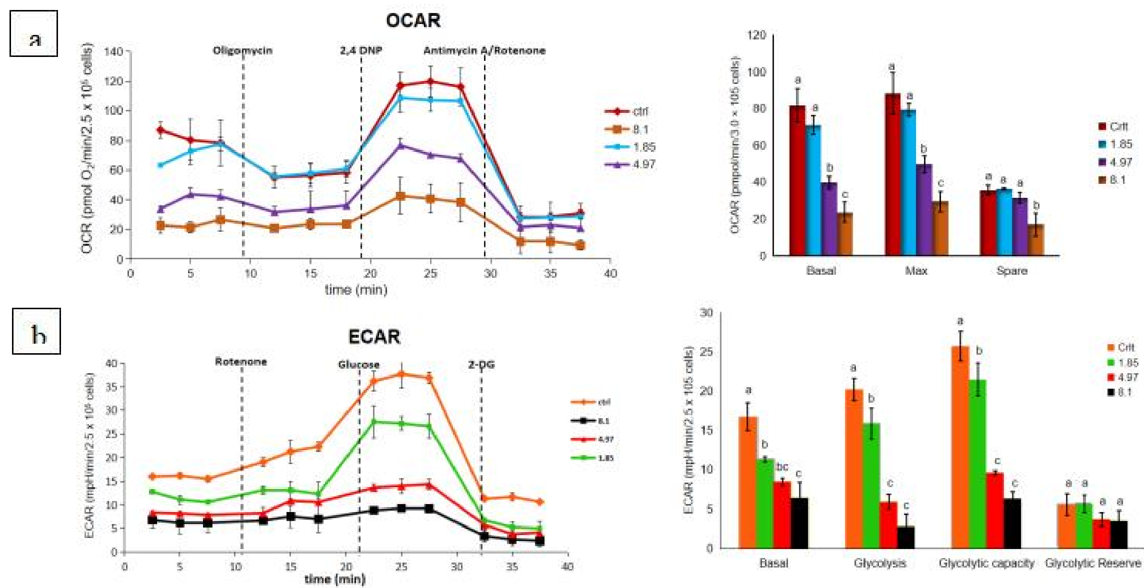


Figure 3.19: Metabolic phenotype and effects of garlic extract on mitochondrial and glycolytic activities in MCF_7 cells. MCF_7 cells were cultured in XF24-well cell culture microplates (Seahorse Bioscience) at a density of 2.5 × 105 cells/per well and then incubated for 24 h at 37 ° C under 5% CO2 atmosphere in proper media. Then MCF_7 cells were treated with different concentrations of garlic extract 1.85 mg/ml, 4.97 mg/ml and 8.1 mg/ml for 48 h. The a) OCR was determined by using the Seahorse XF-24 Extracellular Flux Analyzer after the following sequential injections: oligomycin (3 μg ml^{-1}), 2,4-DNP (300 μM), and rotenone/antimycin (1 μM/ 10 μM) in MCF_7 cells. The basal respiration, maximal respiration capacity and spare respiration capacity were calculated from the XF

cell Mito stress test profile. The b) ECAR was determined by using the Seahorse XF-24 Extracellular Flux Analyzer after the following sequential injections: rotenone (1 μM), glucose (30 mM), and 2-DG (100 mM) in MCF_7 cells. The basal ECAR, glycolysis, glycolytic capacity and glycolytic reserves were calculated from the XF glycolysis stress test profile. Columns graph belongs to the same set of data compare to untreated cells with different superscript letters are significantly different ($p < 0.05$). All data shown were the mean ± SD of three independent experiments.

3.3.6.2. Garlic extract targets on glycols-deficient and imparement of metabolic profiles.

Aerobic glycolysis though in the presence of oxygen and functioning mitochondria (i.e., the Warburg effect) is a pivotal bioenergetic process in cancer cells (Li et al. 2019). We assessed glycolytic function in breast cancer cells based on ECAR, as determined by glycolytic rate assay using antimycin/rotenone mix and 2-deoxy-D-glucose (2-DG). Whereas, MCF_7 cells has the ability to increase glycolysis when mitochondrial function is blocked by rotenone. Additionally, garlic treatment at different concentrations were capable of reduction the ECAR, indicating that garlic has an inhibitory effect on glycolysis, glycolytic capacity and glycplytic reserve compared to untreated cells. As shown in figure 3.19, glycolytic parameters significantly ($p<0.05$) decreased, including basal up to 0.38 fold and compensatory glycolysis up to 0.14 fold, glycolytic capacity up to 0.24 fold and glycolytic reserve up to 0.63 fold were significantly decreased in a dose-dependent manner in tested MCF_7 cells. To elucidate how garlic extract suppresses aerobic glycolysis in MCF7 cells, we observed the gene expression of lactate dehydrogenase A (LDH-A) that involved in the regulation of glycolytic pathways. The expressions of mRNA for LDH-A were significantly ($p < 0.05$) decreased up to 0.27 fold compare with untreated cells, after garlic extract treatment (Figure 3.20).

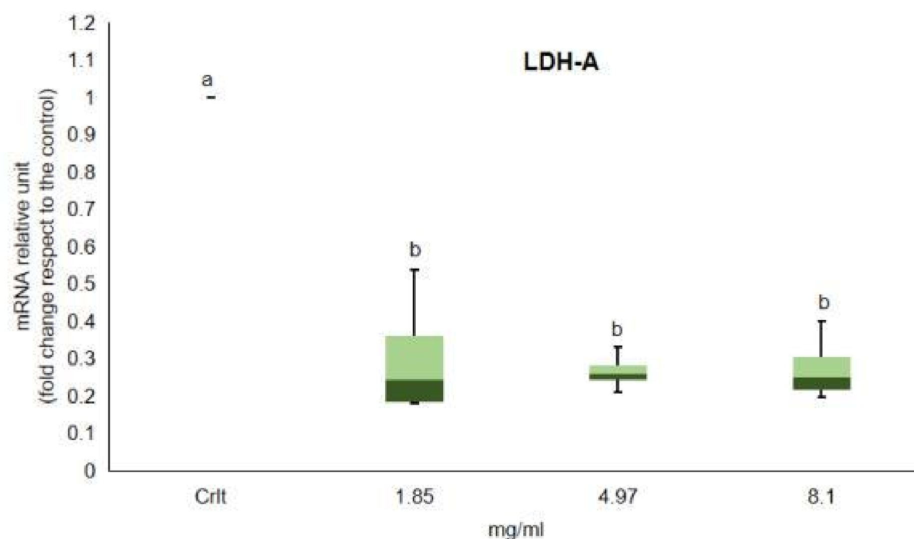


Figure 3.20: Garlic extract induces extrinsic apoptosis on MCF_7 cell. MCF_7 cell were incubates to different concentrations 1.85 mg/ ml, 4.97 mg/ml and 8.1 mg/ml of garlic extract for 48 h. Gene expression of lactate dehydrogenase A (LDH-A) was analysed by real-time PCR. GADPH use as housekeeping gene that was amplified under the same PCR conditions for normalized quantitative data. Results are expressed as median, first quartiles, third quartiles, minimum and maximum values. All data shown were the mean ± SD (n = 3). Different superscript letters for each column indicated significant differences ($p < 0.05$).

LDH-A enzyme the main regulator of lactate balance and metabolism, is mainly associated with pyruvate to lactate conversion and increased glycolysis. Furthermore, the expression of LDH-A is positively correlating with the Warburg effects (Zhao et al. 2013; Shi et al. 2014) Xiao and his colleagues, a high expression of LDHA in breast cancer, which was associated with worse clinical outcomes (Xiao et al. 2016). Also, changes in the expression of the lactate shuttle gene seem to be associated with alterations in oxidative capacity and lactate accumulation within breast cancer cells (Kennedy and Wightman 2011). Finally, our results suggest that garlic extract can inhibit glycolysis which provides electron donors for ATP generation required for cellular biosynthesis pathways.

b

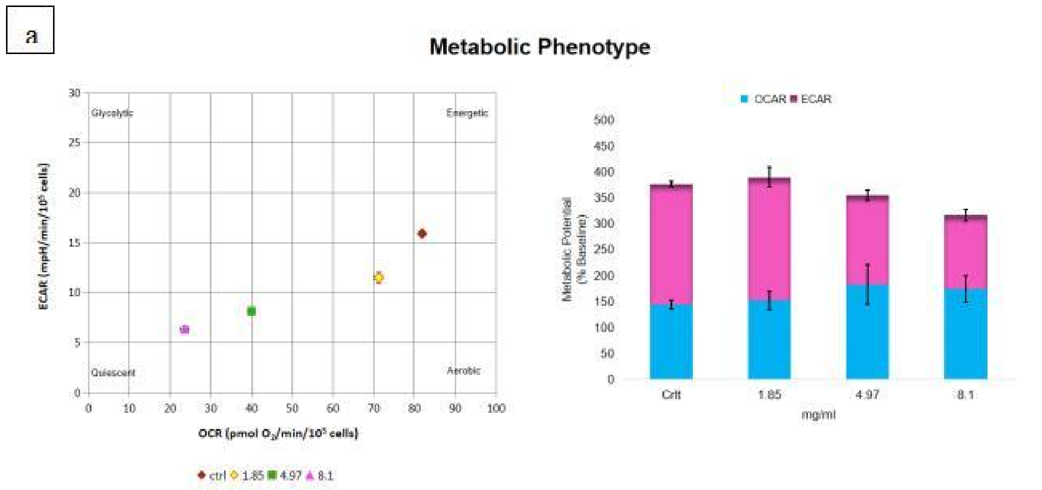


Figure 3.21: The metabolic phenograph and metabolic potential of MCF_7 cells after exposed after 48h of garlic extract. a) The baseline phenograph shows differences in both glycolysis (ECAR) and mitochondrial respiration (OCR). The quadrants were set at arbitrary values to indicate only the direction of bioenergetic changes. b) Column's graph belongs to the same set of data compare to untreated cells with different superscript letters are significantly different ($p < 0.05$). All data shown were the mean ± SD of three independent experiments.

The typical metabolic aberration of cancer cells is amongst the most broadly documented phenotype features along with many therapies and pharmacological treatments. Metabolomics, an innovative science, has unravelled a new way of finding the tumour metabolic phenotype, that can be utilized in drug development. The metabolic active breast tumour cells helped to increase metastatic properties also (Tu et al. 2018a). In this study, for the first time, we observed the metabolic phenotype after garlic extract treatment MCF_7 cells by calculating the cell energy profile, obtained by plotting OCR against ECAR. The graph indicated that garlic extract changes the cell metabolism by shifting MCF_7 cells from an oxidative state to the quiescent stages (metabolically inactive) that are indicating disruptions in both OXPHOS and glycolytic paradigms respectively (Figure 3.21).

3.3.7. Garlic extract suppressing AMPK induced cell survival in metabolic stress conditions

The AMPK signalling pathway is known as a biosynthetic pathway and involves promoting tumour cell survival by elevating mitochondrial biogenesis and fascinating metabolic adaptation under glucose-limiting conditions (Chaube et al. 2015). Recently, several number

of researches show that over expression of LDHA and AMPK promoted glycolysis and cell proliferation in cancers (Huang and Chen 2009; Yao et al. 2013; Cui et al. 2014).

Therefore, we performed Western blot analysis to investigate the expression of proteins related to the AMPK pathway and its downstream targets PGC1α in MCF_7 cells after treatment with different concentrations (1.85 mg/ml, 4.97 mg/ml and 8.1 mg/ml) of garlic extract (Fig) at 48h. We observed that garlic extract significantly ($p < 0.05$) down regulated up to 0.02 fold the AMPK expression in breast cancer MCF_7 cells with respect to the control (Figure 3.22). Xie et al. (2018) demonstrate that DADS inhibits the cell proliferation and glucose metabolism in *in vivo* mouse xenograft via the repression of CD44/PKM2/AMPK pathway in breast cancer stem cells (BCSCs) (Xie et al. 2018). Thus, the suppression of AMPK could also be a therapeutic approach for breast cancer treatment.

Furthermore, activation of AMPK stimulates the expression of downstream targets PGC-1α and its associated genes for cancer cells survive under energetic stress conditions that triggers LDH and mitochondrial biogenesis (LeBleu et al. 2014; Chaube et al. 2015). Interestingly, our data suggest, garlic extract significantly ($p<0.05$) suppressed up to 0.4 fold the expression of PGC1α protein in MCF_7 cells compared to the control in a dose-dependent manner (Figure 3.22).

Based on these particular results suggested that garlic extract decreased AMPK activation via suppression of PGC1α protein expression, which hinders MCF_7 cell survival by inhibiting stress adaptation.

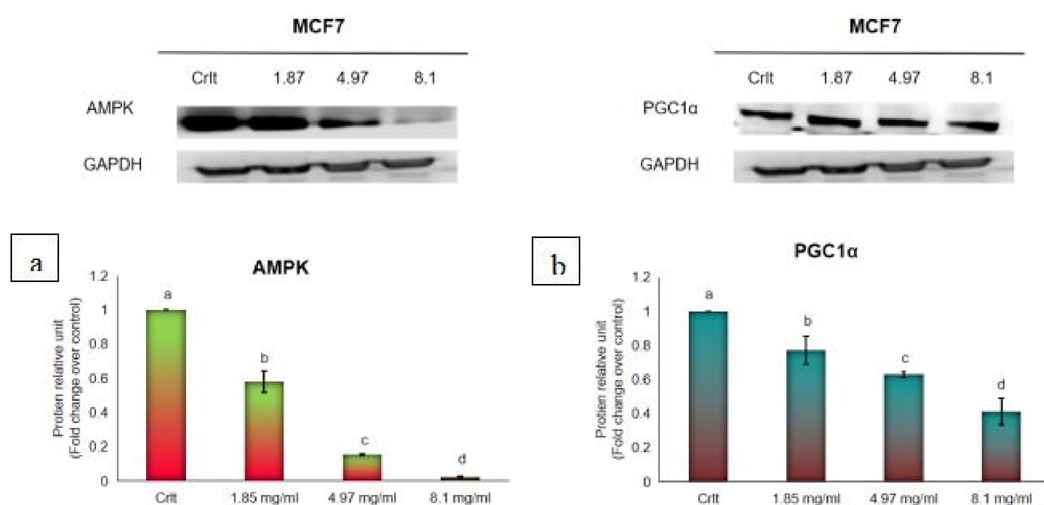


Figure 3.22: Garlic extracts down regulates the MCF_7 cells biogenesis by addressing the AMPK pathway. MCF_7 cell were incubated to different concentrations of Crlt, 1.85 mg/ ml, 4.97 mg/ml and 8.1 mg/ml of garlic extract for 48 h. The expression of a) AMPK and b) PGC1α protein were determined by western blotting analysis. GADPH was used as a loading control protein. All data shown were the mean ± SD of three independent experiments. Different superscripts letter for each column indicated significant differences ($p < 0.05$).

RESULTS AND DISCUSSION: PART IV

The aim of this part of my PhD project was to evaluate the effects of garlic extract and on human breast adenocarcinoma MCF_7 cell line metastasis and focusing on different molecular targets. The past decay, Allium family has received much attention, particularly garlic (Allium sativum), for the treatment of cancer. Until now, there is credible evidence for an association between garlic consumption and prevention of cancers for example, breast, esophageal, colon, prostate and laryngeal cancers (Ban et al. 2007; Kim et al. 2011; Tsubura et al. 2011; Yin et al. 2014). The anticancer activities of garlic are related to the level of Organosufur compound, flavonoids, and phenolic components (Shirzad et al. 2011). Several reports have been published on garlic organosulfur compound and anti-metstatic effect of breast cancer (Chandra-Kuntal et al. 2013; Liu et al. 2015), no work had published on poly phenolic garlic extract and breast cancer tumour microenvironment and autophagy cell death. Moreover, last decay genetic evidence demonstrate that autophagy regulates pathological angiogenesis, a hallmark of solid tumours (Hanahan and Weinberg 2011).

Therefore, it is important to study poly phenolic garlic extract effect on tumour microenvironment in breast cancer. Recent studies have attempted to understand the mechanisms underlying cancer cell metastasis and their prevention by garlic extract through evaluating migration and invasion, colony formation, arrest cell cycle and autophagy induction in MCF_7 cells. To the best of our knowledge, this is the first report to demonstrate the anticancer effects of poly phenol rich garlic extract against breast cancer.

4.1. Anti-metastatic activity of garlic extracts in breast cancer MCF_7 cells

Tumour metastases are accountable for approximately 90% of cancer-related deaths and 25% to 50% of breast cancer patients develop deadly metastases that responsible for the high percentage of deaths among breast cancer patients(Lorusso and Rüegg 2012; Spano et al. 2012; Scully et al. 2012). For cancer growth, the metastatic cascade is a series of biological processes such as angiogenesis, invasion and migration that facilitates the movement of tumour cells from the primary site to a distant location. However, interruption this metastatic cascade via the targeting of tumour cells might be a promising therapeutic strategy (Mego et al. 2010).

4.1.1. Wound healing assay revealed garlic extract induced inhibition of MCF_7 cell migration

For many years, spices have been showed anti-metastatic effect on breast cancer (Zheng et al. 2016). Surprisingly, the therapeutic efficiency and mechanistic action of metastasis of garlic poly phenol in breast cancer cells have not been investigated yet. We studied the effect of garlic extract on cancer cell migration in the breast cancer cell line, MCF_7 after different treated concentrations 1.85 mg/ml, 4.97 mg/ml and 8.1 mg/ml of garlic extract to investigate the possible interference of garlic poly phenol on the mobility of MCF_7 cells by wounding assay. After 48h of treatment, based on the result, untreated MCF_7 cells were able to migrate and partially fill the wound empty area while exposure to garlic extract inhibited the cell migration in a dose-dependent manner (Figure 3.23). Notably, fewer cells migrated across the gap with low garlic extract concentration and with high concentration migration ability of cell significantly decreased. The inhibition of wound closure was monitored starting from the lowest concentration (1.85 mg/ml) of garlic extract tested and appears almost completely inhibited starting from high concentration of garlic extract (8.1 mg/ml) (Figure 3.23).

A quantitative analysis of wound closure was carried out using the Image J software. Our results highlighted that; garlic extract treatment significantly ($p<0.05$) decreased the migration ability of MCF_7 cells from 7.69% to 68% compared to untreated MCF_7 cells in a dose dependent manner. Our result complies with ayakumar and Kanthimathi results, they demonstrated that phenolic extract of Pepper, long pepper and ginger exhibited a high rate of inhibition (up to 90%) and clove and cumin showed low level of inhibition of migration in breast cancer MCF_7 cells. They also found a strong correlation extract phenols and their inhibitory activity (Jayakumar and Kanthimathi 2012). Furthermore, Poly phenol that we identified in garlic extract (Table 3.1) can significantly prevented wound healing with respect to untreated cells in a dose dependent manner. Similarly, caffeine acid, p-Coumaric acid, induces anti-metastasis activity through suppression of migration rate in breast cancer MDA-MB-231 and MCF_7 cells (Kabała-Dzik et al. 2017; Majumder et al. 2019).

Likewise, in a recent study, it was found that poly phenol rich strawberry extract decreased migration of invasive breast cancer A17 cell and inhibited cell growth (Amatori et al. 2016). Remarkably, garlic also showed anti-metastatic activity by disrupting the vimentin filament network in breast cancer MDA-MB-231 cells (Kaschula et al. 2019).

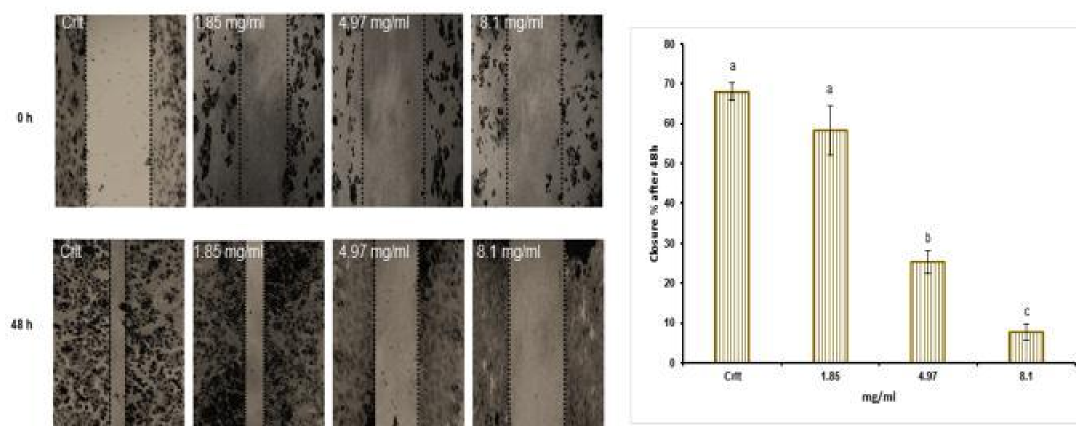


Figure 3.23: Garlic extract showed anti-metastatic effect on MCF_7 cells by wound healing assay. After 24 h incubation, MCF_7 cells was treated with different concentrations of garlic extract for 48 h. The wound closure percentages were analyzed by Image J software. All data shown were the mean ± SD of three independent experiments. Different superscripts letter for each column indicated significant differences ($p < 0.05$).

4.1.2. Garlic extracts down regulated the migration capacity of MCF_7 cells by decreasing migration marker expression.

To further explore the inhibitory effects of garlic, extract on tumour cell migration, we observed the MMP-2 and MMP-9 gene expression, a marker that represents loss of migration potential in breast cancer cell. As expected, after treatment with garlic extract 1.85 mg/ml, 4.97 mg/ml and 8.1 mg/ml at 48h and examined by RT-PCR. Where, garlic extract significantly ($p<0.05$) reduced the expression of MMP-2 and MMP-9 proteins up to 0.72 fold and 0.49 fold respectively, compare to the untreated cells at dose depended manner (Figure 3.24). Interestingly, curcumin, main component of turmeric was significantly decreased MMP-2 and -9 expression in breast cancer MCF_7 and MDA-MB-231 and inhibition migration (Di et al. 2003). Similarly, gallic acid treatment suppressed the MMP-2 and MMP-9 expression in gastric cancer, breast cancer and osteosarcoma cells(Ho et al. 2010; Liao et al. 2012; Chen et al. 2016). In-addition, garlic organosulfur compounds S-Allylcysteine, DADS and DATS also decreased the exporession of MMP-2 and -9 protein in breast cancer cells (Gapter et al. 2008; Huang et al. 2015; Liu et al. 2015).

Finally, aforementioned studies clear that, Inhibition of MMP-2 and -9 protens expression may be involved in the suppression of migration and invasion mediated cell proliferation in breast cancer.

4.1.3. Garlic extract decreased mesenchymal and increased epithelial markers expression in MCF_7 cells by β-Catenin and TGF-β pathway

Breast cancer cells can undergo an epithelial-to-mesenchymal transition (EMT) and develop paracrine-mediated proliferation, migration, and invasion (Kong et al. 2021).
We evaluated the levels of N-cadherin, E-cadherin and β-Catenin by meauriong the mRNA expression of E-cadherin, N-cadherin and β-Catenin gene through RT-PCR. Our data showed a significantly ($p<0.05$) downregulation of N-cadherin ande β-Catenin expression up to 0.27 fold and 0.34 fold respect to the untreated cells. Moreover, a noticeable significant ($p<0.05$) up to 4.05 fold up-regulation of E-cadherin protein expression observed in MCF_7 cells compared to untreated cells (Figure 3.24). Overall, these results indicated that garlic extract decreased the levels of the mesenchymal marker N-cadherin and increased epithelial markers E-cadherin through inhibition of β-Catenin and TGF-β signalling pathway (Figure 3.24) in breast cancer MCF_7 cells. Additionally, it could be inhibited EMT in turn responsible for the suppression of cell mobility, invasion as well as metastasis. Similarly, piperine species used in prevent metastasis effect by decreasing TGF-β1 induced EMT in different type of cancer cells such as HepG2, MDA-MB-231 and A549 cell lines (Marques da Fonseca et al. 2020). Garlic component DADS Induces anti-metastatic activity via inhibition of β-catenin signalling pathway (Huang et al. 2015).

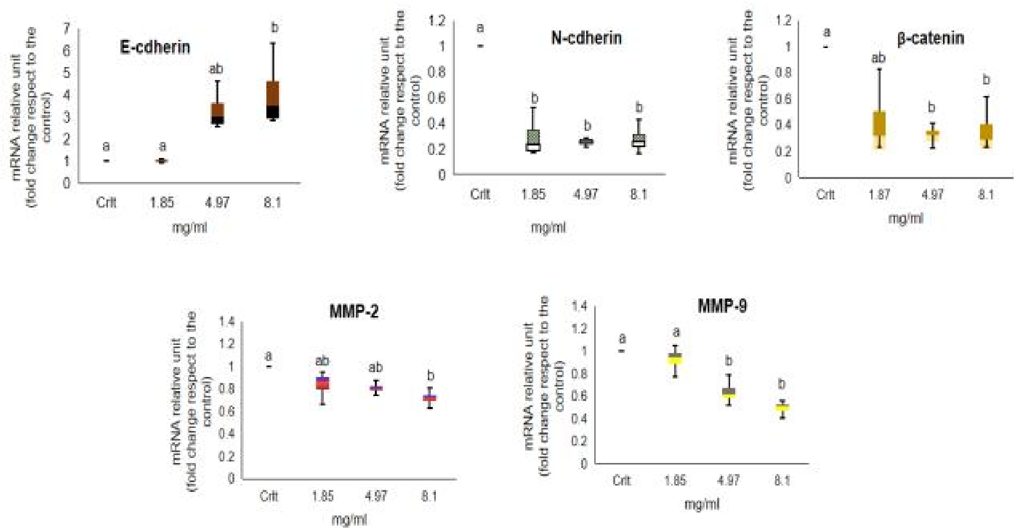


Figure 3.24: Real-time polymerase chain reaction analyses of the expression migration and EMT related gene; E-caherin, N-cadherin, β-catenin, MMP-2 and MMP-9. Garlic extract decreases invasion ability of MCF_7 cells by suppressing MMP-2 and MMP-9 expression. The mRNA expressions of E-caherin, N-cadherin, β-catenin, MMP-2 and MMP-9 gene in MCF_7 cell were determined after 48h treatment with garlic extract compare with untreated cell. GADPH was used as a house keeping gene. All data shown were the mean ± SD of three independent experiments. Different superscripts letter for each column indicated significant differences ($p < 0.05$).

There is an increasing amount of evidence on the anti-metastatic effects of phenolic compounds, with special attention being given to the modulation of the expression of EMT-related genes (E-cadherin, N-cadherin, vimentin, β-catenin, Snail, MMP-2 and MMP-9) in *in vitro* breast cancer models (Belguise et al. 2007; Sun et al. 2019; Pan et al. 2020). Moreover, in our result, significantly increased epithelial markers suggested that phenolic compound enrich garlic extract could be modulated EMT related markers strongly in breast cancer.

4.1.4. Garlic extract inhibits metastasis by blocking Angiogenesis in MCF_7 cells

Early step of the metastatic cascade is formation of new blood vessels from pre-existing vasculature, that help to develop angiogenesis by providing nutrition to the tumour. In cancer cells, nutrition, and oxygen have important role to form new blood vessel that upregulate the angiogenesis as well as tumour progression (Wang et al. 2017). The tumour angiogenesis regulation is depended on balance between pro- and anti-angiogenic factors (Reynolds and

Redmer 2001). Recently, focus on distribution of tumour angiogenesis pathway for treatment of breast cancer, showed potential outcomes to suppress cancer metastatic (Zhou et al. 2017). Several mechanisms have been found to explain the anticancer effects of spices by suppressing angiogenesis (Lin et al. 2009; Freise et al. 2011; Peng et al. 2013), especially, natural polyphenols that inhibit tumour cell proliferation via inhibition of angiogenesis in breast cancer cells (Lin et al. 2009; Karimi et al. 2017; Ghiulai et al. 2020). For example, garlic and lemon aqueous extracts combination prevents angiogenesis by systemic activation in the immune system in breast cancer (Talib 2017). But the mechanism/relation between garlic and angiogenesis is not completely understood, while, angiogenesis is an important factor for breast cancer progression.

To understand the anti-angiogenic effect of poly phenolic garlic extract in molecular level, here, we used Human angiogenesis antibody membrane array (ab134000, Abcam) to evaluate the garlic effect on 20 angiogenic protein factor that involve in angiogenesis processes in breast cancer, after treatment with different concentration of garlic extract (1.85 mg/ml, 4.97 mg/ml and 8. mg/ml) at 48h in MCF_7 cell line.

Proteins for which statistically or no statistically significant differences ($p<0.05$) in expression levels between untreated cells and different treatment conditions have been found will be extensively discussed (Figure 3.25 and 3.26).

Recently, scientists had a greater focus on the role of the tumour microenvironment (TME) and their endogenous inhibitors. Among this, Tissue Inhibitors of Metalloproteinases (TIMPs) has primarily function to prevent degradation of the extracellular matrix (ECM) via inhibition of matrix metalloproteinases (MMPs). Generally, MMPs are prominent molecules which degrades ECM and implicates in migration and invasion of cancer cells, and also induce angiogenesis (Jiang et al. 2000). Therefore, Tissue inhibitor of metalloproteinases-1 (TIMP-1) plays a crucial role on regulation of MMPs in ECM composition, wound healing and inhibition of angiogenesis and the induction of apoptosis (Brew et al. 2000). For example, Japanese quince fruit flavanol preparation (JQFFP) has strongly inhibits MMP-9 by up regulating TIMP-1 expression in MDA-MB-231 human breast cancer cells and shows potent anti proliferative by blocking angiogenesis (Lewandowska et al. 2013). Similarly, Korean Citrus hallabong peels prevents angiogenesis via reduction of tube formation of human umbilical vein vascular endothelial cells and suppress of MMP-9 in MDA-MB-231 breast

cancer cells (Park et al. 2016a). Likewise, garlic component DADS and olive oil polyphenol Oleuropein, increases TIMP-1 expression in human gastric adenocarcinoma AGS cells line and MDA human breast cancer cell line by decreasing MMP-2 and MMP-9 expression (Park et al. 2011; Hassan et al. 2012). In our result showed, poly phenol containing garlic extract significantly ($p<0.05$) up regulated TIMP1 expression up to 2.37 fold with respect to the untreated cells in breast cancer MCF_7 cell (Figure 3.25) that also inhibited MMP-2 and MMP-9 expression (Figure 3.24).

However, Tissue inhibitors of metalloproteinase 2 (TIMP-2) has many specific properties and functions that is a more effective inhibitor of MMP-2. Whichever had been mostly and extensively studied, that could effectively inhibit angiogenesis via reduction of endothelial cell proliferation and migration and the invasion as well as metastasis (Yang et al. 2011; Remillard et al. 2014; Kai et al. 2016). Therefore, reduction of TIMP-2 or over expression of MMP-2 granted to cancer development by altering migration and invasion of tumour cells (Zurac et al. 2016; Sadot et al. 2016; Ukaji et al. 2017). In particular, up regulates of TIMP-2 protein expression that effectively down regulates of the metastatic protein expressions MMP-9 and suppress metastasis and angiogenesis in Hep-2 cells possibly via PI3K/AKT pathway (Shakya et al. 2018). Similar effect also observed, VI-14 and curcumin, phenolic derivative, suppress migration and invasion of human breast cancer MCF_7 and MDA-MB-231 cell lines by increasing TIMP-2 and decreasing MMP-2 expression (Di et al. 2003; Li et al. 2012). In the present report, we observed that the protein levels of TIMP-2 are significantly ($p<0.05$) increased up 1.68 fold following treatment with garlic extract (Figure 3.25). Taken together, we proposed that TIMP family could be inhibited angiogenesis in breast cancer MCF_7 cell after garlic extract treatment at dose depended on manner.

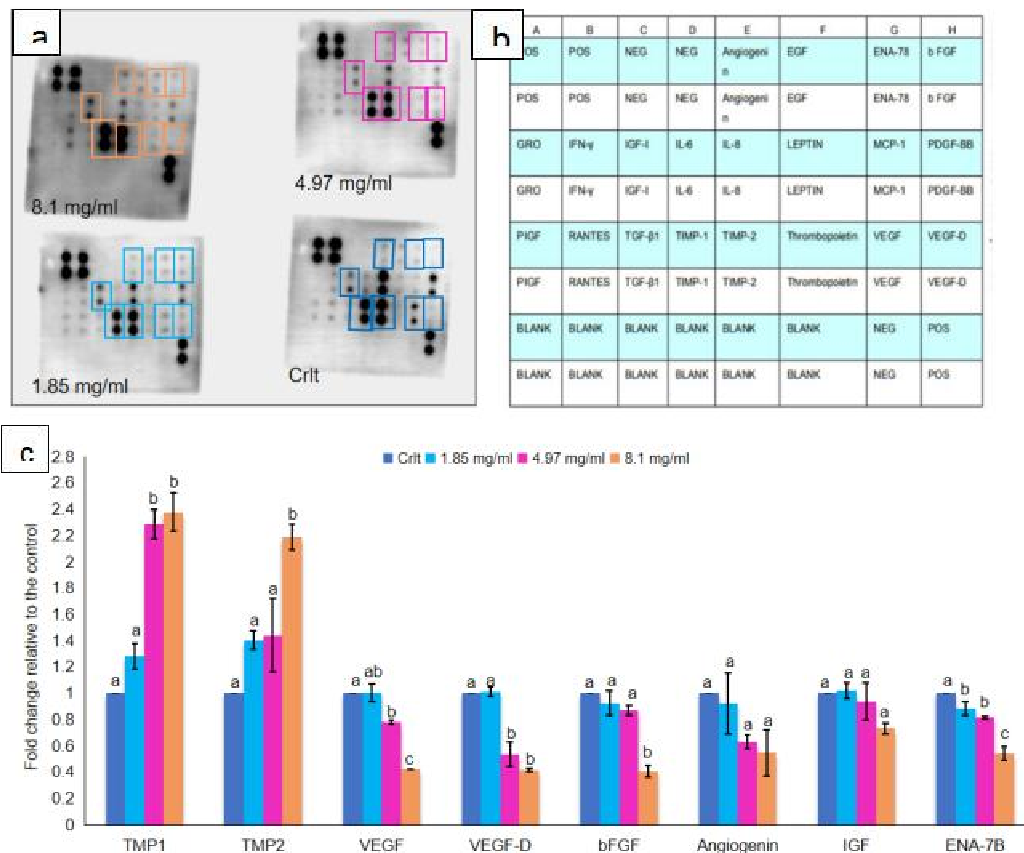


Figure 3.25: Anti-angiogenesis effect of garlic extract on breast cancer MCF_7 cells a) Human Angiogenesis Antibody Array expression. After 24 h incubation, MCF_7 cells was treated with different concentrations of garlic extract 1.87 mg/ml, 4.97 mg/ml and 8.1 mg/ml for 48 h. Positive spots were used as a loading control and for normalization. b) Template showing the location of angiogenic factor spotted onto the abcam Human Angiogenesis antibody array kit. c) Column's graph belongs to the same set of data compare to untreated cells with different superscript letters are significantly ($p<0.05$) different. All data shown are the mean ± SD of two different spots. TIMP-1: Tissue inhibitor of metalloproteinase 1; TIMP-2: Tissue inhibitor of metalloproteinase 2; VEGF: Vascular endothelial growth factor; VEGF-D: Vascular endothelial growth factor-D; b-FGF: basal fibroblast growth factor; IGF-I: Insulin growth factor; ENA-78: Epithelial Neutrophil-Activating peptide 78.

Further, angiogenic factor is Vascular endothelial growth factor (VEGF); plays a promising role in angiogenesis due to its effect on endothelial cell growth (Ravindranath et al. 2001) mediated by receptor tyrosine kinase, such as VEGFR-1, VEGFR-2 and VEGFR-3. Among them VEGFR-2 regulates vascular endothelial proliferation, migration, differentiation, capillary like formation and vascular permeability.

In recent past, great number of natural compounds have been showed to counteract the angiogenic effects by inhibiting VEGF expression (Lin et al. 2009; Xu et al. 2013; Wei et al. 2017; Shakya et al. 2018; Hu et al. 2019). Amongst, spices potentially inhibit angiogenesis formation by decreasing VEGF to target VEGF/VEGFR2 signal pathway for cancer (Xu et al. 2013). In our experiment of protein array, we found garlic extract significantly ($p<0.05$) decreased VEGF protein expression up to 0.42 fold compare to the untreated cells. Here, we demonstrated garlic extract could be a novel candidate inhibitor of angiogenesis to target VEGF signalling pathway and lead to complete inhibition of VEGF (Figure 3.25). Previouly, Diallyl trisulfide (DATS) has the ability to prevent angiogenesis of human endothelial cells by and down-regulating VEGF and inactivating Akt and prevent metastasis (Lai et al. 2015; Xiao et al. 2016; Wei et al. 2017). Similarly, phenolic derivatives resveratrol, epigallocatechin-3-gallate and ellagic acid are inhibited VEGF expression and decreased breast cancer tumour growth (Waleh et al. 2005; Garvin et al. 2006; Wang et al. 2012) that reassembling our results.

As well, Garlic extracts significantly ($p<0.05$) down regulated VEGF-D expression up to 0.41 fold compare to the untreated cells at dose dependent manner (Figure 3.25). VEGF-D expression has a crucial role in formation of breast cancer metastasis (Eroglu et al. 2017). Therefore, Basic fibroblast growth factor (b-FGF) potentially promotes cell proliferation and act as a pro-angiogenesis inducer (Mousa and Mousa 2006) beyond VEGF. It could be regulated migration and tube formation via activation of the receptor tyrosine kinase (Cronauer et al. 2003; Brooks et al. 2012) and has an involvement of b-FGF in the formation of solid tumours (Leunig et al. 1997). However, phenolic compound such as, curcumin, effectively down regulates the response of b-FGF in human breast cancer MCF_7 and MDA-MB-231 cell lines (Di et al. 2003). In this present report, garlic extract significantly ($p<0.05$) decreased b-FGF protein expression up to 0.40 fold in MCF_7 cell compare to the untreated cells in a dose dependent manner (Figure 3.25). Thus, garlic extract was effectively blocked angiogenesis induction by decreasing bFGF, compared to the untreated cell. Likewise, garlic component, Alliin significantly suppress both bFGF2 and VEGF expression in human fibrosarcoma cells in a concentration-dependent manner (Mousa and Mousa 2005). However, VEGF and bFGF could play an important role in the control of angiogenesis and cell proliferation *in vitro* and *in vivo* (Pepper et al. 1992).

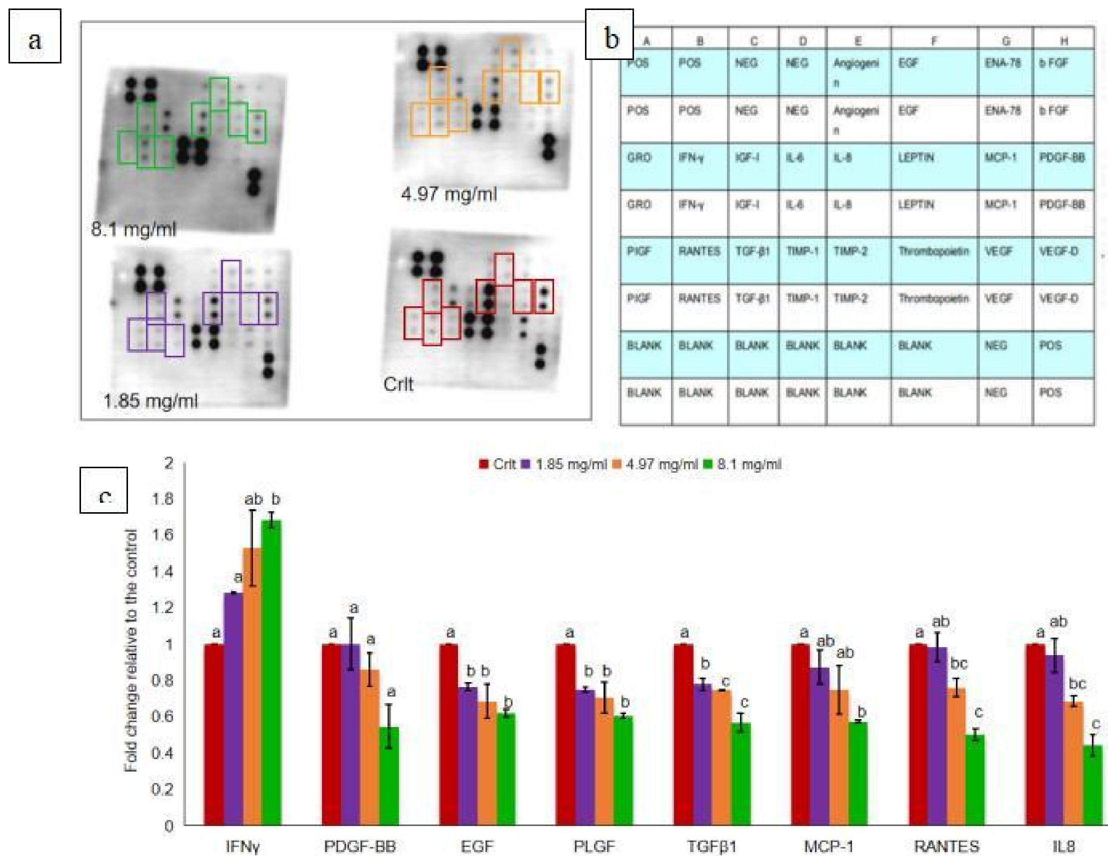
Angiogenin, one of the prominent factors that activates blood vessels formation and significance for angiogenic activity (Shestenko et al. 2001). Usually, it's found in plasma but over expression observed in cancerous condition (Pavlov and Badet 2001). In breast cancer patients, over angiogenin expression associated with the transition of normal breast tissue turns into invasive breast carcinoma. Several studies revelled that down regulation of angiogenin can prevent angiogenesis and suppress the growth of human tumours cells in *in vitro* and *in vivo* studies (Olson et al. 1995; Nilsson et al. 2010). Here, we showed that garlic extract non significantly ($p<0.05$) decreased angiogenin protein up to 0.54 fold in breast cancer MCF_7 cells compare to the untreated cells (figure 3.25).

Insulin-like growth factor (IGF-1) stimulates tumorigenicity of many types of cancer by increasing migration ability of cells. Especially, in breast-cancer, up regulation of IGF-1 protein promotes migration as well as metastasis. It's observing that poly phenol for example, resveratrol, genistein and epigallocatechin-3-gallate (EGCG) inhibits migration and invasion as well as angiogenesis of human breast-cancer MDA-MB 435, MCF_7, T47D and MDA-MB-231 cells via inhibition of IGF-1 expression (Tang et al. 2008; Zeng et al. 2014; Chen et al. 2015). Here, we found garlic extract non significantly ($p<0.05$) decreased IGF-1 protein expression 0.73 fold in MCF_7 cell line with respect to the untreated cells (Figure 3.25).
Epithelial Neutrophil-Activating peptide 78 (ENA78) are crucial contributors to the angiogenic activity and suppression of this chemokine may attempt a novel target therapy for the treatment for cancer (Koch et al. 2001; Pappa et al. 2014). Here, we noticed ENA78 protein expression decreased significantly ($p<0.05$) up to 0.54 fold but not in a dose dependent manner with respect to the untreated cells (Figure 3.25). There is no previous effect of garlic poly phenol on this protein expression. After searching poly phenolic effect on this ENA78 protein, we only found one work; gossypol, a cotton-seed polyphenol, down regulate angiogenic cytokin ENA78 expression in prostate cancer PC-3 and DU-145 cell lines (Karaca et al. 2008).

Then, Interferon gamma (IFNγ) is an anti-angiogenic factor that significantly shows anti proliferative effect on breast cancer by inhibiting tumour angiogenesis (Talib et al. 2019). However, IFNγ prevents angiogenesis by blocking capilaries formation in endothelial cells (Sato et al. 1990). In our study, IFNγ protein expression level significantly ($p<0.05$) higher up to 1.68 fold with respect to the untreated cells at concentration dependent manner (Figure 3.26). Regarding this, after oral administration of resveratrol in mice, dramatically increase

IFN-γ secretion and restricts tumour angiogenesis as well as tumour growth (Lee et al. 2021). Similarly co-administration of edible red clover (Trifolium pratense L.) and doxorubicin (DOX) repress the proliferation of 4T1 tumour cells in dose- and time-dependent manners in 4T1-tumour bearing BALB/c mice via upregulation of IFN-γ (Akbaribazm et al. 2020).

Platelet Derived Growth Factor-BB (PDGF-BB) isomer mitogen, a pro-angiogenic critical factor for angiogenesis development by stimulating *in vitro* capillary formation. It's helps to develop tumour cells and also promising biomarker in breast tumour (Sato et al. 1990; Karimzadeh et al. 2020). In this current study, garlic extract down regulated up to 0.54 fold of PDGF-BB expression with compare to the untreated sample, was noted. The value was not statistically significant ($p<0.05$) (Figure 3.26). Further, EGCG madiated PDGF-BB down regulatory action was observe in A172 glioblastoma and human hepatic stellate cell line LI90 by blocking PDGF-BB binding to its receptor (Sachinidis et al. 2000; Sakata et al. 2004).
After that, Epidermal growth factor (EGF) is an another angiogenic factors in breast cancer patients (Karimzadeh et al. 2020). It's also related to signalling pathways which regulate proliferation, survival, angiogenesis and metastasis of breast cancer (Hardy et al. 2010). Previous studies indicate that garlic bioactive molecules repeals EGF expression and thus, they may be used to inhibit the induction, development and progression of cancer (Rauf et al. 2021). Here, we observed garlic extract significantly ($p<0.05$) decreased up to 0.62 fold EGF protein expression in MCF_7 cells respect to the untreated cells in a dose dependent manner (Figure 3.26). Likewise, grape polyphenol, resveratrol that decreases EGF expression in MDA-MB-231 metastatic human breast cancer cells to prevent cell migration and EMT (Azios and Dharmawardhane 2005; Vergara et al. 2011). Similarly, Delphinidin has a potentially inhibit HIF-1α and VEGF expression by inhibiting EGF expression along with prevention of angiogenesis (Kim et al. 2017).



A	B	C	D	E	F	G	H
POS	POS	NEG	NEG	Angiogenin	EGF	ENA-78	b FGF
POS	POS	NEG	NEG	Angiogenin	EGF	ENA-78	b FGF
GRO	IFN-γ	IGF-I	IL-6	IL-8	LEPTIN	MCP-1	PDGF-BB
GRO	IFN-γ	IGF-I	IL-6	IL-8	LEPTIN	MCP-1	PDGF-BB
PIGF	RANTES	TGF-β1	TIMP-1	TIMP-2	Thrombopoietin	VEGF	VEGF-D
PIGF	RANTES	TGF-β1	TIMP-1	TIMP-2	Thrombopoietin	VEGF	VEGF-D
BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	NEG	POS
BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	NEG	POS

Figure 3.26: Anti-angiogenesis effect of garlic extract on breast cancer MCF_7 cells a) Human Angiogenesis Antibody Array expression. After 24 h incubation, MCF_7 cells was treated with different concentrations of garlic extract 1.87 mg/ml, 4.97 mg/ml and 8.1 mg/ml for 48 h. Positive spots were used as a loading control and for normalization. b) Template showing the location of angiogenic factor spotted onto the abcam Human Angiogenesis antibody array kit. c) Columns graph belongs to the same set of data compare to untreated cells with different superscript letters are significantly different ($p < 0.05$). All data shown are the mean ± SD of two different spots. FN-γ: Interferon gamma; PDGF-BB: Platelet Derived Growth Factor-BB; EGF: epidermal growth factor; PlGF: Placental growth factor; TGFβ1: Transforming growth factor beta 1; MCP-1: Monocyte Chemoattractant Protein-1; IL-8: Interleukin-8.

Placental growth factor (PLGF) is a glycoprotein and also parts of the VEGF family and responsible to endorse migration, invasion and adhesion and triggers angiogenesis of human breast cancer (Coenegrachts et al. 2010; Dewerchin and Carmeliet 2014). Clinical studies demonstrate that over expression of PlGF in breast cancer tissues can plays a major role in the pathogenesis which correlates with cancer patient prognosis (Parr et al. 2005). In present study, we noticed that garlic extract significantly ($p<0.05$) decreased PLGF protein 0.61 fold

compare to the untreated cells (Figure 3.26). There are no studies in the literature that evaluates how polyphenols could affect PlGF factor in cancer except curcumin. Curcumin has blocked arecoline mediated PlGF expression in oral carcinogenesis (Cheng et al. 2013).

The transforming growth factor-beta (TGFβ1) gene are expressed structurally and functionally related to polypeptides and its significant influence on physiological and pathophysiological processes (Govinden and Bhoola 2003). In breast cancer, overexpression of TGF-β1 levels in platelets may be suggested as a possible target for early diagnosis whereas, platelets play a vital role in tumour angiogenesis (Han et al. 2014). In this experiment, the intensity of the observed spots is very weak, but the software used for the detection was able to identify the intensity of the bands. Where, we observed, garlic extract significantly ($p<0.05$) down regulated TGFβ1 expression up to 0.57 fold at a dose dependent manner with respect untreated cells (Figure 3.26). Furthermore, Piperine has cytotoxicity on HepG2, MDA-MB-231 and A549 cell lines via down regulation TGF-β1 protein expression and cell viability (Marques da Fonseca et al. 2020). Similarly, the combination of resveratrol and cisplatin significantly downregulate TGF-β1 and increase anti-tumour effects and decrease side effects of cisplatin in MDA231 xenografts (Yang et al. 2021).

Another factor, MCP-1 (CCL2) is one of the inflammatory mediators that plays a role in breast cancer progression as well as malignancy. It could be a potential therapeutic target for breast cancer treatment (Soria and Ben-Baruch 2008). In this work, we noticed garlic extract significantly ($p<0.05$) decreased MCP-1 protein up to 0.57 fold in compare to the untreated cells (Figure 3.26). In the same way, emodin, a Chinese herb-derived compound that prevents breast cancer in 4T1 breast tumour-bearing mice by blocking the tumour-promoting feed-forward loop via inhibition of MCP-1 expression (Iwanowycz et al. 2016). Furthermore, in *in vivo* studies, inhibits the tumour growth by prevent angiogenesis development, after oral administration of piceatannol, a polyphenol, in mouse 4T1 mammary carcinoma cells bearing female BALB/c mice (Song et al. 2015).

Another inflammatory chemokine is RANTES (CCL5), involves developing breast malignancy activities. Surprisingly, natural poly phenols such as resveratrol, Chrysin, and Hydroxytyrosol have inhibited the expression of RANTES and modulated angiogenesis in different types of cancers (Kim et al. 2008; Sarsour et al. 2014; Yeo et al. 2020). Along with

this, we recognized garlic extract significantly ($p<0.05$) decreased RANTES expression up to 0.50 fold compared to untreated cells in MCF_7 cells in dose dependent manner (Figure 3.26).

The last angiogenic factor is Interleukin-8 (IL-8) is a pro-inflammatory, chemokine has multiple pro-tumourigenic roles within the tumour microenvironment (TME), including appealing proliferation or conversion of tumour cells into a migratory or mesenchymal phenotype. Additionally, IL-8 can increase tumour angiogenesis or recruit larger numbers of immuno suppressive cells to the tumour including breast tumour (Waugh and Wilson 2008; Al-Khalaf et al. 2019; Fousek et al. 2021). Suppression of chemokine family such as IL-8, a potential sight for inhibition angiogenesis and suppresses breast cancer cell growth.

In recent times, several studies underline the ability of phenolic compounds to modulate angiogenesis has been linked to cancer progression. A natural polyphenolic cotton-seed extract, Gossypol, decrease in angiogenic cytokines IL-8 levels secretion in prostate cancer PC-3 and DU-145 cells. Similarly, resveratrol and its analogus suppressed IL-8 expression in breast cancer MCF-7 cells (Karaca et al. 2008; van den Brand et al. 2019). Clove and cinnamon's polyphenol, eugenol, significantly decrease IL-8 cytokines and prevent tumour growth in breast stem cancer cell (Islam et al. 2018). This current experiment, garlic extract significantly ($p<0.05$) suppressed IL-8 protein expression up to 0.44 fold in MCF_7 cells compare to the untreated cells (Figure 3.26). This finding suggested that garlic extract may be down regulated cytokines in cellular process and render the cancer development.

Notably, the intensity of the spots of LEPTIN, GRO and Thrombopoietin were not particularly observed for any sample after garlic extract treatment. The software used for the detection was not able to identify the intensity of the bands and we could not be able to differentiate them from the negative spots (figure 3.25 and 3.26).

Finally, phytochemical screening of garlic extract showed the presence of high concentrations of phenolic compounds and mineral (Table 3.1 and 3.3). It was possible to observe anti-angiogenesis effect may be combined of these physiochemical. Our results demonstrate that garlic ethanolic extract may contains high concentrations of antiangiogenic agents. Furthermore, Human Angiogenesis antibody array results indicate that garlic extract may be a promising candidate for anticancer effect.

Taken together, garlic extract treatment significantly inhibited migration, invasion and decreased the expression of several migration-related genes and inhibited key intracellular signalling pathways in breast cancer cells.

4.2. Effect of garlic extract on the colony growth of MCF_7 cells

The efficacy of the extracts for inhibiting colony formation of the tested MCF_7 cells was determined. Recently, several natural compounds were used for inhibiting colony formation in different cancer cells (Fahrioğlu et al. 2016; Afrin et al. 2018; Cao et al. 2018). In this context, Pelingo juice decreases the tumour-genesis of MDA-MB-231 cells by suppressing colony formation (Schiavano et al. 2015). According to previous data suggested that combination of quercetin-3-methyl ether inhibits colony formation in the hormone-sensitive breast cancer MCF_7, T47D and MDAMB-231 cell lines, via PI3K/Akt pathways (Cao et al. 2018). Likewise, Plantago lanceolata L. extract induced cytotoxic via significantly reduction of colony formation of MCF_7, AMJ13, MDAMB, and CAL51 human breast cancer cells (Alsaraf et al. 2019). Whereas chronic exposure of pterostilbene reduces colony formation up to 40% and 85% in colon cancer cells (HCT-116 and HT-29) (Nutakul et al. 2011) and carnosine decreases colony formation up to 39.9% in gastric cancer cells (SGC-7901) (Shen et al. 2014). Similarly, curcumin and its conjugated reduce colony formation up to 55% and 80% in colon cancer cells (HCT-116) (Waghela et al. 2015).

In our work, we observed chronic exposure to garlic extract could be affected the proliferative ability of MCF_7 cells. The cells were seeded at a low density (1000 cells/well) and allowed to form colonies for 15 days in complete growth media supplemented with different treatments. This study verified that, in *in vitro* cell survival assay is based on the ability of a single cell to grow into a colony. We found, that, garlic extract at 1.85, 4.97 and 8.1 mg/ml, a significantly ($p < 0.05$) decreased colonies formation from 14%, 56% and 83% respectively, with respect to untreated cells in breast cancer MCF_7 cells in a dose dependent manner. In fact, garlic extract showed at a high concentration of 8.1 mg/ml to more efficacy and suppressed colonization of MCF_7 breast cancer cells (Figure 3.27). This data further confirmed the growth inhibitory effect of garlic extract on the proliferation of breast cancer MCF_7 cells.

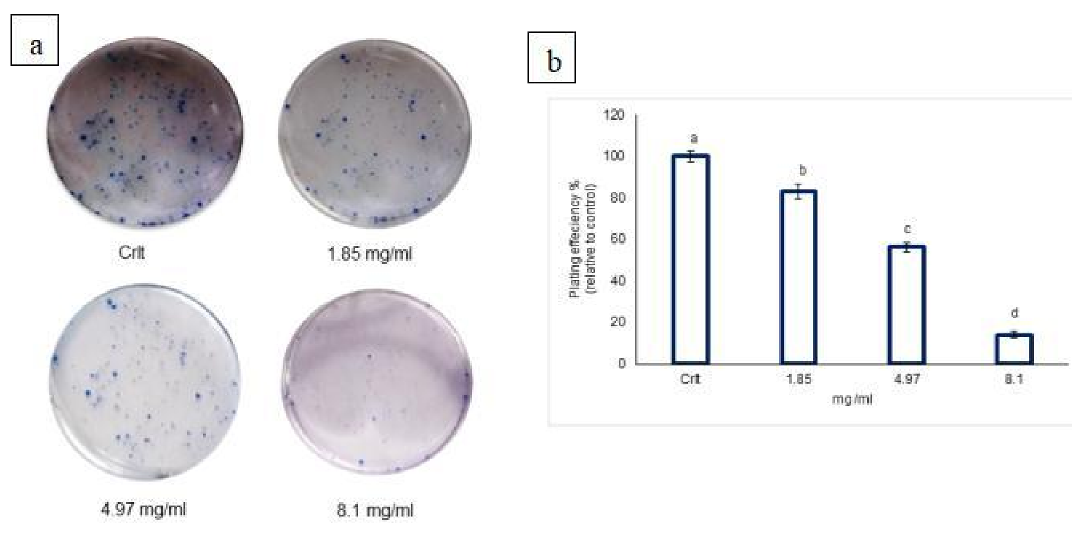


Figure 3.27: Garlic extract inhibited the colony formation of breast cancer MCF_7 cells. a) The representative colony well images showed colony formation in breast cancer cells, treated with 1.87 mg/ml, 4.97 mg/ml and 8.1 mg/ml concentrations of garlic extract and seeded for 12 days. b) Columns graph belongs to the same set of data compare to untreated cells with different superscript letters are significantly different ($p < 0.05$). All data shown were the mean ± SD of three independent experiments.

4.3. Garlic extract suppressing inflammation and prevent tumour growth by down regulating NFκβ/EGFR pathway

Chronic inflammation has prominent role to progress breast cancer by owing to the role of inflammatory factors in genetic instability (Bahiraee et al. 2019). For example, NFκB pathways are play vital role for the inflammatory response and strongly prevent apoptosis of cancer cells and regulates cancer angiogenesis (Fan et al. 2013). NFκB pathways activates through response with increased levels of several inflammatory proteins (COX-2, CRP and LOX- 2) and cytosines (IL-1β, IL-6 and TNF-α) (E. Goldberg and L. Schwertfeger 2010). In recent times, researchers have been focussing on poly phenols for their potential anti-inflammatory and antioxidant effect (Hussain et al. 2016). Interestingly, preclinical and clinical studies shows that spices poly phenol shows anti-inflammatory against breast cancer (Rossi et al. 2018; Martínez et al. 2019; Mahmoud et al. 2021).

In our study, we found garlic extract significantly ($p<0.05$) decreased inflammatory marker expression for NFκB, IL6, IL-1β and p-IκBα up to 0.34 fold, 0.12 fold, 0.13 fold and 0.23 fold respectively, compare to untreated cells in a dose dependent manner (Figure 3.28).

Therefore, garlic are responsible for the anti-inflammation effect, it contains various compounds such as allicin that shows anti-inflammatory effect by reducing TNF-a, IL-1b and IL-8 expression in intestinal epithelial cells (Lang et al. 2004). Similarly, DADS suppresses NF-κB and inhibits colitis induced colorectal cancer by down regulating GSK-3β. In human gastric cancer, DATS suppresses NF-κB signalling as well as cycle arrest and apoptosis, resulting in the prevention of cell proliferation and tumour-genesis in nude mice (Pan et al. 2016). Furthermore, Qin et al. revels that ethanolic garlic extracts can prevent chronic liver injury and inflammation, as well as for the inhibition of the carcinogenesis of fatty livers (Qin et al. 2020). Thus, first time we evaluated anti-inflammatory effect of poly phenol containing garlic extract on breast cancer MCF_7 cell at 48h. Similar effect also observes in turmeric poly phenol; curcumin can reduce metastasis by inhibiting pro inflammatory cytokines in breast cancer (Kronski et al. 2014).

Additionally, Over expression of the EGFR associates with a more aggressive cells growth in human breast carcinomas (Mendelsohn and Baselga 2003). Consequently, in solid tumour, activated EGFR and NF-κB provides oncogenic signals to cancer cells (Shostak and Chariot 2015). Therefore, inhibition of NFkB and the EGFR family of cell surface receptors may suppress proliferation in breast cancer (Chou and Talaly 1977).

In this study, we observed garlic extract significantly ($p<0.05$) down regulated EGFR protein expression up to 0.11 fold respect to the untreated cells at a dose dependent manner (Figure 3.28). Similarly, quercetin, a poly phenol, shows anti proliferative effect by decreasing EGFR expression in human breast cancer cell lines MCF-7 and MDA-MB-231 (Tao et al. 2015). whereas curcumin inhibits the proliferation of MDA-MB-231 cells by Inhibiting of the EGFR signalling pathway (Sun et al. 2012).

4.4. Garlic extract on cell cycle progression in MCF_7 cells

Later, we did an attempt to understand how to MCF_7 cell growth inhibited, first, the effect of the garlic extracts on the cell cycle by using a Tali™ Image-based cytometer with PI staining was determined because cell proliferation is correlated with the regulation of cell cycle progression. Then, we continued to verify and explore whether garlic extract affected cell cycle and related gene expression. After treating MCF_7 cells with 1.85 mg/ml, 4.97mg/ml and 8.1 mg/ml of garlic extract for 48h, we observed that higher number of cells were

accumulated in the G0/G1 phase respect with untreated cells (Figure 3.29). while paralleled a little amount increased of cells in the sub-G1 cell cycle phase.

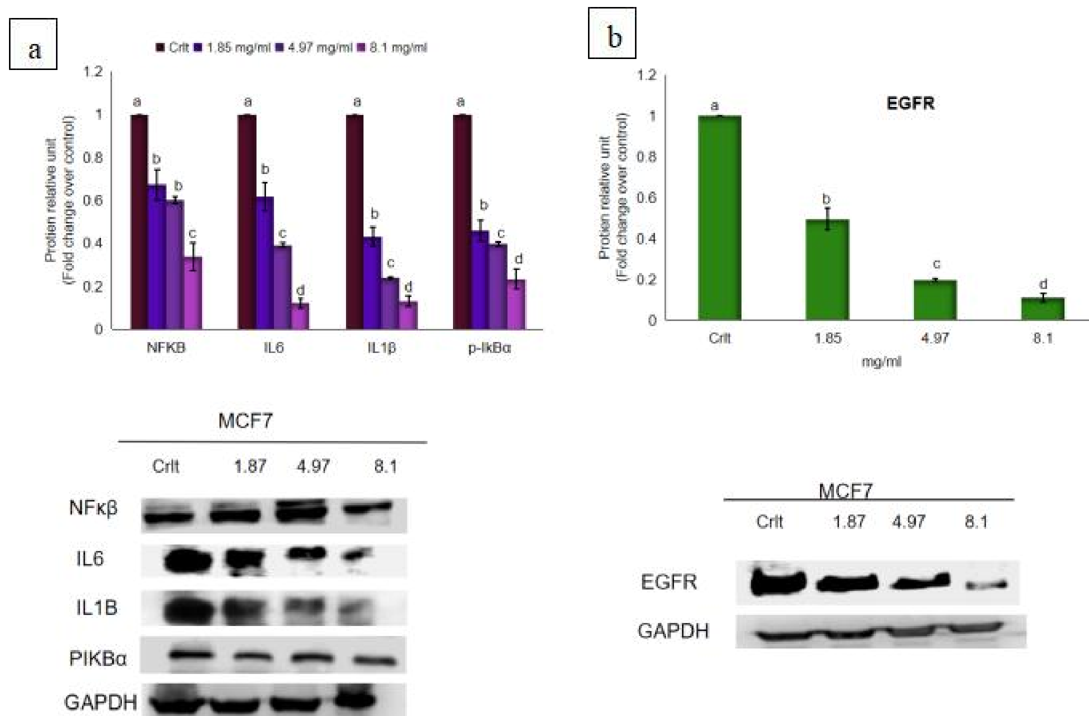


Figure 3.28: Anti-inflammatory effects of garlic extract in MCF_7 cells. MCF_7 cell were incubated different concentrations of garlic extract for 48 h. The concentration of 0 mg/mL corresponds to control (untreated cells). a) The expression of inflammatory markers NFκB, p-IκBα, IL1β and IL-6 were determined by western blotting analysis. b) The expression of anti-proliferative marker EGFR was determined by western blotting analysis. GADPH was used as a loading control. All data shown were the mean ± SD of three independent experiments. Different superscripts letter for each column indicated significant differences ($p < 0.05$)

Treatment of garlic extract significantly ($p < 0.05$) increased the accumulation of cells at G0/G1 phase of about 66% at high concentration (8.1 mg/ml) in MCF_7 cells. While reduced the cells in S phase and G2/M phase compared to untreated cells respectively. In MCF_7 cells, the percentage of cells in the S phase and G2/M were significantly ($p < 0.05$) decreased up to 12.33% and up to 7% after garlic extract treatment compared to untreated sample (up to 21.67%) (Figure 3.29).

Notably, dysregulation of cell cycle is one of the features of cancer progression which contributes to the abounded proliferation in human cancer. The cell cycle can be divided into four different phases G1, S, G2 and M. At the S phase, DNA synthesis takes place, at the M phase the cell divides into two identical daughter cells and both these phases are separated by gap phase G1 and G2 (Williams and Stoeber 2012). At the G1 phase, the cells are response to extracellular signals to progress towards mitosis or withdrawal from the cell cycle into a resting or inactive stage known as G0 phase (Sherr 1994). While cells undergoing apoptosis were known as an accumulated

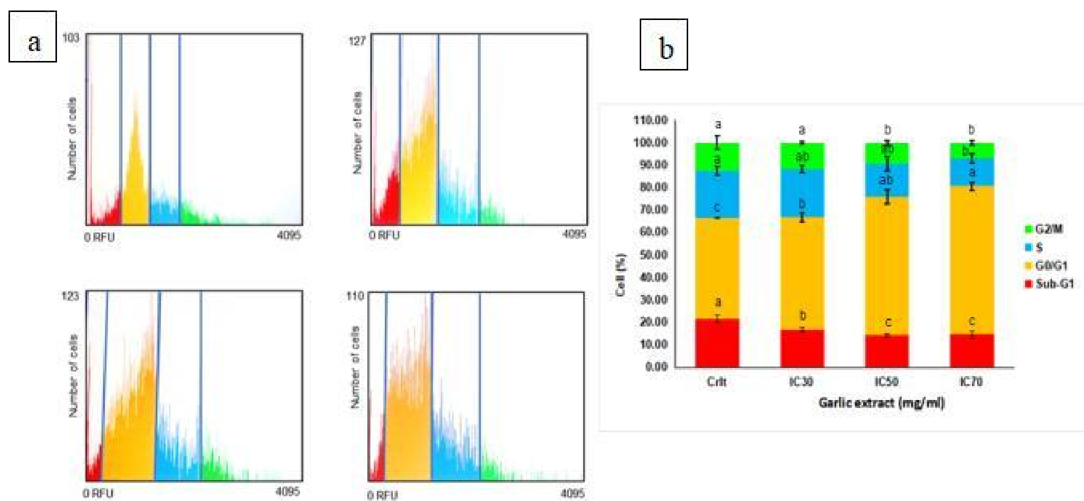


Figure 3.29: Garlic extract induces cell cycle arrest of MCF_7 breast cancer cells. After 24 h seeding, MCF_7 cell was treated with different concentrations of garlic extract for 48 h. Flow cytometer using propidium iodide (PI) assay was used for staining the cells and DNA content of cells was analysed for determination the effect of garlic extract on MCF_7 cell cycle distribution. The percentages of cells in each phase Sub-G1 (apoptotic cells), G0/G1, S and G2/M were analyzed by the Tali® Cell Cycle Assay kit and Tali™ Image-based Cytometer. a) Representative fluorescence image of MCF_7 cycle shows the effect of garlic extract with or without treatment: red colour corresponds to Sub-G1 phase, yellow colour corresponds to G0/G1 phase, cyan, green colour corresponds to S phase and green colour corresponds to G2/M phase. b) Column's graph belongs to the same set of data compare to untreated cells with different superscript letters are significantly different ($p < 0.05$). All data shown were the mean ± SD of three independent experiments.

cell population in the Sub-G1 region (Okuma et al. 2000). Previous studies reported that garlic or its various compounds could arrest the cell cycle at different phases [205,424,549,550]. In our work, garlic extract showed, the accumulation of cells in G1 phase compared to untreated

cells, indicating the percentage of apoptosis in MCF_7 after 48 h incubation. Several studies demonstrated that the cells arrested at G0/G1 phase would be pushed into apoptosis in cancer cell line, for example, methanol extract of Piper capense causes cycle arrest in the G0/G1 in MDA-MB-231-pcDNA3 breast cancer cells and induction of apoptosis. Similarly, Licarin induces apoptosis as well as autophagy in lung cancer NCI–H23 and A549 cells by arrest cell cycle at G1 phase (Maheswari et al. 2018; Mbaveng et al. 2021). In addition, the polyphenol-rich natural compounds such as Ellagic acid, Caffeic Acid, 6-Gingerol have the ability to arrest the cell cycle at G0/G1 phase accumulating the cells at Sub-G1 phase in different cancer cell lines (Chen et al. 2015; Kabała-Dzik et al. 2017; Sp et al. 2021). In our present work, similar results were observed by garlic treatment, with an arrest in the progression of cell cycle at G0/G1 phase for MCF_7 cells (Figure 3.29) compared to untreated cells. This result could be helping to explain, garlic poly phenol may be inhibited cell growth via cell cycle arrest at G0/G1 phase.

Although, monitoring the cell cycle, in addition to the flow cytometer analysis of PI staining used in this study and evaluation of cell cycle markers is also an effective method where, the transformation from one phase to another phase is controlled by several checkpoint genes such as CDKs by uniting with their relevant administrative subunits cyclins that may activate different downstream targets (Vermeulen et al. 2003).

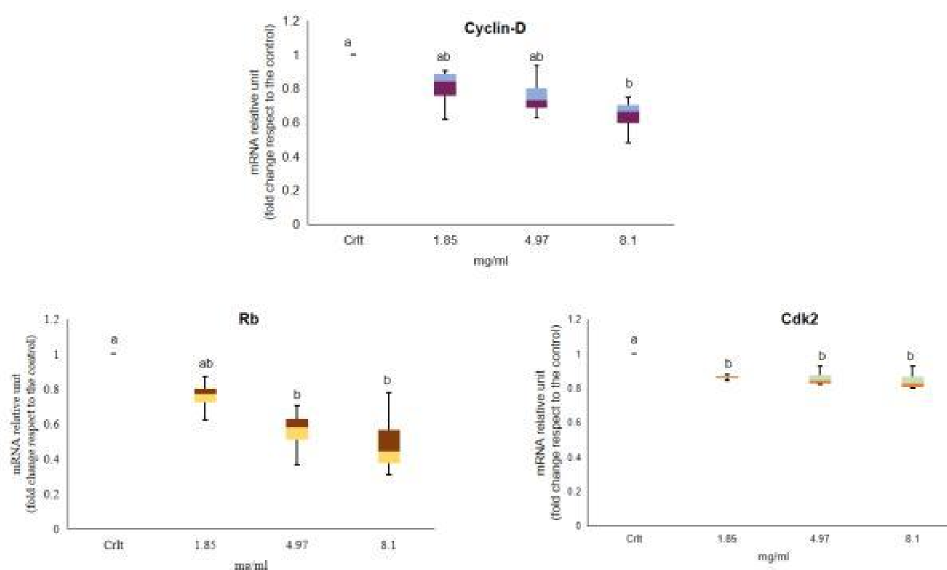


Figure 3.30: Garlic extract induces alteration of cell cycle regulatory mRNA expression in MCF_7 cells. MCF_7 cell were incubated different concentrations of garlic extract for 48 h. Cell cycle regulatory gene cyclin D, dependent kinases CDK2 and cyclin dependent kinase inhibitor Rb were analysed by real-time PCR. GADPH used a house keeping gene under the same PCR conditions for normalized quantitative data. Results are expressed as median, first quartiles, third quartiles, minimum and maximum values. All data shown were the mean ± SD (n = 3). Different superscript letters for each column indicated significant differences ($p < 0.05$).

In our study, we observed that garlic extract treatment significantly ($p < 0.05$) reduced cyclin D1, cyclin E, CDK2, CDK4 and p-Rb gene expression up to 0.76 fold, 0.57 fold, 0.85 fold, 0.50 fold and 0.49 fold respectively, compared to the untreated cells at a dose depended manner in MCF_7 cells (Figure 3.30). In contrast, the gene expression of p21waf1/Cip1 and p27kip1 significantly ($p < 0.05$) increased up to 1.76 fold and 1.72 fold (Figure 3.31).

In recent time, regulation of the G1 phase of the cell cycle has attracted attention as a target for the study and therapy of breast cancer. Two of the most essential proteins involved in the cell cycle machinery are cyclin-dependent kinases and cyclins, among all cyclins D and E mediate progression (Geng et al. 2001). However, D-type cyclins are short-lived proteins that maintain via G1 phase (Hitomi and Stacey, 1999). Cyclin D forms a complex with Cdk4 and Cdk6 (or other types of CDKs) to promote G1-phase progression towards the S phase (Takahashi-Yanaga and Sasaguri 2008). Where, pRb phosphorylation seems to be related to mitogenic signals, which accumulate on the cell cycle machinery, represented by the cyclin D1/cdk4 complex in the early and mid-G1, and composed of cyclin E/cdk2 in late G1 (Giacinti and Giordano 2006).

In contrast, the pRb protein maintaining cell cycle progression and DNA replication by repressing gene transcription that required for transition from G1 to S phase, by directly binding to the trans activation domain of E2F and by binding to the booster of these genes as a complex with E2F (Macaluso et al. 2005) and block S-phase entry and cell growth (Weinberg 1995). In addition, CDK–cyclin complexes are inversely regulated by CDK inhibitors (p21Cip and p27Kip), which are promote dyphosphorylation of Rb proteins for the transcriptional activation of cell cycle regulated genes (Vermeulen et al. 2003). Recently, researcher considered that spices could stopped cell proliferation by modulating of P53, for example, Fenugreek up regulated P53 and arrested cell cycle at G1 phase (Khoja et al.).,

2011 . Similarly, garlic induced cell cycle arrested at G0/G1 phase was associated with the up regulation of p53 gene (Zang et al. 2014). Also, a polyphenol-enriched extract from selenium-enriched Ziyang green tea and herb Radix Sanguisorbae induced cell-cycle arrest at G0/G1 phase by upregulation of p53 and reduced the expression of CDK2 in MCF_7 and MDA-MB-231 cells (Li et al. 2016; Zhu et al. 2016)Consistent with those elaborations, in our study demonstrated that garlic extract arrested the cycle of MCF_7 cells at the G1/G0 phase, along with the reduced gene expression of pRb, cyclin D1, cyclin E, cdk2 and cdk4 and increased gene expression of p21Cip, p27Kip and P53 respect with untreated cells, suggesting that garlic extract suppressed proliferation of breast cancer cells dramatically.

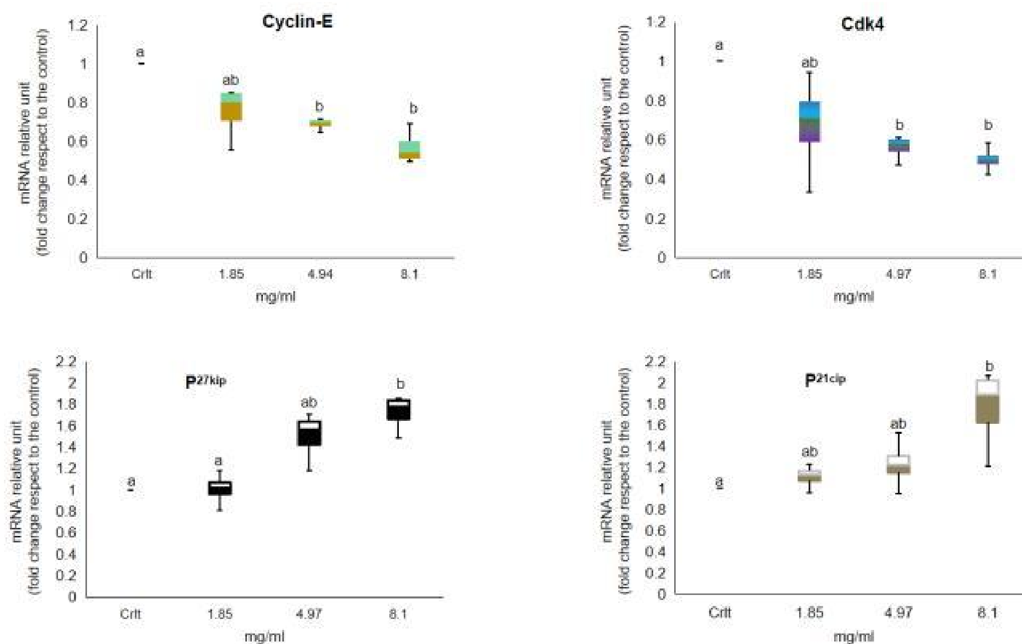


Figure 3.31: Garlic extract induces alteration of cell cycle regulatory mRNA expression in MCF_7 cells. MCF_7 cell were incubated different concentrations of garlic extract for 48 h. Cell cycle regulatory gene cyclin E, dependent kinases Cdk4, cyclin dependent kinase inhibitor p21waf1/Cip1 and p27kip1 awere analyzed by real-time PCR. GADPH used a a house keeping gene under the same PCR conditions for normalized quantitative data. Results are expressed as median, first quartiles, third quartiles, minimum and maximum values. All data shown were the mean ± SD (n = 3). Different superscript letters for each column indicated significant differences ($p < 0.05$).

4.5. Garlic extracts stimulated Autophagy induction in MCF_7 cells

In previous section, we found garlic extract significantly ($p<0.05$) increased dead cells by evaluating after using Annexin V Alexa Fluor® 488 and PI staining. The percentage of dead cells is not only due to apoptosis induction, but it could also be possibilities of autophagy induction. Based on this, in parallel, we evaluated the garlic extract autophagy-inducing capacities via screened 20 autophagy-related proteins in MCF_7 cell lystate by a commercially available human autophagy array kit and found that several players in the autophagy process (Figure 3.32 and 3.33).

Autophagy is an evolutionarily conserved and multi-step lysosomal degradation process, is mainly activated during pathogen infection, nutrient deprivation, accumulation of damaged proteins or organelles. It's play an important role in threatening cancer by regulating a limited number of autophagy-related gene. The process of autophagy 5 steps such as initiation, elongation and autophagosome formation, fusion, and autolysosome formation (Yang et al. 2015).

Last decade, phytochemicals showed a good potential of cancer chemo prevention and garlic is one such potential phytochemical candidate that has been thoroughly studied against various cancer cells (Kim et al. 2012; Modem et al. 2012; Giampieri et al. 2019). However, natural phenolic compounds enabled to trigger autophagy as cell death or survival mechanism in various cancer cells (Chatterjee and Pandey 2011; Li et al. 2011; Park et al. 2016a; Sharma et al. 2020). Above mentioned, identified different type of polyphenol in present in garlic extract that could be possible to induced autophagy in breast cancer MCF_7 cell line (Martino et al. 2019). However, autophagy may be regulated by various canonical and non-canonical pathways.

4.5.1. Garlic extracts induced autophagy in non-canonical pathway

Non-canonical autophagy is a process that is independent from Beclin-1 pathway (Ozpolat et al. 2007; Scarlatti et al. 2008)(.

4.5.1.1. Effect of garlic extract on mTOR signalling

Autophagosome formation is a key initial step for autophagy process; this step is mediated by the serine/threonine protein kinase unc-51-like kinase 1 (ULK1). The activation of ULK1, Atg13, FIP200 and Atg101 to nucleate formation of phagophore (nucleation) (Chen et al. 2014; Zachari and Ganley 2017). While mTOR act as a autophagy inhibitor that suppressed the initiation stage of complex formation in several pathway and blockaged the phosphorylation of ULK1 and ATG13 (Jung et al. 2010; Kim et al. 2011). However, we observed, garlic extract, significantly ($p<0.05$) up regulated ATG13 protein expression up to 2.88-fold compared to the untreated cells (Figure 3.32). Whereas ATG13 protein may help to induction autophagy by activated ULK1 complex and inhibited mTORC1 on the lysosomal surface of breast cancer MCF_7 cells. Moreover, the effect of garlic extracts on the tumour microenvironment that could be a reason for autophagy induction with response to nutrient deficiency such as glucose. In our study, garlic extract decreased glucose/ oxygen uptake via modulation AMPK pathway and may be repressed mTOR in breast cancer MCF_7 cells. Recent studies demonstrate that spices polyphenols activate autophagy via suppression the mTORC1 (Kaur and Moreau 2021).

In addition, Rheb is a potent mTORC1 activator and Rheb-mTORC1 pathway repressed autophagy (Inoki et al. 2003). Interestingly, garlic extract showed significantly ($p<0.05$) down regulation of Rheb protien expression up to 0.66 fold compare to the untreated sample after 48h treatment in MCF_7 cells (Figure 3.32). Subsequently, garlic extract also significantly ($p<0.05$) reduced Mitogen and stress-activated protein kinase (MSK1) protein expression up to 0.74 fold respect to the untreated cells (Figure 3.32). Indeed, inhibition of MSK1 protein promotes autophagy induction (Peng et al. 2021).

4.5.1.2. Effect garlic extract on ATG family protein

ATG family protein are responsible for autophagy regulation, mainly in autophagy initiation and the formation of autolysosome (Xiang et al. 2020). For example, plant lectins induce autophagy by upregulating ATG families (Jiang et al., 2015). Similarly, Canavalia ensiformis (Concanavalin-A, ConA) induced autophagy in non-canonical pathway via up-regulated ATG3 and ATG12 in glioblastoma U87 cells (Pratt et al. 2012).

In our experiment, we found ATG3, ATG4A, ATG34B, ATG5, ATG7, ATG10, ATG12 and ATG13 proteins were significantly ($p<0.05$) up regulated up to 3.51 fold, 3.65 fold, 1.57 fold, 1.70 fold, 1.50 fold, 1.53 fold, 2.66 fold and 2.88 fold respectively, compare to the untreated cells (Figure 3.32).

After initiation, two unique ubiquitin-like conjugation may be occurred by involvement of the covalent conjugation of ATG12 to ATG5 in presence of ATG7 and ATG10 that catalysed the the formation of conjugation and activates LC3/ATG4 protein. Interestingly, activated ULK1 phosphorylated ATG4B that responsible for the conversion of pro-LC3 to LC3-I and LC3-II (Pengo et al. 2017) that is stably inserted into the autophagosomal membrane (Kabeya et al. 2000). Then, LC3 and GABARAPs mediate the phagophore membrane closure (Fusion). Then, autophagosomes fuse with lysosomes under the regulation of cytoskeleton elements of different proteins including lysosomal-associated membrane protein (LAMP)-1 to form the autolysosome, whereas the degradation of macromolecule occurs by the action of lysosomal enzymes (Degradation) (Benvenuto et al. 2020). Recent studies explain that a relation between autophagy and apoptotic signalling via suppression of the binding of lysosomes and autophagy vacuoles (Salminen et al. 2013). Although, induction of autophagy was accompanied with arrest of LAMP-1 expression, that correlates with lysosomal activity.

In present experiment, our result showed that, garlic extract significantly ($p<0.05$) increased LC3A, LC3B and GABARAPs protein expression up to 1.90 fold, 2.42 fold and 2.65 fold respectively and significantly ($p<0.05$) decreased LAMP1 protein up to 0.59 fold, compare to the untreated cells (Figure 3.33).

On the basis of present result, it may be stipulated, garlic extract activated ULK1 by up regulating ATG13 protein that could be phosphorylated ATG4B and ATG4B associated with the vesicle elongation and formation of auto phagolysosome via upregulated ATG7 and ATG3 protein expression. In consistent with this action, our results also showed increased of basal autophagy induction in MCF_7 cells, ones as manifested by up regulated levels in LC3A and LC3B protein. Oppositely, by decreasing LAMP-1protein could be depleted lysosomal activity which involved programmed cell death that agreed with (Ichimura et al. 2000; Chu et al. 2013; Elimam et al. 2020).

However, the expression of another widely used autophagosomal marker p62, a ubiquitin-binding protein that also involved in autophagy induction. In this present study, garlic extract significantly ($p<0.05$) decreased p62 protein expression up to 0.38 fold with respect to the untreated cells (3.32). Our results are comply with previous studies where bioactive compound that shows autophagy induction in different cancer cells (Dhaheri et al. 2014; Park et al. 2016b). Moreover, p62 has been played a negative-regulatory role between Nrf2 and autophagy and promoting oxidative stress status, after garlic extract treatment in MCF_7 breast cancer cells (Zhang et al. 2015; Park et al. 2016b; Li et al. 2017).

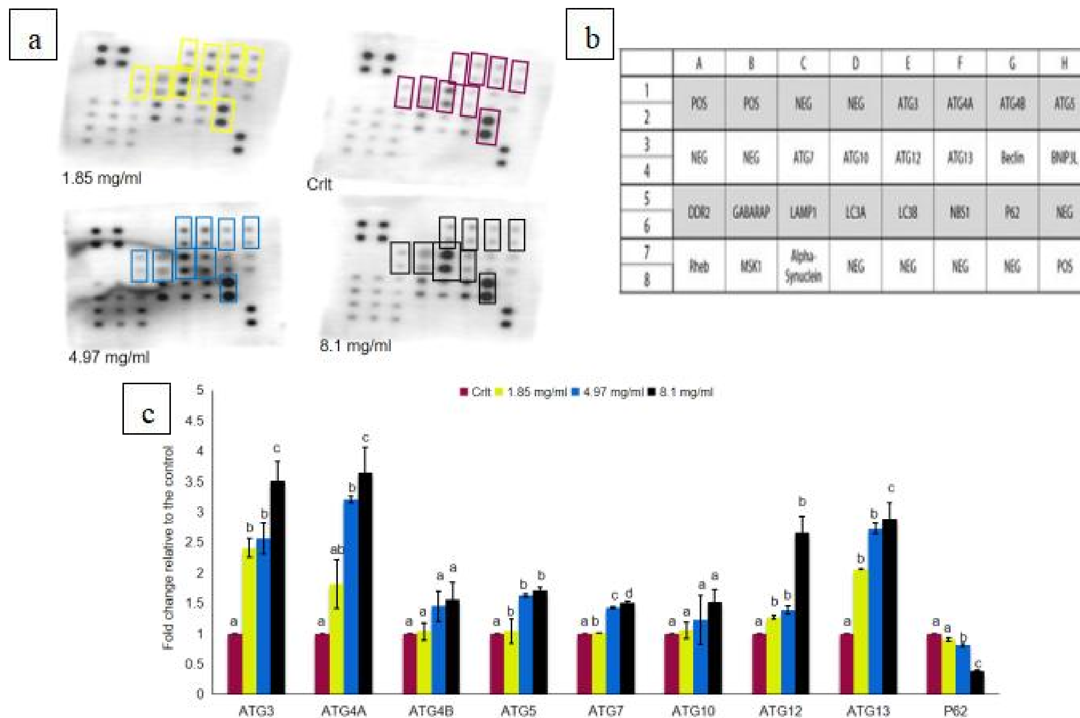


Figure 3.32: Garlic extract induces Autophagy on breast cancer MCF_7 cells a) Human Autophagy Array expression. After 24 h incubation, MCF_7 cells was treated with different concentrations of garlic extract 1.87 mg/ml, 4.97 mg/ml and 8.1 mg/ml for 48 h. Positive spots were used as a loading control and for normalization. b) Template showing the location of autophagy-related proteins spotted onto the Ray Biotech Human Autophagy array kit. c) Columns graph belongs to the same set of data compare to untreated cells with different superscript letters are significantly different ($p < 0.05$). All data shown are the mean ± SD of two different spots.

ATG3: Autophagy related gene 3; ATG4A: Autophagy related gene 4A; ATG4B: Autophagy related gene 4B; ATG5: Autophagy related gene 5; ATG7: Autophagy related gene 7; ATG10: Autophagy related gene 10; ATG 12: Autophagy related gene 12; ATG13: Autophagy related gene13.

There is a crosstalk about P62 dependent autophagy induction. Several literatures demonstrated that increased p62 expression is required for autophagy induction (Robert et al. 2009; Puissant et al. 2012). Though, we found down regulated p62 expression in MCF_7 cell line after garlic treatment that similar with (Li et al. 2013)

4.5.1.3. Effect of BNIP3-mediated mitochondrial autophagy

Autophagy plays a vital role in cellular homo stasis by damaging organelles such as broken mitochondria, to remove misfolded proteins and to eliminate intracellular pathogens (Glick et al. 2010). In recent time, bcl-2 nineteen kilodalton interacting protein (BNIP3), a member of the 'BH3-only' subfamily of pro-apoptotic Bcl-2 family proteins, known as a potent autophagy inducer. In response with ROS generation, BNIP3 induced cell death by modulated mitochondrial membrane potential (Kubli et al. 2007; Burton and Gibson 2009). Where, increased GABARAPL1 protein damaged mitochondria in a BNIP3L-dependent manner (Boyer-Guittaut et al. 2014) by modulating cellular bioenergetic level such as basal OCAR rate. While intracellular ATP that help to inhibit cell proliferation and suppressed breast cancer MDA-MB-436 cell(Boyer-Guittaut et al. 2014). For illustration, silibinin, a flavonolignan induced autophagic cell death via ROS-dependent mitochondrial dysfunction and ATP depletion involving BNIP3 in MCF_7 cells (Jiang et al. 2015). Likewise, ConA induced autophagy via up-regulated BNIP3 in glioblastoma U87 cells (Pratt et al. 2012)(Pratt et al., 2012). We observe garlic extract increased BNIP3 protein significantly ($p<0.05$) up to 3.90 fold compare to untreated cell (Figure 3.33). We hypothesized garlic extract up regulated GABARAPL1 expression that interacted with GABAA or kappa opioid receptors, associated with autophagic vesicles, and suppressed breast cancer cell proliferation by BNIP3 pathway.

4.5.2 Garlic extract induced autophagy in canonical pathway

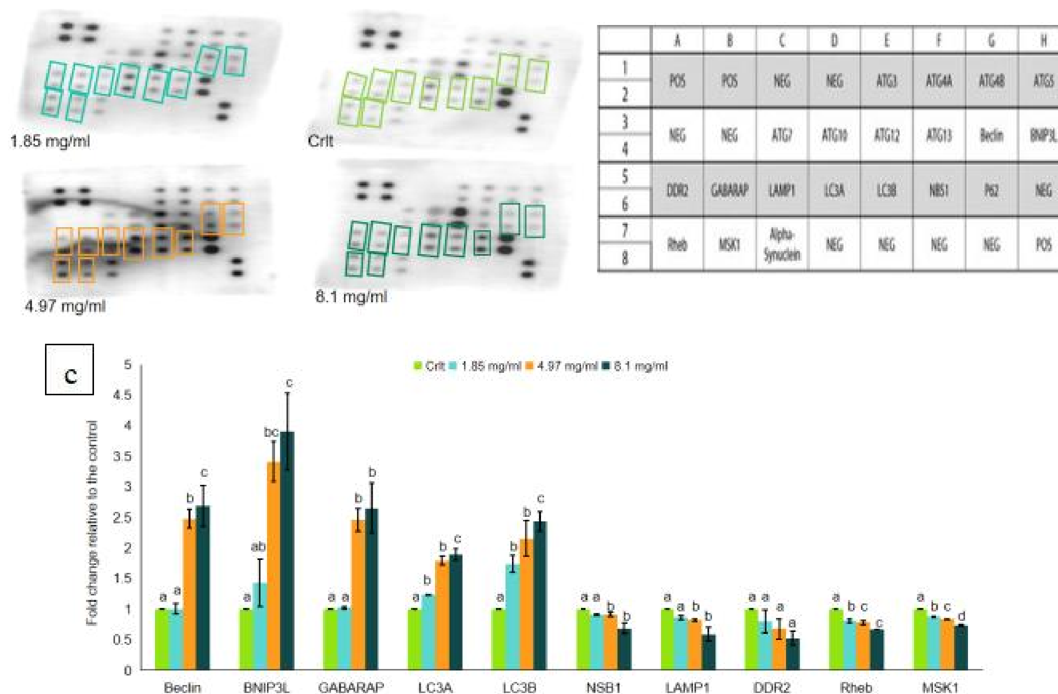
Canonical pathway is Beclin-1 dependent whereas, Beclin-1 acts as a tumour suppressor gene (Aita et al. 1999; Klionsky et al. 2008) that act as an indicator of autophagy induction in ROS dependent pathway in various breast cancer cell lines (Dhaheri et al. 2014; Pandey et al. 2020). Most compelling evidence that, phenolic compound carnosol induces ROS-mediated Beclin1 independent autophagy in breast cancer MDA-MB-231 cells (Dhaheri et al. 2014). Surprisingly, in our Array profile showed significantly ($p<0.05$) up regulation of Beclin-1 protein expression up to 2.68 fold after 48h garlic extract treatment in a dose dependent

manner (3.33). Earlier, we found poly phenol enriched garlic extract has been enhanced intracellular ROS levels and down regulated antioxidant enzyme SOD expression. While inhibition of SOD expression induced autophagy in MCF_7 cells (Sharma et al. 2020). As a consequence, Beclin1 also responsible for the cleavage of LC3 I to LC3 II in autophagic process (Kaowinn et al. 2018). Whereas phenolic compound ellagitannins, urolithin A induces autophagy by increased LC3 expression and decreased MMP-9 in human sw620 colorectal cancer cells (Zhao et al. 2018). Though this induction can be reversed when membrane MT1-MMP gene is silenced (Pratt et al. 2012). In our study we observed, negative correlation between autophagy and MMPs that reflects (Zhao et al. 2018). Even if, the specific roles of MMPs in autophagy after treatment of garlic in breast cancer still remain to be unravelled, here, we first time reported the modulation of MMPs, another well-known autophagy regulator in breast cancer MFC7 cells, after treatment of poly phenolic enrich garlic extract.

However, preclinical and clinical studies demonstrated, discoidin domain receptor 2 (DDR2) has a vital role in breast cancer metastasis (Corsa et al. 2016) by regulating essential cellular processes such as morphogenesis, differentiation, proliferation, adhesion, migration, invasion, and matrix remodeling. Recently, siRNA-mediated downregulation of DDR1 inhibited melanoma cell malignancy, migration, invasion, and survival (Reger de Moura et al. 2019). Thus, down regulated DDR2 inhibited breast cancer metastasis via stabilizing the SNAIL1 protein (Zhang et al. 2013). In our results, garlic extract significantly ($p<0.05$) reduced DDR2 protein expression up to 0.53 fold with respect to the untreated MCF_7 cells and suppressed EMT and metastasis (3.33 and 3.24). Additionally, autophagy has a role in DNA damage response (DDR). At the time of DDR, autophagy plays a role of source of energy that control cell cycle arrest and to defend DNA repair activities (Galati et al. 2019).

a

b



	A	B	C	D	E	F	G	H
1 2	POS	POS	NEG	NEG	ATG3	ATG4A	ATG4B	ATG5
3 4	NEG	NEG	ATG7	ATG10	ATG12	ATG13	Beclin	BNIP3L
5 6	DDR2	GABARAP	LAMP1	LC3A	LC3B	NBS1	P62	NEG
7 8	Rheb	MSK1	Alpha-Synuclein	NEG	NEG	NEG	NEG	POS

Figure 3.33: Garlic extract induces Autophagy on breast cancer MCF_7 cells a) Human Autophagy Array expression. After 24 h incubation, MCF_7 cells was treated with different concentrations of garlic extract 1.87 mg/ml, 4.97 mg/ml and 8.1 mg/ml for 48 h. Positive spots were used as a loading control and for normalization. b) Template showing the location of autophagy-related proteins spotted onto the Ray Biotech Human Autophagy array kit. c) Columns graph belongs to the same set of data compare to untreated cells with different superscript letters are significantly different ($p < 0.05$). All data shown are the mean ± SD of two different spots.

BNIP3: bcl-2 nineteen kilodalton interacting protein; GABARAP: Gamma-aminobutyric acid receptor-associated protein; LC3A: Light chain 3 1A; LC3B: Light chain 3 1B; Lysosomal-associated membrane protein 1; DDR2: Discoidin domain receptor 2; MSK1: Mitogen- and stress-activated protein kinase.

Recent studies showed there is interlink between cell-cycle arrest and autophagy induction. It has been reported that autophagy may be promoted by blocking CDK4/6 in multiple cancer cells (Ezhevsky et al. 1997; Sumi et al. 2015). For example, Apple extract explored autophagy via cell cycle arrest in MCF_7 cell by up regulation of P21 gene and down regulated cyclin dependent kinase (Schiavano et al. 2015).

In DNA repair activities, autophagy inhibits DNA repair proteins. Whereas NBS1 is functioning as a modulator of repairing the DNA damage by promoting homologous

recombination (HR) and activating ATM which essential for cell viability (D'Amours and Jackson 2002; Kobayashi et al. 2004; Chen et al. 2008). Furthermore, NBS1 as a part of MRN complex when cells enter the S and G2 phases of the cell cycle. In addition, NBS1 deficiency abolish the telomere fusions that occur in G1-phase of cell cycle (Dimitrova and de Lange 2009).

In our work, the expression of NBS1 protein was found to reduce by up to 0.68 fold in comparison to untreated cells after 48 h (Figure 3.33). Similar result shows pomegranate extract by decreasing DNA repair NBS1 gene expression and induce apoptosis and autophagy (Shirode et al. 2014).

However, our studies highlight a critical inhibition role of garlic extract on formation of MRN complex in processing of DNA repair. Whereas, CDK activity is important for formation of this complex (Sonoda et al. 2006). Surprisingly, garlic extract modulated CDK activities and arrested cell cycle and induction apoptosis and autophagy. The mechanisms underlying this regulation and the CDK targets that are directly involved in DNA repair are not clear in this study. It needs to be more study to elucidate the exact role of garlic extract on the regulation of HR-mediated DNA repair.

Recent research illustrates that autophagy induction that could be a novel target for cancer treatment associates with anti-angiogenic effect (Kardideh et al. 2019). Our observation, we found garlic extract repressed angiogenesis and boosting autophagy in breast cancer MCF_7 cells. Unfortunately, the software didn't measure the intensity of Alpha-synuclein.

In our work, although we demonstrated garlic extract triggered autophagy in breast cancer MCF_7 cells, we couldn't visualize exact autophagy vacuole formation following garlic exposed at 48h in MCF_7 cell line. Although until now we still cannot explain the exact role of garlic-mediated autophagy in the pathogenesis of breast cancer, its occurrence is to a certain extent in accordance with garlic-mediated anti-apoptotic, anti-angiogenic efect and anti-proliferative roles which deserves further study. Here, we showed for the first time anti proliferative effect of poly phenol enrich garlic extract on breast cancer MCF_7 cells by inducing autophagy and inhibit angiogenesis and metastasis. Our *in vitro* findings provide novel insights into understanding the anti-tumour functions of spices especially garlic.

In summary, we demonstrated that may be used garlic extract-based breast cancer treatments lies in the multiple roles of garlic phenolic molecules including anti-survival, cell cycle arrest and anti-metastasis effect, anti-colonization, inhibition of tumour Angiogenesis and autophagy mediated cell death. Thus, this finding Garlic extract could be potentially tumour suppressive role in Breast cancer MCF_7 cells by blocking tumour cell growth.

CONCLUSIONS, FUTURE PERSPECTIVES AND LIMITATIONS

Based on the results gotten within the assessment of the anti-proliferative potential of garlic extract on *in vitro* breast cancer models, the taking after conclusions can be drawn:
Garlic extract, used in this work, possessed a content of bioactive compounds such as phenolic compounds (caffeic acid, p-coumaric acid, dihydroxybenzoic acid, gallic acid, salicylic acid, sinapic acid, and ferulic acid), high content of total polyphenol, flavonoids, organosulfur compounds and mineral.

The antioxidant activity of this garlic extract was also evaluated through three different methods: FRAP, TEAC and DPPH, it could be a significant impact on the anti-proliferative potential.

In the present study, the effect of garlic on *in vitro* digestion on the phenolic compounds, flavonoids and antioxidant activity was investigated. During the digestion process, it was seen how the content of phenolic compounds and flavonoids changed, a drastic reduction of phenolic compounds and flavonoids was observed in the bioaccessible fractions and higher in the waste fractions. The antioxidant capacity diminished after the simulated gastrointestinal digestion, it could be present of less number of phenolic compounds and flavonoids. Further, bioaccessible fraction of garlic also induced cytotoxic effects in MCF_7 cells that allow an approach to a physiological condition closer to the real one.

Garlic extract can induce anti proliferation by accumulating intracellular ROS in breast cancer MCF_7 cells, associated with an induction of oxidative stress, apoptosis through cell surface death receptors and mitochondria-dependent pathways and reducing antioxidant enzyme activity, inducing DNA, protein and lipid damage. Moreover, garlic extract can be increased ER stress in breast cancer MCF_7 cell line death by increasing XBP1, ATF6α and EIF2α expression. Similarly, garlic extricates can be repressed of mitochondrial respiration by controlling of energy metabolism of MCF_7 cells both in anaerobic and aerobic pathways and show an alternative methodology for the treatment of breast cancer. Moreover, garlic extract can be suppressed the AMPK pathway which plays a vital part in mitochondrial biogenesis in cancer cells to advance metabolism beneath glucose limiting condition. These findings give principal insights into the molecular composition of garlic extract initiated cell death.

Evidence indicated that garlic extract induced cytotoxic effects in MCF_7 cells by suppressing inflammation through decreasing the expression of inflammatory cytokines and pathway signalling NFκB, IL-6, IL-1β, p-IκBα, EGFR

Garlic extract can be inhibited breast cancer cell growth by arresting cells at the G1/G0 phase, along with the reduced gene expression of pRb, cyclin D1, cyclin E, cdk2 and cdk4 and increased gene expression of p21Cip, p27Kip and P53.

Garlic extract can be indicated anti-metastatic effect of against adenocarcinoma MCF_7 cells through inhibition the migration and colony formation ability, as well as suppressed invasion abilities, observed by reducing MMP-2 and MMP-9 expression and regulating the expression of EMT-related genes, including E-cadherin, N-cadherin, and β-catenin. Based on these results, we can be concluded that garlic extract may serve as an effective therapeutic agent for breast cancer anti proliferate effects.

Garlic extract can be repressed angiogenesis by modulation pro-angiogenic factors such as increasing FN-γ, TIMP-1 and TIMP-2 expression and decreasing VEGF, VEGF-D, b-FGF, ENA-78, EGF, PlGF, TGFβ1, MCP-1, IL-8.

This works can be provided new evidence to garlic extract may induce autophagy in breast cancer MCF_7 cells through modulation of autophagy related protein expression by increasing ATG3, ATG4A, ATG5, ATG7, ATG 12, ATG13, Becline, BNIP3, GABARAP, LC3A, LC3B, and decreasing P62, MSK1, NSB1, LMP1, Rheb.

The present work has some limitations, Firstly, in our work, we identified phenolic compound in garlic extract but not able to isolate and purified the main one that is related to the anticancer activity, by the fact that activity may involve multiple phenolic compounds in garlic extract where, Isolation of phenolic compounds could be helpful to formulate modified release dosage and may be improve bioavaiability and increased therapeutic effect of garlic. Next, it should be considered that this study represents a starting point for obtaining preliminary data, for subsequent studies involving *in vitro* simulated digestion processes, where we are failure to evaluate the different composition of phenolic acid and flavonoid after *in vitro* digestion. Among the other limitations there is discuss about growth inhibitory

effect of garlic by modulating autophagy related protein but not evaluate autophagy vacuole formation in breast cancer MCF_7 cell line.

However, the action of bioactive compounds in garlic extract can be attributed not only to their ability to act as antioxidants but also to their ability to interact with different cellular mechanisms. These curiously and promising results encourage our knowledge around and might be valuable for further considers highlighting the phenolic compounds of garlic and the possible molecular components as well as *in vivo* considers against breast cancer to provide a deeper understanding of garlic therapeutic potential.

REFERENCES

Abdul Qadir M, Shahzadi SK, Bashir A, et al (2017) Evaluation of Phenolic Compounds and Antioxidant and Antimicrobial Activities of Some Common Herbs. International Journal of Analytical Chemistry 2017:e3475738. https://doi.org/10.1155/2017/3475738

Abramovič H, Grobin B, Poklar Ulrih N, Cigić B (2018) Relevance and Standardization of In Vitro Antioxidant Assays: ABTS, DPPH, and Folin–Ciocalteu. Journal of Chemistry 2018:e4608405. https://doi.org/10.1155/2018/4608405

Aebi H (1984) [13] Catalase in vitro. In: Methods in Enzymology. Academic Press, pp 121–126

Afrin S, Giampieri F, Gasparrini M, et al (2018) The inhibitory effect of Manuka honey on human colon cancer HCT-116 and LoVo cell growth. Part 1: the suppression of cell proliferation, promotion of apoptosis and arrest of the cell cycle. Food Funct 9:2145–2157. https://doi.org/10.1039/C8FO00164B

Agarwal KC (1996) Therapeutic actions of garlic constituents. Med Res Rev 16:111–124. https://doi.org/10.1002/(SICI)1098-1128(199601)16:1<111::AID-MED4>3.0.CO;2-5

Ahn J, Kim H, Yang KM (2020) ω-hydroxyundec-9-enoic acid induction of breast cancer cells apoptosis through generation of mitochondrial ROS and phosphorylation of AMPK. Arch Pharm Res 43:735–743. https://doi.org/10.1007/s12272-020-01254-x

Aita VM, Liang XH, Murty VVVS, et al (1999) Cloning and Genomic Organization of Beclin 1, a Candidate Tumor Suppressor Gene on Chromosome 17q21. Genomics 59:59–65. https://doi.org/10.1006/geno.1999.5851

Akbaribazm M, Khazaei MR, Khazaei M (2020) Trifolium pratense L. (red clover) extract and doxorubicin synergistically inhibits proliferation of 4T1 breast cancer in tumor-bearing BALB/c mice through modulation of apoptosis and increase antioxidant and anti-inflammatory related pathways. Food Science & Nutrition 8:4276–4290. https://doi.org/10.1002/fsn3.1724

Alayev A, Sun Y, Snyder R, et al (2014) Resveratrol prevents rapamycin-induced upregulation of autophagy and selectively induces apoptosis in TSC2-deficient cells. Cell Cycle 13:371–382. https://doi.org/10.4161/cc.27355

Albeck JG, Burke JM, Spencer SL, et al (2008) Modeling a Snap-Action, Variable-Delay Switch Controlling Extrinsic Cell Death. PLOS Biology 6:e299. https://doi.org/10.1371/journal.pbio.0060299

Algeciras-Schimnich A, Shen L, Barnhart BC, et al (2002) Molecular Ordering of the Initial Signaling Events of CD95. Molecular and Cellular Biology 22:207–220. https://doi.org/10.1128/MCB.22.1.207-220.2002

Al-Khalaf HH, Al-Harbi B, Al-Sayed A, et al (2019) Interleukin-8 Activates Breast Cancer-Associated Adipocytes and Promotes Their Angiogenesis- and Tumorigenesis-Promoting Effects. Molecular and Cellular Biology 39:e00332-18. https://doi.org/10.1128/MCB.00332-18

Alsaraf KM, Mohammad MH, Al-Shammari AM, Abbas IS (2019) Selective cytotoxic effect of Plantago lanceolata L. against breast cancer cells. Journal of the Egyptian National Cancer Institute 31:10. https://doi.org/10.1186/s43046-019-0010-3

Alshammari GM, Balakrishnan A, Alshatwi AA, Al-Khalifa A (2020) Cucurbita ficifolia Fruit Extract Induces Tp53/Caspase-Mediated Apoptosis in MCF-7 Breast Cancer Cells. BioMed Research International 2020:e3712536. https://doi.org/10.1155/2020/3712536

Altonsy MO, Habib TN, Andrews SC (2012) Diallyl Disulfide-Induced Apoptosis in a Breast-Cancer Cell Line (MCF-7) May Be Caused by Inhibition of Histone Deacetylation. Nutrition and Cancer 64:1251–1260. https://doi.org/10.1080/01635581.2012.721156

Altura BM, Altura BT (1991) Cardiovascular risk factors and magnesium: relationships to atherosclerosis, ischemic heart disease and hypertension. Magnes Trace Elem 10:182–192

Amagase H (2006) Clarifying the Real Bioactive Constituents of Garlic. The Journal of Nutrition 136:716S-725S. https://doi.org/10.1093/jn/136.3.716S

Amatori S, Mazzoni L, Alvarez-Suarez JM, et al (2016) Polyphenol-rich strawberry extract (PRSE) shows in vitro and in vivo biological activity against invasive breast cancer cells. Sci Rep 6:30917. https://doi.org/10.1038/srep30917

Ariza MT, Forbes-Hernández TY, Reboredo-Rodríguez P, et al (2018) Strawberry and Achenes Hydroalcoholic Extracts and Their Digested Fractions Efficiently Counteract the AAPH-Induced Oxidative Damage in HepG2 Cells. International Journal of Molecular Sciences 19:2180. https://doi.org/10.3390/ijms19082180

Ariza MT, Reboredo-Rodríguez P, Mazzoni L, et al (2016) Strawberry Achenes Are an Important Source of Bioactive Compounds for Human Health. International Journal of Molecular Sciences 17:1103. https://doi.org/10.3390/ijms17071103

Atsumi T, Tonosaki K, Fujisawa S (2007) Comparative Cytotoxicity and ROS Generation by Curcumin and Tetrahydrocurcumin Following Visible-light Irradiation or Treatment with Horseradish Peroxidase. Anticancer Research 27:363–371

Azios NG, Dharmawardhane SF (2005) Resveratrol and Estradiol Exert Disparate Effects on Cell Migration, Cell Surface Actin Structures, and Focal Adhesion Assembly in MDA-MB-231 Human Breast Cancer Cells. Neoplasia 7:128–140. https://doi.org/10.1593/neo.04346

Bagci EZ, Vodovotz Y, Billiar TR, et al (2006) Bistability in Apoptosis: Roles of Bax, Bcl-2, and Mitochondrial Permeability Transition Pores. Biophysical Journal 90:1546–1559. https://doi.org/10.1529/biophysj.105.068122

Bagul M, Kakumanu S, Wilson TA (2015) Crude Garlic Extract Inhibits Cell Proliferation and Induces Cell Cycle Arrest and Apoptosis of Cancer Cells In Vitro. Journal of Medicinal Food 18:731–737. https://doi.org/10.1089/jmf.2014.0064

Baharetha HM, Nassar ZD, Aisha AF, et al (2013) Proapoptotic and Antimetastatic Properties of Supercritical CO2 Extract of Nigella sativa Linn. Against Breast Cancer Cells. Journal of Medicinal Food 16:1121–1130. https://doi.org/10.1089/jmf.2012.2624

Bahiraee A, Ebrahimi R, Halabian R, et al (2019) The role of inflammation and its related microRNAs in breast cancer: A narrative review. Journal of Cellular Physiology 234:19480–19493. https://doi.org/10.1002/jcp.28742

Ballweg R, Paek AL, Zhang T (2017) A dynamical framework for complex fractional killing. Sci Rep 7:8002. https://doi.org/10.1038/s41598-017-07422-2

Ban JO, Yuk DY, Woo KS, et al (2007) Inhibition of Cell Growth and Induction of Apoptosis via Inactivation of NF-κB by a Sulfurcompound Isolated From Garlic in Human Colon Cancer Cells. Journal of Pharmacological Sciences 104:374–383. https://doi.org/10.1254/jphs.FP0070789

Bar-Eli M (1999) Role of Interleukin-8 in Tumor Growth and Metastasis of Human Melanoma. PAT 67:12–18. https://doi.org/10.1159/000028045

Baroni S, Romero-Cordoba S, Plantamura I, et al (2016) Exosome-mediated delivery of miR-9 induces cancer-associated fibroblast-like properties in human breast fibroblasts. Cell Death Dis 7:e2312–e2312. https://doi.org/10.1038/cddis.2016.224

Basaiyye SS, Naoghare PK, Kanojiya S, et al (2018) Molecular mechanism of apoptosis induction in Jurkat E6-1 cells by Tribulus terrestris alkaloids extract. Journal of Traditional and Complementary Medicine 8:410–419. https://doi.org/10.1016/j.jtcme.2017.08.014

Basile A, Rigano D, Loppi S, et al (2015) Antiproliferative, Antibacterial and Antifungal Activity of the Lichen Xanthoria parietina and Its Secondary Metabolite Parietin. International Journal of Molecular Sciences 16:7861–7875. https://doi.org/10.3390/ijms16047861

Batlle E, Sancho E, Francí C, et al (2000) The transcription factor Snail is a repressor of E-cadherin gene expression in epithelial tumour cells. Nat Cell Biol 2:84–89. https://doi.org/10.1038/35000034

Baublis A, Decker EA, Clydesdale FM (2000) Antioxidant effect of aqueous extracts from wheat based ready-to-eat breakfast cereals. Food Chemistry 68:1–6. https://doi.org/10.1016/S0308-8146(99)00142-9

Bayir H, Kagan VE (2008) Bench-to-bedside review: Mitochondrial injury, oxidative stress and apoptosis – there is nothing more practical than a good theory. Critical Care 12:206. https://doi.org/10.1186/cc6779

Beato VM, Orgaz F, Mansilla F, Montaño A (2011) Changes in Phenolic Compounds in Garlic (Allium sativum L.) Owing to the Cultivar and Location of Growth. Plant Foods Hum Nutr 66:218–223. https://doi.org/10.1007/s11130-011-0236-2

Belguise K, Guo S, Sonenshein GE (2007) Activation of FOXO3a by the Green Tea Polyphenol Epigallocatechin-3-Gallate Induces Estrogen Receptor α Expression

Reversing Invasive Phenotype of Breast Cancer Cells. Cancer Research 67:5763–5770. https://doi.org/10.1158/0008-5472.CAN-06-4327

Benvenuto M, Albonici L, Focaccetti C, et al (2020) Polyphenol-Mediated Autophagy in Cancer: Evidence of In Vitro and In Vivo Studies. International Journal of Molecular Sciences 21:6635. https://doi.org/10.3390/ijms21186635

Benzie IFF, Strain JJ (1996) The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. Analytical Biochemistry 239:70–76. https://doi.org/10.1006/abio.1996.0292

Beral, V., Bull, D., Doll, R., et al (2004) Collaborative Group on Hormonal Factors in Breast cancer: Breast cancer and abortion: collaborative reanalysis of data from 53 epidemiological studies, including 83000 women with breast cancer from 16 countries. Lancet 363:1007–1016. https://doi.org/10.1016/s0140-6736(04)15835-2

Berger RG (2007) Flavours and Fragrances: Chemistry, Bioprocessing and Sustainability. Springer Science & Business Media

Berginc K, Milisav I, Kristl A (2010) Garlic Flavonoids and Organosulfur Compounds: Impact on the Hepatic Pharmacokinetics of Saquinavir and Darunavir. Drug Metabolism and Pharmacokinetics 25:521–530. https://doi.org/10.2133/dmpk.DMPK-10-RG-053

Berlett BS, Stadtman ER (1997) Protein oxidation in aging, disease, and oxidative stress. J Biol Chem 272:20313–20316. https://doi.org/10.1074/jbc.272.33.20313

Bermúdez-Soto M-J, Tomás-Barberán F-A, García-Conesa M-T (2007) Stability of polyphenols in chokeberry (Aronia melanocarpa) subjected to in vitro gastric and pancreatic digestion. Food Chemistry 102:865–874. https://doi.org/10.1016/j.foodchem.2006.06.025

Berse B, Brown LF, Van de Water L, et al (1992) Vascular permeability factor (vascular endothelial growth factor) gene is expressed differentially in normal tissues, macrophages, and tumors. MBoC 3:211–220. https://doi.org/10.1091/mbc.3.2.211

Bertolini F, Petit J-Y, Kolonin MG (2015) Stem cells from adipose tissue and breast cancer: hype, risks and hope. Br J Cancer 112:419–423. https://doi.org/10.1038/bjc.2014.657

Bhanja T, Kumari A, Banerjee R (2009) Enrichment of phenolics and free radical scavenging property of wheat koji prepared with two filamentous fungi. Bioresource Technology 100:2861–2866. https://doi.org/10.1016/j.biortech.2008.12.055

Bhardwaj M, Kim N-H, Paul S, et al (2016) 5-Hydroxy-7-Methoxyflavone Triggers Mitochondrial-Associated Cell Death via Reactive Oxygen Species Signaling in Human Colon Carcinoma Cells. PLOS ONE 11:e0154525. https://doi.org/10.1371/journal.pone.0154525

Bhatt A, Patel V (2013) Antioxidant activity of garlic using conventional extraction and in vitro gastrointestinal digestion. Free Radicals and Antioxidants 3:30–34. https://doi.org/10.1016/j.fra.2013.03.003

Bhowmik S, Chowdhury SD, Kabir MH, Ali MA (2008) Chemical composition of some medicinal plant products of indigenous origin. Bangladesh Veterinarian 25:32–39. https://doi.org/10.3329/bvet.v25i1.4616

Bhuiyan AI, Papajani VT, Paci M, Melino S (2015) Glutathione-Garlic Sulfur Conjugates: Slow Hydrogen Sulfide Releasing Agents for Therapeutic Applications. Molecules 20:1731–1750. https://doi.org/10.3390/molecules20011731

Bhuvaneswari V, Velmurugan B, Abraham SK, Nagini S (2004) Tomato and garlic by gavage modulate 7,12-dimethylbenz[a]anthracene-induced genotoxicity and oxidative stress in mice. Braz J Med Biol Res 37:1029–1034. https://doi.org/10.1590/s0100-879x2004000700012

Biswas S, Chida AS, Rahman I (2006) Redox modifications of protein–thiols: Emerging roles in cell signaling. Biochemical Pharmacology 71:551–564. https://doi.org/10.1016/j.bcp.2005.10.044

Block E (1992) The Organosulfur Chemistry of the Genus Allium – Implications for the Organic Chemistry of Sulfur. Angewandte Chemie International Edition in English 31:1135–1178. https://doi.org/10.1002/anie.199211351

Bonnomet A, Brysse A, Tachsidis A, et al (2010) Epithelial-to-Mesenchymal Transitions and Circulating Tumor Cells. J Mammary Gland Biol Neoplasia 15:261–273. https://doi.org/10.1007/s10911-010-9174-0

Borek C (2001) Antioxidant Health Effects of Aged Garlic Extract. The Journal of Nutrition 131:1010S-1015S. https://doi.org/10.1093/jn/131.3.1010S

Bouayed J, Hoffmann L, Bohn T (2011) Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: Bioaccessibility and potential uptake. Food Chemistry 128:14–21. https://doi.org/10.1016/j.foodchem.2011.02.052

Boyd NF, Lockwood GA, Byng JW, et al (1998) Mammographic densities and breast cancer risk. Cancer Epidemiology, Biomarkers & Prevention 7:1133–1144

Boyer-Guittaut M, Poillet L, Liang Q, et al (2014) The role of GABARAPL1/GEC1 in autophagic flux and mitochondrial quality control in MDA-MB-436 breast cancer cells. Autophagy 10:986–1003. https://doi.org/10.4161/auto.28390

Bozin B, Mimica-Dukic N, Samojlik I, et al (2008) Phenolics as antioxidants in garlic (Allium sativum L., Alliaceae). Food Chemistry 111:925–929. https://doi.org/10.1016/j.foodchem.2008.04.071

Brady NJ, Chuntova P, Bade LK, Schwertfeger KL (2013) The FGF/FGF receptor axis as a therapeutic target in breast cancer. Expert Review of Endocrinology & Metabolism 8:391–402. https://doi.org/10.1586/17446651.2013.811910

Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology 28:25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Brew K, Dinakarpandian D, Nagase H (2000) Tissue inhibitors of metalloproteinases: evolution, structure and function11Dedicated to Professor H. Neurath on the occasion of his 90th birthday. Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology 1477:267–283. https://doi.org/10.1016/S0167-4838(99)00279-4

Brocker C, Thompson D, Matsumoto A, et al (2010) Evolutionary divergence and functions of the human interleukin (IL) gene family. Human Genomics 5:30. https://doi.org/10.1186/1479-7364-5-1-30

Brooks AN, Kilgour E, Smith PD (2012) Molecular Pathways: Fibroblast Growth Factor Signaling: A New Therapeutic Opportunity in Cancer. Clinical Cancer Research 18:1855–1862. https://doi.org/10.1158/1078-0432.CCR-11-0699

Burstein HJ, Polyak K, Wong JS, et al (2004) Ductal Carcinoma in Situ of the Breast. New England Journal of Medicine 350:1430–1441. https://doi.org/10.1056/NEJMra031301

Burton TR, Gibson SB (2009) The role of Bcl-2 family member BNIP3 in cell death and disease: NIPping at the heels of cell death. Cell Death Differ 16:515–523. https://doi.org/10.1038/cdd.2008.185

Cabioglu N, Gong Y, Islam R, et al (2007) Expression of growth factor and chemokine receptors: new insights in the biology of inflammatory breast cancer. Annals of Oncology 18:1021–1029. https://doi.org/10.1093/annonc/mdm060

Cai Y, Luo Q, Sun M, Corke H (2004) Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sciences 74:2157–2184. https://doi.org/10.1016/j.lfs.2003.09.047

Cao L, Yang Y, Ye Z, et al (2018) Quercetin-3-methyl ether suppresses human breast cancer stem cell formation by inhibiting the Notch1 and PI3K/Akt signaling pathways. International Journal of Molecular Medicine 42:1625–1636. https://doi.org/10.3892/ijmm.2018.3741

Carlberg I, Mannervik B (1985) [59] Glutathione reductase. In: Methods in Enzymology. Academic Press, pp 484–490

Cecchi L, Ieri F, Vignolini P, et al (2020) Characterization of Volatile and Flavonoid Composition of Different Cuts of Dried Onion (Allium cepa L.) by HS-SPME-GC-MS, HS-SPME-GC×GC-TOF and HPLC-DAD. Molecules 25:408. https://doi.org/10.3390/molecules25020408

Çelik SE, Özyürek M, Güçlü K, Apak R (2010) Solvent effects on the antioxidant capacity of lipophilic and hydrophilic antioxidants measured by CUPRAC, ABTS/persulphate and FRAP methods. Talanta 81:1300–1309. https://doi.org/10.1016/j.talanta.2010.02.025

Chaffer CL, Weinberg RA (2011) A Perspective on Cancer Cell Metastasis. Science 331:1559–1564. https://doi.org/10.1126/science.1203543

Chandra-Kuntal K, Lee J, Singh SV (2013) Critical role for reactive oxygen species in apoptosis induction and cell migration inhibition by diallyl trisulfide, a cancer

chemopreventive component of garlic. Breast Cancer Res Treat 138:69–79. https://doi.org/10.1007/s10549-013-2440-2

Charron CS, Dawson HD, Albaugh GP, et al (2015) A Single Meal Containing Raw, Crushed Garlic Influences Expression of Immunity- and Cancer-Related Genes in Whole Blood of Humans. The Journal of Nutrition 145:2448–2455. https://doi.org/10.3945/jn.115.215392

Chatterjee SJ, Pandey S (2011) Chemo-resistant melanoma sensitized by tamoxifen to low dose curcumin treatment through induction of apoptosis and autophagy. Cancer Biology & Therapy 11:216–228. https://doi.org/10.4161/cbt.11.2.13798

Chaube B, Malvi P, Singh SV, et al (2015) AMPK maintains energy homeostasis and survival in cancer cells via regulating p38/PGC-1α-mediated mitochondrial biogenesis. Cell Death Discovery 1:1–11. https://doi.org/10.1038/cddiscovery.2015.63

Chelouche-Lev D, Miller CP, Tellez C, et al (2004) Different signalling pathways regulate VEGF and IL-8 expression in breast cancer: implications for therapy. European Journal of Cancer 40:2509–2518. https://doi.org/10.1016/j.ejca.2004.05.024

Chen C, Pung D, Leong V, et al (2004) Induction of detoxifying enzymes by garlic organosulfur compounds through transcription factor Nrf2: effect of chemical structure and stress signals. Free Radical Biology and Medicine 37:1578–1590. https://doi.org/10.1016/j.freeradbiomed.2004.07.021

Chen G, Ding X-F, Bouamar H, et al (2019) Everolimus induces G1 cell cycle arrest through autophagy-mediated protein degradation of cyclin D1 in breast cancer cells. American Journal of Physiology-Cell Physiology 317:C244–C252. https://doi.org/10.1152/ajpcell.00390.2018

Chen J, Duan Y, Zhang X, et al (2015) Genistein induces apoptosis by the inactivation of the IGF-1R/p-Akt signaling pathway in MCF-7 human breast cancer cells. Food Funct 6:995–1000. https://doi.org/10.1039/C4FO01141D

Chen L, Nievera CJ, Lee AY-L, Wu X (2008) Cell Cycle-dependent Complex Formation of BRCA1·CtIP·MRN Is Important for DNA Double-strand Break Repair *. Journal of Biological Chemistry 283:7713–7720. https://doi.org/10.1074/jbc.M710245200

Chen S, Shen X, Cheng S, et al (2013) Evaluation of Garlic Cultivars for Polyphenolic Content and Antioxidant Properties. PLOS ONE 8:e79730. https://doi.org/10.1371/journal.pone.0079730

Chen Y, He J, Tian M, et al (2014) UNC51-like kinase 1, autophagic regulator and cancer therapeutic target. Cell Proliferation 47:494–505. https://doi.org/10.1111/cpr.12145

Chen Y-J, Lin K-N, Jhang L-M, et al (2016) Gallic acid abolishes the EGFR/Src/Akt/Erk-mediated expression of matrix metalloproteinase-9 in MCF-7 breast cancer cells. Chemico-Biological Interactions 252:131–140. https://doi.org/10.1016/j.cbi.2016.04.025

Cheng S-J, Ko H-H, Cheng S-L, et al (2013) Arecoline-stimulated placenta growth factor production in gingival epithelial cells: modulation by curcumin. Oral Diseases 19:513–518. https://doi.org/10.1111/odi.12034

Cho S-J, Ryu J-H, Surh Y-J (2019) Ajoene, a Major Organosulfide Found in Crushed Garlic, Induces NAD(P)H:quinone Oxidoreductase Expression Through Nuclear Factor E2-related Factor-2 Activation in Human Breast Epithelial Cells. J Cancer Prev 24:112–122. https://doi.org/10.15430/JCP.2019.24.2.112

Chou TC, Talaly P (1977) A simple generalized equation for the analysis of multiple inhibitions of Michaelis-Menten kinetic systems. Journal of Biological Chemistry 252:6438–6442. https://doi.org/10.1016/S0021-9258(17)39978-7

Choudhury J, Kumar S, Mayank V, et al (2011) A REVIEW ON APOPTOSIS & ITS DIFFERENT PATHWAY. International Journal of Biological & Pharmaceutical Research 3:848–861

Choumessi AT, Loureiro R, Silva AM, et al (2012) Toxicity evaluation of some traditional African spices on breast cancer cells and isolated rat hepatic mitochondria. Food and Chemical Toxicology 50:4199–4208. https://doi.org/10.1016/j.fct.2012.08.008

Christiaens V, Lijnen HR (2010) Angiogenesis and development of adipose tissue. Molecular and Cellular Endocrinology 318:2–9. https://doi.org/10.1016/j.mce.2009.08.006

Chu Y-L, Raghu R, Lu K-H, et al (2013) Autophagy therapeutic potential of garlic in human cancer therapy. J Tradit Complement Med 3:159–162. https://doi.org/10.4103/2225-4110.114895

Chung YM, Bae YS, Lee SY (2003) Molecular ordering of ROS production, mitochondrial changes, and caspase activation during sodium salicylate-induced apoptosis. Free Radical Biology and Medicine 34:434–442. https://doi.org/10.1016/S0891-5849(02)01301-1

Chuturgoon AA, Phulukdaree A, Moodley D (2015) Fumonisin B1 inhibits apoptosis in HepG2 cells by inducing Birc-8/ILP-2. Toxicology Letters 235:67–74. https://doi.org/10.1016/j.toxlet.2015.03.006

Cianciosi D, Forbes-Hernández TY, Giampieri F, et al (2019) Effect of In vitro Gastrointestinal Digestion on the Bioaccessibility of Phenolic Compounds and Antioxidant Activity of Manuka Honey. eFood 1:85–93. https://doi.org/10.2991/efood.k.191011.001

Clifford MN, Scalbert A (2000) Ellagitannins – nature, occurrence and dietary burden. Journal of the Science of Food and Agriculture 80:1118–1125. https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<1118::AID-JSFA570>3.0.CO;2-9

Clinton SK, Giovannucci EL, Hursting SD (2020) The World Cancer Research Fund/American Institute for Cancer Research Third Expert Report on Diet, Nutrition, Physical Activity, and Cancer: Impact and Future Directions. J Nutr 150:663–671. https://doi.org/10.1093/jn/nxz268

Coenegrachts L, Maes C, Torrekens S, et al (2010) Anti–Placental Growth Factor Reduces Bone Metastasis by Blocking Tumor Cell Engraftment and Osteoclast Differentiation. Cancer Research 70:6537–6547. https://doi.org/10.1158/0008-5472.CAN-09-4092

Coghlin C, Murray GI (2010) Current and emerging concepts in tumour metastasis. The Journal of Pathology 222:1–15. https://doi.org/10.1002/path.2727

Cohen N, Shani O, Raz Y, et al (2017) Fibroblasts drive an immunosuppressive and growth-promoting microenvironment in breast cancer via secretion of Chitinase 3-like 1. Oncogene 36:4457–4468. https://doi.org/10.1038/onc.2017.65

Colditz GA, Kaphingst KA, Hankinson SE, Rosner B (2012) Family history and risk of breast cancer: nurses' health study. Breast Cancer Res Treat 133:1097–1104. https://doi.org/10.1007/s10549-012-1985-9

Colpaert CG, Vermeulen PB, Benoy I, et al (2003) Inflammatory breast cancer shows angiogenesis with high endothelial proliferation rate and strong E-cadherin expression. Br J Cancer 88:718–725. https://doi.org/10.1038/sj.bjc.6600807

Corsa CAS, Brenot A, Grither WR, et al (2016) The Action of Discoidin Domain Receptor 2 in Basal Tumor Cells and Stromal Cancer-Associated Fibroblasts Is Critical for Breast Cancer Metastasis. Cell Reports 15:2510–2523. https://doi.org/10.1016/j.celrep.2016.05.033

Cronauer MV, Schulz WA, Seifert H-H, et al (2003) Fibroblast Growth Factors and Their Receptors in Urological Cancers: Basic Research and Clinical Implications. European Urology 43:309–319. https://doi.org/10.1016/S0302-2838(03)00005-8

Cui J, Shi M, Xie D, et al (2014) FOXM1 Promotes the Warburg Effect and Pancreatic Cancer Progression via Transactivation of LDHA Expression. Clinical Cancer Research 20:2595–2606. https://doi.org/10.1158/1078-0432.CCR-13-2407

da Silva PM, Gauche C, Gonzaga LV, et al (2016) Honey: Chemical Composition, Stability and Authenticity. Food Chemistry 196:309–323. https://doi.org/10.1016/j.foodchem.2015.09.051

D'Amours D, Jackson SP (2002) The Mre11 complex: at the crossroads of dna repair and checkpoint signalling. Nat Rev Mol Cell Biol 3:317–327. https://doi.org/10.1038/nrm805

D'Archivio AA, Foschi M, Aloia R, et al (2019) Geographical discrimination of red garlic (Allium sativum L.) produced in Italy by means of multivariate statistical analysis of ICP-OES data. Food Chemistry 275:333–338. https://doi.org/10.1016/j.foodchem.2018.09.088

Das A, Banik NL, Ray SK (2007) Garlic compounds generate reactive oxygen species leading to activation of stress kinases and cysteine proteases for apoptosis in human glioblastoma T98G and U87MG cells. Cancer 110:1083–1095. https://doi.org/10.1002/cncr.22888

Davies E, Hiscox S (2011) New therapeutic approaches in breast cancer. Maturitas 68:121–128. https://doi.org/10.1016/j.maturitas.2010.10.012

De AK, Muthiyan R, Mondal S, et al (2019) A Natural Quinazoline Derivative from Marine Sponge Hyrtios erectus Induces Apoptosis of Breast Cancer Cells via ROS Production and Intrinsic or Extrinsic Apoptosis Pathways. Marine Drugs 17:658. https://doi.org/10.3390/md17120658

de Castro Junior G, Puglisi F, de Azambuja E, et al (2006) Angiogenesis and cancer: A cross-talk between basic science and clinical trials (the “do ut des” paradigm). Critical Reviews in Oncology/Hematology 59:40–50. https://doi.org/10.1016/j.critrevonc.2006.02.007

de Souza Grinevicius VMA, Kviecinski MR, Santos Mota NSR, et al (2016) Piper nigrum ethanolic extract rich in piperamides causes ROS overproduction, oxidative damage in DNA leading to cell cycle arrest and apoptosis in cancer cells. Journal of Ethnopharmacology 189:139–147. https://doi.org/10.1016/j.jep.2016.05.020

Del Rio D, Rodriguez-Mateos A, Spencer JPE, et al (2013) Dietary (Poly)phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases. Antioxidants & Redox Signaling 18:1818–1892. https://doi.org/10.1089/ars.2012.4581

Dewangan J, Tandon D, Srivastava S, et al (2017) Novel combination of salinomycin and resveratrol synergistically enhances the anti-proliferative and pro-apoptotic effects on human breast cancer cells. Apoptosis 22:1246–1259. https://doi.org/10.1007/s10495-017-1394-y

Dewerchin M, Carmeliet P (2014) Placental growth factor in cancer. Expert Opinion on Therapeutic Targets 18:1339–1354. https://doi.org/10.1517/14728222.2014.948420

Dhaheri YA, Attoub S, Ramadan G, et al (2014) Carnosol Induces ROS-Mediated Beclin1-Independent Autophagy and Apoptosis in Triple Negative Breast Cancer. PLOS ONE 9:e109630. https://doi.org/10.1371/journal.pone.0109630

Di G, Li H, Shen Z, Shao Z (2003) [Analysis of anti-proliferation of curcumin on human breast cancer cells and its mechanism]. Zhonghua Yi Xue Za Zhi 83:1764–1768

Dimitrova N, de Lange T (2009) Cell Cycle-Dependent Role of MRN at Dysfunctional Telomeres: ATM Signaling-Dependent Induction of Nonhomologous End Joining (NHEJ) in G1 and Resection-Mediated Inhibition of NHEJ in G2. Molecular and Cellular Biology 29:5552–5563. https://doi.org/10.1128/MCB.00476-09

Ding H, Han C, Guo D, et al (2009) Selective Induction of Apoptosis of Human Oral Cancer Cell Lines by Avocado Extracts Via a ROS-Mediated Mechanism. Nutrition and Cancer 61:348–356. https://doi.org/10.1080/01635580802567158

Dinkova-Kostova AT, Abramov AY (2015) The emerging role of Nrf2 in mitochondrial function. Free Radical Biology and Medicine 88:179–188. https://doi.org/10.1016/j.freeradbiomed.2015.04.036

Diretto G, Rubio-Moraga A, Argandoña J, et al (2017) Tissue-Specific Accumulation of Sulfur Compounds and Saponins in Different Parts of Garlic Cloves from Purple and White Ecotypes. Molecules 22:1359. https://doi.org/10.3390/molecules22081359

Dirsch VM, Gerbes AL, Vollmar AM (1998) Ajoene, a Compound of Garlic, Induces Apoptosis in Human Promyeloleukemic Cells, Accompanied by Generation of Reactive Oxygen Species and Activation of Nuclear Factor κB. Mol Pharmacol 53:402–407. https://doi.org/10.1124/mol.53.3.402

Dong C, Yuan T, Wu Y, et al (2013) Loss of FBP1 by Snail-Mediated Repression Provides Metabolic Advantages in Basal-like Breast Cancer. Cancer Cell 23:316–331. https://doi.org/10.1016/j.ccr.2013.01.022

Dreher ML (2018) Dietary Patterns and Whole Plant Foods in Aging and Disease. Humana Press

Drygin D, Ho CB, Omori M, et al (2011) Protein kinase CK2 modulates IL-6 expression in inflammatory breast cancer. Biochemical and Biophysical Research Communications 415:163–167. https://doi.org/10.1016/j.bbrc.2011.10.046

Du C, Fang M, Li Y, et al (2000) Smac, a Mitochondrial Protein that Promotes Cytochrome c–Dependent Caspase Activation by Eliminating IAP Inhibition. Cell 102:33–42. https://doi.org/10.1016/S0092-8674(00)00008-8

Dziri S, Hassen I, Fatnassi S, et al (2012) Phenolic constituents, antioxidant and antimicrobial activities of rosy garlic (Allium roseum var. odoratissimum). Journal of Functional Foods 4:423–432. https://doi.org/10.1016/j.jff.2012.01.010

E. Goldberg J, L. Schwertfeger K (2010) Proinflammatory Cytokines in Breast Cancer: Mechanisms of Action and Potential Targets for Therapeutics. Current Drug Targets 11:1133–1146. https://doi.org/10.2174/138945010792006799

Eissing T, Conzelmann H, Gilles ED, et al (2004) Bistability Analyses of a Caspase Activation Model for Receptor-induced Apoptosis *. Journal of Biological Chemistry 279:36892–36897. https://doi.org/10.1074/jbc.M404893200

Elimam H, El-Say KM, Cybulsky AV, Khalil H (2020) Regulation of Autophagy Progress via Lysosomal Depletion by Fluvastatin Nanoparticle Treatment in Breast Cancer Cells. ACS Omega 5:15476–15486. https://doi.org/10.1021/acsomega.0c01618

Ellman GL (1958) ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS 82, 70-77 (1959) Tissue Su yd l Groups

Elmore S (2007) Apoptosis: A Review of Programmed Cell Death. Toxicol Pathol 35:495–516. https://doi.org/10.1080/01926230701320337

Elston C w., Ellis I o. (1991) pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 19:403–410. https://doi.org/10.1111/j.1365-2559.1991.tb00229.x

Enayat S, Şeyma Ceyhan M, Taşkoparan B, et al (2016) CHNQ, a novel 2-Chloro-1,4-naphthoquinone derivative of quercetin, induces oxidative stress and autophagy both in vitro and in vivo. Archives of Biochemistry and Biophysics 596:84–98. https://doi.org/10.1016/j.abb.2016.03.004

Eroglu A, Ersoz C, Karasoy D, Sak S (2017) Vascular Endothelial Growth Factor (Vegf)-C, Vegf-D, Vegfr-3 And D2-40 Expressions In Primary Breast Cancer: Association With Lymph Node Metastasis. https://doi.org/10.17219/acem/58784

Ezhevsky SA, Nagahara H, Vocero-Akbani AM, et al (1997) Hypo-phosphorylation of the retinoblastoma protein (pRb) by cyclin D:Cdk4/6 complexes results in active pRb. Proceedings of the National Academy of Sciences 94:10699–10704. https://doi.org/10.1073/pnas.94.20.10699

Fahrioğlu U, Dodurga Y, Elmas L, Seçme M (2016) Ferulic acid decreases cell viability and colony formation while inhibiting migration of MIA PaCa-2 human pancreatic cancer cells in vitro. Gene 576:476–482. https://doi.org/10.1016/j.gene.2015.10.061

Fan Y, Mao R, Yang J (2013) NF-κB and STAT3 signaling pathways collaboratively link inflammation to cancer. Protein Cell 4:176–185. https://doi.org/10.1007/s13238-013-2084-3

Fang J (2014) Bioavailability of anthocyanins. Drug Metabolism Reviews 46:508–520. https://doi.org/10.3109/03602532.2014.978080

Fang J, Huang S, Liu H, et al (2003) Role of FGF-2/FGFR signaling pathway in cancer and its signification in breast cancer. Chin Sci Bull 48:1539–1547. https://doi.org/10.1007/BF03183956

Fantl V, Smith R, Brookes S, et al (1993) Chromosome 11q13 abnormalities in human breast cancer. Cancer Surv 18:77–94

Farhat Z, Hershberger PA, Freudenheim JL, et al (2021) Types of garlic and their anticancer and antioxidant activity: a review of the epidemiologic and experimental evidence. Eur J Nutr 60:3585–3609. https://doi.org/10.1007/s00394-021-02482-7

Faria A, Pestana D, Teixeira D, et al (2010) Blueberry anthocyanins and pyruvic acid adducts: anticancer properties in breast cancer cell lines. Phytotherapy Research 24:1862–1869. https://doi.org/10.1002/ptr.3213

Fendt S-M, Frezza C, Erez A (2020) Targeting Metabolic Plasticity and Flexibility Dynamics for Cancer Therapy. Cancer Discovery 10:1797–1807. https://doi.org/10.1158/2159-8290.CD-20-0844

Fenwick GR, Hanley AB, Whitaker JR (1985) The genus allium. Part 2. C R C Critical Reviews in Food Science and Nutrition 22:273–377. https://doi.org/10.1080/10408398509527417

Filomeni G, Aquilano K, Rotilio G, Ciriolo MR (2003) Reactive Oxygen Species-dependent c-Jun NH2-terminal Kinase/c-Jun Signaling Cascade Mediates Neuroblastoma Cell Death Induced by Diallyl Disulfide1. Cancer Research 63:5940–5949

Fousek K, Horn LA, Palena C (2021) Interleukin-8: A chemokine at the intersection of cancer plasticity, angiogenesis, and immune suppression. Pharmacology & Therapeutics 219:107692. https://doi.org/10.1016/j.pharmthera.2020.107692

Franco R, DeHaven WI, Sifre MI, et al (2008) Glutathione Depletion and Disruption of Intracellular Ionic Homeostasis Regulate Lymphoid Cell Apoptosis *. Journal of Biological Chemistry 283:36071–36087. https://doi.org/10.1074/jbc.M807061200

Freeman F, Koder Y (1995) Garlic chemistry: stability of S-(2-propenyl) 2-propene-1-sulfinothioate (allicin) in blood, solvents, and simulated physiological fluids. Journal of agricultural and food chemistry 43:2332–2338. https://doi.org/10.1021/jf00057a004

Freise C, Ruehl M, Erben U, et al (2011) A hepatoprotective Lindera obtusiloba extract suppresses growth and attenuates insulin like growth factor-1 receptor signaling and NF-kappaB activity in human liver cancer cell lines. BMC Complementary and Alternative Medicine 11:39. https://doi.org/10.1186/1472-6882-11-39

Fu Y, Chang H, Peng X, et al (2014) Resveratrol Inhibits Breast Cancer Stem-Like Cells and Induces Autophagy via Suppressing Wnt/β-Catenin Signaling Pathway. PLOS ONE 9:e102535. https://doi.org/10.1371/journal.pone.0102535

Fuentes-Retamal S, Sandoval-Acuña C, Peredo-Silva L, et al (2020) Complex Mitochondrial Dysfunction Induced by TPP+-Gentisic Acid and Mitochondrial Translation Inhibition by Doxycycline Evokes Synergistic Lethality in Breast Cancer Cells. Cells 9:407. https://doi.org/10.3390/cells9020407

Fulda S, Wick W, Weller M, Debatin K-M (2002) Smac agonists sensitize for Apo2L/TRAIL- or anticancer drug-induced apoptosis and induce regression of malignant glioma in vivo. Nat Med 8:808–815. https://doi.org/10.1038/nm735

Fussenegger M, Bailey JE, Varner J (2000) A mathematical model of caspase function in apoptosis. Nat Biotechnol 18:768–774. https://doi.org/10.1038/77589

Gail MH, You W-C (2006) A Factorial Trial Including Garlic Supplements Assesses Effect in Reducing Precancerous Gastric Lesions. The Journal of Nutrition 136:813S-815S. https://doi.org/10.1093/jn/136.3.813S

Galati S, Boni C, Gerra MC, et al (2019) Autophagy: A Player in response to Oxidative Stress and DNA Damage. Oxidative Medicine and Cellular Longevity 2019:e5692958. https://doi.org/10.1155/2019/5692958

Gao Y, Yang F, Yang X-A, et al (2019) Mitochondrial metabolism is inhibited by the HIF1α-MYC-PGC-1β axis in BRAF V600E thyroid cancer. The FEBS Journal 286:1420–1436. https://doi.org/10.1111/febs.14786

Gapter LA, Yuin OZ, Ng K (2008) S-Allylcysteine reduces breast tumor cell adhesion and invasion. Biochemical and Biophysical Research Communications 367:446–451. https://doi.org/10.1016/j.bbrc.2007.12.175

Garrido-Armas M, Corona JC, Escobar ML, et al (2018) Paraptosis in human glioblastoma cell line induced by curcumin. Toxicol In Vitro 51:63–73. https://doi.org/10.1016/j.tiv.2018.04.014

Garvin S, Öllinger K, Dabrosin C (2006) Resveratrol induces apoptosis and inhibits angiogenesis in human breast cancer xenografts in vivo. Cancer Letters 231:113–122. https://doi.org/10.1016/j.canlet.2005.01.031

Gasparini G, Harris AL REVIEW ARTICLE Clinical Importance of the Determination of Tumor Angiogenesis in Breast Carcinoma: Much More Than a New Prognostic Tool

Gatt ME, Strahilevitz J, Sharon N, et al (2015) A Randomized Controlled Study to Determine the Efficacy of Garlic Compounds in Patients With Hematological Malignancies at Risk for Chemotherapy-Related Febrile Neutropenia. Integr Cancer Ther 14:428–435. https://doi.org/10.1177/1534735415588928

Geng Y, Yu Q, Whoriskey W, et al (2001) Expression of cyclins E1 and E2 during mouse development and in neoplasia. Proceedings of the National Academy of Sciences 98:13138–13143. https://doi.org/10.1073/pnas.231487798

George ML, Tutton MG, Janssen F, et al (2001) VEGF-A, VEGF-C, and VEGF-D in Colorectal Cancer Progression. Neoplasia 3:420–427. https://doi.org/10.1038/sj.neo.7900186

Germain E, Auger J, Ginies C, et al (2002) In vivo metabolism of diallyl disulphide in the rat: identification of two new metabolites. Xenobiotica 32:1127–1138. https://doi.org/10.1080/0049825021000017902

Ghazanfari T, Yaraee R, Rahmati B, et al (2011) In vitro cytotoxic effect of garlic extract on malignant and nonmalignant cell lines. Immunopharmacology and Immunotoxicology 33:603–608. https://doi.org/10.3109/08923973.2011.551832

Ghiulai R, Avram S, Stoian D, et al (2020) Lemon Balm Extracts Prevent Breast Cancer Progression In Vitro and In Ovo on Chorioallantoic Membrane Assay. Evidence-Based Complementary and Alternative Medicine 2020:e6489159. https://doi.org/10.1155/2020/6489159

Giacinti C, Giordano A (2006) RB and cell cycle progression. Oncogene 25:5220–5227. https://doi.org/10.1038/sj.onc.1209615

Giampieri F, Afrin S, Forbes-Hernandez TY, et al (2019) Autophagy in Human Health and Disease: Novel Therapeutic Opportunities. Antioxidants & Redox Signaling 30:577–634. https://doi.org/10.1089/ars.2017.7234

Giampieri F, Afrin S, Stewart D, et al (2018) Phytochemical Composition and Cytotoxic Effects on Liver Hepatocellular Carcinoma Cells of Different Berries Following a Simulated In Vitro Gastrointestinal Digestion. Molecules 23:1918. https://doi.org/10.3390/molecules23081918

Giampieri F, Alvarez-Suarez JM, Cordero MD, et al (2017) Strawberry consumption improves aging-associated impairments, mitochondrial biogenesis and functionality through the AMP-activated protein kinase signaling cascade. Food Chemistry 234:464–471. https://doi.org/10.1016/j.foodchem.2017.05.017

Gibcus JH, Menkema L, Mastik MF, et al (2007) Amplicon Mapping and Expression Profiling Identify the Fas-Associated Death Domain Gene as a New Driver in the

11q13.3 Amplicon in Laryngeal/Pharyngeal Cancer. Clinical Cancer Research 13:6257–6266. https://doi.org/10.1158/1078-0432.CCR-07-1247

Gil-Izquierdo A, Zafrilla P, Tomás-Barberán FA (2002) An in vitro method to simulate phenolic compound release from the food matrix in the gastrointestinal tract. Eur Food Res Technol 214:155–159. https://doi.org/10.1007/s00217-001-0428-3

Glick D, Barth S, Macleod KF (2010) Autophagy: cellular and molecular mechanisms. The Journal of Pathology 221:3–12. https://doi.org/10.1002/path.2697

Golub TR, Slonim DK, Tamayo P, et al (1999) Molecular Classification of Cancer: Class Discovery and Class Prediction by Gene Expression Monitoring. Science 286:531–537. https://doi.org/10.1126/science.286.5439.531

Gorinstein S, Leontowicz H, Leontowicz M, et al (2008) Comparison of the Main Bioactive Compounds and Antioxidant Activities in Garlic and White and Red Onions after Treatment Protocols. J Agric Food Chem 56:4418–4426. https://doi.org/10.1021/jf800038h

Gorrini C, Harris IS, Mak TW (2013) Modulation of oxidative stress as an anticancer strategy. Nat Rev Drug Discov 12:931–947. https://doi.org/10.1038/nrd4002

Govinden R, Bhoola KD (2003) Genealogy, expression, and cellular function of transforming growth factor-β. Pharmacology & Therapeutics 98:257–265. https://doi.org/10.1016/S0163-7258(03)00035-4

Griffith OW (1980) Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Analytical Biochemistry 106:207–212. https://doi.org/10.1016/0003-2697(80)90139-6

Guan F, Ding Y, Zhang Y, et al (2016) Curcumin Suppresses Proliferation and Migration of MDA-MB-231 Breast Cancer Cells through Autophagy-Dependent Akt Degradation. PLOS ONE 11:e0146553. https://doi.org/10.1371/journal.pone.0146553

Gued LR, Thomas RD, Green M (2003) Diallyl sulfide inhibits diethylstilbestrol-induced lipid peroxidation in breast tissue of female ACI rats: Implications in breast cancer prevention. Oncology Reports 10:739–743. https://doi.org/10.3892/or.10.3.739

Guillon F, Renard CMGC, Hospers J, et al (1995) Characterisation of residual fibres from fermentation of pea and apple fibres by human faecal bacteria. Journal of the Science of Food and Agriculture 68:521–529. https://doi.org/10.1002/jsfa.2740680417

Gump FE, Jicha DL, Ozello L (1987) Ductal carcinoma in situ (DCIS): A revised concept. Surgery 102:790–795. https://doi.org/10.5555/uri:pii:0039606087901577

Gunasekaran S, Venkatachalam K, Namasivayam N (2015) p-Methoxycinnamic acid, an active phenylpropanoid induces mitochondrial mediated apoptosis in HCT-116 human colon adenocarcinoma cell line. Environmental Toxicology and Pharmacology 40:966–974. https://doi.org/10.1016/j.etap.2015.09.013

Guo R, Chang X, Guo X, et al (2017) Phenolic compounds, antioxidant activity, antiproliferative activity and bioaccessibility of Sea buckthorn (Hippophaë

rhamnoides L.) berries as affected by in vitro digestion. Food Funct 8:4229–4240. https://doi.org/10.1039/C7FO00917H

Guo S, Colbert LS, Fuller M, et al (2010) Vascular endothelial growth factor receptor-2 in breast cancer. Biochimica et Biophysica Acta (BBA) - Reviews on Cancer 1806:108–121. https://doi.org/10.1016/j.bbcan.2010.04.004

Guo T, Wei L, Sun J, et al (2011) Antioxidant activities of extract and fractions from Tuber indicum Cooke & Massee. Food Chemistry 127:1634–1640. https://doi.org/10.1016/j.foodchem.2011.02.030

Gupta SC, Hevia D, Patchva S, et al (2012) Upsides and Downsides of Reactive Oxygen Species for Cancer: The Roles of Reactive Oxygen Species in Tumorigenesis, Prevention, and Therapy. Antioxidants & Redox Signaling 16:1295–1322. https://doi.org/10.1089/ars.2011.4414

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-Transferases: THE FIRST ENZYMATIC STEP IN MERCAPTURIC ACID FORMATION. Journal of Biological Chemistry 249:7130–7139. https://doi.org/10.1016/S0021-9258(19)42083-8

Halliwell B, Rafter J, Jenner A (2005) Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects? Antioxidant or not? The American Journal of Clinical Nutrition 81:268S-276S. https://doi.org/10.1093/ajcn/81.1.268S

Hamacher-Brady A (2012) Autophagy Regulation and Integration with Cell Signaling. Antioxidants & Redox Signaling 17:756–765. https://doi.org/10.1089/ars.2011.4410

Hamlaoui-Gasmi S, Limam# N, Mokni M, et al (2011) Garlic-mode treatment effects on rat brain redox status. JMPR 5:5094–5098. https://doi.org/10.5897/JMPR.9000662

Han H, Cao F-L, Wang B-Z, et al (2014) Expression of Angiogenesis Regulatory Proteins and Epithelial-Mesenchymal Transition Factors in Platelets of the Breast Cancer Patients. The Scientific World Journal 2014:e878209. https://doi.org/10.1155/2014/878209

Han H, Li J, Feng X, et al (2017) Autophagy-related genes are induced by histone deacetylase inhibitor suberoylanilide hydroxamic acid via the activation of cathepsin B in human breast cancer cells. Oncotarget 8:53352–53365. https://doi.org/10.18632/oncotarget.18410

Hanahan D, Folkman J (1996) Patterns and Emerging Mechanisms of the Angiogenic Switch during Tumorigenesis. Cell 86:353–364. https://doi.org/10.1016/S0092-8674(00)80108-7

Hanahan D, Weinberg RA (2011) Hallmarks of Cancer: The Next Generation. Cell 144:646–674. https://doi.org/10.1016/j.cell.2011.02.013

Hanby AM, Hughes TA (2008) In situ and invasive lobular neoplasia of the breast. Histopathology 52:58–66. https://doi.org/10.1111/j.1365-2559.2007.02891.x

Hance KW, Anderson WF, Devesa SS, et al (2005) Trends in Inflammatory Breast Carcinoma Incidence and Survival: The Surveillance, Epidemiology, and End Results Program at the National Cancer Institute. JNCI: Journal of the National Cancer Institute 97:966–975. https://doi.org/10.1093/jnci/dji172

Hardy KM, Booth BW, Hendrix MJC, et al (2010) ErbB/EGF Signaling and EMT in Mammary Development and Breast Cancer. J Mammary Gland Biol Neoplasia 15:191–199. https://doi.org/10.1007/s10911-010-9172-2

Hassan ZK, Elamin MH, Daghestani MH, et al (2012) Oleuropein Induces Anti-metastatic Effects in Breast Cancer. Asian Pacific Journal of Cancer Prevention 13:4555–4559. https://doi.org/10.7314/APJCP.2012.13.9.4555

Hayano-Kanashiro C, Gámez-Meza N, Medina-Juárez LÁ (2016) Wild Pepper Capsicum annuum L. var. glabriusculum: Taxonomy, Plant Morphology, Distribution, Genetic Diversity, Genome Sequencing, and Phytochemical Compounds. Crop Science 56:1–11. https://doi.org/10.2135/cropsci2014.11.0789

He C, Klionsky DJ (2009) Regulation Mechanisms and Signaling Pathways of Autophagy. Annu Rev Genet 43:67–93. https://doi.org/10.1146/annurev-genet-102808-114910

Hedges JC, Singer CA, Gerthoffer WT (2000) Mitogen-Activated Protein Kinases Regulate Cytokine Gene Expression in Human Airway Myocytes. Am J Respir Cell Mol Biol 23:86–94. https://doi.org/10.1165/ajrcmb.23.1.4014

Helal A, Tagliazucchi D, Verzelloni E, Conte A (2014) Bioaccessibility of polyphenols and cinnamaldehyde in cinnamon beverages subjected to in vitro gastro-pancreatic digestion. Journal of Functional Foods 7:506–516. https://doi.org/10.1016/j.jff.2014.01.005

Heppner KJ, Matrisian LM, Jensen RA, Rodgers WH (1996) Expression of most matrix metalloproteinase family members in breast cancer represents a tumor-induced host response. Am J Pathol 149:273–282

Hinkula M, Pukkala E, Kyyrönen P, Kauppila A (2001) Grand multiparity and the risk of breast cancer: population-based study in Finland. Cancer Causes Control 12:491–500. https://doi.org/10.1023/A:1011253527605

Hirsch K, Danilenko M, Giat J, et al (2000) Effect of Purified Allicin, the Major Ingredient ofFreshly Crushed Garlic, on Cancer Cell Proliferation. Nutrition and Cancer 38:245–254. https://doi.org/10.1207/S15327914NC382_14

Ho H-H, Chang C-S, Ho W-C, et al (2010) Anti-metastasis effects of gallic acid on gastric cancer cells involves inhibition of NF-κB activity and downregulation of PI3K/AKT/small GTPase signals. Food and Chemical Toxicology 48:2508–2516. https://doi.org/10.1016/j.fct.2010.06.024

Hobbs GA, Zhou B, Cox AD, Campbell SL (2014) Rho GTPases, oxidation, and cell redox control. Small GTPases 5:e28579. https://doi.org/10.4161/sgtp.28579

Hrbek V, Rektorisova M, Chmelarova H, et al (2018) Authenticity assessment of garlic using a metabolomic approach based on high resolution mass spectrometry. Journal of Food Composition and Analysis 67:19–28. https://doi.org/10.1016/j.jfca.2017.12.020

Hsu C-L, Hurang S-L, Chen W, et al (2004) Qualities and antioxidant properties of bread as affected by the incorporation of yam flour in the formulation. International Journal of Food Science & Technology 39:231–238. https://doi.org/10.1046/j.0950-5423.2003.00770.x

Hu L, Huang W, Ding Y, et al (2019) PHMH, a diarylheptanoid from Alpinia officinarum attenuates VEGF-induced angiogenesis via inhibition of the VEGFR-2 signaling pathway. Food Funct 10:2605–2617. https://doi.org/10.1039/C8FO01809J

Hu W, Kavanagh JJ (2003) Anticancer therapy targeting the apoptotic pathway. The Lancet Oncology 4:721–729. https://doi.org/10.1016/S1470-2045(03)01277-4

Huang J, Yang B, Xiang T, et al (2015) Diallyl disulfide inhibits growth and metastatic potential of human triple-negative breast cancer cells through inactivation of the β-catenin signaling pathway. Molecular Nutrition & Food Research 59:1063–1075. https://doi.org/10.1002/mnfr.201400668

Huang X-F, Chen J-Z (2009) Obesity, the PI3K/Akt signal pathway and colon cancer. Obesity Reviews 10:610–616. https://doi.org/10.1111/j.1467-789X.2009.00607.x

Hulka BS (1996) Epidemiology of susceptibility to breast cancer. Prog Clin Biol Res 395:159–174

Husain I, Ahmad R, Chandra A, et al (2018) Phytochemical characterization and biological activity evaluation of ethanolic extract of Cinnamomum zeylanicum. Journal of Ethnopharmacology 219:110–116. https://doi.org/10.1016/j.jep.2018.02.001

Hussain T, Tan B, Yin Y, et al (2016) Oxidative Stress and Inflammation: What Polyphenols Can Do for Us? Oxidative Medicine and Cellular Longevity 2016:e7432797. https://doi.org/10.1155/2016/7432797

Ichimura Y, Kirisako T, Takao T, et al (2000) A ubiquitin-like system mediates protein lipidation. Nature 408:488–492. https://doi.org/10.1038/35044114

IKEDA H, NISHI S, SAKAI M (2004) Transcription factor Nrf2/MafK regulates rat placental glutathione S-transferase gene during hepatocarcinogenesis. Biochemical Journal 380:515–521. https://doi.org/10.1042/bj20031948

Inoki K, Li Y, Xu T, Guan K-L (2003) Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. Genes Dev 17:1829–1834. https://doi.org/10.1101/gad.1110003

Isbilen O, Rizaner N, Volkan E (2018) Anti-proliferative and cytotoxic activities of Allium autumnale P. H. Davis (Amaryllidaceae) on human breast cancer cell lines MCF-7 and MDA-MB-231. BMC Complement Altern Med 18:30. https://doi.org/10.1186/s12906-018-2105-0

Ishikawa H, Saeki T, Otani T, et al (2006) Aged Garlic Extract Prevents a Decline of NK Cell Number and Activity in Patients with Advanced Cancer. The Journal of Nutrition 136:816S-820S. https://doi.org/10.1093/jn/136.3.816S

Islam SS, Al-Sharif I, Sultan A, et al (2018) Eugenol potentiates cisplatin anti-cancer activity through inhibition of ALDH-positive breast cancer stem cells and the NF-κB signaling pathway. Molecular Carcinogenesis 57:333–346. https://doi.org/10.1002/mc.22758

Ivanova D, Zhelev Z, Semkova S, et al (2019) Resveratrol Modulates the Redox-status and Cytotoxicity of Anticancer Drugs by Sensitizing Leukemic Lymphocytes and Protecting Normal Lymphocytes. Anticancer Res 39:3745–3755. https://doi.org/10.21873/anticanres.13523

Iwanowycz S, Wang J, Hodge J, et al (2016) Emodin Inhibits Breast Cancer Growth by Blocking the Tumor-Promoting Feedforward Loop between Cancer Cells and Macrophages. Molecular Cancer Therapeutics 15:1931–1942. https://doi.org/10.1158/1535-7163.MCT-15-0987

Jacobs AL, Schär P (2012) DNA glycosylases: in DNA repair and beyond. Chromosoma 121:1–20. https://doi.org/10.1007/s00412-011-0347-4

Jang E-K, Seo J-H, Lee S-P (2008) Physiological Activity and Antioxidative Effects of Aged Black Garlic (Allium sativum L.) Extract. Korean Journal of Food Science and Technology 40:443–448

Jaramillo MC, Zhang DD (2013) The emerging role of the Nrf2–Keap1 signaling pathway in cancer. Genes Dev 27:2179–2191. https://doi.org/10.1101/gad.225680.113

Jayakumar R, Kanthimathi MS (2012) Dietary spices protect against hydrogen peroxide-induced DNA damage and inhibit nicotine-induced cancer cell migration. Food Chemistry 134:1580–1584. https://doi.org/10.1016/j.foodchem.2012.03.101

Jeong W-S, Jun M, Kong A-NT (2006) Nrf2: A Potential Molecular Target for Cancer Chemoprevention by Natural Compounds. Antioxidants & Redox Signaling 8:99–106. https://doi.org/10.1089/ars.2006.8.99

Jia J, Yang M, Chen Y, et al (2014) Inducing apoptosis effect of caffeic acid 3,4-dihydroxy-phenethyl ester on the breast cancer cells. Tumor Biol 35:11781–11789. https://doi.org/10.1007/s13277-014-2304-3

Jiang C, Ganther H, Lu J (2000) Monomethyl selenium–specific inhibition of MMP-2 and VEGF expression: Implications for angiogenic switch regulation. Molecular Carcinogenesis 29:236–250. https://doi.org/10.1002/1098-2744(200012)29:4<236::AID-MC1006>3.0.CO;2-E

Jiang Q-L, Zhang S, Tian M, et al (2015) Plant lectins, from ancient sugar-binding proteins to emerging anti-cancer drugs in apoptosis and autophagy. Cell Proliferation 48:17–28. https://doi.org/10.1111/cpr.12155

Jiang X, Zhu X, Xu H, et al (2017) Diallyl trisulfide suppresses tumor growth through the attenuation of Nrf2/Akt and activation of p38/JNK and potentiates cisplatin efficacy

in gastric cancer treatment. Acta Pharmacol Sin 38:1048–1058. https://doi.org/10.1038/aps.2016.176

Jiao C, Chen W, Tan X, et al (2020) Ganoderma lucidum spore oil induces apoptosis of breast cancer cells in vitro and in vivo by activating caspase-3 and caspase-9. Journal of Ethnopharmacology 247:112256. https://doi.org/10.1016/j.jep.2019.112256

Jin Z-Y, Wu M, Han R-Q, et al (2013) Raw Garlic Consumption as a Protective Factor for Lung Cancer, a Population-Based Case–Control Study in a Chinese Population. Cancer Prevention Research 6:711–718. https://doi.org/10.1158/1940-6207.CAPR-13-0015

Johnson VL, Ko SC, Holmstrom TH, et al (2000) Effector caspases are dispensable for the early nuclear morphological changes during chemical-induced apoptosis. Journal of Cell Science 113:2941–2953. https://doi.org/10.1242/jcs.113.17.2941

Joukov V, Pajusola K, Kaipainen A, et al (1996) A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. EMBO J 15:290–298

Joyce JA, Pollard JW (2009) Microenvironmental regulation of metastasis. Nat Rev Cancer 9:239–252. https://doi.org/10.1038/nrc2618

Jung CH, Ro S-H, Cao J, et al (2010) mTOR regulation of autophagy. FEBS Letters 584:1287–1295. https://doi.org/10.1016/j.febslet.2010.01.017

Jung S, Wang M, Anderson K, et al (2016) Alcohol consumption and breast cancer risk by estrogen receptor status: in a pooled analysis of 20 studies. International Journal of Epidemiology 45:916–928. https://doi.org/10.1093/ije/dyv156

Kabała-Dzik A, Rzepecka-Stojko A, Kubina R, et al (2017) Migration Rate Inhibition of Breast Cancer Cells Treated by Caffeic Acid and Caffeic Acid Phenethyl Ester: An In Vitro Comparison Study. Nutrients 9:1144. https://doi.org/10.3390/nu9101144

Kabeya Y, Mizushima N, Ueno T, et al (2000) LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. EMBO J 19:5720–5728. https://doi.org/10.1093/emboj/19.21.5720

Kai AK-L, Chan LK, Lo RC-L, et al (2016) Down-regulation of TIMP2 by HIF-1α/miR-210/HIF-3α regulatory feedback circuit enhances cancer metastasis in hepatocellular carcinoma. Hepatology 64:473–487. https://doi.org/10.1002/hep.28577

Kakkar P, Das B, Viswanathan PN (1984) A Modified Spectrophotometric Assay of Superoxide Dismutase. IJBB Vol21(2) [April 1984]

Kamanna VS, Chandrasekhara N (1980) Fatty acid composition of garlic (Allium sativum Linnaeus) lipids. Journal of the American Oil Chemists' Society 57:175. https://doi.org/10.1007/BF02883781

Kang Y-H, Park Y-K, Lee G-D (1996) The Nitrite Scavenging and Electron Donating Ability of Phenolic Compounds. Korean Journal of Food Science and Technology 28:232–239

Kaowinn S, Kaewpiboon C, Kim JE, et al (2018) N-Benzyl-N-methyl-dodecan-1-amine, a novel compound from garlic, exerts anti-cancer effects on human A549 lung cancer cells overexpressing cancer upregulated gene (CUG)2. Eur J Pharmacol 841:19–27. https://doi.org/10.1016/j.ejphar.2018.09.035

Karaca B, Kucukzeybek Y, Gorumlu G, et al (2008) Profiling of angiogenic cytokines produced by hormone- and drug-refractory prostate cancer cell lines, PC-3 and DU-145 before and after treatment with gossypol. European Cytokine Network 19:176–184. https://doi.org/10.1684/ecn.2008.0139

Karadag A, Ozcelik B, Saner S (2009) Review of Methods to Determine Antioxidant Capacities. Food Anal Methods 2:41–60. https://doi.org/10.1007/s12161-008-9067-7

Kardideh B, Samimi Z, Norooznezhad F, et al (2019) Autophagy, cancer and angiogenesis: where is the link? Cell Biosci 9:65. https://doi.org/10.1186/s13578-019-0327-6

Karimi M, Babaahmadi-Rezaei H, Mohammadzadeh G, ghaffari M-A (2017) Effect of Silibinin on Maspin and ERα Gene Expression in MCF-7 Human Breast Cancer Cell Line. Iran J Pathol 12:135–143

Karimzadeh P, Faghih Z, Rahmani N, et al (2020) Quantification of angiogenic factors and their clinicopathological associations in breast cancer. Eur Cytokine Netw 31:68–75. https://doi.org/10.1684/ecn.2020.0447

Karppanen H (1991) Minerals and Blood Pressure. Annals of Medicine 23:299–305. https://doi.org/10.3109/07853899109148064

Kaschula CH, Hunter R, Cotton J, et al (2016) The garlic compound ajoene targets protein folding in the endoplasmic reticulum of cancer cells. Molecular Carcinogenesis 55:1213–1228. https://doi.org/10.1002/mc.22364

Kaschula CH, Tuveri R, Ngarande E, et al (2019) The garlic compound ajoene covalently binds vimentin, disrupts the vimentin network and exerts anti-metastatic activity in cancer cells. BMC Cancer. https://doi.org/10.1186/s12885-019-5388-8

Kaur H, Moreau R (2021) Curcumin represses mTORC1 signaling in Caco-2 cells by a two-sided mechanism involving the loss of IRS-1 and activation of AMPK. Cellular Signalling 78:109842. https://doi.org/10.1016/j.cellsig.2020.109842

Kello M, Takac P, Kubatka P, et al (2020) Oxidative Stress-Induced DNA Damage and Apoptosis in Clove Buds-Treated MCF-7 Cells. Biomolecules 10:139. https://doi.org/10.3390/biom10010139

Kennedy DO, Wightman EL (2011) Herbal Extracts and Phytochemicals: Plant Secondary Metabolites and the Enhancement of Human Brain function. Advances in Nutrition 2:32–50. https://doi.org/10.3945/an.110.000117

Kessenbrock K, Plaks V, Werb Z (2010) Matrix Metalloproteinases: Regulators of the Tumor Microenvironment. Cell 141:52–67. https://doi.org/10.1016/j.cell.2010.03.015

Key TJ, Verkasalo PK, Banks E (2001) Epidemiology of breast cancer. The Lancet Oncology 2:133–140. https://doi.org/10.1016/S1470-2045(00)00254-0

Key TJA, Pike MC (1988) The role of oestrogens and progestagens in the epidemiology and prevention of breast cancer. European Journal of Cancer and Clinical Oncology 24:29–43. https://doi.org/10.1016/0277-5379(88)90173-3

Khalid N, Ahmed I, Latif MSZ, et al (2014) Comparison of antimicrobial activity, phytochemical profile and minerals composition of garlic Allium sativum and Allium tuberosum. J Korean Soc Appl Biol Chem 57:311–317. https://doi.org/10.1007/s13765-014-4021-4

Khoja KK, Shafi G, Hasan TN, et al Fenugreek, a Naturally Occurring Edible Spice, Kills MCF-7 Human Breast Cancer Cells via an Apoptotic Pathway. 6

Kiesel VA, Stan SD (2017) Diallyl trisulfide, a chemopreventive agent from Allium vegetables, inhibits alpha-secretases in breast cancer cells. Biochemical and Biophysical Research Communications 484:833–838. https://doi.org/10.1016/j.bbrc.2017.01.184

Kim HS, Moon H-G, Han W, et al (2012) COX2 overexpression is a prognostic marker for Stage III breast cancer. Breast Cancer Res Treat 132:51–59. https://doi.org/10.1007/s10549-011-1521-3

Kim J, Kundu M, Viollet B, Guan K-L (2011) AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol 13:132–141. https://doi.org/10.1038/ncb2152

Kim JE, Kim HS, Shin Y-J, et al (2008) LYR71, a derivative of trimeric resveratrol, inhibits tumorigenesis by blocking STAT3-mediated matrix metalloproteinase 9 expression. Exp Mol Med 40:514–522. https://doi.org/10.3858/emm.2008.40.5.514

Kim JY, Kwon O (2009) Garlic intake and cancer risk: an analysis using the Food and Drug Administration's evidence-based review system for the scientific evaluation of health claims. The American Journal of Clinical Nutrition 89:257–264. https://doi.org/10.3945/ajcn.2008.26142

Kim M-H, Jeong Y-J, Cho H-J, et al (2017) Delphinidin inhibits angiogenesis through the suppression of HIF-1α and VEGF expression in A549 lung cancer cells. Oncology Reports 37:777–784. https://doi.org/10.3892/or.2016.5296

Kim SR, Jung YR, An HJ, et al (2013) Anti-Wrinkle and Anti-Inflammatory Effects of Active Garlic Components and the Inhibition of MMPs via NF-κB Signaling. PLOS ONE 8:e73877. https://doi.org/10.1371/journal.pone.0073877

Kimura K (1996) [Role of essential trace elements in the disturbance of carbohydrate metabolism]. Nihon Rinsho 54:79–84

Kispert S, McHowat J (2017) Recent insights into cigarette smoking as a lifestyle risk factor for breast cancer. Breast Cancer (Dove Med Press) 9:127–132. https://doi.org/10.2147/BCTT.S129746

Klionsky DJ, Abeliovich H, Agostinis P, et al (2008) Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes. Autophagy 4:151–175. https://doi.org/10.4161/auto.5338

Knight JA, Fan J, Malone KE, et al (2017) Alcohol consumption and cigarette smoking in combination: A predictor of contralateral breast cancer risk in the WECARE study. International Journal of Cancer 141:916–924. https://doi.org/10.1002/ijc.30791

Kobayashi J, Antoccia A, Tauchi H, et al (2004) NBS1 and its functional role in the DNA damage response. DNA Repair 3:855–861. https://doi.org/10.1016/j.dnarep.2004.03.023

Koch AE, Polverini PJ, Kunkel SL, et al (1992) Interleukin-8 as a Macrophage-Derived Mediator of Angiogenesis. Science 258:1798–1801. https://doi.org/10.1126/science.1281554

Koch AE, Volin MV, Woods JM, et al (2001) Regulation of angiogenesis by the C-X-C chemokines interleukin-8 and epithelial neutrophil activating peptide 78 in the rheumatoid joint. Arthritis & Rheumatism 44:31–40. https://doi.org/10.1002/1529-0131(200101)44:1<31::AID-ANR5>3.0.CO;2-4

Kong D, Zhou H, Neelakantan D, et al (2021) VEGF-C mediates tumor growth and metastasis through promoting EMT-epithelial breast cancer cell crosstalk. Oncogene 40:964–979. https://doi.org/10.1038/s41388-020-01539-x

Kong S-Y, Kim K-S, Kim J, et al (2016) The ELK3-GATA3 axis orchestrates invasion and metastasis of breast cancer cells in vitro and in vivo. Oncotarget 7:65137–65146. https://doi.org/10.18632/oncotarget.11427

Kourounakis PN, Rekka EA (1991) Effect on active oxygen species of alliin and Allium sativum (garlic) powder. Res Commun Chem Pathol Pharmacol 74:249–252

Kronski E, Fiori ME, Barbieri O, et al (2014) miR181b is induced by the chemopreventive polyphenol curcumin and inhibits breast cancer metastasis via down-regulation of the inflammatory cytokines CXCL1 and -2. Molecular Oncology 8:581–595. https://doi.org/10.1016/j.molonc.2014.01.005

Kubli DA, Ycaza JE, Gustafsson ÅB (2007) Bnip3 mediates mitochondrial dysfunction and cell death through Bax and Bak. Biochemical Journal 405:407–415. https://doi.org/10.1042/BJ20070319

Kumar PS, Febriyanti RM, Sofyan FF, et al (2014) Anticancer potential of Syzygium aromaticum L. in MCF-7 human breast cancer cell lines. Pharmacognosy Res 6:350–354. https://doi.org/10.4103/0974-8490.138291

Kumaran A, Joel Karunakaran R (2007) In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT - Food Science and Technology 40:344–352. https://doi.org/10.1016/j.lwt.2005.09.011

Lacerda L, Debeb BG, Smith D, et al (2015) Mesenchymal stem cells mediate the clinical phenotype of inflammatory breast cancer in a preclinical model. Breast Cancer Res 17:42. https://doi.org/10.1186/s13058-015-0549-4

Lai K-C, Hsu S-C, Yang J-S, et al (2015) Diallyl trisulfide inhibits migration, invasion and angiogenesis of human colon cancer HT-29 cells and umbilical vein endothelial cells,

and suppresses murine xenograft tumour growth. Journal of Cellular and Molecular Medicine 19:474–484. https://doi.org/10.1111/jcmm.12486

Lang A, Lahav M, Sakhnini E, et al (2004) Allicin inhibits spontaneous and TNF-α induced secretion of proinflammatory cytokines and chemokines from intestinal epithelial cells. Clinical Nutrition 23:1199–1208. https://doi.org/10.1016/j.clnu.2004.03.011

Laparra JM, Sanz Y (2010) Interactions of gut microbiota with functional food components and nutraceuticals. Pharmacological Research 61:219–225. https://doi.org/10.1016/j.phrs.2009.11.001

Latz E, Xiao TS, Stutz A (2013) Activation and regulation of the inflammasomes. Nat Rev Immunol 13:397–411. https://doi.org/10.1038/nri3452

Lau A, Villeneuve NF, Sun Z, et al (2008) Dual roles of Nrf2 in cancer. Pharmacological Research 58:262–270. https://doi.org/10.1016/j.phrs.2008.09.003

Lawson LD, Hunsaker SM (2018) Allicin Bioavailability and Bioequivalence from Garlic Supplements and Garlic Foods. Nutrients 10:812. https://doi.org/10.3390/nu10070812

Layde PM, Webster LA, Baughman AL, et al (1989) The independent associations of parity, age at first full term pregnancy, and duration of breastfeeding with the risk of breast cancer. Journal of Clinical Epidemiology 42:963–973. https://doi.org/10.1016/0895-4356(89)90161-3

LeBleu VS, O'Connell JT, Gonzalez Herrera KN, et al (2014) PGC-1α mediates mitochondrial biogenesis and oxidative phosphorylation in cancer cells to promote metastasis. Nat Cell Biol 16:992–1003. https://doi.org/10.1038/ncb3039

Lee AS (2005) The ER chaperone and signaling regulator GRP78/BiP as a monitor of endoplasmic reticulum stress. Methods 35:373–381. https://doi.org/10.1016/j.ymeth.2004.10.010

Lee G-Y, Lee JJ, Lee S-M (2015) Antioxidant and Anticoagulant Status Were Improved by Personalized Dietary Intervention Based on Biochemical and Clinical Parameters in Cancer Patients. Nutrition and Cancer 67:1083–1092. https://doi.org/10.1080/01635581.2015.1073754

Lee J, Banu SK, Burghardt RC, et al (2013) Selective Inhibition of Prostaglandin E2 Receptors EP2 and EP4 Inhibits Adhesion of Human Endometriotic Epithelial and Stromal Cells Through Suppression of Integrin-Mediated Mechanisms1. Biology of Reproduction 88:77, 1–11. https://doi.org/10.1095/biolreprod.112.100883

Lee JS, Kim YJ, Kim CL, Lee GM (2012) Differential induction of autophagy in caspase-3/7 down-regulating and Bcl-2 overexpressing recombinant CHO cells subjected to sodium butyrate treatment. Journal of Biotechnology 161:34–41. https://doi.org/10.1016/j.jbiotec.2012.05.011

Lee Y, Shin H, Kim J (2021) In vivo Anti-Cancer Effects of Resveratrol Mediated by NK Cell Activation. JIN 13:94–106. https://doi.org/10.1159/000510315

Legewie S, Blüthgen N, Herzel H (2006) Mathematical Modeling Identifies Inhibitors of Apoptosis as Mediators of Positive Feedback and Bistability. PLOS Computational Biology 2:e120. https://doi.org/10.1371/journal.pcbi.0020120

Lei S, Zheng R, Zhang S, et al (2021) Global patterns of breast cancer incidence and mortality: A population-based cancer registry data analysis from 2000 to 2020. Cancer Communications 41:1183–1194. https://doi.org/10.1002/cac2.12207

Lei X, Yao S, Zu X, et al (2008) Apoptosis induced by diallyl disulfide in human breast cancer cell line MCF-71. Acta Pharmacologica Sinica 29:1233–1239. https://doi.org/10.1111/j.1745-7254.2008.00851.x

Leopoldini M, Russo N, Toscano M (2011) The molecular basis of working mechanism of natural polyphenolic antioxidants. Food Chemistry 125:288–306. https://doi.org/10.1016/j.foodchem.2010.08.012

Leunig A, Spaett R, Tauber S, et al (1997) [Fibroblast growth factor (b-FGF) in serum and urine of patients with head-neck malignancies]. Laryngorhinootologie 76:421–424. https://doi.org/10.1055/s-2007-997455

Lewandowska U, Szewczyk K, Owczarek K, et al (2013) Flavanols from Japanese Quince (Chaenomeles Japonica) Fruit Inhibit Human Prostate and Breast Cancer Cell Line Invasiveness and Cause Favorable Changes in Bax/Bcl-2 mRNA Ratio. Nutrition and Cancer 65:273–285. https://doi.org/10.1080/01635581.2013.749292

Li B, Takeda T, Tsuiji K, et al (2013) Curcumin induces cross-regulation between autophagy and apoptosis in uterine leiomyosarcoma cells. Int J Gynecol Cancer 23:803–808. https://doi.org/10.1097/IGC.0b013e31828c9581

Li F, Li C, Zhang H, et al (2012) VI-14, a novel flavonoid derivative, inhibits migration and invasion of human breast cancer cells. Toxicology and Applied Pharmacology 261:217–226. https://doi.org/10.1016/j.taap.2012.04.011

Li G, Qiao C, Lin R, et al (1995) ANTIPROLIFERATIVE EFFECTS OF GARLIC CONSTITUENTS IN CULTURED HUMAN BREAST-CANCER CELLS. Oncology Reports 2:787–791. https://doi.org/10.3892/or.2.5.787

LI H, LI H, WANG Y, et al An intervention study to prevent gastric cancer by micro-selenium and large dose of allitridum. Chinese Medical Journal 117:1155–1160. https://doi.org/10.3760/cma.j.issn.0366-6999.2004.08.106

Li M, Liu J, Li S, et al (2019) Autophagy-related 7 modulates tumor progression in triple-negative breast cancer. Lab Invest 99:1266–1274. https://doi.org/10.1038/s41374-019-0249-2

Li S, Yang G, Zhu X, et al (2017) Combination of rapamycin and garlic-derived S-allylmercaptocysteine induces colon cancer cell apoptosis and suppresses tumor growth in xenograft nude mice through autophagy/p62/Nrf2 pathway. Oncology Reports 38:1637–1644. https://doi.org/10.3892/or.2017.5849

Li W, He N, Tian L, et al (2016) Inhibitory effects of polyphenol-enriched extract from Ziyang tea against human breast cancer MCF-7 cells through reactive oxygen species-

dependent mitochondria molecular mechanism. Journal of Food and Drug Analysis 24:527–538. https://doi.org/10.1016/j.jfda.2016.01.005

Li W, Zhu S, Li J, et al (2011) EGCG stimulates autophagy and reduces cytoplasmic HMGB1 levels in endotoxin-stimulated macrophages. Biochemical Pharmacology 81:1152–1163. https://doi.org/10.1016/j.bcp.2011.02.015

Liang T, Zhang X, Xue W, et al (2014) Curcumin Induced Human Gastric Cancer BGC-823 Cells Apoptosis by ROS-Mediated ASK1-MKK4-JNK Stress Signaling Pathway. International Journal of Molecular Sciences 15:15754–15765. https://doi.org/10.3390/ijms150915754

Liang Y, Zhang H, Song X, Yang Q (2020) Metastatic heterogeneity of breast cancer: Molecular mechanism and potential therapeutic targets. Seminars in Cancer Biology 60:14–27. https://doi.org/10.1016/j.semcancer.2019.08.012

Liao C-L, Lai K-C, Huang A-C, et al (2012) Gallic acid inhibits migration and invasion in human osteosarcoma U-2 OS cells through suppressing the matrix metalloproteinase-2/-9, protein kinase B (PKB) and PKC signaling pathways. Food and Chemical Toxicology 50:1734–1740. https://doi.org/10.1016/j.fct.2012.02.033

Liekens S, De Clercq E, Neyts J (2001) Angiogenesis: regulators and clinical applications. Biochemical Pharmacology 61:253–270. https://doi.org/10.1016/S0006-2952(00)00529-3

Liemburg-Apers DC, Willems PHGM, Koopman WJH, Grefte S (2015) Interactions between mitochondrial reactive oxygen species and cellular glucose metabolism. Arch Toxicol 89:1209–1226. https://doi.org/10.1007/s00204-015-1520-y

Lin L, Hron JD, Peng SL (2004) Regulation of NF-κB, Th Activation, and Autoinflammation by the Forkhead Transcription Factor Foxo3a. Immunity 21:203–213. https://doi.org/10.1016/j.immuni.2004.06.016

Lin S-S, Lai K-C, Hsu S-C, et al (2009) Curcumin inhibits the migration and invasion of human A549 lung cancer cells through the inhibition of matrix metalloproteinase-2 and -9 and Vascular Endothelial Growth Factor (VEGF). Cancer Letters 285:127–133. https://doi.org/10.1016/j.canlet.2009.04.037

Lina N, Roy R, Ahamed Z, et al (2020) Relative Proximate Composition and Mineral Analysis of Three Garlic Varieties Available in Bangladesh. 31:1–9. https://doi.org/10.9734/EJMP/2020/v31i930265

Linardou H, Kalogeras KT, Kronenwett R, et al (2012) The prognostic and predictive value of mRNA expression of vascular endothelial growth factor family members in breast cancer: a study in primary tumors of high-risk early breast cancer patients participating in a randomized Hellenic Cooperative Oncology Group trial. Breast Cancer Res 14:R145. https://doi.org/10.1186/bcr3354

Linderholm B, Tavelin B, Grankvist K, Henriksson R (1999) Does vascular endothelial growth factor (VEGF) predict local relapse and survival in radiotherapy-treated node-negative breast cancer? Br J Cancer 81:727–732. https://doi.org/10.1038/sj.bjc.6690755

Liu Y, Zhu P, Wang Y, et al (2015) Antimetastatic Therapies of the Polysulfide Diallyl Trisulfide against Triple-Negative Breast Cancer (TNBC) via Suppressing MMP2/9 by Blocking NF-κB and ERK/MAPK Signaling Pathways. PLOS ONE 10:e0123781. https://doi.org/10.1371/journal.pone.0123781

Liyana-Pathirana CM, Shahidi F (2005) Antioxidant Activity of Commercial Soft and Hard Wheat (Triticum aestivum L.) as Affected by Gastric pH Conditions. J Agric Food Chem 53:2433–2440. https://doi.org/10.1021/jf049320i

Londhe V, Gavasane A, S.S N, et al (2011) Role of garlic (Allium sativum) in various diseases: An overview. JOURNAL OF PHARMACEUTICAL RESEARCH AND OPINION 1:129–134

Lorusso G, Rüegg C (2012) New insights into the mechanisms of organ-specific breast cancer metastasis. Seminars in Cancer Biology 22:226–233. https://doi.org/10.1016/j.semcancer.2012.03.007

Lou Y, Preobrazhenska O, auf dem Keller U, et al (2008) Epithelial–mesenchymal transition (EMT) is not sufficient for spontaneous murine breast cancer metastasis. Developmental Dynamics 237:2755–2768. https://doi.org/10.1002/dvdy.21658

Lu Y, Qin T, Li J, et al (2020) Correction: MicroRNA-140-5p inhibits invasion and angiogenesis through targeting VEGF-A in breast cancer. Cancer Gene Ther 27:838–839. https://doi.org/10.1038/s41417-020-00240-1

Lunetti P, Di Giacomo M, Vergara D, et al (2019) Metabolic reprogramming in breast cancer results in distinct mitochondrial bioenergetics between luminal and basal subtypes. The FEBS Journal 286:688–709. https://doi.org/10.1111/febs.14756

Lushnikova AA, Nasunova IB, Parokonnaya AA, et al (2010) VEGFR-2 expression in tumor tissue of breast cancer patients. Dokl Biol Sci 434:363–367. https://doi.org/10.1134/s0012496610050194

Lv Z-D, Liu X-P, Zhao W-J, et al (2014) Curcumin induces apoptosis in breast cancer cells and inhibits tumor growth in vitro and in vivo. Int J Clin Exp Pathol 7:2818–2824

Ma J-L, Zhang L, Brown LM, et al (2012) Fifteen-Year Effects of Helicobacter pylori, Garlic, and Vitamin Treatments on Gastric Cancer Incidence and Mortality. JNCI: Journal of the National Cancer Institute 104:488–492. https://doi.org/10.1093/jnci/djs003

Macaluso M, Montanari M, Cinti C, Giordano A (2005) Modulation of Cell Cycle Components by Epigenetic and Genetic Events. Seminars in Oncology 32:452–457. https://doi.org/10.1053/j.seminoncol.2005.07.009

Madu CO, Wang S, Madu CO, Lu Y (2020) Angiogenesis in Breast Cancer Progression, Diagnosis, and Treatment. J Cancer 11:4474–4494. https://doi.org/10.7150/jca.44313

Magalhães LM, Segundo MA, Reis S, Lima JLFC (2008) Methodological aspects about in vitro evaluation of antioxidant properties. Analytica Chimica Acta 613:1–19. https://doi.org/10.1016/j.aca.2008.02.047

Maharani MG, Lestari SR, Lukiati B (2020) Molecular docking studies flavonoid (quercetin, isoquercetin, and kaempferol) of single bulb garlic (Allium sativum) to inhibit lanosterol synthase as anti-hypercholesterol therapeutic strategies. AIP Conference Proceedings 2231:040021. https://doi.org/10.1063/5.0002531

Maheswari U, Ghosh K, Sadras SR (2018) Licarin A induces cell death by activation of autophagy and apoptosis in non-small cell lung cancer cells. Apoptosis 23:210–225. https://doi.org/10.1007/s10495-018-1449-8

Mahmoud MA, Okda TM, Omran GA, Abd-Alhaseeb MM (2021) Rosmarinic acid suppresses inflammation, angiogenesis, and improves paclitaxel induced apoptosis in a breast cancer model via NF3 κB-p53-caspase-3 pathways modulation. J Appl Biomed 19:202–209. https://doi.org/10.32725/jab.2021.024

Mahmoud SS, Torchilin VP (2013) Hormetic/Cytotoxic Effects of Nigella sativa Seed Alcoholic and Aqueous Extracts on MCF-7 Breast Cancer Cells Alone or in Combination with Doxorubicin. Cell Biochem Biophys 66:451–460. https://doi.org/10.1007/s12013-012-9493-4

Majumder M, Debnath S, Gajbhiye RL, et al (2019) Ricinus communis L. fruit extract inhibits migration/invasion, induces apoptosis in breast cancer cells and arrests tumor progression in vivo. Sci Rep 9:14493. https://doi.org/10.1038/s41598-019-50769-x

Makarem N, Chandran U, Bandera EV, Parekh N (2013) Dietary Fat in Breast Cancer Survival. Annual Review of Nutrition 33:319–348. https://doi.org/10.1146/annurev-nutr-112912-095300

Malki A, El-Saadani M, Sultan AS (2009) Garlic constituent diallyl trisulfide induced apoptosis in MCF7 human breast cancer cells. Cancer Biology & Therapy 8:2174–2184. https://doi.org/10.4161/cbt.8.22.9882

Mantovani A, Barajon I, Garlanda C (2018) IL-1 and IL-1 regulatory pathways in cancer progression and therapy. Immunological Reviews 281:57–61. https://doi.org/10.1111/imr.12614

Marjon PL, Bobrovnikova-Marjon EV, Abcouwer SF (2004) Expression of the pro-angiogenic factors vascular endothelial growth factor and interleukin-8/CXCL8 by human breast carcinomas is responsive to nutrient deprivation and endoplasmic reticulum stress. Molecular Cancer 3:4. https://doi.org/10.1186/1476-4598-3-4

Markopoulos C (2013) Overview of the use of Oncotype DX® as an additional treatment decision tool in early breast cancer. Expert Review of Anticancer Therapy 13:179–194. https://doi.org/10.1586/era.12.174

Marques da Fonseca L, Jacques da Silva LR, Santos dos Reis J, et al (2020) Piperine Inhibits TGF-β Signaling Pathways and Disrupts EMT-Related Events in Human Lung Adenocarcinoma Cells. Medicines 7:19. https://doi.org/10.3390/medicines7040019

Martin D, Galisteo R, Gutkind JS (2009) CXCL8/IL8 Stimulates Vascular Endothelial Growth Factor (VEGF) Expression and the Autocrine Activation of VEGFR2 in Endothelial Cells by Activating NFκB through the CBM (Carma3/Bcl10/Malt1)

Complex *. Journal of Biological Chemistry 284:6038–6042. https://doi.org/10.1074/jbc.C800207200

Martínez N, Herrera M, Frías L, et al (2019) A combination of hydroxytyrosol, omega-3 fatty acids and curcumin improves pain and inflammation among early stage breast cancer patients receiving adjuvant hormonal therapy: results of a pilot study. Clin Transl Oncol 21:489–498. https://doi.org/10.1007/s12094-018-1950-0

Martino E, Vuoso DC, D'Angelo S, et al (2019) Annurca apple polyphenol extract selectively kills MDA-MB-231 cells through ROS generation, sustained JNK activation and cell growth and survival inhibition. Sci Rep 9:13045. https://doi.org/10.1038/s41598-019-49631-x

Masuelli L, Benvenuto M, Fantini M, et al (2013) Curcumin induces apoptosis in breast cancer cell lines and delays the growth of mammary tumors in neu transgenic mice. J Biol Regul Homeost Agents 27:105–119

Mbaveng AT, Wamba BEN, Bitchagno GTM, et al (2021) Bioactivity of fractions and constituents of Piper capense fruits towards a broad panel of cancer cells. Journal of Ethnopharmacology 271:113884. https://doi.org/10.1016/j.jep.2021.113884

McCaskill ML, Rogan E, Thomas RD (2014) Diallyl sulfide inhibits diethylstilbestrol induced DNA damage in human breast epithelial cells (MCF-10A). Steroids 92:96–100. https://doi.org/10.1016/j.steroids.2014.09.005

McDougall GJ, Kulkarni NN, Stewart D (2009) Berry polyphenols inhibit pancreatic lipase activity in vitro. Food Chemistry 115:193–199. https://doi.org/10.1016/j.foodchem.2008.11.093

Mego M, Mani SA, Cristofanilli M (2010) Molecular mechanisms of metastasis in breast cancer—clinical applications. Nat Rev Clin Oncol 7:693–701. https://doi.org/10.1038/nrclinonc.2010.171

Mendelsohn J, Baselga J (2003) Status of Epidermal Growth Factor Receptor Antagonists in the Biology and Treatment of Cancer. JCO 21:2787–2799. https://doi.org/10.1200/JCO.2003.01.504

Miller KD, Nogueira L, Mariotto AB, et al (2019) Cancer treatment and survivorship statistics, 2019. CA: A Cancer Journal for Clinicians 69:363–385. https://doi.org/10.3322/caac.21565

Miraghajani M, Rafie N, Hajianfar H, et al (2018) Aged Garlic and Cancer: A Systematic Review. Int J Prev Med 9:84. https://doi.org/10.4103/ijpvm.IJPVM_437_17

Mitchell L, Hobbs GA, Aghajanian A, Campbell SL (2013) Redox Regulation of Ras and Rho GTPases: Mechanism and Function. Antioxidants & Redox Signaling 18:250–258. https://doi.org/10.1089/ars.2012.4687

Mizushima N, Komatsu M (2011) Autophagy: Renovation of Cells and Tissues. Cell 147:728–741. https://doi.org/10.1016/j.cell.2011.10.026

Modem S, DiCarlo SE, Reddy TR (2012) Fresh Garlic Extract Induces Growth Arrest and Morphological Differentiation of MCF7 Breast Cancer Cells. Genes & Cancer 3:177–186. https://doi.org/10.1177/1947601912458581

Moghtaderi H, Sepehri H, Delphi L, Attari F (2018) Gallic acid and curcumin induce cytotoxicity and apoptosis in human breast cancer cell MDA-MB-231. Bioimpacts 8:185–194. https://doi.org/10.15171/bi.2018.21

Mohme M, Riethdorf S, Pantel K (2017) Circulating and disseminated tumour cells — mechanisms of immune surveillance and escape. Nat Rev Clin Oncol 14:155–167. https://doi.org/10.1038/nrclinonc.2016.144

Montaño A, Beato VM, Mansilla F, Orgaz F (2011) Effect of Genetic Characteristics and Environmental Factors on Organosulfur Compounds in Garlic (Allium sativum L.) Grown in Andalusia, Spain. J Agric Food Chem 59:1301–1307. https://doi.org/10.1021/jf104494j

Moreno-Sánchez R, Rodríguez-Enríquez S, Marín-Hernández A, Saavedra E (2007) Energy metabolism in tumor cells. The FEBS Journal 274:1393–1418. https://doi.org/10.1111/j.1742-4658.2007.05686.x

Mousa AS, Mousa SA (2005) Anti-Angiogenesis Efficacy of the Garlic Ingredient Alliin and Antioxidants: Role of Nitric Oxide and p53. Nutrition and Cancer 53:104–110. https://doi.org/10.1207/s15327914nc5301_12

Mousa S, Mousa SA (2006) Cellular and molecular mechanisms of nicotine's pro-angiogenesis activity and its potential impact on cancer. Journal of Cellular Biochemistry 97:1370–1378. https://doi.org/10.1002/jcb.20741

Mousavi SH, Tavakkol-Afshari J, Brook A, Jafari-Anarkooli I (2009) Role of caspases and Bax protein in saffron-induced apoptosis in MCF-7 cells. Food and Chemical Toxicology 47:1909–1913. https://doi.org/10.1016/j.fct.2009.05.017

Murkovic M, Adam U, Pfannhauser W (2000) Analysis of anthocyane glycosides in human serum. Fresenius J Anal Chem 366:379–381. https://doi.org/10.1007/s002160050077

Nagae S, Ushijima M, Hatono S, et al (1994) Pharmacokinetics of the Garlic Compound S-Allylcysteine. Planta Med 60:214–217. https://doi.org/10.1055/s-2006-959461

NAVARRO GARCIA G, SIMÓN NG (2013) Química agrícola: química del suelo y de los nutrientes esenciales para las plantas. Mundi-Prensa Libros

Nencini C, Menchiari A, Franchi GG, Micheli L (2011) In vitro Antioxidant Activity of Aged Extracts of some Italian Allium Species. Plant Foods Hum Nutr 66:11–16. https://doi.org/10.1007/s11130-010-0204-2

Ng WK, Yazan LS, Ismail M (2011) Thymoquinone from Nigella sativa was more potent than cisplatin in eliminating of SiHa cells via apoptosis with down-regulation of Bcl-2 protein. Toxicology in Vitro 25:1392–1398. https://doi.org/10.1016/j.tiv.2011.04.030

Ngo SNT, Williams DB, Cobiac L, Head RJ (2007) Does Garlic Reduce Risk of Colorectal Cancer? A Systematic Review. The Journal of Nutrition 137:2264–2269. https://doi.org/10.1093/jn/137.10.2264

Nguyen C, Baskaran K, Pupulin A, et al (2019) Hibiscus flower extract selectively induces apoptosis in breast cancer cells and positively interacts with common chemotherapeutics. BMC Complementary and Alternative Medicine 19:98. https://doi.org/10.1186/s12906-019-2505-9

Nguyen DX, Bos PD, Massagué J (2009) Metastasis: from dissemination to organ-specific colonization. Nat Rev Cancer 9:274–284. https://doi.org/10.1038/nrc2622

Nilsson UW, Abrahamsson A, Dabrosin C (2010) Angiogenin Regulation by Estradiol in Breast Tissue: Tamoxifen Inhibits Angiogenin Nuclear Translocation and Antiangiogenin Therapy Reduces Breast Cancer Growth In vivo. Clinical Cancer Research 16:3659–3669. https://doi.org/10.1158/1078-0432.CCR-10-0501

Nioi P, Nguyen T (2007) A mutation of Keap1 found in breast cancer impairs its ability to repress Nrf2 activity. Biochemical and Biophysical Research Communications 362:816–821. https://doi.org/10.1016/j.bbrc.2007.08.051

Nishitoh H (2012) CHOP is a multifunctional transcription factor in the ER stress response. The Journal of Biochemistry 151:217–219. https://doi.org/10.1093/jb/mvr143

Nogueira V, Hay N (2013) Molecular Pathways: Reactive Oxygen Species Homeostasis in Cancer Cells and Implications for Cancer Therapy. Clinical Cancer Research 19:4309–4314. https://doi.org/10.1158/1078-0432.CCR-12-1424

Norgauer J, Metzner B, Schraufstätter I (1996) Expression and growth-promoting function of the IL-8 receptor beta in human melanoma cells. The Journal of Immunology 156:1132–1137

Noy R, Pollard JW (2014) Tumor-Associated Macrophages: From Mechanisms to Therapy. Immunity 41:49–61. https://doi.org/10.1016/j.immuni.2014.06.010

Nutakul W, Sobers HS, Qiu P, et al (2011) Inhibitory Effects of Resveratrol and Pterostilbene on Human Colon Cancer Cells: A Side-by-Side Comparison. J Agric Food Chem 59:10964–10970. https://doi.org/10.1021/jf202846b

Nuutila AM, Puupponen-Pimiä R, Aarni M, Oksman-Caldentey K-M (2003a) Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. Food Chemistry 81:485–493. https://doi.org/10.1016/S0308-8146(02)00476-4

Nuutila AM, Puupponen-Pimiä R, Aarni M, Oksman-Caldentey K-M (2003b) Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. Food Chemistry 81:485–493. https://doi.org/10.1016/S0308-8146(02)00476-4

Ogrodzinski MP, Bernard JJ, Lunt SY (2017) Deciphering metabolic rewiring in breast cancer subtypes. Translational Research 189:105–122. https://doi.org/10.1016/j.trsl.2017.07.004

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry 95:351–358. https://doi.org/10.1016/0003-2697(79)90738-3

Ohkubo S, Dalla Via L, Grancara S, et al (2018) The antioxidant, aged garlic extract, exerts cytotoxic effects on wild-type and multidrug-resistant human cancer cells by altering mitochondrial permeability. International Journal of Oncology 53:1257–1268. https://doi.org/10.3892/ijo.2018.4452

Okuma E, Saeki K, Shimura M, et al (2000) Induction of apoptosis in human hematopoietic U937 cells by granulocyte–macrophage colony-stimulating factor: possible existence of caspase 3-like pathway. Leukemia 14:612–619. https://doi.org/10.1038/sj.leu.2401716

Okwu DE, Omodamiro OD (2005) Effects of hexane extract and phytochemical content of Xylopia aethiopica and Ocimum gratissimum on the uterus of guinea pig. Bio-Research 3:40–44. https://doi.org/10.4314/br.v3i2.28589

Oloyede OI (2005) Chemical profile of unripe pulp of carica papaya. Pak J Nutr 379–381

Olson KA, Fett JW, French TC, et al (1995) Angiogenin antagonists prevent tumor growth in vivo. Proceedings of the National Academy of Sciences 92:442–446. https://doi.org/10.1073/pnas.92.2.442

Orlowski RZ (1999) The role of the ubiquitin-proteasome pathway in apoptosis. Cell Death Differ 6:303–313. https://doi.org/10.1038/sj.cdd.4400505

Orozco-Morales M, Hernández-Pedro NY, Barrios-Bernal P, et al (2021) S-allylcysteine induces cytotoxic effects in two human lung cancer cell lines via induction of oxidative damage, downregulation of Nrf2 and NF-κB, and apoptosis. Anti-Cancer Drugs 32:117–126. https://doi.org/10.1097/CAD.0000000000001015

Osellame LD, Blacker TS, Duchen MR (2012) Cellular and molecular mechanisms of mitochondrial function. Best Practice & Research Clinical Endocrinology & Metabolism 26:711–723. https://doi.org/10.1016/j.beem.2012.05.003

Otunola GA, Oloyede OB, Oladiji AT, Afolayan AJ (2010) Comparative analysis of the chemical composition of three spices – Allium sativum L. Zingiber officinale Rosc. and Capsicum frutescens L. commonly consumed in Nigeria. African Journal of Biotechnology 9:6927–6931. https://doi.org/10.4314/ajb.v9i41

Ovadje P, Ammar S, Guerrero J-A, et al (2016) Dandelion root extract affects colorectal cancer proliferation and survival through the activation of multiple death signalling pathways. Oncotarget 7:73080–73100. https://doi.org/10.18632/oncotarget.11485

Ozpolat B, Akar U, Mehta K, Lopez-Berestein G (2007) PKCδ and Tissue Transglutaminase are Novel Inhibitors of Autophagy in Pancreatic Cancer Cells. Autophagy 3:480–483. https://doi.org/10.4161/auto.4349

Pan L, Duan Y, Ma F, Lou L (2020) Punicalagin inhibits the viability, migration, invasion, and EMT by regulating GOLPH3 in breast cancer cells. Journal of Receptors and Signal Transduction 40:173–180. https://doi.org/10.1080/10799893.2020.1719152

Pan Y, Lin S, Xing R, et al (2016) Epigenetic Upregulation of Metallothionein 2A by Diallyl Trisulfide Enhances Chemosensitivity of Human Gastric Cancer Cells to Docetaxel Through Attenuating NF-κB Activation. Antioxidants & Redox Signaling 24:839–854. https://doi.org/10.1089/ars.2014.6128

Pandey N, Tyagi G, Kaur P, et al (2020) Allicin Overcomes Hypoxia Mediated Cisplatin Resistance in Lung Cancer Cells through ROS Mediated Cell Death Pathway and by Suppressing Hypoxia Inducible Factors. Cell Physiol Biochem 54:748–766. https://doi.org/10.33594/000000253

Pantel K, Speicher MR (2016) The biology of circulating tumor cells. Oncogene 35:1216–1224. https://doi.org/10.1038/onc.2015.192

Papetti M, Herman IM (2002) Mechanisms of normal and tumor-derived angiogenesis. American Journal of Physiology-Cell Physiology 282:C947–C970. https://doi.org/10.1152/ajpcell.00389.2001

Pappa CA, Tsirakis G, Devetzoglou M, et al (2014) Bone marrow mast cell density correlates with serum levels of VEGF and CXC chemokines ENA-78 and GRO-α in multiple myeloma. Tumor Biol 35:5647–5651. https://doi.org/10.1007/s13277-014-1747-x

Park HS, Kim G-Y, Choi I-W, et al (2011) Inhibition of Matrix Metalloproteinase Activities and Tightening of Tight Junctions by Diallyl Disulfide in AGS Human Gastric Carcinoma Cells. Journal of Food Science 76:T105–T111. https://doi.org/10.1111/j.1750-3841.2011.02114.x

Park J-H, Kim KP, Ko J-J, Park K-S (2016a) PI3K/Akt/mTOR activation by suppression of ELK3 mediates chemosensitivity of MDA-MB-231 cells to doxorubicin by inhibiting autophagy. Biochemical and Biophysical Research Communications 477:277–282. https://doi.org/10.1016/j.bbrc.2016.06.057

Park JY, Shin MS, Kim SN, et al (2016b) Polysaccharides from Korean Citrus hallabong peels inhibit angiogenesis and breast cancer cell migration. International Journal of Biological Macromolecules 85:522–529. https://doi.org/10.1016/j.ijbiomac.2016.01.015

Parr C, Watkins G, Boulton M, et al (2005) Placenta growth factor is over-expressed and has prognostic value in human breast cancer. European Journal of Cancer 41:2819–2827. https://doi.org/10.1016/j.ejca.2005.07.022

Parzych KR, Klionsky DJ (2014) An Overview of Autophagy: Morphology, Mechanism, and Regulation. Antioxidants & Redox Signaling 20:460–473. https://doi.org/10.1089/ars.2013.5371

Pastò A, Bellio C, Pilotto G, et al (2014) Cancer stem cells from epithelial ovarian cancer patients privilege oxidative phosphorylation, and resist glucose deprivation. Oncotarget 5:4305–4319

Patel A, Soni A, Siddiqi NJ, Sharma P (2019) An insight into the anticancer mechanism of Tribulus terrestris extracts on human breast cancer cells. 3 Biotech 9:58. https://doi.org/10.1007/s13205-019-1585-z

Patya M, Zahalka MA, Vanichkin A, et al (2004) Allicin stimulates lymphocytes and elicits an antitumor effect: a possible role of p21ras. International Immunology 16:275–281. https://doi.org/10.1093/intimm/dxh038

Paulsen CE, Carroll KS (2013) Cysteine-Mediated Redox Signaling: Chemistry, Biology, and Tools for Discovery. Chem Rev 113:4633–4679. https://doi.org/10.1021/cr300163e

Pavlov N, Badet J (2001) [Angiogenin: involvement in angiogenesis and tumour growth]. Bull Cancer 88:725–732

Peng L, Liu A, Shen Y, et al (2013) Antitumor and anti-angiogenesis effects of thymoquinone on osteosarcoma through the NF-κB pathway. Oncology Reports 29:571–578. https://doi.org/10.3892/or.2012.2165

Peng Y-C, Wang S-R, Lai Y-F, et al (2021) Isoamylamine Induces B16-F1 Melanoma Cell Autophagy by Upregulating the 5′ Adenosine Monophosphate-Activated Protein Pathway. Journal of Medicinal Food 24:188–196. https://doi.org/10.1089/jmf.2020.4777

Pengo N, Agrotis A, Prak K, et al (2017) A reversible phospho-switch mediated by ULK1 regulates the activity of autophagy protease ATG4B. Nat Commun 8:294. https://doi.org/10.1038/s41467-017-00303-2

Pepper MS, Ferrara N, Orci L, Montesano R (1992) Potent synergism between vascular endothelial growth factor and basic fibroblast growth factor in the induction of angiogenesis in vitro. Biochemical and Biophysical Research Communications 189:824–831. https://doi.org/10.1016/0006-291X(92)92277-5

Pérez-Jiménez J, Saura-Calixto F (2005) Literature Data May Underestimate the Actual Antioxidant Capacity of Cereals. J Agric Food Chem 53:5036–5040. https://doi.org/10.1021/jf050049u

Pérez-Vicente A, Gil-Izquierdo A, García-Viguera C (2002) In Vitro Gastrointestinal Digestion Study of Pomegranate Juice Phenolic Compounds, Anthocyanins, and Vitamin C. J Agric Food Chem 50:2308–2312. https://doi.org/10.1021/jf0113833

Perou CM, Sørlie T, Eisen MB, et al (2000) Molecular portraits of human breast tumours. Nature 406:747–752. https://doi.org/10.1038/35021093

Petropoulos S, Fernandes Â, Barros L, Ferreira ICFR (2018) Chemical composition, nutritional value and antioxidant properties of Mediterranean okra genotypes in relation to harvest stage. Food Chemistry 242:466–474. https://doi.org/10.1016/j.foodchem.2017.09.082

Petrovic V, Nepal A, Olaisen C, et al (2018) Anti-Cancer Potential of Homemade Fresh Garlic Extract Is Related to Increased Endoplasmic Reticulum Stress. Nutrients 10:450. https://doi.org/10.3390/nu10040450

Phan ADT, Netzel G, Chhim P, et al (2019) Phytochemical Characteristics and Antimicrobial Activity of Australian Grown Garlic (Allium Sativum L.) Cultivars. Foods 8:358. https://doi.org/10.3390/foods8090358

Phang C-W, Karsani SA, Sethi G, Malek SNA (2016) Flavokawain C Inhibits Cell Cycle and Promotes Apoptosis, Associated with Endoplasmic Reticulum Stress and Regulation of MAPKs and Akt Signaling Pathways in HCT 116 Human Colon Carcinoma Cells. PLOS ONE 11:e0148775. https://doi.org/10.1371/journal.pone.0148775

Pharoah PDP, Day NE, Duffy S, et al (1997) Family history and the risk of breast cancer: A systematic review and meta-analysis. International Journal of Cancer 71:800–809. https://doi.org/10.1002/(SICI)1097-0215(19970529)71:5<800::AID-IJC18>3.0.CO;2-B

Philion C, Ma D, Ruvinov I, et al (2017) Cymbopogon citratus and Camellia sinensis extracts selectively induce apoptosis in cancer cells and reduce growth of lymphoma xenografts in vivo. Oncotarget 8:110756–110773. https://doi.org/10.18632/oncotarget.22502

Polyak K (2007) Breast cancer: origins and evolution. J Clin Invest 117:3155–3163. https://doi.org/10.1172/JCI33295

Prakash D, Singh BN, Upadhyay G (2007) Antioxidant and free radical scavenging activities of phenols from onion (Allium cepa). Food Chemistry 102:1389–1393. https://doi.org/10.1016/j.foodchem.2006.06.063

Pratt J, Roy R, Annabi B (2012) Concanavalin-A-induced autophagy biomarkers requires membrane type-1 matrix metalloproteinase intracellular signaling in glioblastoma cells. Glycobiology 22:1245–1255. https://doi.org/10.1093/glycob/cws093

Puissant A, Fenouille N, Auberger P (2012) When autophagy meets cancer through p62/SQSTM1. Am J Cancer Res 2:397–413

Qadir MA, Kwok B, Dragowska WH, et al (2008) Macroautophagy inhibition sensitizes tamoxifen-resistant breast cancer cells and enhances mitochondrial depolarization. Breast Cancer Res Treat 112:389–403. https://doi.org/10.1007/s10549-007-9873-4

Qi JH, Ebrahem Q, Moore N, et al (2003) A novel function for tissue inhibitor of metalloproteinases-3 (TIMP3): inhibition of angiogenesis by blockage of VEGF binding to VEGF receptor-2. Nat Med 9:407–415. https://doi.org/10.1038/nm846

Qin X-Y, Su T, Kojima S (2020) Prevention of arachidonic acid-induced liver injury by controlling oxidative stress-mediated transglutaminase activation with garlic extracts. Exp Ther Med 19:1522–1527. https://doi.org/10.3892/etm.2019.8384

Rabinkov A, Miron T, Mirelman D, et al (2000) S-Allylmercaptoglutathione: the reaction product of allicin with glutathione possesses SH-modifying and antioxidant properties. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 1499:144–153. https://doi.org/10.1016/S0167-4889(00)00119-1

Rakha EA, El-Sayed ME, Green AR, et al (2007) Prognostic markers in triple-negative breast cancer. Cancer 109:25–32. https://doi.org/10.1002/cncr.22381

Rana SV, Pal R, Vaiphei K, et al (2011) Garlic in health and disease. Nutrition Research Reviews 24:60–71. https://doi.org/10.1017/S0954422410000338

Rauf A, Abu-Izneid T, Thiruvengadam M, et al (2021) Garlic (Allium sativum L.): Its Chemistry, Nutritional Composition, Toxicity and Anticancer Properties. Curr Top Med Chem. https://doi.org/10.2174/1568026621666211105094939

Ravindranath N, Wion D, Brachet P, Djakiew D (2001) Epidermal Growth Factor Modulates the Expression of Vascular Endothelial Growth Factor in the Human Prostate. Journal of Andrology 22:432–443. https://doi.org/10.1002/j.1939-4640.2001.tb02199.x

Razmkhah M, Jaberipour M, Hosseini A, et al (2010) Expression profile of IL-8 and growth factors in breast cancer cells and adipose-derived stem cells (ASCs) isolated from breast carcinoma. Cellular Immunology 265:80–85. https://doi.org/10.1016/j.cellimm.2010.07.006

Re R, Pellegrini N, Proteggente A, et al (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26:1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Reboredo-Rodríguez P, González-Barreiro C, Cancho-Grande B, et al (2018) Effect of pistachio kernel extracts in MCF-7 breast cancer cells: Inhibition of cell proliferation, induction of ROS production, modulation of glycolysis and of mitochondrial respiration. Journal of Functional Foods 45:155–164. https://doi.org/10.1016/j.jff.2018.03.045

Reger de Moura C, Battistella M, Sohail A, et al (2019) Discoidin domain receptors: A promising target in melanoma. Pigment Cell & Melanoma Research 32:697–707. https://doi.org/10.1111/pcmr.12809

Reis-Filho JS, Tutt ANJ (2008) Triple negative tumours: a critical review. Histopathology 52:108–118. https://doi.org/10.1111/j.1365-2559.2007.02889.x

Relf M, LeJeune S, Scott PAE, et al (1997) Expression of the Angiogenic Factors Vascular Endothelial Cell Growth Factor, Acidic and Basic Fibroblast Growth Factor, Tumor Growth Factor β-1, Platelet-derived Endothelial Cell Growth Factor, Placenta Growth Factor, and Pleiotrophin in Human Primary Breast Cancer and Its Relation to Angiogenesis1. Cancer Research 57:963–969

Remillard TC, Bratslavsky G, Jensen-Taubman S, et al (2014) Molecular mechanisms of tissue inhibitor of metalloproteinase 2 in the tumor microenvironment. Mol and Cell Ther 2:17. https://doi.org/10.1186/2052-8426-2-17

Reynolds LP, Redmer DA (2001) Angiogenesis in the Placenta1. Biology of Reproduction 64:1033–1040. https://doi.org/10.1095/biolreprod64.4.1033

Ribas V, García-Ruiz C, Fernández-Checa JC (2014) Glutathione and mitochondria. Frontiers in Pharmacology 5:

Rice-Evans CA, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine 20:933–956. https://doi.org/10.1016/0891-5849(95)02227-9

Ristimäki A, Sivula A, Lundin J, et al (2002) Prognostic Significance of Elevated Cyclooxygenase-2 Expression in Breast Cancer1. Cancer Research 62:632–635

Ritota M, Casciani L, Han B-Z, et al (2012) Traceability of Italian garlic (Allium sativum L.) by means of HRMAS-NMR spectroscopy and multivariate data analysis. Food Chemistry 135:684–693. https://doi.org/10.1016/j.foodchem.2012.05.032

Rivlin RS (2009) Can garlic reduce risk of cancer?123. Am J Clin Nutr 89:17–18. https://doi.org/10.3945/ajcn.2008.27181

Robert G, Ben Sahra I, Puissant A, et al (2009) Acadesine Kills Chronic Myelogenous Leukemia (CML) Cells through PKC-Dependent Induction of Autophagic Cell Death. PLoS One 4:e7889. https://doi.org/10.1371/journal.pone.0007889

Rochín-Wong CS, Gámez-Meza N, Montoya-Ballesteros LC, Medina-Juárez LA (2013) Effect of drying and pickling processes on antioxidant capacity of phytochemicals of chiltepin (Capsicum annuum L. var. glabriusculum). Revista mexicana de ingeniería química 12:227–239

Rossi EL, Khatib SA, Doerstling SS, et al (2018) Resveratrol inhibits obesity-associated adipose tissue dysfunction and tumor growth in a mouse model of postmenopausal claudin-low breast cancer. Molecular Carcinogenesis 57:393–407. https://doi.org/10.1002/mc.22763

Rozeboom B, Dey N, De P (2019) ER+ metastatic breast cancer: past, present, and a prescription for an apoptosis-targeted future. Am J Cancer Res 9:2821–2831

Russo J, Hu Y-F, Yang X, Russo IH (2000) Chapter 1: Developmental, Cellular, and Molecular Basis of Human Breast Cancer. JNCI Monographs 2000:17–37. https://doi.org/10.1093/oxfordjournals.jncimonographs.a024241

Ryan L, Prescott SL (2010) Stability of the antioxidant capacity of twenty-five commercially available fruit juices subjected to an in vitro digestion. International Journal of Food Science & Technology 45:1191–1197. https://doi.org/10.1111/j.1365-2621.2010.02254.x

Sachinidis A, Seul C, Seewald S, et al (2000) Green tea compounds inhibit tyrosine phosphorylation of PDGF β-receptor and transformation of A172 human glioblastoma. FEBS Letters 471:51–55. https://doi.org/10.1016/S0014-5793(00)01360-0

Sadot E, Lee SY, Sofocleous CT, et al (2016) Hepatic resection or ablation for isolated breast cancer liver metastasis: A case-control study with comparison to medically treated patients. Ann Surg 264:147–154. https://doi.org/10.1097/SLA.0000000000001371

Saha T, Rih JK, Rosen EM (2009) BRCA1 down-regulates cellular levels of reactive oxygen species. FEBS Letters 583:1535–1543. https://doi.org/10.1016/j.febslet.2009.04.005

Sajid M, Butt M, Shehzad A, Tanweer S (2014) Chemical and mineral analysis of garlic: a golden herb. 24:108–110

Sakata R, Ueno T, Nakamura T, et al (2004) Green tea polyphenol epigallocatechin-3-gallate inhibits platelet-derived growth factor-induced proliferation of human hepatic stellate cell line LI90. Journal of Hepatology 40:52–59. https://doi.org/10.1016/S0168-8278(03)00477-X

Salminen A, Kaarniranta K, Kauppinen A (2013) Beclin 1 interactome controls the crosstalk between apoptosis, autophagy and inflammasome activation: Impact on the aging process. Ageing Research Reviews 12:520–534. https://doi.org/10.1016/j.arr.2012.11.004

Sano R, Reed JC (2013) ER stress-induced cell death mechanisms. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 1833:3460–3470. https://doi.org/10.1016/j.bbamcr.2013.06.028

Santandreu FM, Valle A, Oliver J, Roca P (2011) Resveratrol Potentiates the Cytotoxic Oxidative Stress Induced by Chemotherapy in Human Colon Cancer Cells. CPB 28:219–228. https://doi.org/10.1159/000331733

Sarsour EH, Goswami M, Kalen AL, et al (2014) Hydroxytyrosol inhibits chemokine C-C motif ligand 5 mediated aged quiescent fibroblast-induced stimulation of breast cancer cell proliferation. AGE 36:9645. https://doi.org/10.1007/s11357-014-9645-0

Sato N, Nariuchi H, Tsuruoka N, et al (1990) Actions of TNF and INF-γ on Angiogenesis In Vitro. Journal of Investigative Dermatology 95:S85–S89. https://doi.org/10.1111/1523-1747.ep12874809

Sauer SJ, Tarpley M, Shah I, et al (2017) Bisphenol A activates EGFR and ERK promoting proliferation, tumor spheroid formation and resistance to EGFR pathway inhibition in estrogen receptor-negative inflammatory breast cancer cells. Carcinogenesis 38:bgx003. https://doi.org/10.1093/carcin/bgx003

Saura-Calixto F, Serrano J, Goñi I (2007) Intake and bioaccessibility of total polyphenols in a whole diet. Food Chemistry 101:492–501. https://doi.org/10.1016/j.foodchem.2006.02.006

Scarlatti F, Maffei R, Beau I, et al (2008) Non-canonical autophagy: An exception or an underestimated form of autophagy? Autophagy 4:1083–1085. https://doi.org/10.4161/auto.7068

Schiavano GF, Santi MD, Brandi G, et al (2015) Inhibition of Breast Cancer Cell Proliferation and In Vitro Tumorigenesis by a New Red Apple Cultivar. PLOS ONE 10:e0135840. https://doi.org/10.1371/journal.pone.0135840

Schmidt T, Ben-Batalla I, Schultze A, Loges S (2012) Macrophage–tumor crosstalk: role of TAMR tyrosine kinase receptors and of their ligands. Cell Mol Life Sci 69:1391–1414. https://doi.org/10.1007/s00018-011-0863-7

Schnitt SJ, Silen W, Sadowsky NL, et al (2010) Ductal Carcinoma in Situ (Intraductal Carcinoma) of the Breast. In: http://dx.doi.org/10.1056/NEJM198804073181406. https://www.nejm.org/doi/pdf/10.1056/NEJM198804073181406. Accessed 17 Feb 2022

Scully OJ, Bay B-H, Yip G, Yu Y (2012) Breast Cancer Metastasis. Cancer Genomics & Proteomics 9:311–320

Seraglio SKT, Valese AC, Daguer H, et al (2016) Development and validation of a LC-ESI-MS/MS method for the determination of phenolic compounds in honeydew honeys

with the diluted-and-shoot approach. Food Research International 87:60–67. https://doi.org/10.1016/j.foodres.2016.06.019

Setiawan VW, Yu G-P, Lu Q-Y, et al (2005) Allium Vegetables and Stomach Cancer Risk in China. Asian Pac J Cancer Prev 6:387–395

Sevrioukova IF (2011) Apoptosis-Inducing Factor: Structure, Function, and Redox Regulation. Antioxidants & Redox Signaling 14:2545–2579. https://doi.org/10.1089/ars.2010.3445

Sgroi DC (2010) Preinvasive Breast Cancer. Annual Review of Pathology: Mechanisms of Disease 5:193–221. https://doi.org/10.1146/annurev.pathol.4.110807.092306

Shakya G, Balasubramanian S, Hoda M, Rajagopalan R (2018) Inhibition of metastasis and angiogenesis in Hep-2 cells by wheatgrass extract – an in vitro and in silico approach. Toxicology Mechanisms and Methods 28:205–218. https://doi.org/10.1080/15376516.2017.1388460

Shankar S, Srivastava RK (2007) Involvement of Bcl-2 family members, phosphatidylinositol 3’-kinase/AKT and mitochondrial p53 in curcumin (diferulolylmethane)-induced apoptosis in prostate cancer. International Journal of Oncology 30:905–918. https://doi.org/10.3892/ijo.30.4.905

Sharma A, Mishra T, Thacker G, et al (2020) Chebulinic acid inhibits MDA-MB-231 breast cancer metastasis and promotes cell death through down regulation of SOD1 and induction of autophagy. Cell Biology International 44:2553–2569. https://doi.org/10.1002/cbin.11463

Sharma H, Stephen NM, Gopal SS, et al (2021) Phenolic Extract of Seagrass, Halophila ovalis Activates Intrinsic Pathway of Apoptosis in Human Breast Cancer (MCF-7) Cells. Nutrition and Cancer 73:307–317. https://doi.org/10.1080/01635581.2020.1743874

Shen Y, Yang J, Li J, et al (2014) Carnosine Inhibits the Proliferation of Human Gastric Cancer SGC-7901 Cells through Both of the Mitochondrial Respiration and Glycolysis Pathways. PloS one 9:e104632. https://doi.org/10.1371/journal.pone.0104632

Sherr CJ (1994) G1 phase progression: Cycling on cue. Cell 79:551–555. https://doi.org/10.1016/0092-8674(94)90540-1

Shestenko OP, Nikonov SD, Mertvetsov NP (2001) [Angiogenin and its role in angiogenesis]. Mol Biol (Mosk) 35:349–371

Shi M, Cui J, Du J, et al (2014) A Novel KLF4/LDHA Signaling Pathway Regulates Aerobic Glycolysis in and Progression of Pancreatic Cancer. Clinical Cancer Research 20:4370–4380. https://doi.org/10.1158/1078-0432.CCR-14-0186

Shirode AB, Kovvuru P, Chittur SV, et al (2014) Antiproliferative effects of pomegranate extract in MCF-7 breast cancer cells are associated with reduced DNA repair gene expression and induction of double strand breaks. Molecular Carcinogenesis 53:458–470. https://doi.org/10.1002/mc.21995

Shirzad H, Taji F, Rafieian-Kopaei M (2011) Correlation Between Antioxidant Activity of Garlic Extracts and WEHI-164 Fibrosarcoma Tumor Growth in BALB/c Mice. Journal of Medicinal Food 14:969–974. https://doi.org/10.1089/jmf.2011.1594

Shostak K, Chariot A (2015) EGFR and NF-κB: partners in cancer. Trends in Molecular Medicine 21:385–393. https://doi.org/10.1016/j.molmed.2015.04.001

Siegel RL, Miller KD, Jemal A (2018) Cancer statistics, 2018. CA: A Cancer Journal for Clinicians 68:7–30. https://doi.org/10.3322/caac.21442

Sies H, Koch OR, Martino E, Boveris A (1979) Increased biliary glutathione disulfide release in chronically ethanol-treated rats. FEBS Letters 103:287–290. https://doi.org/10.1016/0014-5793(79)81346-0

Sigounas G, Hooker J, Anagnostou A, Steiner M (1997) S-allylmercaptocysteine inhibits cell proliferation and reduces the viability of erythroleukemia, breast, and prostate cancer cell lines. Nutrition and Cancer 27:186–191. https://doi.org/10.1080/01635589709514523

Simstein R, Burow M, Parker A, et al (2003) Apoptosis, Chemoresistance, and Breast Cancer: Insights From the MCF-7 Cell Model System. Exp Biol Med (Maywood) 228:995–1003. https://doi.org/10.1177/153537020322800903

Singh RK, Gutman M, Bucana CD, et al (1995) Interferons alpha and beta down-regulate the expression of basic fibroblast growth factor in human carcinomas. PNAS 92:4562–4566. https://doi.org/10.1073/pnas.92.10.4562

Singletary SE, Connolly JL (2006) Breast Cancer Staging: Working With the Sixth Edition of the AJCC Cancer Staging Manual. CA: A Cancer Journal for Clinicians 56:37–47. https://doi.org/10.3322/canjclin.56.1.37

Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In: Methods in Enzymology. Academic Press, pp 152–178

Siziopikou KP (2013) Ductal Carcinoma In Situ of the Breast: Current Concepts and Future Directions. Archives of Pathology & Laboratory Medicine 137:462–466. https://doi.org/10.5858/arpa.2012-0078-RA

Slattery ML, John EM, Stern MC, et al (2013) Associations with growth factor genes (FGF1, FGF2, PDGFB, FGFR2, NRG2, EGF, ERBB2) with breast cancer risk and survival: the Breast Cancer Health Disparities Study. Breast Cancer Res Treat 140:587–601. https://doi.org/10.1007/s10549-013-2644-5

Sommer S, Fuqua SAW (2001) Estrogen receptor and breast cancer. Seminars in Cancer Biology 11:339–352. https://doi.org/10.1006/scbi.2001.0389

Song H, Jung JI, Cho HJ, et al (2015) Inhibition of tumor progression by oral piceatannol in mouse 4T1 mammary cancer is associated with decreased angiogenesis and macrophage infiltration. The Journal of Nutritional Biochemistry 26:1368–1378. https://doi.org/10.1016/j.jnutbio.2015.07.005

Song H, Lu Y, Qu Z, et al (2016) Effects of aged garlic extract and FruArg on gene expression and signaling pathways in lipopolysaccharide-activated microglial cells. Sci Rep 6:35323. https://doi.org/10.1038/srep35323

Sonoda E, Hochegger H, Saberi A, et al (2006) Differential usage of non-homologous end-joining and homologous recombination in double strand break repair. DNA Repair 5:1021–1029. https://doi.org/10.1016/j.dnarep.2006.05.022

Sørensen V, Nilsen T, Więdłocha A (2006) Functional diversity of FGF-2 isoforms by intracellular sorting. BioEssays 28:504–514. https://doi.org/10.1002/bies.20405

Soria G, Ben-Baruch A (2008) The inflammatory chemokines CCL2 and CCL5 in breast cancer. Cancer Letters 267:271–285. https://doi.org/10.1016/j.canlet.2008.03.018

Sorice A, Guerriero E, Volpe MG, et al (2016) Differential Response of Two Human Breast Cancer Cell Lines to the Phenolic Extract from Flaxseed Oil. Molecules 21:319. https://doi.org/10.3390/molecules21030319

Soto C, González R, Sance M, Galmarini C (2016) Organosulfur and phenolic content of garlic (Allium sativum L.) and onion (Allium cepa L.) and its relationship with antioxidant activity. Acta Horticulturae 277–290. https://doi.org/10.17660/ActaHortic.2016.1143.39

Sp N, Kang DY, Lee J-M, et al (2021) Potential Antitumor Effects of 6-Gingerol in p53-Dependent Mitochondrial Apoptosis and Inhibition of Tumor Sphere Formation in Breast Cancer Cells. International Journal of Molecular Sciences 22:4660. https://doi.org/10.3390/ijms22094660

Spano D, Heck C, De Antonellis P, et al (2012) Molecular networks that regulate cancer metastasis. Seminars in Cancer Biology 22:234–249. https://doi.org/10.1016/j.semcancer.2012.03.006

Sroka Z, Cisowski W (2003) Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food and Chemical Toxicology 41:753–758. https://doi.org/10.1016/S0278-6915(02)00329-0

Steeg PS (2006) Tumor metastasis: mechanistic insights and clinical challenges. Nat Med 12:895–904. https://doi.org/10.1038/nm1469

Stefani T, Romo-Mancillas A, Carrizales-Castillo JJJ, et al (2021) Cytotoxic Fractions from Hechtia glomerata Extracts and p-Coumaric Acid as MAPK Inhibitors. Molecules 26:1096. https://doi.org/10.3390/molecules26041096

Steller H (1995) Mechanisms and Genes of Cellular Suicide. Science 267:1445–1449. https://doi.org/10.1126/science.7878463

Su S, Liu Q, Chen J, et al (2014) A Positive Feedback Loop between Mesenchymal-like Cancer Cells and Macrophages Is Essential to Breast Cancer Metastasis. Cancer Cell 25:605–620. https://doi.org/10.1016/j.ccr.2014.03.021

Sultana B, Anwar F (2008) Flavonols (kaempeferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants. Food Chemistry 108:879–884. https://doi.org/10.1016/j.foodchem.2007.11.053

Sumi NJ, Kuenzi BM, Knezevic CE, et al (2015) Chemoproteomics Reveals Novel Protein and Lipid Kinase Targets of Clinical CDK4/6 Inhibitors in Lung Cancer. ACS chemical biology 10:2680–2686. https://doi.org/10.1021/acschembio.5b00368

Sun X-D, Liu X-E, Huang D-S (2012) Curcumin induces apoptosis of triple-negative breast cancer cells by inhibition of EGFR expression. Molecular Medicine Reports 6:1267–1270. https://doi.org/10.3892/mmr.2012.1103

Sun Y, Zhou Q-M, Lu Y-Y, et al (2019) Resveratrol Inhibits the Migration and Metastasis of MDA-MB-231 Human Breast Cancer by Reversing TGF-β1-Induced Epithelial-Mesenchymal Transition. Molecules 24:1131. https://doi.org/10.3390/molecules24061131

Sundaram SG, Milner JA (1996) Diallyl disulfide inhibits the proliferation of human tumor cells in culture. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1315:15–20. https://doi.org/10.1016/0925-4439(95)00088-7

Sunu P, Sunarti D, Mahfudz LD, Yunianto VD (2019) Prebiotic activity of garlic (Allium sativum) extract on Lactobacillus acidophilus. Vet World 12:2046–2051. https://doi.org/10.14202/vetworld.2019.2046-2051

Tagliazucchi D, Verzelloni E, Bertolini D, Conte A (2010) In vitro bio-accessibility and antioxidant activity of grape polyphenols. Food Chemistry 120:599–606. https://doi.org/10.1016/j.foodchem.2009.10.030

Takahashi-Yanaga F, Sasaguri T (2008) GSK-3β regulates cyclin D1 expression: A new target for chemotherapy. Cellular Signalling 20:581–589. https://doi.org/10.1016/j.cellsig.2007.10.018

Talavéra S, Felgines C, Texier O, et al (2003) Anthocyanins Are Efficiently Absorbed from the Stomach in Anesthetized Rats. The Journal of Nutrition 133:4178–4182. https://doi.org/10.1093/jn/133.12.4178

Talib WH (2017) Consumption of garlic and lemon aqueous extracts combination reduces tumor burden by angiogenesis inhibition, apoptosis induction, and immune system modulation. Nutrition 43–44:89–97. https://doi.org/10.1016/j.nut.2017.06.015

Talib WH, Al-Noaimi M, Alsultan ES, et al (2019) A new acetylacetone derivative inhibits breast cancer by apoptosis induction and angiogenesis inhibition. J Cancer Res Ther 15:1141–1146. https://doi.org/10.4103/jcrt.JCRT_948_17

Tang F-Y, Su Y-C, Chen N-C, et al (2008) Resveratrol inhibits migration and invasion of human breast-cancer cells. Molecular Nutrition & Food Research 52:683–691. https://doi.org/10.1002/mnfr.200700325

Tao S, He H, Chen Q (2015) Quercetin inhibits proliferation and invasion acts by up-regulating miR-146a in human breast cancer cells. Mol Cell Biochem 402:93–100. https://doi.org/10.1007/s11010-014-2317-7

Tebay LE, Robertson H, Durant ST, et al (2015) Mechanisms of activation of the transcription factor Nrf2 by redox stressors, nutrient cues, and energy status and the pathways through which it attenuates degenerative disease. Free Radical Biology and Medicine 88:108–146. https://doi.org/10.1016/j.freeradbiomed.2015.06.021

Telli ML, Carlson RW (2009) First-Line Chemotherapy for Metastatic Breast Cancer. Clinical Breast Cancer 9:S66–S72. https://doi.org/10.3816/CBC.2009.s.007

Thiery JP, Acloque H, Huang RYJ, Nieto MA (2009) Epithelial-Mesenchymal Transitions in Development and Disease. Cell 139:871–890. https://doi.org/10.1016/j.cell.2009.11.007

Tímár J, Döme B, Fazekas K, et al (2001) Angiogenesis-dependent diseases and angiogenesis therapy. Pathol Oncol Res 7:85–94. https://doi.org/10.1007/BF03032573

Tommonaro G, Speranza G, De Prisco R, et al (2017) Antioxidant activity and bioactive compound contents before and after in vitro digestion of new tomato hybrids. Journal of the Science of Food and Agriculture 97:5241–5246. https://doi.org/10.1002/jsfa.8408

Tsubura A, Lai Y-C, Kuwata M, et al (2011) Anticancer Effects of Garlic and Garlic-derived Compounds for Breast Cancer Control. Anti-Cancer Agents in Medicinal Chemistry-Anti-Cancer Agents) 11:249–253. https://doi.org/10.2174/187152011795347441

Tu X, Kahila MM, Zhou Q, et al (2018a) ATR Inhibition Is a Promising Radiosensitizing Strategy for Triple-Negative Breast Cancer. Molecular Cancer Therapeutics 17:2462–2472. https://doi.org/10.1158/1535-7163.MCT-18-0470

Tu Y, Johnstone CN, Ryall JG, et al (2018b) Altered energy metabolism and metabolic gene expression associated with increased metastatic capacity identified in MDA-MB-231 cell line variants. Journal of Cancer Metastasis and Treatment 4:58. https://doi.org/10.20517/2394-4722.2018.67

Tulotta C, Ottewell P (2018) The role of IL-1B in breast cancer bone metastasis. Endocrine-Related Cancer 25:R421–R434. https://doi.org/10.1530/ERC-17-0309

Ukaji T, Lin Y, Okada S, Umezawa K (2017) Inhibition of MMP-2-mediated cellular invasion by NF-κB inhibitor DHMEQ in 3D culture of breast carcinoma MDA-MB-231 cells: A model for early phase of metastasis. Biochemical and Biophysical Research Communications 485:76–81. https://doi.org/10.1016/j.bbrc.2017.02.022

Vadalà R, Mottese AF, Bua GD, et al (2016) Statistical Analysis of Mineral Concentration for the Geographic Identification of Garlic Samples from Sicily (Italy), Tunisia and Spain. Foods 5:20. https://doi.org/10.3390/foods5010020

Valko M, Rhodes CJ, Moncol J, et al (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactions 160:1–40. https://doi.org/10.1016/j.cbi.2005.12.009

Vallejo F, Gil-Izquierdo A, Pérez-Vicente A, García-Viguera C (2004) In Vitro Gastrointestinal Digestion Study of Broccoli Inflorescence Phenolic Compounds,

Glucosinolates, and Vitamin C. J Agric Food Chem 52:135–138. https://doi.org/10.1021/jf0305128

Valtola R, Salven P, Heikkilä P, et al (1999) VEGFR-3 and Its Ligand VEGF-C Are Associated with Angiogenesis in Breast Cancer. The American Journal of Pathology 154:1381–1390. https://doi.org/10.1016/S0002-9440(10)65392-8

van den Brand AD, Villevoye J, Nijmeijer SM, et al (2019) Anti-tumor properties of methoxylated analogues of resveratrol in malignant MCF-7 but not in non-tumorigenic MCF-10A mammary epithelial cell lines. Toxicology 422:35–43. https://doi.org/10.1016/j.tox.2019.04.009

Varela NM, Guevara-Ramírez P, Acevedo C, et al (2021) A New Insight for the Identification of Oncogenic Variants in Breast and Prostate Cancers in Diverse Human Populations, With a Focus on Latinos. Frontiers in Pharmacology 12:

Vaya J, Tamir S (2004) The relation between the chemical structure of flavonoids and their estrogen-like activities. Curr Med Chem 11:1333–1343. https://doi.org/10.2174/0929867043365251

Vazquez-Martin A, Oliveras-Ferraros C, Menendez JA (2009) Autophagy Facilitates the Development of Breast Cancer Resistance to the Anti-HER2 Monoclonal Antibody Trastuzumab. PLOS ONE 4:e6251. https://doi.org/10.1371/journal.pone.0006251

Vega-Rubín-de-Celis S, Zou Z, Fernández ÁF, et al (2018) Increased autophagy blocks HER2-mediated breast tumorigenesis. PNAS 115:4176–4181. https://doi.org/10.1073/pnas.1717800115

Vergara D, Valente CM, Tinelli A, et al (2011) Resveratrol inhibits the epidermal growth factor-induced epithelial mesenchymal transition in MCF-7 cells. Cancer Letters 310:1–8. https://doi.org/10.1016/j.canlet.2011.04.009

Verhagen AM, Ekert PG, Pakusch M, et al (2000) Identification of DIABLO, a Mammalian Protein that Promotes Apoptosis by Binding to and Antagonizing IAP Proteins. Cell 102:43–53. https://doi.org/10.1016/S0092-8674(00)00009-X

Vermeulen K, Van Bockstaele DR, Berneman ZN (2003) The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. Cell Proliferation 36:131–149. https://doi.org/10.1046/j.1365-2184.2003.00266.x

Vermeulen PB, Van Laere SJ, Dirix LY (2009) Inflammatory breast carcinoma as a model of accelerated self-metastatic expansion by intravascular growth. Br J Cancer 101:1028–1029. https://doi.org/10.1038/sj.bjc.6605251

Vijayan S, Loganathan C, Sakayanathan P, Thayumanavan P (2021) In silico and in vitro investigation of anticancer effect of newly synthesized nonivamide-s-allyl cysteine ester. Journal of Biomolecular Structure and Dynamics 0:1–15. https://doi.org/10.1080/07391102.2021.1959404

Vivek R (2018) Tactics of Breast Cancer Therapeutics and Future Outlooks

Vlodavsky I, Korner G, Ishai-Michaeli R, et al (1990) Extracellular matrix-resident growth factors and enzymes: possible involvement in tumor metastasis and angiogenesis. Cancer Metast Rev 9:203–226. https://doi.org/10.1007/BF00046361

Voelkel NF, Cool C, Taraceviene-Stewart L, et al (2002) Janus face of vascular endothelial growth factor: The obligatory survival factor for lung vascular endothelium controls precapillary artery remodeling in severe pulmonary hypertension. Critical Care Medicine 30:S251

Waghela BN, Sharma A, Dhumale S, et al (2015) Curcumin Conjugated with PLGA Potentiates Sustainability, Anti-Proliferative Activity and Apoptosis in Human Colon Carcinoma Cells. PLOS ONE 10:e0117526. https://doi.org/10.1371/journal.pone.0117526

Waleh NS, Chao W-R, Bensari A, Zaveri NT (2005) Novel D-ring Analog of Epigallocatechin-3-gallate Inhibits Tumor Growth and VEGF Expression in Breast Carcinoma Cells. Anticancer Research 25:397–402

Wan X, Polyakova Y, Row KH (2007) Determination of diallyl disulfide in garlic by reversed-phase high performance liquid chromatography. Analytical Science and Technology 20:442–447

Wang N, Wang Z-Y, Mo S-L, et al (2012) Ellagic acid, a phenolic compound, exerts anti-angiogenesis effects via VEGFR-2 signaling pathway in breast cancer. Breast Cancer Res Treat 134:943–955. https://doi.org/10.1007/s10549-012-1977-9

Wang W, Nag SA, Zhang R (2015) Targeting the NFKB Signaling Pathways for Breast Cancer Prevention and Therapy. Current Medicinal Chemistry 22:264–289

Wang X, Reyes ME, Zhang D, et al (2017) EGFR signaling promotes inflammation and cancer stem-like activity in inflammatory breast cancer. Oncotarget 8:67904–67917. https://doi.org/10.18632/oncotarget.18958

Wang X-J, Sun Z, Villeneuve NF, et al (2008) Nrf2 enhances resistance of cancer cells to chemotherapeutic drugs, the dark side of Nrf2. Carcinogenesis 29:1235–1243. https://doi.org/10.1093/carcin/bgn095

Wang Y, Guan M, Zhao X, Li X (2018) Effects of garlic polysaccharide on alcoholic liver fibrosis and intestinal microflora in mice. Pharmaceutical Biology 56:325–332. https://doi.org/10.1080/13880209.2018.1479868

Wang Y, Zhang L, Moslehi R, et al (2009) Long-Term Garlic or Micronutrient Supplementation, but Not Anti-Helicobacter pylori Therapy, Increases Serum Folate or Glutathione Without Affecting Serum Vitamin B-12 or Homocysteine in a Rural Chinese Population. The Journal of Nutrition 139:106–112. https://doi.org/10.3945/jn.108.091389

Waugh DJJ, Wilson C (2008) The Interleukin-8 Pathway in Cancer. Clinical Cancer Research 14:6735–6741. https://doi.org/10.1158/1078-0432.CCR-07-4843

Wawruszak A, Halasa M, Okla K (2021) Chapter 35 - Lycium barbarum (goji berry), human breast cancer, and antioxidant profile. In: Preedy VR, Patel VB (eds) Cancer (Second Edition). Academic Press, San Diego, pp 399–406

Wei Z, Shan Y, Tao L, et al (2017) Diallyl trisulfides, a natural histone deacetylase inhibitor, attenuate HIF-1α synthesis, and decreases breast cancer metastasis. Molecular Carcinogenesis 56:2317–2331. https://doi.org/10.1002/mc.22686

Weidner N, Semple JP, Welch WR, Folkman J (1991) Tumor Angiogenesis and Metastasis — Correlation in Invasive Breast Carcinoma. New England Journal of Medicine 324:1–8. https://doi.org/10.1056/NEJM199101033240101

Weigelt B, Peterse JL, van't Veer LJ (2005) Breast cancer metastasis: markers and models. Nat Rev Cancer 5:591–602. https://doi.org/10.1038/nrc1670

Weinberg RA (1995) The retinoblastoma protein and cell cycle control. Cell 81:323–330. https://doi.org/10.1016/0092-8674(95)90385-2

Williams GH, Stoeber K (2012) The cell cycle and cancer. The Journal of Pathology 226:352–364. https://doi.org/10.1002/path.3022

Williams MM, Cook RS (2015) Bcl-2 family proteins in breast development and cancer: could Mcl-1 targeting overcome therapeutic resistance? Oncotarget 6:3519–3530

Williamson G, Manach C (2005) Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. The American Journal of Clinical Nutrition 81:243S-255S. https://doi.org/10.1093/ajcn/81.1.243S

WOESSNER J (1993) The family of matrix metalloproteinases. Ann NY Acad Sci 732:11–21

Wolff B, Burns AR, Middleton J, Rot A (1998) Endothelial Cell "Memory" of Inflammatory Stimulation: Human Venular Endothelial Cells Store Interleukin 8 in Weibel-Palade Bodies. Journal of Experimental Medicine 188:1757–1762. https://doi.org/10.1084/jem.188.9.1757

Wong Y-H, Tan C-P, Long K, Nyam K-L (2014) In vitro simulated digestion on the biostability of Hibiscus cannabinus L. seed extract. Czech Journal of Food Sciences 32 (2014):177–181. https://doi.org/10.17221/222/2013-CJFS

Wu C-F, Lee M-G, El-Shazly M, et al (2018) Isoaaptamine Induces T-47D Cells Apoptosis and Autophagy via Oxidative Stress. Marine Drugs 16:18. https://doi.org/10.3390/md16010018

Wu X, Shi J, Fang W, et al (2019) Allium vegetables are associated with reduced risk of colorectal cancer: A hospital-based matched case-control study in China. Asia-Pacific Journal of Clinical Oncology 15:e132–e141. https://doi.org/10.1111/ajco.13133

Xiang H, Zhang J, Lin C, et al (2020) Targeting autophagy-related protein kinases for potential therapeutic purpose. Acta Pharmaceutica Sinica B 10:569–581. https://doi.org/10.1016/j.apsb.2019.10.003

Xiao X, Huang X, Ye F, et al (2016) The miR-34a-LDHA axis regulates glucose metabolism and tumor growth in breast cancer. Sci Rep 6:21735. https://doi.org/10.1038/srep21735

Xie X, Huang X, Tang H, et al (2018) Diallyl Disulfide Inhibits Breast Cancer Stem Cell Progression and Glucose Metabolism by Targeting CD44/PKM2/AMPK Signaling. Current Cancer Drug Targets 18:592–599. https://doi.org/10.2174/1568009617666171024165657

Xu H-Y, Pan Y-M, Chen Z-W, et al (2013) 12-Deoxyphorbol 13-palmitate inhibit VEGF-induced angiogenesis via suppression of VEGFR-2-signaling pathway. Journal of Ethnopharmacology 146:724–733. https://doi.org/10.1016/j.jep.2013.01.007

Yagdi E, Cerella C, Dicato M, Diederich M (2016) Garlic-derived natural polysulfanes as hydrogen sulfide donors: Friend or foe? Food and Chemical Toxicology 95:219–233. https://doi.org/10.1016/j.fct.2016.07.016

Yang M, Soga T, Pollard PJ (2013) Oncometabolites: linking altered metabolism with cancer. J Clin Invest 123:3652–3658. https://doi.org/10.1172/JCI67228

Yang M-D, Sun Y, Zhou W-J, et al (2021) Resveratrol Enhances Inhibition Effects of Cisplatin on Cell Migration and Invasion and Tumor Growth in Breast Cancer MDA-MB-231 Cell Models In Vivo and In Vitro. Molecules 26:2204. https://doi.org/10.3390/molecules26082204

Yang SW, Cody JJ, Rivera AA, et al (2011) Conditionally Replicating Adenovirus Expressing TIMP2 for Ovarian Cancer Therapy. Clinical Cancer Research 17:538–549. https://doi.org/10.1158/1078-0432.CCR-10-1628

Yang X, Yu D-D, Yan F, et al (2015) The role of autophagy induced by tumor microenvironment in different cells and stages of cancer. Cell Biosci 5:14. https://doi.org/10.1186/s13578-015-0005-2

Yao F, Zhao T, Zhong C, et al (2013) LDHA is necessary for the tumorigenicity of esophageal squamous cell carcinoma. Tumor Biol 34:25–31. https://doi.org/10.1007/s13277-012-0506-0

Yarden Y (2001) Biology of HER2 and Its Importance in Breast Cancer. OCL 61:1–13. https://doi.org/10.1159/000055396

Yedjou CG, Tchounwou PB (2012a) In vitro assessment of oxidative stress and apoptotic mechanisms of garlic extract in the treatment of acute promyelocytic leukemia. J Cancer Sci Ther 2012:006. https://doi.org/10.4172/1948-5956.S3-006

Yedjou CG, Tchounwou PB (2012b) In vitro assessment of oxidative stress and apoptotic mechanisms of garlic extract in the treatment of acute promyelocytic leukemia. J Cancer Sci Ther 2012:006. https://doi.org/10.4172/1948-5956.S3-006

Yeo H, Lee YH, Koh D, et al (2020) Chrysin Inhibits NF-κB-Dependent CCL5 Transcription by Targeting IκB Kinase in the Atopic Dermatitis-Like Inflammatory Microenvironment. International Journal of Molecular Sciences 21:7348. https://doi.org/10.3390/ijms21197348

Yiangou C, Gomm JJ, Coope RC, et al (1997) Fibroblast growth factor 2 in breast cancer: occurrence and prognostic significance. Br J Cancer 75:28–33. https://doi.org/10.1038/bjc.1997.5

Yin X, Zhang J, Li X, et al (2014) DADS Suppresses Human Esophageal Xenograft Tumors through RAF/MEK/ERK and Mitochondria-Dependent Pathways. International Journal of Molecular Sciences 15:12422–12441. https://doi.org/10.3390/ijms150712422

Yoshida S, Ono M, Shono T, et al (1997) Involvement of interleukin-8, vascular endothelial growth factor, and basic fibroblast growth factor in tumor necrosis factor alpha-dependent angiogenesis. Molecular and Cellular Biology 17:4015–4023. https://doi.org/10.1128/MCB.17.7.4015

You W, Brown LM, Zhang L, et al (2006) Randomized Double-Blind Factorial Trial of Three Treatments To Reduce the Prevalence of Precancerous Gastric Lesions. JNCI: Journal of the National Cancer Institute 98:974–983. https://doi.org/10.1093/jnci/djj264

Yu L, Chen Y, Tooze SA (2018) Autophagy pathway: Cellular and molecular mechanisms. Autophagy 14:207–215. https://doi.org/10.1080/15548627.2017.1378838

Zachari M, Ganley IG (2017) The mammalian ULK1 complex and autophagy initiation. Essays in Biochemistry 61:585–596. https://doi.org/10.1042/EBC20170021

Zang S, Liu T, Shi J, Qiao L (2014) Curcumin: A Promising Agent Targeting Cancer Stem Cells. Anti-Cancer Agents in Medicinal Chemistry- Anti-Cancer Agents) 14:787–792

Zedan AMG, Sakran MI, Bahattab O, et al (2021) Oriental Hornet (Vespa orientalis) Larval Extracts Induce Antiproliferative, Antioxidant, Anti-Inflammatory, and Anti-Migratory Effects on MCF7 Cells. Molecules 26:3303. https://doi.org/10.3390/molecules26113303

Zelenay S, van der Veen AG, Böttcher JP, et al (2015) Cyclooxygenase-Dependent Tumor Growth through Evasion of Immunity. Cell 162:1257–1270. https://doi.org/10.1016/j.cell.2015.08.015

Zeng L, Holly JMP, Perks CM (2014) Effects of Physiological Levels of the Green Tea Extract Epigallocatechin-3-Gallate on Breast Cancer Cells. Frontiers in Endocrinology 5:

Zhang D, LaFortune TA, Krishnamurthy S, et al (2009) EGFR Tyrosine Kinase Inhibitor Reverses Mesenchymal to Epithelial Phenotype and Inhibits Metastasis in Inflammatory Breast Cancer. Clin Cancer Res 15:6639–6648. https://doi.org/10.1158/1078-0432.CCR-09-0951

Zhang H, Wang K, Lin G, Zhao Z (2014) Antitumor mechanisms of S-allyl mercaptocysteine for breast cancer therapy. BMC Complementary and Alternative Medicine 14:270. https://doi.org/10.1186/1472-6882-14-270

Zhang L, Li J, Ma J, et al (2015) The Relevance of Nrf2 Pathway and Autophagy in Pancreatic Cancer Cells upon Stimulation of Reactive Oxygen Species. Oxidative

Medicine and Cellular Longevity 2016:e3897250. https://doi.org/10.1155/2016/3897250

Zhang L, Zhou F, ten Dijke P (2013) Signaling interplay between transforming growth factor-β receptor and PI3K/AKT pathways in cancer. Trends in Biochemical Sciences 38:612–620. https://doi.org/10.1016/j.tibs.2013.10.001

Zhang Z, Li X, Sun W, et al (2017) Loss of exosomal miR-320a from cancer-associated fibroblasts contributes to HCC proliferation and metastasis. Cancer Letters 397:33–42. https://doi.org/10.1016/j.canlet.2017.03.004

Zhao D, Zou S-W, Liu Y, et al (2013) Lysine-5 Acetylation Negatively Regulates Lactate Dehydrogenase A and Is Decreased in Pancreatic Cancer. Cancer Cell 23:464–476. https://doi.org/10.1016/j.ccr.2013.02.005

Zhao W, Shi F, Guo Z, et al (2018) Metabolite of ellagitannins, urolithin A induces autophagy and inhibits metastasis in human sw620 colorectal cancer cells. Molecular Carcinogenesis 57:193–200. https://doi.org/10.1002/mc.22746

Zheng J, Zhou Y, Li Y, et al (2016) Spices for Prevention and Treatment of Cancers. Nutrients 8:495. https://doi.org/10.3390/nu8080495

Zhou Z, Yao H, Hu H (2017) Disrupting Tumor Angiogenesis and “the Hunger Games” for Breast Cancer. Adv Exp Med Biol 1026:171–195. https://doi.org/10.1007/978-981-10-6020-5_8

Zhu F (2018) Interactions between cell wall polysaccharides and polyphenols. Critical Reviews in Food Science and Nutrition 58:1808–1831. https://doi.org/10.1080/10408398.2017.1287659

Zhu X, Wang K, Zhang K, et al (2016) Ziyuglycoside I Inhibits the Proliferation of MDA-MB-231 Breast Carcinoma Cells through Inducing p53-Mediated G2/M Cell Cycle Arrest and Intrinsic/Extrinsic Apoptosis. International Journal of Molecular Sciences 17:1903. https://doi.org/10.3390/ijms17111903

Zuniga KE, Parma DL, Muñoz E, et al (2019) Dietary intervention among breast cancer survivors increased adherence to a Mediterranean-style, anti-inflammatory dietary pattern: the Rx for Better Breast Health Randomized Controlled Trial. Breast Cancer Res Treat 173:145–154. https://doi.org/10.1007/s10549-018-4982-9

Zurac S, Neagu M, Constantin C, et al (2016) Variations in the expression of TIMP1, TIMP2 and TIMP3 in cutaneous melanoma with regression and their possible function as prognostic predictors. Oncology Letters 11:3354–3360. https://doi.org/10.3892/ol.2016.4391

ACKNOWLEDGMENTS

Firstly, all of my praises be to Allah alone, the creator, the supreme authority of the universe for bestowing me the good health, strength and perseverance needed to accomplish this piece of work.

I would like to express my sincere acknowledgement and deepest appreciation to my tutor, Prof. Dr. Maurizio Battino for his supervision and tremendous support over the past three years of my PhD. The most importantly, he provided me unflinching encouragement in various ways during my bad period and providing me with an excellent atmosphere for doing research. He sacrifices many of his precious hours and extensive preoccupation with professional and academic responsibilities and also helped me in many ways outside of the lab as a guardian. Special thanks to him again form my bottom of heart, without his cordial help I could not get back my normal life and complete this work or become a research scientist that I wanted to be.

I would also like to give special thanks to Dr. Francesca Giampieri for her constant guidance, encouragement, discussions and suggestions in day-to-day life in the lab. Most need, she helped me in many ways outside of the lab as an elder sister that made my foreign life so smooth in Ancona. I genuinely regard and appreciate her for her humility, tirelessness and her excitement for science. I have acquired a lot of knowledge from her in all these years especially the importance of hard work, passion and commitment to be a successful scientist. I have learned from her “never give up”. I always felt peace when she told me “Don’t worry”.

Special thanks to Dr. Danila Cianciosi for her continuing guidance, generous technical support and were always willing to help and give her best accompany as good friend in my worst time. It would have been a lonely lab without you. I am particularly grateful to Dr. Tamara Y. Forbes-Hernandez for having done the HPLC analysis for me. I would always remember you for your care and sensibility for me.I would also like to acknowledge Dr. Sadia and Dr. Golam Gousul Azam since it was a great privilege for me to get their suggestions/advice to carry out my research work. I am sincerely grateful to Prof. Dr. Mohammad Ali Hossain who provided us the Garlic samples from Bangladesh. I would like to express my gratitude to dear friend, Dr Tushar Chandra Sarkar, with whom I shared pleasantly, most of my free time, for their sweetness and amazing friendship.

I am very thankful to Lucia and yasmany (PhD student) who were a nice person, good luck guys also for your journey! Allessia and Michelle (Master's student) were always willing to help and give their best accompany, together we enjoyed a lot, especially in our coffee time.
I thank all the people I met during this magnificent journey and with them and I spent special moments of my days, and which cheered me up with a smile and supported without knowing it: Mattia, Stefano, Jerry, Armando, Rossana. I would also like to acknowledge Dr. Francesco Orlandoni who help me to get back my healthy condition and always encouraged me for complete this work when I was disappointed.
I would like to thank the most amazing person in my life, my daughter, who sacrificed her childhood 3 years for my degree in Italy. Her patience, support, understanding and faith in me and have been my greatest source of motivation that always stimulate me to do something better.
Last but most importantly, I want to thank from core of my heart to my parents and Madhusudan Shome sir for their endless love, well-wishes and support

Ancona, May 14, 2022