ANTI-CANCER AND ANTI-ANGIOGENIC ACTIVITIES OF NATURAL COMPOUND ZERUMBONE FROM ZINGIBER ZERUMBET – A SYSTEMATIC REVIEW

BY
REHAB M. H. ELFAGIE

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (HEALTH TOXICOLOGY)

ADVANCED MEDICAL AND DENTAL INSTITUTE
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DECLARATION

Here, I declare that this research has been sent to University Sains Malaysia for degree of Master of Science. It is also not being send to any other universities. With that, this research might be used for the consultation and can be photocopied as reference.

REHAB M. H. ELFAGIE

P-IPM0015/18

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Finally, endless appreciation to USM staff, and all my colleagues, who support me with what they can throughout this academic year and especially during my daughter's illness. Only God can reimburse them for all they have done for me.

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LIST OF ABBREVIATIONS

A375	Melanoma cells
A549	Non-small cell lung cancer cell Embryonic
A293	Kidney carcinoma cell
Apaf-1	Apoptotic protease activating factor 1
AsPC-1	Pancreatic adenocarcinoma
Bad	Bcl-2-associated death promoter
Bak	Bcl-2 homologous antagonist/killer
Bax	Bcl-2-associated X protein
Bcl-2	B cell lymphoma 2
Caki-1	Human renal carcinoma cell line
CARD	Caspase activation and recruitment domain
Caov-3	Ovarian cancer
Calpain I	Calcium-dependent proteolytic enzyme
CEMss	T4-Lymphoplastoid cell line
cH2AX	DNA damage biomarker
CHL-1	Human melanoma cell line
CHO	Chinese Hamster Ovary
CML	CML chronic myeloid leukaemia
CML-K562	Chronic myelogenous leukaemia
CXCR4	C-X-C MOTIF) receptor 4
CXCL12	CXCR4 ligand
DED	Death effector domain
DFF45/ICAD	DNA fragmentation factor45 kD subunit

DISC	Death-inducing signaling complex
DR4 and DR5	Death receptor 4 and 5
DU145	Pancreatic cancer cells
E-cadherin	Cell adhesion proteins
ECs	Endothelial cells
EC-109	Esophagus cancer cells
EMT	Endothelial mesenchymal transition
ERK	Extracellular signal-regulated kinase
FLIP	FLICE-Like Inhibitor Protein
FADD	Fas-associated death domain
GSH	Glutathione
HCT116 cells	Human CRC cell lines
Hela	Human cervical cancer
Hep-2	Laryngeal carcinoma cells
H1299	Lung adenocarcinoma
Hs578T	Breast cell cancer
HT29	Human CRC cell lines
HtrA2	Human protein Serine protease
HUVEC	Human umbilical vein endothelial cell
IAP	Inhibitors of Apoptosis
IGF-1	Insulin-like growth factor 1
IKB	Inhibitor of Kappa B
IKK	Inhibitor of Kappa B Kinase
IL-6	Interleukin-6
KBM-5	Myelogenous cell line

MCF-7	Human breast cancer cell line
MCF-10A	Breast cancer cell line
MDA-MB-468	Breast cancer cell line
MDA-MB-361	Breast cancer cell line
MDA-MB-231	Mammary Breast cancer cell line
MIAP	Microscopy and Image Analysis Platform
MMP	Mitochondrial membrane permeabilization
MPM-2	Monoclonal antibody raised against HeLa cells
MPTP	Mitochondrial permeability transition pore
NCI-H1299	Human non–small lung cancer cell lines
NCI-H460	Human non–small lung cancer cell lines
NDUFS1	NADH-ubiquinone-oxidoreductase 75 KD
NFκB	Nuclear Factor Kappa B
NIK NFκB	Inducing Kinase
NKT cells	Natural Killer T cells
NMR	Nuclear magnatic resonance spectroscopy
786-O	Human renal carcinoma cell line
OPN	Osteopontin
ORL-48	Oral squamous cell carcinoma (OSCC)
OSCC cells	Oral squamous cell carcinoma
Ovaca8	Ovarian cancer cell
PaCa	Pancreatic carcinoma
PANC-1 cells	Pancreatic cancer cells
PANC-28	Pancreatic carcinoma
PARP	Poly ADP ribose polymerase

PBS	Phosphate-buffered saline
PC-12	Pheochromocytoma cells
PC-3	Prostate cancer cells
769-P cell lines	Human renal carcinoma cell line
PI3Ks	Phosphatidylinositol-3- kinases
PKB AKT	Protein kinase B
PKCd	Protein kinase C delta type
P53	Tumour suppressor gene
Rac1	Ras-related C3 botulinum toxin substrate1
RIP	Ribosome Inactivating Protein
ROS	Reactive oxygen species
ROCK/LIMK	Rho-associated kinase and activate LIM kinases
RXRα	Retinoid-x-receptor-α
SCC4	Squamous cell carcinoma
SEM	Scanning electron microscopy
SGC-7901 cells	Gastric cancer
SiHa	Human cervical carcinoma lines
siRNA	Small-interfering ribonucleic acid
SMAC	Second-mitochondria-derived activator of caspase
STAT3	Signal transducer and activator of transcription
SW620 cells	Human CRC cell lines
SW480	Human colon cancer cell lines
SW1990	Human pancreatic carcinoma cell lines
T-47D	Breast cancer cell line
3T3	Mouse fibroblast

TNBC cells	Breast cancer cell line
TNF-α	Tumour necrosis factor alpha
TRAIL	Tumour necrosis factor-related apoptosis-inducing ligand
U266	Multiple myeloma
VEGF-A	Vascular endothelial growth factor - A
WEHI-3B	Murine monomyelocytic leukaemia
WEHI 7.2	Wild type murine thymoma
WM1552C	Human melanoma cell line
WRL68 cells	Normal cell
ZER	Zerumbone

ABSTRAK

Beberapa kajian penting telah menunjukkan kesan anti-percambahan bagi Zerumbone serta peranannya sebagai anti-angiogenesis. Tujuan utama ulasan sistematik ini adalah untuk menilai kesan anti-kanser dan peranan kompoun ini sebagai anti-angiogenesis dalam mengubati kanser. Artikel-artikel yang berkaitan telah dipilih berdasarkan kriteria kemasukan spesifik. Artikel-artikel yang dipilih adalah antara Januari 2008 sehngga Disember 2018. Artikel-artikel yang berkaitan telah dikenalpasti secara carian meluas di dalam Science Direct, PubMed, Google Scholar dan Scopus. Carian literatur yang digunakan dalam pengkalan data elektronik adalah dengan gabungan kata kunci-kata kunci seperti berikut : 'anti-angiogenic, anticancer, Zerumbone dan *Zingiber zerumbet*'. Kajian-kajian yang telah dipilih bagi ulasan ini termasuk kajian *in vitro*, *in vivo* dan *ex vivo*. Perolehan permulaan dalam pencarian bagi kajian literatur ini adalah sebanyak 352 rekod dan jumlah kajian terakhir yang memenuhi kriteria kemasukan bagi ulasan ini adalah sebanyak 43 kajian. Kajian *In vitro* merupakan jenis kajian yang tertinggi. Secara jelasnya, Zerumbone berpotensi sebagai anti-percambahan dan anti-angiogenesis. Aktiviti anti-percambahan bagi Zerumbone telah ditunjukkan oleh pelbagai isyarat laluan yang berbeza. Zerumbone dengan kesan anti-angiogenesisnya telah memainkan peranan yang penting dalam mengurangkan penyerangan dan perebakan dalam kanser. Beberapa kajian terpilih bagi Zerumbone didapati mempunyai limitasi-limitasi antaranya kurang kejelasan bagi nilai ambang toksik yang diperlukan bagi kompoun ini dalam peringkat ujikaji klinikal.

ABSTRACT

Significant number of literature has demonstrated the antiproliferative effect of Zerumbone and its role as anti-angiogenesis. The aims of this systematic review were to evaluate the anti-cancer effects of Zerumbone and the role of its antiangiogenic properties in treating cancer. Relevant articles were selected based on specific inclusion criteria. Articles chosen for this systematic review were between January 2008 and December 2018. Relevant articles were identified through an extensive search in Science Direct, PubMed, Google Scholar and Scopus. The literature searches of the electronic databases combined the following key words: anti-angiogenic, anticancer, Zerumbone and *Zingiber zerumbet*. Studies chosen for this review includes the following designs *in vitro*, *in vivo* and *ex vivo*. The initial literature search obtained a total of 352 related records and the final number of studies that met the inclusion criteria in the current review was 43 studies. *In vitro* studies was the commonest study design. Evidently, Zerumbone demonstrate a potential antiproliferative and antiangiogenic. The antiproliferative activities of Zerumbone was shown to induce by different signalling pathway. Zerumbone through its antiangiogenic effect play a great role in reducing invasion and metastasis. Some selected studies on Zerumbone were found to plague with limitation such as lack of toxic threshold value which may be needed for the clinical trials on this compound.

CHAPTER 1

INTRODUCTION

1.1 Background

Cancer is the second most common cause of death in the US and worldwide. Dysregulation of the molecular pathways which alters protein expression resulting in uncontrolled cellular proliferation is the most identified underlying mechanism of cancer (Xia, 2006). There has been significantly improve in anticancer drug discovery herbal medicine. Poor efficacy, undesirable side effects, and tumor resistance were associated with drugs that target specific signaling pathways, while drugs that interfere with multiple molecules responsible for tumor growth have more efficacy and targeting different cancer cell line. Multi-targets drugs are considered to be more effective and less susceptible to drug-resistance (Adel S Al-Zubairi et al., 2010).

Angiogenesis, a process where new vasculature initiated from pre-existing normal vessels. Initiation of angiogenesis starts with localized release of pro and anti-angiogenesis growth factors by endothelial cells (ECs) (Prasannan et al., 2012).

New blood vessel inhibition properties of angiogenic proteins in cancer cells will induce endothelial cell apoptosis or inhibiting endothelial receptors. Plant derived anti-angiogenesis agents are unlike conventional chemotherapeutic agents. It can be used in lower doses with less cytotoxicity, and over a period of months to years to prevent recurrence from latent micro metastases (Adel S Al-Zubairi et al., 2010).

Zingiber zerumbet from Zingiberaceae family consisted of a range of different phytochemical groups. This plant is highly consumed in the traditional Asian dietary. Zerumbone is one of these phytochemicals (Wahab et al., 2009). It possesses antiproliferative properties towards various cancer cell lines with minimal side effect

on normal cells and it also exhibits antiangiogenic activities in cancer (N. A. Samad et al., 2015).

1.2 Problem statement

Despite numerous research focused on the effects of Zerumbone as anti-cancer and anti-angiogenesis compound, limited evidence-based systematic review regarding these topics available.

1.3 Significance of the study

Zerumbone extracted from *Zingiber zerumbet* is proven to have anti-cancer and anti-angiogenesis properties. The effect of Zerumbone on cancer cells has been established through *in vitro*. *in vivo* and *ex vivo* researches. Zerumbone displays promising anticancer effects through several mechanisms such as cell cycle arrest, apoptosis, modulate multiple molecular targets and inhibition of tumour angiogenesis (Rahman et al., 2014; Taha et al., 2010).

This systematic review aimed to systematically summarize studies done on Zerumbone, including its mechanisms of actions, therapeutic and prophylactic potential against different cancers and its role as angiogenesis inhibitor.

1.4 Objectives of the study.

Main Objective

To review the efficacy of Zerumbone extracted from *Zingiber zerumbet* as an anti-cancer and anti-angiogenesis agent.

Specific objectives

1. To review the *in vitro*, *ex vivo* and *in vivo* studies of Zerumbone
2. To determine the most effective dose of Zerumbone for the anti-cancer and anti-angiogenesis treatment.
3. To review the mechanism of action of Zerumbone.

CHAPTER 2

LITERATURE REVIEW

2.0 Cancer

Cancer, also known as malignant neoplasm consists of a spectrum of diseases defined by unregulated cell growth in the body mainly due to function failure of tumour suppressor genes. If cancer cell growths are not arrested, the cells will divide uncontrollably and form malignant tumours, which may invade nearby organs. The metastasize cancer cells could also affect distant parts of the body via lymphatics or circulation and cause morbidity and mortality (Adel S Al-Zubairi et al., 2010). An approximately 200 different types of cancers that can grow from almost any type of cell of more 60 different organs in the body. Although any organ or tissue can develop more than one type of cancer, more often a particular organ manifests one type of cancer only. Among the most common cancers is lung cancer; however, the best biggest treat, particularly to females, is breast cancer (Kim et al., 2009; Xia, 2006). Most cancers are detected in patients at the late stages. This may be due delay in diagnosis, or the cancer is at a location that evades detection at an early stage. The delay in diagnosis may also be due patients sourcing unorthodox medical remedies at the initial presentation of sickness (Xia, 2006).

The incidence of cancer especially breast, lung, and colorectal cancers continues to increase largely as a result of aging and fast expanding world population and adoption of cancer-causing lifestyle of economically developing countries (Adel S Al-Zubairi et al., 2010).

2.1 Natural Therapeutic Compounds

Natural therapeutic compound is a substance or compound which has been extracted or isolated by living organisms and contain of pharmacological or biological activity. This substance or compound consist of promising properties which can be developed into new pharmaceuticals. Plant tissues, marine organisms or microorganism fermentation broths are of natural products that proven to have a therapeutic properties in combating many diseases (Sobhan et al., 2013).

2.2 Natural Dietary Products as Anticancer Agents

Fruits, vegetables, grains, and legumes have held a historically place in preventing diseases. Convincing evidence has mentioned that diet containing beneficial antioxidants, macronutrients and/ or other micronutrients potentially preventing and curing cancer incidence and mortalities. Studies has shown that many cancer can be avoided by taking a balanced diet (Debatin, 2004).

Plants have been shown to be a considerable source for the new anticancer drugs development. However 25% of medicines prescribed around the world are extracted from plants and a lot of these drugs are semi-synthetic derivatives from a natural compound. One of the famous example is paclitaxel or taxol, paclitaxel is a potent anticancer drug which derived from natural compounds named Pacific yew (Gurubasavaraj & Charantimath, 2019; Ramondetta et al., 2001).

Around 100 new natural products have been established for cancer therapy and this type of research is still on going and needed in cancer therapeutic research area (Jin & El-Deiry, 2005). To date only small part of rainforest plant have been studied

in cancer research even the potential of this work have been more relevant and pressing from times to times (Hanke, 2017).

2.3 Zerumbone

Zerumbone (ZER), a pure compound from *Zingiber zerumbet* was first isolated in 1956. In 1960, Zerumbone has been determined and characterised by NMR and X-ray diffraction (Jin & El-Deiry, 2005). Researchers have found that rhizomes of *Zingiber zerumbet* are rich with this compound. Figure 2.1 showing the plant and its rhizome. This compound comprises of three double bonds, two conjugated and one isolated and a conjugated carbonyl group in 11-membrane ring structure (Figure 2.2). Researchers have found that rhizomes of *Zingiber zerumbet* are rich with Zerumbone (Rahman et al., 2014; Taha et al., 2010).

Several pharmacological activities of Zerumbone have been documented using *in vivo* and *in vitro* study models. These studies have shown that Zerumbone possesses antitumor, antinociceptive, anti-inflammatory, antioxidant, anti-platelet aggregation, antimicrobial, anti-atherosclerosis, hepatoprotective and immunomodulatory activities (N A Samad, Abdul, Abdullah, Rahman, & Chartrand, 2018).

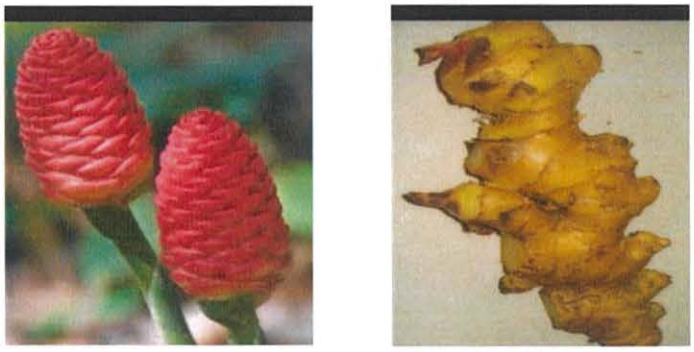

Figure 2.1 Zingiber zerumbet Smith (wild ginger) plant and its rhizomes adapted from (Rahman et al., 2014)

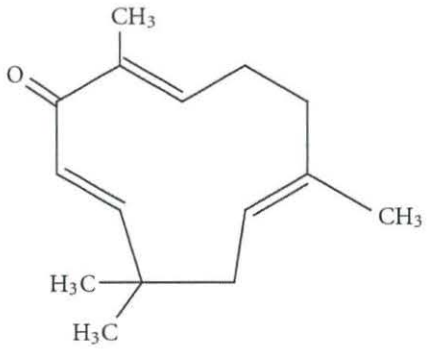

Figure 2.2 Zerumbone chemical structure (adapted from Rahman et al. (2014))

2.4 Apoptosis

Apoptosis, also well known as programmed cell death is defined as a highly preserved and controlled energy-dependent process categorized by distinct morphological and biochemical processes. Apoptosis can ocurre via a caspases-dependent and caspases-independent process. This programme of cell death is a normal and continuous process from fundamental and complex physiological events to various cellular processes. Abnormal regulation of this process has been detected in many human diseases, including cancers, ischemic damage, neurodegenerative diseases, and autoimmune disorders. Thus, one of the hallmarks of cancer progression is abnormality in apoptosis process (Hanahan & Weinberg, 2011). Majority of the key apoptotic proteins that involved in the apoptotic pathways have been identified but the molecular mechanisms aspects of these proteins activation remains unclear (Ferrari et al., 2009).

The capability to control cell apoptosis becomes the key for immense therapeutic potential. Thus, many studies focus on emphasizing the elucidation and analysis of the cell cycle machinery and signalling pathways that control cell cycle arrest and apoptosis particularly after treatment with potential anticancer compounds. Considering the main role of apoptosis in the maintenance of health and development of disease, understanding the mechanisms involved in this process is crucial for therapeutic intervention purposes. Apoptotic induction is the most applied approach to restrict the growth and proliferation of tumours. Thus studies has proven that pure compounds or crude extracts of plants that can be consider as promising potent inducers of apoptosis of cancer cells have such a high potential in the preventing and treating cancers (Ferrari et al., 2009; Xia, 2006).

2.5 Cell cycle stages

Somatic cells can divide through a process name cell cycle which consist of four phases: G1 (interphase), S (DNA synthesis phase), G2 (interphase) and M (mitosis phase). Cells that continue to past the restriction point in the G1 phase enter the S phase. At this stage, DNA will duplicate. Some of undivided cells can be taken out from the cell cycle and enter in G0 phase, where cells are termed inactive. M phase is the last phase where nucleotide and cytoplasmic divisions happen. Some of the apoptotic stimuli can cause cell cycle arrest which will bring to cell death. Thus apoptotic stimuli can disturb apoptotic and cell cycle process (De Palma, Biziato, & Petrova, 2017). Figure 2.3 illustrate the cell cycle stages.

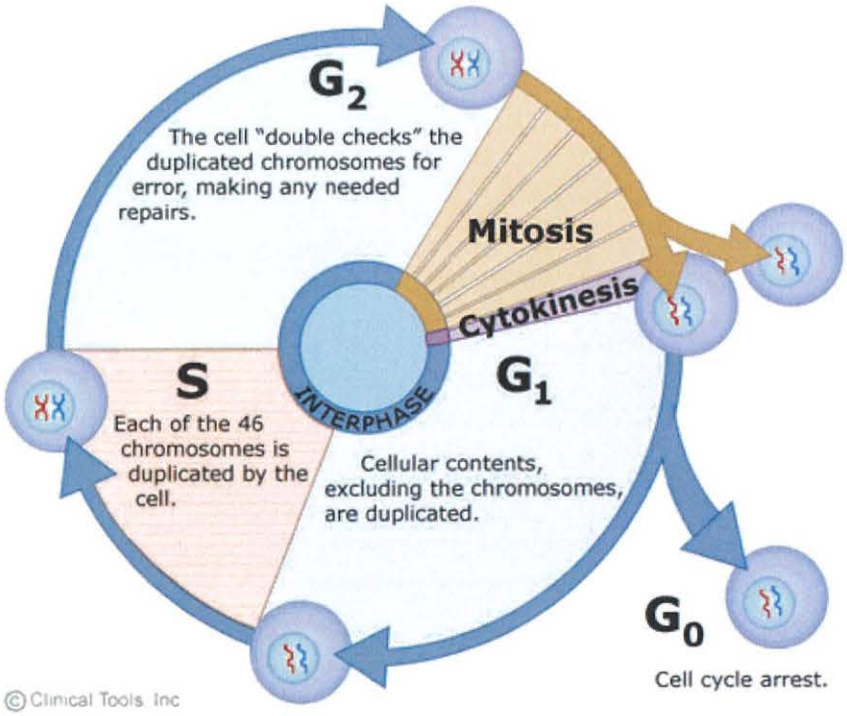

Figure 2.3 Stages of cell cycle (adapted from www2.le.ac.uk/projects/vgec/highereducation/topics/cellcycle-mitosis-meiosis, accessed on 12 June 2019)

2.6 Angiogenesis

Angiogenesis is the formation of new blood vessels from existing vasculature. This process normally happens during wound healing and development of embryonic. Although angiogenesis can follow some pathological event such as chronic inflammation, immune reaction, pre malignancy and malignancy, this process also plays a crucial in tumour invasion and metastasis and represents a vital process in the control of cancer growth (N. A. Samad et al., 2015). For cancer angiogenesis, there is an imbalance between pro-angiogenic and anti-angiogenic factor, called an "angiogenic switch". This switch involves formation of microvasculature surrounded

by multiple layers of tumour cells, creating a capillary "cuff." This alteration in formation of new blood vessels occur through the disturbance in the local balance of proangiogenic and antiangiogenic factors. Tumour angiogenesis regulates oxygen supply and nutrients. It can affect tumour growth, invasion as well as metastatic. Angiogenic factors were found to be crucial in regulating angiogenesis (Figure 2.4). The level of expression of angiogenic factors directly related to the aggressiveness of cancer cells (Stephen, 2012, Aggarwal, Sethi, Baladandayuthapani, Krishnan, & Shishodia, 2007).

The mechanism by which angiogenic proteins regulate promoting and developing of the angiogenic switch, involves secretion of vascular endothelial growth factor and matrix metalloprotease-9 (MMP-9). is predominantly reflected early distribution of malignant cells, while are proteolytic enzymes are vital in the degradation of extracellular matrix and basement membrane during this process. This route will facilitate angiogenesis (Adel S Al-Zubairi et al., 2010).

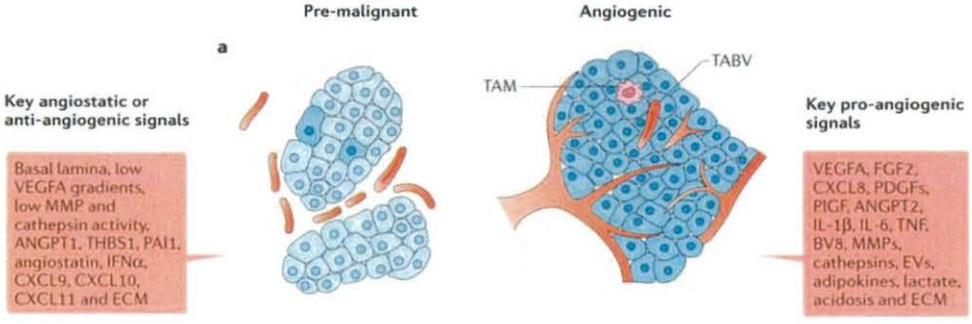

Figure 2.4 Tumour angiogenesis and its regulators (adapted from De Palma et al. (2017)).

2.4 Anti-angiogenesis as Therapeutic Agent in Cancer

Tumor neovascularization differ from normal blood vessels. This tumour vasculature is weak vessels fails to stabilize, undergoes constant growth, and is characterized by disorganization with unclear morphological structure, lake of definitive venules, arterioles, or capillaries. Furthermore, the shape of these vessels is irregular, tortuous, dilated, sometimes with blind ends, may be patent or latent, and are frequently had high permeability with poorly associated pericytes. These features make the tumour vasculature to be clearly distinguished from normal vascular networks. Additionally, phenotypical differences in tumour-associated endothelial cells which include incorporation of tumour associated macrophages, dendritic cells, and CD45+ leukocytes instead of normal endothelial (Dudley, 2012 , Aggarwal et al., 2007).

Recognition of the differences between normal blood and blood vessels tumor may help in reliable targets in the treatment of tumor. Therefore, anti-angiogenic therapy may involve different mechanisms such as inhibiting the angiogenic proteins, suppressing production of angiogenic proteins by cancerous cells, inhibiting the endothelial receptors for angiogenic proteins and directly promoting the endothelial cell apoptosis. The new role of anti-angiogenic agents in normalization of tumor vascularity is an important target to improve the efficacy of cancer radiotherapy and chemotherapy (Aggarwal et al., 2007).

2.5 Signaling pathways in cancer therapy

The receptor and mitochondrial signaling pathway are the first target in the cancer therapy. Studies has shown that the most cytotoxic drugs trigger cell death by targeting cytochrome-c/Apaf-1/caspase-9–dependent multiple proapoptotic and antiapoptotic signaling pathways. Various signaling pathways can modulate apoptosis and angiogenesis by converging on, thereby altering the activity of, common central control points within the apoptotic and angiogenic signaling pathways, which involve related proteins or mediators such as caspases, Bcl-2 proteins,VEGF and chemokines. (Nakamura-Ishizu, Takizawa, & Suda, 2014).

2.5.1 Caspases

Caspases are of the key effectors proteins of apoptosis. Their activation is important in initiating apoptosis. Caspases can be grouped into three which includes (1) inflammatory caspases, including caspase-1, -4, -5, -11, -12, -13 and -14, which are implicated in inflammation. (2) Apoptotic initiator caspases which had a long prodomains comprising of caspase-8 and 9. (3) Apoptotic effector caspases such as caspase 3, -6, -7 possesses a short prodomain. Normally these effectors will be activated by upstream caspases and act upon the downstream execution steps. This process is through apoptosis by cleaving multiple intracellular substrates (Ferrari et al., 2009).

Caspase-3 plays a vital role as an effector on the signaling components cleavage that regulate the morphologic apoptosis associated changes. Caspases predominantly targeting DNA fragmentation factor 45kD subunit (DFF45/ICAD).

Caspase cleavage eliminates the N-terminal interaction domain in DNA fragmentation factor 45 kD subunit which cause internucleosomal DNA degradation. This is the important characteristic of apoptosis. Caspase cleavage of NDUFS1 (the 75 kD subunit of respiratory complex) is imparted to several mitochondrial changes which linked to apoptosis. This then bring to the loss of mitochondrial transmembrane potential, production of reactive oxygen species (ROS), and will also cause interruption of electron transport (Nakamura-Ishizu et al., 2014).

2.5.2 Bcl-2 Family of Proteins

Mitochondrial membrane permeabilization (MMP) is one of the important steps in the induction of apoptosis. Bax/Bcl-2 family proteins, pro- and anti-apoptotic proteins regulates MMP. Bcl-2 family of proteins was the first to be identified. The first link between Bcl-2 signaling and regulation of cell survival was first determined in human B cell lymphoma (Xia, 2006). Bcl-2 family of proteins consists of both pro-apoptotic and antiapoptotic proteins, Bcl-2 Bax, Bak, and Bok are pro-apoptotic proteins comprises of three BH (BH1-3) domains and they are referred as multi-domain pro-apoptotic proteins. Bax and Bak upgrade apoptosis by hetero- and homo-oligomerizing at the mitochondrial membrane follow-on the formation of mitochondrial pores, which facilitate the release of inner mitochondrial proteins to cytosol (Indra Priyadharshini, Saranyan, Madhavan Nirmal, & Sekar, 2018).

2.5.3 CXCR4

Chemokine receptor 4 is a transmembrane G-protein coupled receptor. It is the main chemokine receptor recognized in tumors. It consists of four groups of chemokine receptors: C, CC, CXC, and CX3C. Chemokines usually implicates in immune cell function and migration of stem cells; however, in cancer, they involve in regulation of invasion, angiogenesis, migration, and metastasis. Activation of CXCR4 leads to the triggering in intracellular signaling cascades and inhibited both MMP1 and VEGFA. VEGFA has also been shown to upregulate CXCR4 expression. The ligand for CXCR4 is (CXCL12). CXCR4 and its ligand CXCL12 are responsible on tumor metastasis through eliciting proliferation and migration of cancer cells and enhance tumor angiogenesis (Folkman, 2002).

2.5.4 Vascular endothelial growth factors (VEGFs)

VEGFs are families of proteins which plays a vital role in the formation of new blood vessels. VEGFs can increase permeability of the blood vessel, development of the endothelium, and cancer cell proliferation, differentiation and migration. VEGFs comprises of 6 members with different biological activity. VEGF-A is principally implicated in angiogenesis while VEGF-C and VEGF-D are responsible on lymphogenesis. Patients with overexpressing of VEGF-A and VEGF-C factors tend to show early lymph node metastases and associated with decreased survival rate. VEGF-C and VEGF-D have identical structure and their main receptors (VEGFR-2 and VEGFR-3) demonstrated in most of cancer. These types of VEGF may control lymphangiogenesis process by facilitating the signaling network between tumor cells

and endothelial. VEGF-D has been found to contribute in the final stage of neoangiogenesis stabilization. It portrays an antagonistic effect on other VEGFs (Samad et al., 2018; Nakamura-Ishizu et al., 2014).

VEGF a key factors in angiogenesis can induce this process by upgrading the integrin expression, plasminogen activator, interstitial collagenase expression, plasminogen activator receptor expression. It also has the potential in the increasing of the vascular permeability (Adel S Al-Zubairi et al., 2010).

2.5.5 PI3K/AKT signaling

Phosphatidylinositol-3- kinases (PI3Ks) are kinases with lipid substrate, PI3Ks have been linked to a very distinct group of key cellular functions, involving cell growth, proliferation, motility, differentiation, survival and intracellular processes. There was arising links between PI3Ks and many human diseases, including human cancer. The upregulation of PI3K signaling can be consequence of gene mutations, amplification and protein overexpression, AKT also known as protein kinase B (PKB) is the most important down regulator of PI3K, AKT1 had a vital role in cellular survival pathways through phosphorylating the Bad component of the Bad/Bcl-XL complex, AKT1 upregulation has been found in primary gastric cancer, ovarian cancer, oral squamous cell carcinoma, pancreatic cancer and melanoma , PI3K/AKT signaling pathway has been involved in the intracellular effects of other endothelial cell stimuli. Therefore this signaling has a very important role in controlling endothelial cell survival, adhesion and migration which are important processes in angiogenesis (Kamstock, 2007).

2.5.6 The reactive oxygen species

A reactive oxygen species referred to superoxide anion, hydrogen peroxide and hydroxyl radical. Superoxide anion is generated during the aerobic metabolic process and reduction of triplet-state molecular oxygen ($3\ O_2$) by NADPH oxidases. Reactive oxygen species (ROS) can cause damages to all major cellular components at high concentrations. It plays an important role as second messengers in various physiological processes for instance in the regulation of fibroblast proliferation and can implicates cancer development (Kamstock, 2007).

CHAPTER 3

METHODOLOGY

3.4 Search strategy

For identifying articles used in this systematic review, two separated searches were conducted, both searches were performed through four databases which were Science Direct, PubMed, Google Scholar and Scopus. The last search was performed on 11/01/2019. The combinations of the following keywords were used in the first survey: "anticancer, Zerumbone, *Zingiber zerumbet*". The keywords for second search included "anti-angiogenesis, anti-angiogenic, anticancer, Zerumbone and *Zingiber zerumbet*".

3.5 Inclusion and exclusion criteria

The inclusion criteria for selecting relevant studies were as follows: article published between 1/01/2008 to 31/12/2018, articles on anticancer properties of Zerumbone extracted from *Zingiber zerumbet*, articles on antiangiogenic activity of Zerumbone as one of the roles for their anticancer effect, study designs using *in vitro*, *in vivo* and *ex vivo*. Articles which were written in language other than English, and articles that reported the activity of Zerumbone from unknown source were excluded.

3.6 Data extraction

All selected articles that reported the effect of Zerumbone on tumor cell, and role of their angiogenic properties in anticancer effect were further analyzed. These following information were gathered: authors, publication year, design of a study,

country, sample size, dose and duration, cytotoxic effect on cancer and normal cells and mechanism of its cytotoxic effect.

3.7 Article selection

The steps for selecting the manuscripts were illustrated in the Figure 3.1. The articles retrieved from the databases were screened for duplication. After removing the duplicated articles, a reviewer independently screened the retrieved relevant articles by reading the full copy or abstract; inclusion and exclusion criteria were considered at this stage. In addition to that, manual search was conducted from the bibliography section of the selected articles. Finally, supervisor and co-supervisor reviewed the selected article.

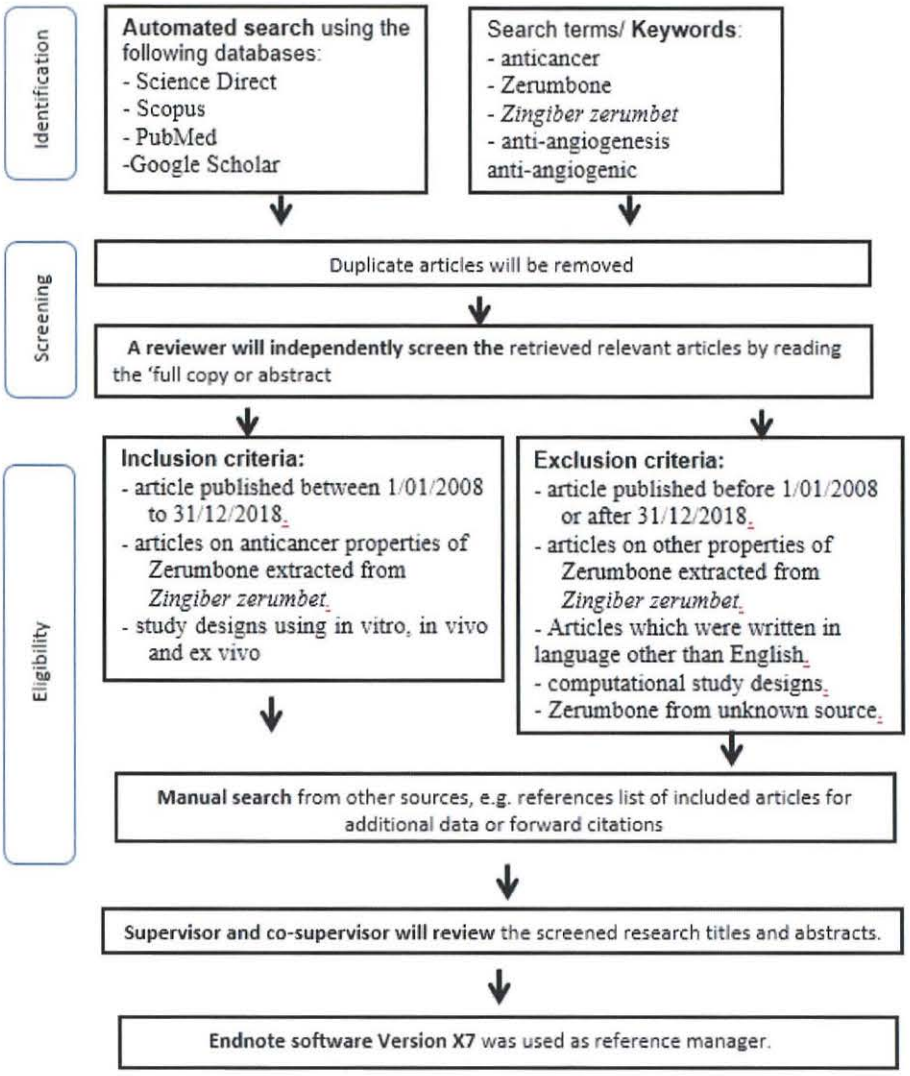

Figure 3.1 Methods used for identification, screening and eligibility of the articles

CHAPTER 4

RESULT AND DISCUSSION

4.1 Search literature:

The literature searched conceded a total of 352 records (from January 2008 to December 2018). The numbers of articles obtained from the corresponding databases are as follows; Google scholar (n=142), Science Direct (n=134), PubMed (n=48) and Scopus (n=28). After the online search, duplicate articles were removed. The remaining 146 articles were screened by referring to the title and abstract. 84 articles were excluded due to nonfulfillment of inclusion criteria (refer to Figure 3.1). From which 62 articles were further evaluated based on the full text, and a total of 19 articles were removed; two were using Zerumbone derivatives, two articles were review articles, six articles did not mention source of Zerumbone from *Zingiber zerumbet* clearly, one not related to anticancer nor antiangiogenic effect of Zerumbone, three were computational study and five no full text available. Finally, only 43 articles met the inclusion criteria and were further analyzed in this review. The summary of search strategy was depicted in Figure 4.1.

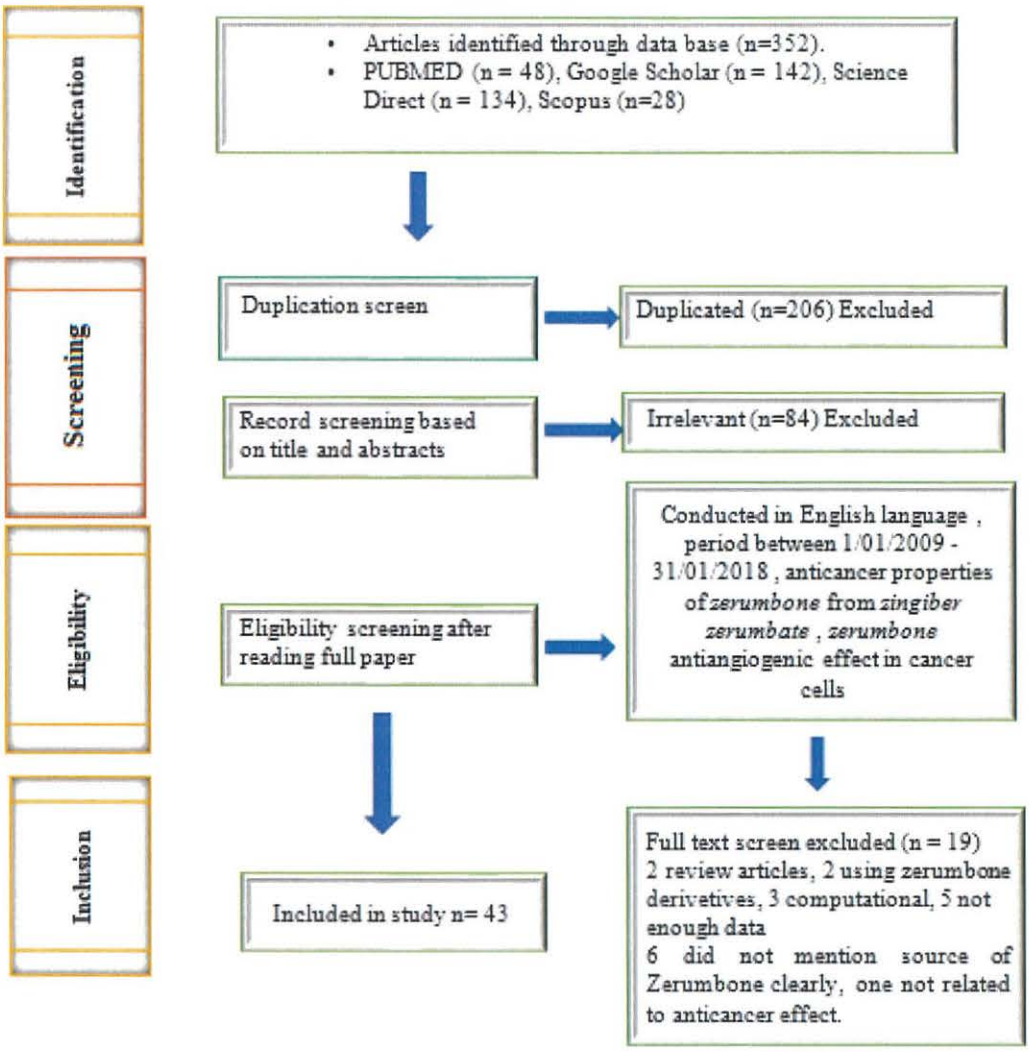

Figure 4.1 Summary of search methodology

The number of study conducted on Zerumbone is strongly correlated to the geographical distribution of *Zingiber zerumbet.* The highest number of study conducted on Zerumbone was recorded from Malaysia (15 studies), followed by India

(7 studies), China (7 studies), USA (5 studies), Korea (4 studies), Japan (1 study) and Taiwan and Saudia (each of them 2 studies) as shown in Figure 4.2.

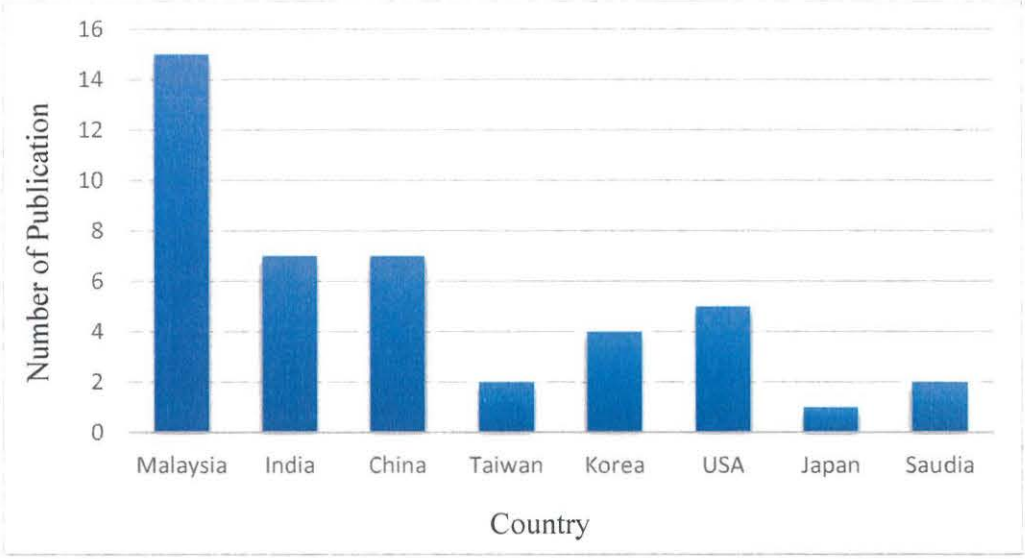

Figure 4.2 Distribution of the selected manuscript for the systematic review according to country

4.2 Classification of included articles describing anticancer and anti-angiogenic activities of Zerumbone:

The total number of articles included in this systematic review is 43. The articles were classified according to the models of study applied; animal *in vivo, in vitro* and *ex vivo* (Table 1). Two *in vivo* studies using animal models, 34 *in vitro* studies, and 7 studies combining *in vitro/in vivo* models. Two of the articles had combined *ex vivo, in vivo and in vitro* studies reported the anti-angiogenic effect of Zerumbone. The classification of the study model have been outlined in Table 4.1.

Table 4.1 Classification of included articles according to study model

Study model	Number	Articles (reference)
***In vitro* study**	34	A S Al-Zubairi (2018), Ma, Lei, Zhang, and Wang (2018), Sithara et al. (2018), Zainal et al. (2018), Hamid, Rajab, Shu, and Nasrom (2017), Nozlena Abdul Samad et al. (2017), Shyanti, Sehrawat, Singh, Mishra, and Singh (2017), Thiyam and Narasu (2017), Yan et al. (2017), Zhou et al. (2017), Adel Sharaf Al-Zubairi (2016), Jegannathan, Arul, and Dayalan (2016), D. Wang et al. (2016), Wang et al., (2016b), Chan et al. (2015), Deorukhkar et al. (2015), Hseu et al. (2015), Jorvig and Chakraborty (2015), Kang, Lee, Kim, and Lee (2015), Park, Park, and Kim (2015); (S. Wang et al., 2016), Rajan, Jayasree, and Kumar (2015), N. Samad et al. (2015), Shanmugam et al. (2015), Han et al. (2014), Hosseinpour et al. (2014), Sobhan et al. (2013),Sun et al. (2013), S. I. Abdel Wahab, Abdul, Zain, and Hadi (2012), Zhang, Liu, Liu, Qiao, and Liu (2012), S. I. Abdel Wahab et al. (2011), I. S. Abdel Wahab, Abdul, Alzubairi, Mohamed Elhassan, and Mohan (2009), Yodkeeree, Sung, Limtrakul, and Aggarwal (2009), Abdul et al. (2008), Sung et al. (2008).

***In vivo* study**	2	S. I. Abdel Wahab et al. (2010), Kim et al. (2009).
***Ex vivo* study**	2	Nozlena Abdul Samad et al. (2017),Park et al. (2015).
Combination *in vivo & in vitro* study	7	Nozlena A Samad et al. (2018), Park et al. (2015), Ni (2013), Sehrawat, Arlotti, Murakami, and Singh (2012), Choi et al. (2011), Adel S Al-Zubairi et al. (2010),Taha et al. (2010).