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THE HEALING POWER OF CULINARY SPICES AND HERBS IN TRIPLE NEGATIVE BREAST CANCER

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ABSTRACT

Breast cancer remains as a major cause of cancer related deaths in women worldwide which accounts for about 25% of all cancers. Gradual increment in mortality rate of breast cancer can be seen due to high metastatic potential. Triple-negative breast cancer (TNBC) is a highly aggressive and metastatic group of breast cancers which lack the expression of estrogen, progesterone and human epidermal growth factor-2 receptors. Due to the lack of therapeutic targets and due to the drawbacks of available treatment methods, treating TNBC has become challenging. Hence, to overcome these drawbacks new treatment strategies are experimenting with the use of natural products such as culinary spices & herbs and their bioactive agents. The bioactive agents in natural compounds like Rosemary (*Rosmarinus officinalis*), Turmeric (*Curcuma longa*), Ginger (*Zingiber officinale*), Garlic (*Allium sativum*) and Red onion (*Allium cepa*) found to have pro-apoptotic, anti-proliferative, anti-angiogenic, anti-metastatic and anti-migratory effects in triple negative breast cancer cells. Moreover, certain bioactive agents shown to have synergistic effects with other bioactive agents and with conventional chemotherapeutics. The effectiveness of bioactive agents such as betulinic acid, curcumin, gingerols, shogaol, diallyl sulfides and quercetin was discussed showing that they are efficacious in triple negative breast cancer cell line MDA-MB-231. Thereby, concluding that the bioactive agents in natural spices and herbs may be potential therapeutic agents in treatment of TNBC and in order to develop novel treatment strategies, it is important to experiment more about their therapeutic effects, mechanism of action, synergistic effects and side effects.

Key words: Triple negative breast cancer, spices & herbs, bioactive agents, efficacy

INTRODUCTION

Breast cancer can be simply shown as an abnormal growth and proliferation of cells in the breast tissue. This is the most common cancer with rising incidence in women worldwide which accounts for about 25% of all cancers. Figure 1 shows the distribution of incidence rate of breast cancer in the world and incidence rate in Sri Lanka is between 24.1-33.9% (Ghoncheh, Pournamdar and Salehiniya, 2016). In average, one third of women develop breast cancer metastasis resulting in increased mortality rates. According to the statistical analysis of American Cancer Society, the estimated values of new cases and deaths due to breast cancer for the year 2018 are 266,120 and 40,920 respectively (Seigel, Naishadham and Jemal, 2018).

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Considerable improvements have been made in treating breast cancer over years in receptor positive breast cancers. However, regardless of these advancements yet it is a frequent cause of death in women worldwide (Saadat, 2008).

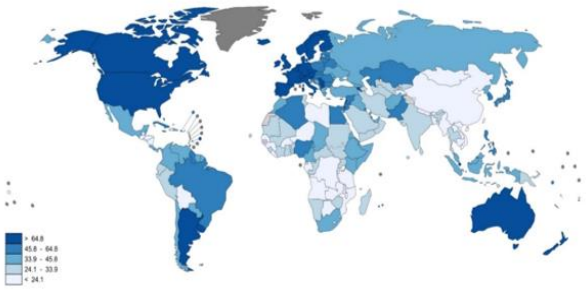

Figure 1. *Distribution of the incidence rate of breast cancer in world. Incidence rate includes approximately 1.7 new cases per year and it ranges from 19.4 per 100,000 people in East Africa to 89.7 per 100,000 in West Europe. A growing incidence can be seen in South America, Africa and Asia* (Ghoncheh, Pournamdar, and Salehiniya, 2016)

Intrinsic subtypes of breast cancer

Based on immunohistochemical (IHC) profiling breast cancer can be divided as estrogen hormone receptor, progesterone hormone receptor and human epidermal growth factor receptor-2 sensitive categories and the category which lacks all the three receptors is known as triple negative category. Based on IHC and gene expression profiling (GEP) breast cancer can be divided into 6 intrinsic molecular subtypes as basal-like, claudin-low, luminal A, luminal B, HER2-enriched and normal-like as shown in figure 2. Each intrinsic subtype has its own gene/molecular expression based on the receptor expression and other molecular expressions such as cyto-keratins, cell-adhesion molecules etc. Receptors are considered as the classical biomarkers in IHC profiling which determine the tumor size, grade, prognosis and survival rate (Dai et al., 2015; Ribelles et al., 2013).

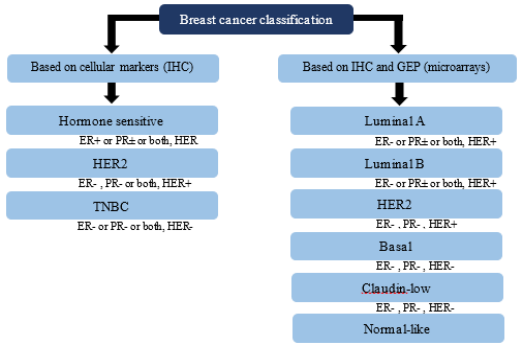

Figure 2. *IHC and GEP based intrinsic classification of breast cancer* (Adapted from Uscanga-Perales, Santuario-Facio and Ortiz-Lopez, 2016)

Triple negative breast cancer (TNBC)

TNBC is the type of breast cancers which lack the expression of estrogen receptor (ER-), progesterone receptor (PR-) and human epidermal growth factor receptor-2 (HER2-). This accounts for approximately 20% of all breast cancers and more common in younger women. Especially, young women are of Hispanic and African-American descent. It is more likely to occur in BRCA1 mutation carriers and women with TP53 alterations. GEP shows that TNBC is a heterogeneous group of breast cancers which consist of different distinct molecular sub-types with distinct patterns of metastasis including lung, bone and liver metastasis. As shown in the figure 3, TNBCs mainly fall under basal-like and claudin-low molecular subtype according to the GEP (Berrocal and Chagpar, 2017; Robertson et al., 2012; Bauer et al., 2011; Kreike et al., 2007).

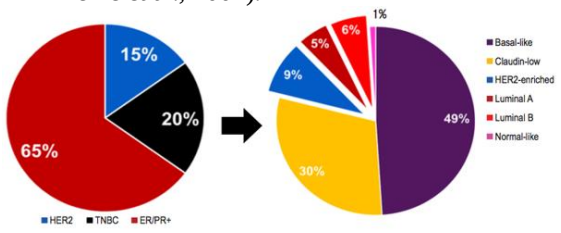

Figure 3. *Heterogeneity of the TNBC. Approximately 65% is HER2 positive, 15% is positive for ER and PR while 20% of the breast cancers lack all the three receptors namely TNBC. Majority of TNBCs are basal-like and claudin-low tumors* (Berrocal and Chagpar, 2017)

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Basal like tumors accounts for 49% of TNBCs and characterized by the triple-negative phenotype, expression of basal cyto-keratins such as CK 5,6,14,17 and over-expression of epidermal growth factor receptor (EFGR) (Badowska-Kozakiewicz and Budzik, 2017). Whereas, claudin-low tumors are typify by triple-negative phenotype, low expression of cell-cell adhesion molecules like claudin 3, 4, 7 and E-cadherin. Claudin-low tumors are associated with young age of onset, larger tumor size, higher tumor grade, poor prognosis & low survival outcomes (Bane et al., 2017). TNBCs are highly aggressive, invasive, metastatic and has a poor prognosis due to the absence of therapeutic targets. It tends to be more invasive and metastatic than the other breast cancer types due to its unique molecular profile (Maegawa and Tang, 2010; Bauer et al., 2007).

Available treatment methods

Commonly available treatment options for breast cancer are chemotherapy, hormone therapy, targeted therapy, radiation therapy, gene therapy and surgery. The treatment options are determined based upon IHC profiling of a tumor (Ribelles et al., 2013). As shown in the figure 4, since TNBC lacks the receptor expression it does not respond to hormone therapy and targeted therapy. Hence the main treatment option is chemotherapy.

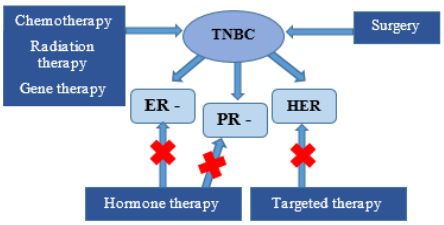

Figure 4. *Available treatment options for TNBC* (Adapted from PDQ Adult Treatment Editorial Board, 2018; Huang et al., 2015)

Limitations of available treatments and TNBC cell lines

To develop new treatment strategies for TNBC, numerous researches have been focused on breast cancer cell lines. The oldest triple-negative cell line was BT20. Later on many triple-negative cell lines were established and studied over years to observe the cell behavior and to explore new drug targets. To date there are more than 30 triple-negative cell lines available. Some of them are BT-549, MDA- MDA-MB-435, MDA-MB-436 and so on (Chavez, Garimella and Lipkowitz, 2010). The current review is focused on claudin-low like triple-negative MDA-MB-231 cell line because it is one of the most widely used triple-negative cell line in many research studies due to its highly aggressive, invasive and metastatic nature. The figure 5 shows an epitome to prove the acquired drug resistance of triple-negative cell lines towards chemotherapeutic agents (Smith et al., 2006). Therefore, to overcome these drawbacks new treatment strategies are experimenting with the use of natural products such as culinary spices & herbs and their bioactive agents.

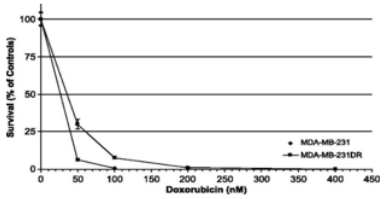

Figure 5. *The dose-response of relative resistance of MDA-MB-231 and MDA-MB-231DR towards doxorubicin over the concentration range 0 to 400 nmol/L. Doxorubicin is a first-line chemotherapeutic agent in breast cancer treatment. The IC_{50} value of MDA-MB-231DR (25 nmol/L) is significantly less than the IC_{50} value of MDA-MB-231(35 nml/L) showing the acquired resistance of MDA-MD-231 towards doxorubicin* (Smith et al., 2006)

Bioactive agents in culinary spices and herbs

Hippocrates is frequently quoted having said “Let food be thy medicine and medicine be thy food” showing the importance of well-being diet.

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Among the potential alternatives, natural spices and herb products contribute for high efficacy in cancer therapy due to their chemo-preventive properties. Culinary spices and herbs are plant substances such as seed, leaf, root, fruit, bark, bud, stigma or flower which are used in insignificant levels in culinary purposes as flavourants, colourants or as preservatives. They exhibit wide range of medicinal benefits due to the presence of phytochemicals and the predominant phytochemical constituent is polyphenols. They contribute in chemo-preventive property of culinary spices and herbs by inhibiting the carcinogen bio-activation, decrease free radical generation, inhibits angiogenesis, cancer cell proliferation, induce apoptosis and exert anti-obese effects in cancer cells. Advantages of these natural agents over available chemotherapeutics are, induction of apoptosis instead of necrosis, reduces drug resistance, induces chemo-sensitization, increases drug accumulation in breast cancer cells and they reduce the side effects of chemotherapeutics as well (Opara and Chohan, 2014; Kaefer and Milner, 2011; Somers-Edgar et al., 2008). The current review discusses the chemo-preventive properties and the effectiveness of culinary spices & herbs and their bioactive agents in treating TNBC.

Rosemary (*Rosmarinus officinalis*)

Rosemary is a member of mint family which is native to Mediterranean region. It contains betulinic acid (BA/BetA) which is a pentacyclic triterpene (Figure 6). Fresh and dried forms of rosemary leaves are used for culinary purposes (Liburdi et al., 2017).

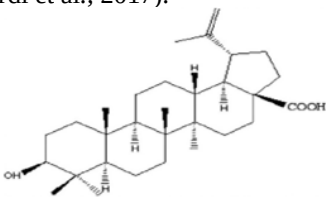

Figure 6. *Chemical structure of BA* (Aggarwal and Takada, 2003)

A study was performed to check the cytotoxic effects of BA towards MDA-MB-231 cells and according to the results obtained from the study, BA induced apoptosis in MDA-MB-231 cells via intrinsic pathway as shown in the Figure 7 (Yazan et al., 2009).

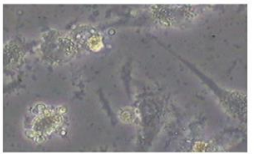

Figure 7. *Cytotoxic effects in MDA-MB-231 cells induced by BA. Characteristic changes in cell morphology was observed after the treatment with BA such as cell blebbing and apoptotic body formation in MDA-MB-231 cells (*Adapted from Yazan et al.. 2009)

According to Yazan et al, BA induces apoptosis, inhibits cell proliferation and angiogenesis. These outcomes were achieved by decreasing the expression of specificity protein (Sp) transcription factors as shown in the figure 8. Usually Sp transcription factors and Sp regulated gene products are not over-expressed in non-tumor tissues. Since these proteins regulate the expression of genes which controls the growth, survival, angiogenesis and metastasis of MDA-MB-231 cells, oncogenic microRNA-27a targets the genes that regulate the Sp levels such as zinc finger ZBTB10 gene which is a putative Sp repressor. BA down regulates the microRNA-27a and up-regulates the ZBTB10 expression. Thereby, it inhibits Sp transcription factors such as Sp1, Sp3 & Sp4 and inhibits the expression of Sp regulated angiogenic products such as vascular endothelial growth factor (VEGF), VEGFR & c-MET. Moreover, BA induces Myt-1 which is another target gene for microRNA-27. Myt-1 enhances the phosphorylation of cdc2 and inactivates it. Thereby, it results in G2/M arrest (Mertens-Talcott et al., 2013). Figure 9 summarizes the outcomes of the study.

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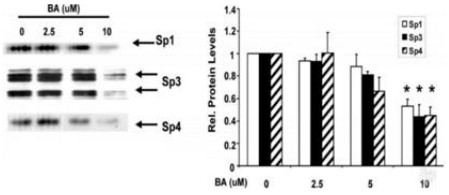

Figure 8. *Decreased protein expression of Sp transcription factors were assessed by western blots. MDA-MB-231 cells were treated with different concentrations of BA for 24 hours and Sp expression was determined. Sp1, Sp3 and Sp4 expression has decreased with the increased BA concentration* (Mertens-Talcott et al., 2013)

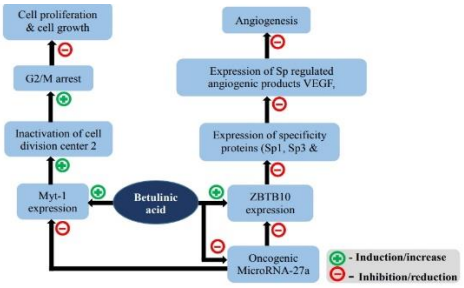

Figure 9. *Summary of the outcomes. BA inhibit the MicroRNA expression and induce ZBTB10 gene expression leading to inhibition of angiogenesis. BA also induces the Myt-1 expression leading to G2/M arrest* (Adapted from Mertens-Talcott et al., 2013)

metastasis by reducing matrix metalloproteinases (MPPs) and myeloid derived suppressor cells (MDSCs). MPPs play a major role in cell proliferation, migration and metastasis. Certain breast cancer cells increase the expression of MPPs via transfecting with certain oncogenes and involve in breast cancer invasion and metastasis. BA activates tissue inhibitor of metalloproteinase-2 (TIMP2) and inhibits the MPP-2 & MPP-9. As well BA reduces myeloid-derived suppressor cells (MDSCs) which are important constituents in tumor microenvironment that promote metastasis. Therefore, by inhibiting MPPs and by reducing MDSCs BA inhibits metastasis. Figure 10 shows the percentage of migrated cell number after the treatment with BA. Whereas, figure 11 summarizes the outcomes of the study (Zeng et al., 2018)

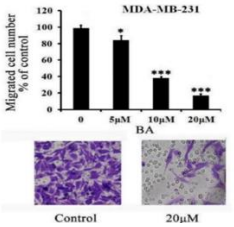

Figure 10. *Inhibition of migration of breast cancer cells by BA, assessed via trans-well migration assays. MDA-MB-231 cells were treated with BA in dose-dependent manner for 48 hours. After 48 hours, migrated cells were fixed, stained, graphed and quantified. With the increased BA dose migrated cell number has reduced* (Zeng et al., 2018)

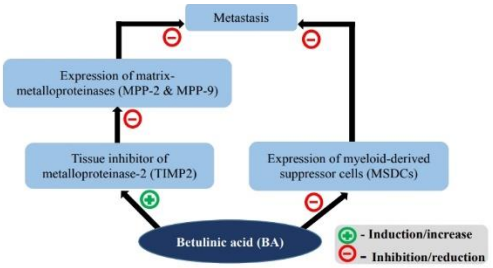

Figure 11. *Summary of the outcomes of the study. BA induces TIMP2 and inhibits MPPs. Thereby, inhibits metastasis. BA also inhibits the expression of MSDCs and promote metastasis inhibition* (Adapted from Zeng et al., 2018)

The natural compound BA shows potent anti-cancer activities through inducing apoptosis, inhibiting cell proliferation, migration and metastasis in TNBC. BA can also be used in combination therapy in order to enhance the efficacy of TNBC treatments. Due to the relative cytotoxicity BA exerts against MDA-MB-231 cells compared to normal cells, BA can be shown as a promising new agent for the treatment of TNBC.

Turmeric (*Curcuma longa*)

Turmeric is commonly used by Asians in order to flavor and color food. It contains phytochemical constituent curcumin (Figure 12) (Prasad and Aggarwal, 2011).

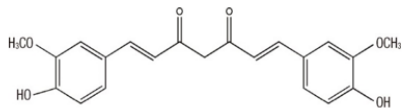

Figure 12. *The chemical structure of curcumin* (Liu and Chen, 2013)

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Curcumin induces p53 activation in MDA-MB-231 cells which results in growth arrest, DNA repair and apoptosis. Figure 13 shows the apoptosis induction in MDA-MB-231 cells by curcumin. TP53 is known as a principle regulator of the cell cycle and a tumor suppressor. It stimulates p21 which is a cyclin dependent kinase inhibitor (CDKI) which interacts with CDK2 and involves in growth arrest. p53 up-regulates the expression of pro-apoptotic BaX and decreases anti-apoptotic Bcl-2 level as well. Thereby, curcumin induces apoptosis and inhibits cell proliferation. Inhibition of migration of MDA-MB-231 cells were achieved by curcumin via suppressing NF-κBp65 which is a major transcription factor of MPPs (Khosropanah et al., 2016; Chiu and Su, 2009; el-Deiry et al., 1993). Figure 14 shows the summary of the outcomes of the study.

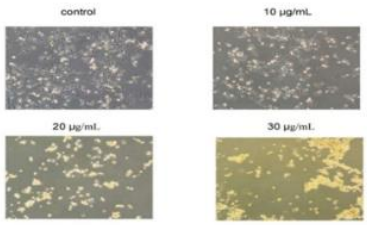

Figure 13: *The dose-dependent effect of curcumin on cellular morphology of MDA-MB-231 cells. The number of MDA-MB-231 cells have reduced when treated with curcumin in dose-dependent manner (Chiu and Su, 2009)*

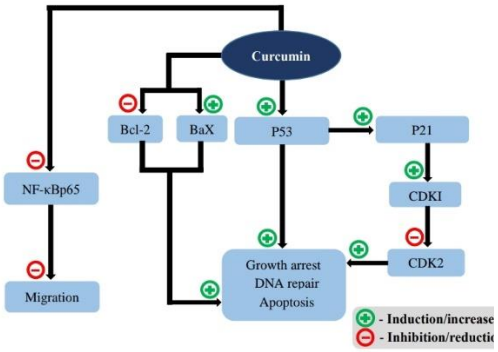

Figure 14. *The summary of the outcomes of the study. Curcumin induces P53, BaX and inhibits Bcl-2 causing growth arrest and apoptosis of MDA-MB-231 cells. It also suppresses NF-κBp65 inhibiting the migration* (Adapted from Chiu and Su, 2009)

Curcumin suppresses MDA-MB-231 cell proliferation and migration via degradation of autophagy dependent Akt pathway which involves in apoptosis, growth, proliferation and metastasis. Aberrant over-expression of Akt proteins such as Akt1, 2 & 3 can be seen in breast cancer. According to Guan et al, curcumin increased the expression of autophagy markers such as LC3-I and LC3-II confirming the induction of autophagy which involves in degradation of intracellular proteins including Akt degradation. Thereby, curcumin suppresses MDA-MB-231 proliferation and migration. Figure 15 shows the migrated cell number after the treatment with curcumin (Guan et al., 2016). Whereas, figure 16 summarizes the outcomes of the study.

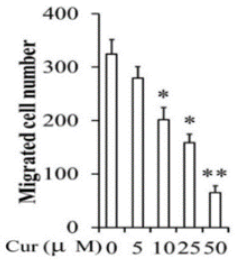

Figure 15. *Effect of curcumin on migration of MDA-MB-231 cells. With the increased dose of curcumin, the migrated cell number has gradually reduced* (Guan et al., 2016)

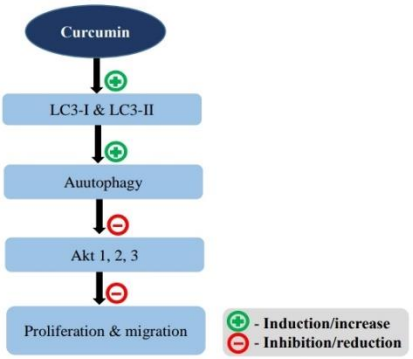

Figure 16. *The summary of the outcomes of the study. Curcumin inhibits the Akt pathway and Akt proteins which promote tumor cell proliferation and migration* (Adapted from Guan et al., 2016)

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Curcumin increases the drug efficacy of docetaxel in metastatic breast cancer patients (Bayet-Robert et al., 2010) and it chemo-sensitizes paclitaxel, doxorubicin and 5-fluorouracil. These synergistic effects of curcumin suggest that it may achieve high therapeutic index when used in combination (Seca and Pinto, 2018). Curcumin in combination with epigallocatechine gallate (EGCG) synergistically inhibits the tumor growth by reducing the expression of VEGF and by cell cycle arrest (Somers-Edgar et al., 2008). The data suggests that curcumin has a significant anti-breast cancer activity and synergistic effects in MDA-MB-231 cells. Hence it might be a potential agent in treating TNBC.

Ginger (*Zingiber officinale*)

Ginger is indigenous to Indian subcontinent and the areas of Southeast Asia. Usually the root of *Zingiber officinale* is used for culinary purposes. Apart from the culinary use ginger exhibits chemo-preventive properties by its constituent phytochemicals such as 6-Shogaol (Figure 17), 6-gingerol (Figure 18) and 10-gingerol (Wallace, 2016).

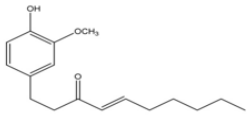

Figure 17. *Chemical structure of 6-Shogaol* (Zheng et al., 2016)

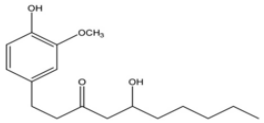

Figure 18. *Chemical structure of 6-Gingerol* (Zheng et al., 2016)

6-Shogaol reduces matrix metalloproteinase-9 expression through blockade of nuclear factor-κB and inhibits the production of tumor-associated dendritic cell (TADs) derived inflammatory mediator CC-chemokine ligand 2 (CCL2) (Hsu et al., 2015; Ling et al., 2010). CCL2 mediates recruitment of tumor-associated macrophages (TAMs) which are promoters of tumor micro-environment. TAMs secrete growth factors, angiogenic factors and inflammatory mediators. Furthermore, CCL2 stimulates angiogenesis independent of TAMs (Steiner and Murphy, 2012). Inhibition of MMP-9 and TADs by 6-shogaol contributes in inhibition of angiogenesis, migration and metastasis.

Another study showed that 6-Shogaol inhibits cancer stem cells (CSCs) via altering the Notch signaling pathway and induces autophagy in MDA-MB-231 cells. CSCs increase the risk of tumor relapse and result in poor prognosis. Notch pathway usually involves in self renewal of stem cells. Deregulated notch pathway is associated with breast cancer. 6-Shogaol cleaves the Notch1 and down-regulates the Notch1 target proteins such as cyclin D1 which involves in cell cycle progression (Ray, Vasudevan and Sengupta, 2015). Over-expressed Notch1 levels are prominent in breast cancer and Notch1 regulates proliferation, motility, cell-cell adhesion, epithelial-mesenchymal-transition (EMT) and invasion (Yuan et al., 2015). Figure 19 shows the summary of the outcomes of 6-shogaol.

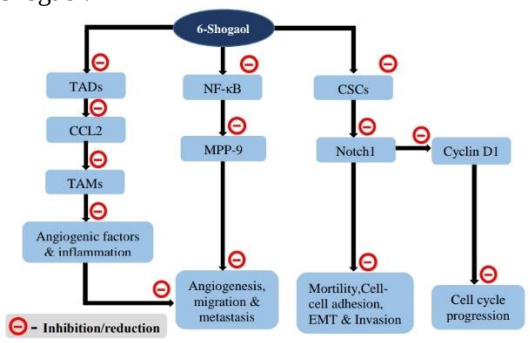

Figure 19. *The summary of the outcomes of the studies. 6-shogaol inhibits MDA-MB-231 cell proliferation, migration, metastasis, EMT and cell-cell adhesion via inhibiting Notch pathway, NF-κB and TADs* (Adapted from Hsu et al., 2015; Ray, Vasudevan and Sengupt, 2015; Ling et al., 2010)

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6-gingerol reduces the expression of MMP-2 and MMP-9 inhibiting proliferation, invasion and metastasis in MDA-MB-231 cells (Lee et al., 2008). Whereas, 10-gingerol down-regulate the cell cycle regulator proteins such as cyclins and CDKs resulting in G2/M arrest inhibiting the proliferation. Moreover, it suppresses the expression of EGFR which is over-expressed in ER- breast cancers. Suppression of EGFR down-regulate the EGFR dependent signaling pathways such as PI3K/Akt and p38 MAPK which promotes cell proliferation, survival, invasion and migration (Joo et al., 2015). Figure 20 shows the inhibition of cell invasion by 10-gingerol. Whereas, Figure 21 shows the summary of the outcomes of 10-gingerol.

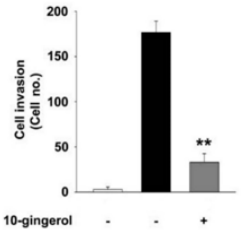

Figure 20. *10-gingerol inhibits MDA-MB-231 cell invasion. After the treatment with 10-gingerol, cell invasion has significantly reduced* (Joo et al., 2015)

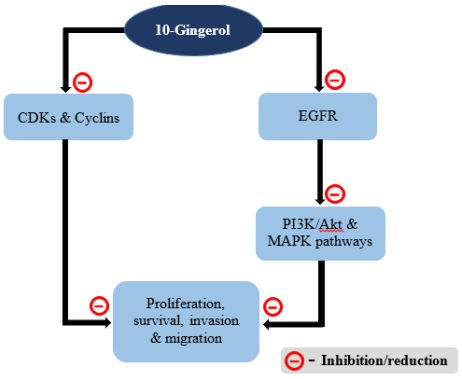

Figure 21. *The summary of the outcomes of the study. 10-Gingerol dow regulate the cell cycle regulator proteins and EGFR which involves in MDA-MB-231 cell proliferation, survival, invasion and migration* (Adap from Joo et al., 2015)

These findings show that 6-Shogaol, 6-Gingerol and 10-Gingerol of ginger are potent anti-tumor agents in MDA-MB-231 cells. Further evaluation and pre-clinical development of these bioactive agents in combination with conventional or molecular targeted therapies could be effective in TNBC treatment.

Garlic (*Allium sativum*)

Garlic is a bulbous plant which is native to Central Asia and its bulb is the most common part used in culinary purposes due to its pungent flavor (Nicastro, Ross and Milner, 2015). Garlic contains bioactive agents such as diallyl diulfide (DADS) and dially trisulfide (DATS). DADS is an oil-soluble organo-sulfur compound which exhibits anti-cancer properties via apoptosis induction and growth inhibition of MDA-MB-231 cells. It up-regulates the apoptotic Bax, down-regulates anti-apoptotic Bcl-XL and activates caspase-3 mediated apoptotic pathway. Figure 22 shows the cleaved caspase-3 in MDA-MB-231 cells by DADS (Nakagawa et al., 2001). Another study showed that DADS induces apoptosis and inhibits metastasis in triple-negative MDA-MB-231 cells via inhibition of ß-catenine pathway which involves in the regulation of Bcl-2 proteins, MMP-9 and epithelial-mesenchymal transition (EMT) (Huang et al., 2015). Figure 23 summarizes the outcomes obtained by DADS.

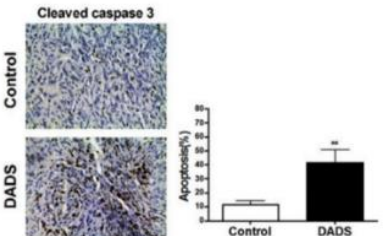

Figure 22. *DADS induced apoptosis was detected by immunohistochemistry staining for cleaved caspases-3. Cleaved caspase-3 levels have significantly increased after the treatment with DADS* (Huang et al., 2015).

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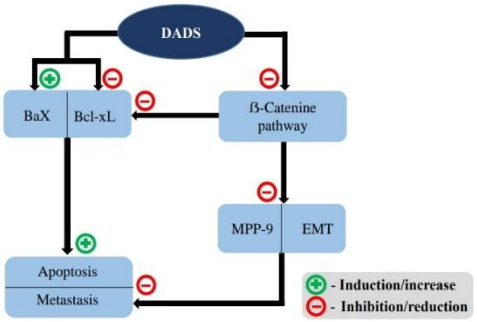

Figure 23. *The summary of the outcomes produced by DADS. DADS induces apoptosis and inhibits metastasis via down-regulating ß-Catenine pathway, Bcl-xL and by up-regulating BaX* (Adapted from Huang et al., 2015).

Diallyl trisulfide (DATS) is another bioactive agent present in garlic which is effective in TNBC. DATS inhibits the MDA-MB-231 proliferation, invasion, migration and metastasis via down-regulating lactose dehydrogenase A (LDHA) which is the key enzyme of Warburg effect. Most cancer cells acquire their energy requirement through aerobic glycolysis in the presence of abound oxygen along with lactic acid fermentation, a process known as Warburg effect. This provides energy for growing tumors promoting proliferation and survival. In glycolysis, glucose is converted to pyruvate and under limited oxygen conditions pyruvate transform into lactate. This transformation is mainly based on LDHA. Therefore, targeting of LDHA by DATS can be shown as an important target in developing novel treatment strategy for TNBC. Figure 24 shows the cell viability after the treatment with DATS and figure 25 summarizes the outcomes of the study (Cheng et al., 2016).

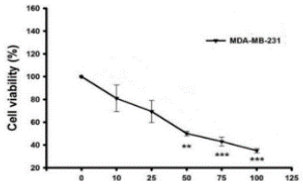

Figure 24. *Inhibitory effects of DATS on cell viability by MTT assay. MDA-MB-231 cell viability has gradually reduced after the treatment with DATS in dose-dependent manner* (Cheng et al., 2016)

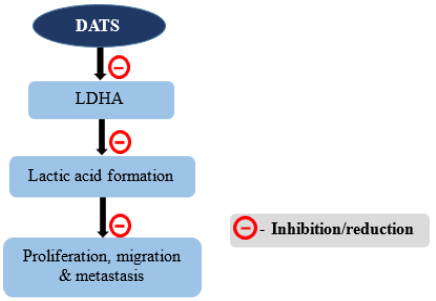

Figure 25. *The summary of the outcomes of the study. DATS inhibits proliferation, migration and metastasis by down-regulation of LDHA* (Adapted from Cheng et al., 2016)

According to the data, DADS and DATS can be shown as potent anti-breast cancer agents which promote apoptosis and inhibits metastatic potential. Hence, DADS and DATS might be potential chemotherapeutic agents in TNBC.

Red Onion (*Allium cepa*)

Red onion is commonly used in culinary purposes as a flavourant. Usually the bulbous part of the Allium cepa plant is used. One of the main bioactive agent of red onion is quercetin (Corea et al., 2005). Quercetin induces apoptosis in MDA-MB-231 cells via intrinsic pathway by promoting the activation of caspase-3, 8 and 9. It was achieved by increasing the Bax which is a pro-apoptotic protein and by decreasing Bcl-2 which is an anti-apoptotic protein (Chien et al., 2009). Another study showed that quercetin induces apoptosis through inhibition of fatty acid synthase (FASN) and ß-catenin. FASN involves in fatty acid biosynthesis. Figure 26 shows the apoptosis induced by quercetin in MDA-MB-31 cells. Elevated levels of FASN is associated with breast cancer which promotes estrogen receptor signaling and enhance breast cancer cell growth (Sultan et al., 2017; Menendez and Lupu, 2017).

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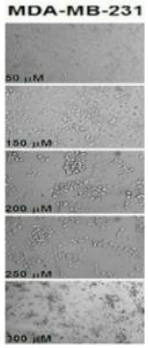

Figure 26. *Quercetin induced morphological changes in MDA-MB-231. With the increased dose of quercetin, apoptosis in MDA-MB-231 cells has increased* (Sultan et al., 2017).

Quercetin induces growth arrest through modulation of Foxo3a activity. It was found to be achieved via increased p51 and p21 expression (Nguyen et al., 2017). Moreover, it has the ability to inhibit breast cancer stem cells (CSCs) via down-regulating aldehyde dehydrogenase (ALDH1A1), epithelial cell adhesion molecule (EpCAM), chemokine receptor CXCR4 and Mucin 1 (MUC1). In majority of the patients, breast cancer recurrence occur as a result of CSCs. They differentiate and proliferate increasing the metastatic potential. ALDH1A1 is an important marker of CSCs which has high capability to initiate tumor recurrence and increase the drug resistance. MUC1 is a trans-membrane glycoprotein which interacts with EFGR and ß-catenine to activate growth-related signaling pathways. Over-expression of MUC1 involves in increased proliferation and metastasis while EpCAM up-regulates the cyclin A and activates Wnt signaling pathway that involves in tumor cell migration. CXCR4 also plays an important role in promoting breast cancer carcinogenesis via increasing proliferation, migration and metastasis (Wang et al., 2018). Therefore, targeting of ALDH1A1, EpCAM, MUC1 and CXCR4 by quercetin inhibits MDA-MB-231 cell proliferation, migration, metastasis and induces apoptosis. Figure 27 shows inhibition of migration by quercetin and figure 28 shows the summary the outcomes of studies.

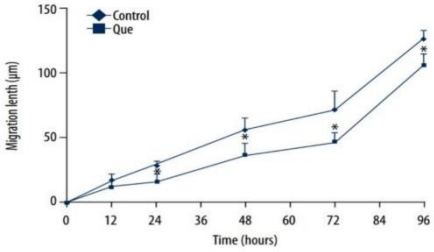

Figure 27. *Analysis of migration via scratch assay. After the treatment with quercetin in dose-dependent manner migration has reduced when compared with the control* (Wang et al., 2018).

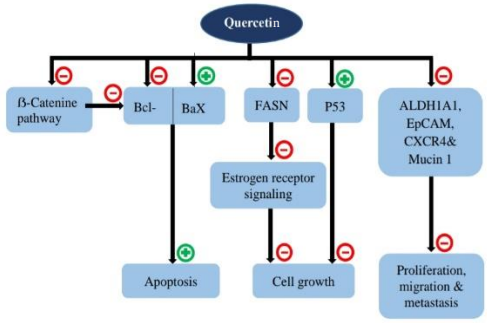

Figure 28. *The summary of outcomes produced by quercetin. Quercetin up-regulates BaX and P53 expression inducing apoptosis and growth arrest. It also inhibits FASN and inhibits the estrogen receptor signaling which stimulate the breast cancer cell growth. Quercetin inhibits MDA-MB-231 proliferation, migration and metastasis via down-regulating ALDH1A1, EpCAM, CXCR4 and mucin 1* (Adapted from Wang et al., 2018; Sultan et al., 2017)

Synergistic effects of quercetin have been proven by exposing quercetin along with topotecan which is a chemotherapeutic agent. According to the study, combination with quercetin increased the efficacy of topotecan in breast cancer treatment (Akbas, Timur and Ozben, 2005). These studies show that, quercetin is highly effective in treating MDA-MB-231 cells suggesting that quercetin might be a potential therapeutic agent in developing novel therapies for TNBC.

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Although culinary spices provide a wide range of medicinal benefits, appropriate doses should be consumed and high doses may produce toxic effects in the human body. Up to now, none of the studies have been reported with any negative outcomes in MDA-MB-231 cell line in response to treatment with different bioactive agents. Majority of the bioactive agents have showed high efficacies in MDA-MB-231 cell line. However, unlike in MDA-MB-231 cell line the efficacy may vary in other triple-negative cell lines. Hence, there might be side effects arise with response to different doses of different bioactive agents in other breast cancer cell lines.

A study reported the side effects of curcumin after being studied for a long period in other cancer cell lines. In a long-term study (2 years) curcumin has induced toxic and carcinogenic effects in rats and mice under specific conditions while it was found to be effective in short-term studies of 3 months (Lopez-Lazaro, 2008). These data suggests that despite the advantages, there might be therapeutic limitations in these bioactive agents. Hence, further experimentations are necessary to reveal negative effects and how to overcome those. It may include structural modifications, alternative formulations, enhancing pharmacological potential and increased use off effective drug delivery systems.

SUMMARY

This review scratches the healing power of culinary spices and herbs in treatment of TNBC. Many studies have suggested that culinary spices and herbs show anti-breast cancer activities and its effectiveness in TNBC. Clinical heterogeneity of TNBC results in limitations to available treatment methods.

Therefore it is necessary to explore promising treatment strategies for TNBC. Hence, there is a growing amount of literature concerning the potential chemo-preventive properties of natural spices and herbs. In the current review the effects of betulinic acid, curcumin, gingerols, shogaol, diallyl sulfides and quercetin were discussed elaborating their effectiveness in triple-negative MDA-MB-231 cell line suggesting that they are potential chemotherapeutic agents in treatment of TNBC. In order to develop novel treatment strategies, it is important to experiment more about their therapeutic effects, mechanism of action, synergistic effects and side effects. Steps should be taken to increase the consumption natural products such as culinary spices and herbs which may promote breast cancer prevention.

REFERENCES

Aggarwal, B.B. and Takada, Y. (2003) "Betulinic acid suppresses carcinogenic-induced NF-κB activation through inhibition of IκBa kinase and p65 phosphorylation: Abrogation of cyclooxygenase-2 and matrix metalloprotease", The Journal of Immunology, Vol. 171, No. 6, pp.3278-3286.

Akbas, S.H., Timur, M. and Ozben, T. (2005) "The effect of quercetin on topotecan cytotoxicity in MCF-7 and MDA-MB-231 human breast cancer cells", Journal of Surgical Research, Vol. 125, No. 1, pp. 49-55.

Badowska-Kozakiewicz, A.M. and Budzik, M.P. (2016) "Immunohistochemical characteristics of basal-like breast cancer", Contemporary Oncology, Vol. 20, No. 6, pp.436-443.

Bauer, J.A., Chakravarthy, A.B., Chen, X., Lehmann, B.D., Pitenpol, J.A., Sanders, M.E., Shyr, Y. (2011) "Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies", Journal of Clinical Investigations, Vol. 121, No. 7, pp. 2750-2767.

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Bauer, K.R., Brown, M., Cress, R.D., Parise, C.A. and Caggiano, V. and (2007) "Descriptive analysis of estrogen receptor (ER)- negative, progesterone receptor (PR)-negative and HER2 negative invasive breast cancer, the so-called triple-negative phenotype", Cancer, Vol. 109, No. 9, pp. 1721-1727.

Bayet-Robert, M., Kwiatkowski, F., Leheurteur, M., Gachon, F., Planchat, E., Abrial, C., Mouret-Reynier, M.A., Durando, X., Barthomeuf, C. and Cohollet, P. (2010) "Phase I dose escalation trail of docetaxel plus curcumin in patients with advanced and metastatic breast cancer", Cancer Biology & Therapy, Vol. 9, No. 1, pp.8-14.

Berrocal, J.K. and Chagpar, A.B. (2017) "Current approaches to triple negative breast cancer", The American Journal of Hematology/Oncology, Vol. 13, No. 6, pp.16-19.

Chavez, K.J., Garimella, S.V. and Lipkivitz, S. (2010) "Triple negative breast cancer cell lines: One tool in the search for better treatment of triple negative breast cancer", Breast Disease, Vol. 32, No. 2, pp.35-48.

Cheng, S.Y., Yang, Y.C., Ting, K.L., Wen, S.Y., Viswanadha, V.P., Huang, C.Y. and Kuo, W.W. (2016) "Lactate dehydrogenase down-regulation mediates the inhibitory effect of diallyl trisulfide on proliferation, metastasis and invasion in triple-negative breast cancer", Environmental Toxicology, Vol. 32, No. 4, pp. 1390-1398.

Chien, S.Y., Wu, Y.C., Chung, J.G., Yang, J.S., Lu, H.F., Tsou, M.F., Wood, W.G., Kuo, S. and Chen, D.R. (2009) "Quercetin-induced apoptosis acts through mitochondrial- and caspase-3-dependent pathways in human breast cancer MDA-MB-231 cells", Human & Experimental Toxicology, Vol. 28, No. 8, pp.493-503.

Chiu, T.L. and Su, C.C. (2009) "Curcumin inhibits proliferation and migration by increasing the Bax to Bcl-2 ratio and decreasing NF-κBp65 expression in breast cancer MDA-MB-231 cells", International Journal of Molecular Medicine, Vol. 23, pp.469-475.

Corea, G., Fattorusso, E., Lanzotti, V., Capasso, R. and Izzo, A.A. (2005) "Antispasmodic saponins from bulbs of red onion, Allium cepa L. Var. Tropea", Journal of Agricultural and Food Chemistry, Vol. 53, No. 4, pp. 935-940.

Dai, X., Li, T., Bai, Z., Yang, Y., Liu, X., Zhan, J. and Shi, B. (2015) "Breast cancer intrinsic subtype classification, clinical use and future trends", American Journal of Cancer Research, Vol. 5, No. 10, pp.2929-2943.

Dias, K., Dvorkin-Gheva, A., Hallett, R.M., Wu, Y., Hassell, J., Pond. G.R., Levine, M., Whelan, T. and Bane, A.L. (2017) "Caludin-low breast cancer; clinical & pathological characteristics", PLoS ONE, Vol. 12, No. 1.

el-Deiry, W.A., Tokino, T., Velculescu, V.E., Levy, D.B., Parsons, R., Trent, J.M., Lin, D., Mercer, W.E., Kinzler, K.W. and Vogelstein, B. (1993) "WAF1, a potential mediator of p53 tumor suppression", Cell, Vol. 75, pp.817-825.

Ghoncheh, M., Pournamdar, Z. and Salehiniya, H. (2016) "Incidence and mortality and epidemiology of breast cancer in the world", Asian Pacific Journal of Cancer Preview, Vol. 17, pp.43-46.

Guan, F., Ding, Y., Zhang, Y., Zhou, Y., Li, M. and Wang, C. (2016) "Curcumin suppresses proliferation and migration of MDA-MB-231 breast cancer cells through autophagy-dependent Akt degradation", PLoS ONE, Vol. 11, No. 1, pp.1-18.

Hsu, Y.L., Hung, J.Y., Tsai, E.M., Huang, M.S., Hou, M.F. and Kuo, P.L. (2015) "6-Shogaol, an active constituent of dietary ginger, impairs cancer development and lung metastasis by inhibiting the secretion of CC-Chemokine Ligand 2 (CCL2) in tumor-associated dendritic cells", Journal of Agricultural and Food Chemistry, Vol. 63, No. 3, pp.1730-1738.

Huang, J., Yang, B., Xiang, T., Peng, W., Qui, Z., Wan, J., Zhang, L., Li, H., Li, H. and Ren, G. (2015) "Diallyl disulfide inhibits growth and metastatic potential of human triple-negative breast cancer cells through inactivation of the ß-catenin signaling pathway", Molecular Nutrition & Food Research, Vol. 59, No. 6, pp.1063-1075.

Joo, J., Hong, S., Cho, Y.R. and Seo, D.W. (2015) "10-Gingerol inhibits proliferation and invasion of MDA-MB-231 breast cancer cells through suppression of Akt and p38MAPK activity", Oncology Reports, Vol. 35, pp. 779-784.

Kaefer, C.M. and Milner, J.A. (2011) "The role of herbs and spices in cancer prevention and treatment", Journal of Nutritional Biochemistry, Vol. 19, No. 6, pp.347-361.

Khosropanah, M.H., Dinarvand, A., Nezhadhosseini, A., Haghighi, A., Hashemi, M., Nirouzad, A., Khatamsaz, S., Entezari, M., Hashemi, S. and Dehghani, H. (2016) "Analysis of the antiproliferative effects of curcumin and nanocurcumin in MDA-MB-231 as a breast cancer cell line", Iranian Journal of Pharmaceutical Research, Vol. 15, No. 1, pp.231-239.

Kreike, B., Kouwenhove, M.V., Horlings, H., Weigelt, B., Peterse, H., Bartelink, H., and van de Vijver, M.J. (2007) "Gene expression profiling and histopathological characterization of triple-negative/basal-like breast carcinomas", Breast Cancer Research, Vol. 9, No. 5.

Lee, H.S., Seo, E.Y., Kang, N.E. and Kim, W.K. (2008) "[6]-Gingerol inhibits metastasis of MDA-MB-231 human breast cancer cells", Journal of Nutritional Biochemistry, Vol. 19, No. 5, pp.313-319.

Liburdi, K., Benucci, I., Lombardelli, C. and Esti, M. (2017) "Identification and characterization of lipoxygenase in fresh culinary herbs", International Journal of Food Therapeutics, Vol. 20, No. 7, pp. 1470-1478.

Ling, H., Yang, S.H., Tan, S.H., Chui, W.K. and Chew, E.H. (2010) "6-Sogaol, an active constituent of ginger, inhibits breast cancer cell invasion by reducing matrix metalloproteinase-9 expression via blockade of nuclear factor-κB activation", British Journal of Pharmacology, Vol. 161, No. 8, pp.1763-1777.

Liu, D. and Chen, Z. (2013) "The effect of curcumin on breast cancer cells', Journal of Breast Cancer", Vol.16, No. 2, pp.133-137.

Lopez-Lazaro, M. (2008) "Anticancer and carcinogenic properties of curcuma: Considerations for its clinical development as a cancer chemopreventive and chemotherapeutic agent", Molecular Nutrition & Food Research, Vol. 52, No. 1, pp.103-127.

Maegawa, R.O.B. and Tang, S. (2010) "Triple negative breast cancer: unique biology and its management", Cancer Investigation, Vol. 28, No. 8, pp. 878-883.

Menendez, J.A. and Lupu, R. (2017) "Fatty acid synthase regulates estrogen receptor-α signaling in breast cancer cells", Oncogenesis, Vol. 6, No. 2.

Mertens-Talcott, S.U., Noratto, G.D., Li, X., Angel-Morales, G., Bertoldi, M.C. and Safe, S. (2013) "Betulinic acid decreases ER-negative breast cancer cell growth in vitro and in vivo: role of Sp transcription factors and microRNA-27:ZBTB10", Molecular Carcinogenesis, Vol. 52, No. 8, pp.591-602.

Mertens-Talcott, S.U., Noratto, G.D., Li, X., Angel-Morales, G., Bertoldi, M.C. and Safe, S. (2013) "Betulinic acid decreases ER-negative breast cancer cell growth in vitro and in vivo: role of Sp transcription factors and microRNA-27:ZBTB10", Molecular Carcinogenesis, Vol. 52, No. 8, pp.591-602.

Nakagawa, H., Tsuta, K., Kiuchi, K., Senzaki, H., Tanaka, K., Hioki, K. and Tsubura, A. (2001) "Growth inhibitory effects of diallyl disulfide on human breast cancer cell lines", Carcinogenesis, Vol. 22, No. 6, pp.891-897.

Nguyen, L.T., Lee, Y., Sharma, A.R., Park, J., Jagga, S., Sharma, G., Lee, S. and Nam, J. (2017) "Quercetin induces apoptosis and cell cycle arrest in triple negative breast cancer cells through modulation of Foxo3a activity", Korean Journal of Physiology & Pharmacology, Vol. 21, No. 2, pp.205-213.

Nicastro, H.L., Ross, S.A. and Milner, J.A. (2015) "Garlic and onions: Their cancer prevention properties", Cancer Prevention Research, Vol. 8, No. 3, pp.181-189.

Opara, E.I. and Cjohan, M. (2014) "Culinary herbs and spices: Their bioactive properties, the contribution of polyphenols and the challenges in deducing their true health benefits", International Journal of Molecular Sciences, Vol. 15, No. 10, pp.19183-19202.

Prasad, S. and Aggarwal, B.B. (2011) "Turmeric, the golden spice", in Benzie, F.F. and Galor-Wachtel, S. (ed.) Herbal Medicine. Taylor & Francis.

Ray, A., Vasudevan, S. and Sengupta, S. (2015) "6-Shogaol inhibits breast cancer cells and stem cell-like spheroids by modulation of notch signaling pathway and induction of autophagic cell death", PLoS ONE, Vol. 10, No. 9, pp. 1-22.

Ribelles, N., Perez-Villa, L., Jerez, M., Pajares, B., Vicioso, L., Jimenez, B., Luque,

A., Franco, L., Gallego, E., Marquez, A., Alvarez, M., Sanchez-Munoz, A., Perez-Rivas, L. Perez-Rivas, L. and Alba, E. (2013) "Pattern of recurrence of early breast cancer is different according to intrinsic subtype and proliferation index", Breast Cancer Research, Vol. 15, No. 5.

Robertson, L., Hanson, H., Seal, S., Warren-Perry, M., Hughes, D., Howell, I., Turnbull, C., Houlston, R., Shanley, S., Butler, S., Evans, D.G., Ross, G., Eccles, D., Tutt, A., and Rahman, N. (2012) "BRCA1 testing be offered to individual with triple-negative breast cancer diagnosed below 50 years", British Journal of Cancer, 1 Vol. 06, pp. 1234-1238.

Saadat, S. (2015) "Can we prevent breast cancer", International Journal of Health Sciences, Vol. 2, No. 2, pp.167-170.

Seca, A.M.L. and Pinto, D.C.G.A. (2018) "Plant secondary metabolites as anticancer agents: Successes in clinical trials and therapeutic application", International Journal of Molecular Sciences, Vol. 19, No. 1, pp.1-22.

Seigel, R., Naishadham, D. and Jemal, A. (2013) "Cancer statistics 2013", Cancer Journal for Clinicians, Vol. 63, No. 1, pp.11-30.

Smith, L., Watson, M.B., O'Kane, S.L., Drew, P.J., Lind, M.J. and Cawkwell, L. (2006) 'The analysis of doxorubicin resistance in human breast cancer cells using antibody microarrays', Molecular Cancer Therapeutics, 5(8), pp. 2115-2120.

Somers-Edgar, T.J, Scandlyn, M.J., Stuart, E.C., Nedelec, M.J., Valentine, S.P. and Rosengren, R.J. (2008) "The combination of epigallocatechin gallate and curcumin suppresses ERα-breast cancer cell growth in vitro and in vivo", International Journal of Cancer, Vol. 122, pp. 1966-1971.

Steiner, J.L and Murphy, E.A. (2012) "Importance of chemokine (CC-motif) ligand 2 in breast cancer", International Journal of Biomarkers, Vol. 27, No. 3, pp.179-185.

Sultan, A. S., Khail, M.I.M., Sami, B.M., Alkhuriji, A.F. and Sadek, O. (2017) "Quercetin induces apoptosis in triple-negative breast cancer cells via inhibiting fatty acid synthase and ß-catenin", International Journal of Experimental Pathology, Vol. 10, No. 1, pp.156-171.

Uscanga-Perales, G.I., Santuario-Facio, S.K. and Ortiz-Lopez, R. (2016) "Triple negative breast cancer: Deciphering the biology and heterogeneity", Medicina Universitaria, Vol. 18, No. 71, pp.105-114.

Wallace, D. (2016) "Natural products as a source of anti-cancer lead compounds: Ginger and breast cancer", International Journal of Clinical Pharmacology Research, Vol. 1, No. 3, pp. 1-6.

Wang, R., Yang, L., Li, S., Ye, D., Yang, L., Liu, Q., Zhao, Z., Cai, Q., Tan, J. and Li, X. (2018) "Quercetin inhibits breast cancer stem cells via downregulation of aldehyde dehydrogenase 1A1 (ALDH1A1), chemokine receptor type 4 (CXCR4), mucin 1 (MUC1) and epithelial cell adhesion molecule (EpCAM)", International Medical Journal of Experimental and Clinical Research, Vol. 24, pp.412-420.

Yazan, L.S., Ahmad, F.H., Li, O.C., Rahim, R.A., Hamid, H.A. and Sze, L.P. (2009) "Betulinic acid was more cytotoxic towards the human breast cancer cell lines MDA-MB-231 than the human promyelocytic leukemia cell line HL-60", Malaysian Journal of Pharmaceutical Sciences, Vol. 7, No. 1, pp.23-37.

Yuan, X., Zhang, M., Wu, H., Xu, H., Han, N., Chu, Q., Yu, S., Chen, Y. and Wu, K. (2015) "Expression of Notch1 correlates with breast cancer progression and prognosis", PLoS ONE, Vol. 10, No. 6, pp. 1-13.

Zeng, A., Yu, Y., Yao, Y., Yang, F., Liao, M., Song, L., Li, Y., Yu, Y., Li, Y., Deng, Y., Yang, S., Zeng, C., Liu, P., Xie, Y., Yang, J., Zhang, Y., Ye, T. and Wei, Y. (2018) "Betulinic acid impairs metastasis and reduces immunosuppressive cells in breast cancer models", Oncotarget, Vol. 9, No. 3, pp. 3794-3804.

Zheng, J., Zhou, Y., Li, Y., Xu, D. P., Li, S. and Li, H.B. (2016) "Spices for prevention and treatment of cancers", Nutrients, Vol. 8, pp.1-35.