Food & Function

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: M. A. Haque, I. Jantan, L. Arshad and S. N. A. BUKHARI, *Food Funct.*, 2017, DOI: 10.1039/C7FO00595D.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/food-function

View Article Online
DOI: 10.1039/C7FO00595D

Food & Function

ARTICLE

Exploring immunomodulatory and anticancer properties of zerumbone

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Md. Areeful Haque, Ibrahim Jantan*, Laiba Arshad and Syed Nasir Abbas Bukhari

Plant-derived immunomodulators and anti-cancer agents have attracted a lot of interest from natural products scientists for their efficacy and safety, and their significant contribution towards understanding the targeted drug action and drug delivery mechanisms. Zerumbone, the main constituent of *Zingiber zerumbet* rhizomes, has been investigated for its wide spectrum role in treating multitargeted diseases. The rhizomes have been used as food flavoring agents in various cuisines and herbal medicine. Many *in vivo* and *in-vitro* studies have provided evidence of zerumbone as a potent immunomodulator as well as a potential anti-cancer agent. This review is an interesting compilation of all those significant outcomes from investigations carried out to date to explore the immunomodulatory and anticancer properties of zerumbone. The ultimate objective of this comprehensive review is to provide updated information and a critical assessment on zerumbone including its chemistry, immunomodulating and anticancer properties which may be of paramount importance to provide a new path for ensuing research to discover new agents to treat cancers and immune-related diseases. In addition, updated information on the toxicology of zerumbone has also been summarized to provide its safety profile.

1. Introduction

Immunity is the systematic defense mechanism of a living organism. The coordinated innate and adaptive defense mechanisms along with the requisite balance of immune cells maintain the homeostasis within the body of a healthy organism. Any imbalance of the immune system and cells lead to an abnormal immune system resulting in impaired immune responses mainly as seen in inflammation, autoimmune diseases and immune deficiency diseases as well as cancers.[1] Immunomodulators are natural or synthetic substances envisaged restoring the normal immune responses. These substances usually aid by stimulating natural and adaptive immune systems like cytokines that empower the body to maintain itself. Immunomodulators are mainly classified as immunosuppressants and immunostimulants. Immunosuppressants are favored in autoimmune diseases to control the pathological immune responses and suppress the immune system while immunostimulants are selected in immune deficiency diseases and several types of cancers to enhance the body's ability to fight invaders, causing less vulnerability to infections.[2] Cancer is a complex disease producing accelerated effects both at the cellular and molecular levels and has become one of the leading causes of death around the globe as well as the exasperating challenge of the modern era. Persistent autocrine and paracrine activation of various pro-inflammatory transcription factors in patient samples and various tumor cell lines is considered as one of the main hindrances of treating cancer.[3]

To overcome the problems associated with immunity and cancer, natural immunomodulators and anticancer agents are the appropriate choices besides the synthetic agents. Abundant bioactive natural metabolites have been reported as anticancer agents for the prevention and treatment of cancer by modulating several signaling molecules and pathways.[4] Among the anticancer agents, some produce their effects by modulating the inflammation associated molecular targets; thus, numerous lines of evidence suggest the causal relationship of cancer with inflammation.[5, 6] Generally, inflammation is segregated as acute inflammation and chronic inflammation and beside immune related syndromes, these inflammatory responses play an imperative role at various stages of different cancers. To understand the causal relationship between inflammation and cancer, it is important to explain how inflammation is involved in wound healing and infection. In response to any injury to the body, the protective response, inflammation is mediated by the innate immune system in which a series of steps initiate, involving the activation and migration of different immune cells (neutrophils, monocytes, and eosinophils) to the site of infection. As tissue homeostasis is disturbed, dendritic cells, mast cells, and tissue resident macrophages cause the release of certain cytokines, inflammatory mediators, histamine and reactive oxygen species (ROS) which further accelerate the movement of more and more monocytes and neutrophils to the affected tissue.[7, 8] In addition, vascular and fibroblast

Drug and Herbal Research Centre, Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.
E-mail: profibj@gmail.com; Fax: +6-032 6983271; Tel: +6-016 2886445

View Article Online
DOI: 10.1039/C7FO00595D

responses are also activated by these cells to eliminate the invading microbes and to commence local tissue repair. Once pervading agents are removed, immune cells cause the critical step of cell proliferation and cell death pathways back to normal that ultimately leading to re-epithelialization and synthesis of new extracellular matrix. Consequently, inflammation stops and tissue homeostasis is normalized. Contrary to these sequential steps, chronic inflammation is not resolved neither the normal homeostasis is executed.

Several types of inflammation with varying causes and mechanisms contribute to tumor and cancer development, as tumors are said to be wounds that do not heal. In such a scenario, the previously mentioned stages are not well coordinated and the altered epithelial and stromal cells may initiate the mutation and proliferation of epithelial cells. This remodeling starts an inflammatory response, which enhances tumor growth and development, particularly through neovascularization eventually leading to a neoplastic state.[8] As a result of such inflammations, the tumor microenvironment contains cancer cells and their surrounding stroma along with the innate immune cells and adaptive immune cells.[9] Most often in a cancerous situation, both systems that are innate and adaptive invade the tumor cells while the tumor cells employing the innate system promote its growth and development and keep protected itself from being resolved. Moreover, tumor cells also produce various cytokines and chemokines that attract leukocytes. Some tumors produce pro-inflammatory cytokines while others produce anti-inflammatory cytokines and the balance of cytokines in any given tumors determines the size and type of inflammatory infiltrate formed. Therefore, tumors producing more anti-inflammatory cytokines produce less inflammatory and vascular responses, ultimately the tumor growth is restricted while in the case of raised pro-inflammatory cytokines production, increased inflammation enhanced angiogenesis, and neoplastic growth is resulted.[10, 11]

Since ancient times different parts of plants, plant extracts, and plant-derived natural products have been used by all cultures and civilizations for the treatment of various ailments. Paclitaxel from pacific yew (*Taxus brevifolia*), capsaicin from chili peppers (Capsicum species), vinblastine and vincristine from Madagascar periwinkle (*Catharanthus roseus*), and galantamine from the Caucasian snowdrop (*Galanthus caucasicus*), bromelain from pineapple (*Ananas comosus*), aescin from European horse-chestnut (*Aesculus hippocastanum*), betulinic acid from warty birch (*Betula pendula*), podophyllotoxin from mayapple (*Podophyllum peltatum*), demecolcine, colchicine and colchiceine amide from autumn crocus (*Colchicum autumnale*), irinotecan and camptothecin from cancer tree (*Camptotheca acuminata*), curcumin from turmeric (*Curcuma longa*), are among the examples of plant-based therapeutics as natural immunomodulators and anti-cancer agents.[12, 13] Plant-derived agents or phytochemicals with immunomodulatory and anticancer properties have become an area of great interest for researchers. A number of plant species with immunomodulatory properties have been instrumental towards the development of therapies used for different ailments.[14] Moreover, plants and plant-based natural products possess significant therapeutic potential; likewise, approximately over 3000 plant species have been documented to be used in the treatment of cancer. In addition, it has also been reported that more than 60% of anticancer agents are derived from natural sources.[15] Brazilian propolis (a waxy substance produced by honeybees) is a potent immunomodulator and anticancer agent. It was found that propolis exerted anti-cancer effects mainly by affecting the players of the adaptive immune system like CD4+ T cells. Therefore, based on the *in vitro* test, the study concluded that this immunomodulatory agent not only inhibited the activation of T cells but also impeded the proliferation by inducing apoptosis in CD4+ T-cells. Moreover, the suppression of pro-inflammatory cytokines (IL-6, IL-12) has also been reported.[16] Correspondingly, in recent studies, *Tinospora* species (family, Menispermaceae) have been reported as a potent source for compounds with modulating effects on the immune system through various signaling cascades.[17]

Zerumbone is an α,β-unsaturated carbonyl-based component of *Zingiber* species mainly found in the rhizomes of *Zingiber zerumbet*. The rhizomes have been widely used in traditional medicine and as food flavoring and appetizers in various cuisines. Studies have shown that zerumbone contributed a paramount role in treating multipurpose diseases especially related to immune system and cancer. This review stockpiled the potential immunomodulatory and anticancer roles of zerumbone and critically assessed its importance in providing a new lead for the development of a new anticancer agent, which produce its effect by modulating the immune associated molecular targets. Additionally, toxicological information was also gathered to prove its safety profile. Detailed chemistry and isolation techniques along with the sources of this natural chemical marker were also reviewed to support future scientific investigations.

2. Chemistry of zerumbone

Zerumbone (**1**), (2,6,9,9-tetramethyl-[2E,6E,10E]-cycloundeca-2,6,10-trien-1-one), is a cyclic eleven-membered sesquiterpenoid (three isoprene units) isolated from the rhizomes of many Zingiberaceae species especially *Zingiber* and *Curcuma* species. It contains α,β-carbonyl based moiety along with three double bonds; an isolated one at position C-6 and two at positions C-2 and C-10. The double bonds at position C-2 and C-10 are part of a conjugated dienone system.[18] By employing different schemes and derivatization strategies, a number of derivatives have been reported in different studies, which led to increased versatility in the bioactivities of zerumbone. Numerous lines of evidence suggest that the chemistry and reactivity of isolated double bond and reactions of the carbonyl group in zerumbone have exceptional significance. By exploiting the reactive carbonyl moiety, azazerumbone 1 (**2**) and azazerumbone 2 (**3**) have been synthesized. On the other hand, zerumbal (**4**) (with additional aldehyde function) and zerumbenone (**5**) (with

View Article Online
DOI: 10.1039/C7FO00595D

additional enone) were prepared by oxidation of zerumbone. Activated esters like oxime esters have been reported by utilizing the carbonyl functionality of zerumbone. In addition, zerumbone oxime (**6**) and its esters with short & long chain fatty acids (C4 to C16) and benzoic acid have been prepared. By using 1-ethyl-3-(3-dimethylaminopropyl)carbinodiimide (EDCI) as a dehydrating agent, oxime esters and corresponding esters of zerumbone oximes, called zerumbone oxime fatty acid (ZOFA) esters, have been prepared. Aldoxime and ketoxime-esters of alkyl and aryl substituted carboxylic acids have also been synthesized. Another zerumbone derivative (**7**), a white solid primary amine, has also been synthesized by stirring a mixture of zerumbone with excess ammonia (30% in H_2O, 2 mL) at room temperature for 5 days. Analogously, several derivatives have been prepared by using different methods like conjugation addition method, transannular reaction, ring cleavage, ring expansion, and asymmetric induction.[19, 20] Chart 1 represents zerumbone and its derivatives.

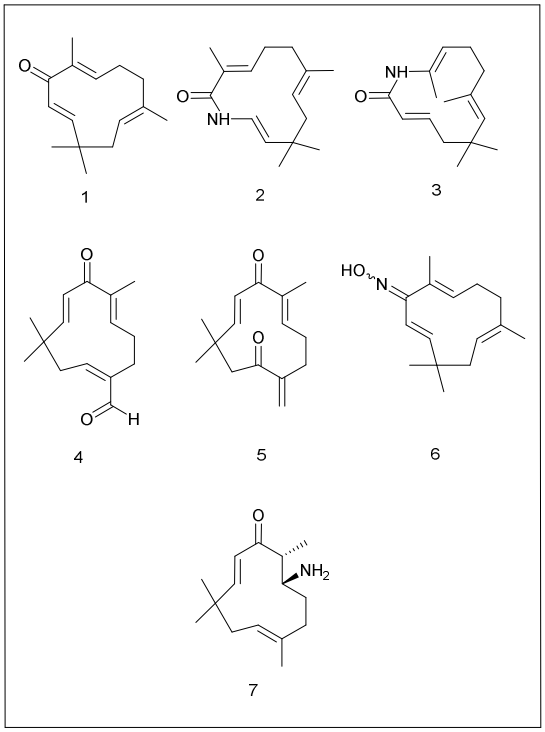


Chart 1 Structures of zerumbone and zerumbone derivatives

3. Sources of zerumbone

The multitargeting agent, zerumbone, has been isolated mainly from Zingiberaceae species especially *Zingiber* and *Curcuma* species as tabulated in Table 1. From *Zingiber zerumbet,* the yields of zerumbone were documented from 12.6 to 73.1%, isolated mainly from the whole plant and rhizome from various geographic locations. As demonstrated by one study, it was isolated as the main antiviral and cytotoxic constituent from the organic extract of *Z. aromaticum*.[21] Correspondingly, from the methanol extract of the rhizomes of *Z. spectabile*, zerumbone was isolated as the major compound at 30% yield.[22] Notably, Gupta and coworkers managed to isolate zerumbone from *Z. rerumbei*.[23] Zerumbone was also found as the main component (0.42%) of the rhizomes of *Z. ottensii*.[24] In addition, zerumbone was also isolated from *Z. cassumunar Roxb*. and *Curcuma zedoaria*.[25, 26] Moreover, zerumbone was identified as one of the constituents isolated from *C. heyneana*.[27] *C. ochrorhiza*, a common traditional medicine in Malaysia and Indonesia was also reported to contain zerumbone (0.6%) as an antibacterial and cytotoxic chemical constituent.[28] Kaushik et al. reported that chemical investigation of *Alpinia galanga* revealed zerumbone as the dominant constituent at 44.9% yield.[29] Ogunwande et al. described this sesquiterpene ketone (zerumbone) for the first time as a constituent of *Xylopia aethiopica* belonging to the family Annonaceae and it was isolated at a yield of 4%[30]. *Syringa pinnatifolia* belonging to the family Oleaceae and *Rhaphidophora korthalsii* (Araceae) are also known to have this important chemical constituent.[31, 32] The plant sources for zerumbone have been summarized in Table 1 with corresponding parts of the plants, family and percentage amount.

A number of studies illustrated the isolation of zerumbone from the rhizomes of *Z. zerumbet*. The process of isolation involved oven drying of small pieces of rhizomes at 37 ^{o}C for a period of 3 weeks. After grinding the sample was soaked in methanol or 80% ethanol for another 3 days and ultimately filtered. This process was repeated for three times and then evaporated *in vacuo* to obtain the crude extract. The crude extract was then subjected to column chromatography in silica gel, eluted with *n*-hexane and ethyl acetate to yield zerumbone. Pure zerumbone was obtained by recrystallization. Its structure was elucidated and confirmed by

Table 1 Sources of zerumbone

Species	Family	Plant Parts	Extract	Amount (%)	References
Zingiber zerumbet	Zingiberaceae	rhizome, whole plant	diethyl ether, n-hexane, methanol	12.6 to 73.1	18, 21, 26, 33-43
Zingiber aromaticum	Zingiberaceae	rhizome	methanol		21
Zingiber rerumbei Sm	Zingiberaceae				23
Zingiber spectabile	Zingiberaceae	rhizome	methanol	30	22
Curcuma heyneana	Zingiberaceae	rhizome	hexane		27, 28
Curcuma ochrorhiza	Zingiberaceae	rhizome	hexane	0.6	28
Zingiber cassumunar Roxb.	Zingiberaceae	rhizome	methanol		25, 26

View Article Online
DOI: 10.1039/C7FO00595D

Zingiber ottensii	Zingiberaceae	rhizome		0.42	24
Syringa pinnatifolia	Oleaceae				31
Curcuma zedoaria	Zingiberaceae	rhizome	petroleum ether		44, 45
Xylopia aethiopica	Annonaceae			4	30
Rhaphidophora korthalsii	Araceae				32
Alpinia galanga	Zingiberaceae	rhizome		44.9	29

using various spectroscopic techniques. Qualitative and quantitative analyses of zerumbone were carried out by high-performance liquid chromatography (HPLC) (Fig. 1).[43, 46]

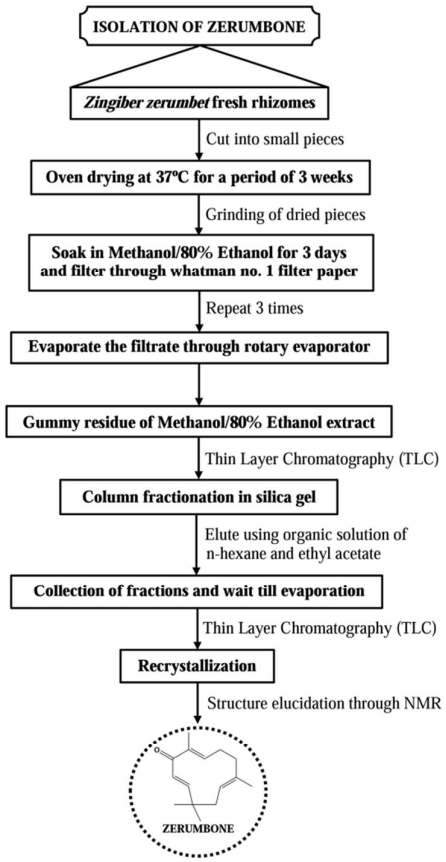


Figure 1 General technique for isolating zerumbone from *Z. zerumbet* rhizomes

4. Immunomodulating properties

Innumerable investigations have revealed zerumbone as a potent immunomodulator (Table 2). Although the particular mechanisms for potent immunomodulatory properties of the compound still need to be explicated, current scientific evidence suggests that it can be a prospective molecular target for the treatment of immune disorders (Fig. 2). The literature review revealed that the immunomodulatory properties of this potent phytoconstituent might be due to the presence of a α, β-unsaturated carbonyl-based moiety in its structure.[47] All the investigations, involving both *in vivo* and *in-vitro* models, conducted to evaluate the immunomodulatory effects of zerumbone until now have been mainly focused on mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NFκB) pathways, nitric oxide (NO) production, and inflammation. In this review, all the data investigated have been piled up constructively.

4.1. Effects of zerumbone on modulation of innate and adaptive immune responses through MAPK and NFκB pathways

Signaling molecules associated with MAPK and NFκB signaling pathways perform central roles in immune responses and inflammation. In a model of LPS- and IFN-γ-induced RAW 264.7 mouse macrophages, zerumbone was found to significantly suppress the protein expressions of iNOS synthase and COX-2. Interestingly, it also showed the release of TNF-α in all executed experiments. On the other hand, α-humulene, a structural analog of zerumbone remained inactive which was considered to be associated with the absence of carbonyl group in its structure.[48] Furthermore, it was reported that zerumbone showed immunosuppressive activity in RAW 264.7 macrophages where it was found to repress the LPS-stimulated COX-2 gene expression.[49] They further reported that zerumbone did not show a significant effect on NF-kB expression in RAW 264.7 macrophages at a dose of 20 mM. Moreover, zerumbone displayed activation of LPS-stimulated MAPKs/Akt and down-regulation of IkBα protein. Taken together these results, the authors appraised that zerumbone can be a potent molecular target of MAPK-activated protein kinase-2 and downstream molecules of MAPK-activated protein kinase-2 in degraded COX-2 mRNA pathway. According to a study, zerumbone showed the immunosuppressive effects via inhibition of expression of activator protein (AP-1) and NF-κB in THP1 cell line.[50] 3-O-Methyl kaempferol in addition to zerumbone has also been evaluated for the inhibitory effects on PGE2, COX-2 production in LPS-stimulated RAW 264.7 macrophages, and it was observed that PGE2 was suppressed at high doses of 20 and 40 μM, respectively, which was in contrast to COX-2 that remained unaffected.[39] Moreover, it was also revealed that zerumbone exhibited increased anti-inflammatory effects in contrast to 3-O-methyl kaempferol. Oral administration of zerumbone at a dose of 10 mg/kg or indomethacin with 100 mg/kg manifested ($p<0.05$) remarkably diminished paw edema when compared with non-treated controls.[35] This compound also blocked the production of pro-inflammatory genes COX-2 and iNOS in RAW 264.7 macrophages.[51]

View Article Online
DOI: 10.1039/C7FO00595D

Food & Function

ARTICLE

Table 2 Key immunomodulating properties of zerumbone

Pharmacological Activity	Subject	Dose	Effect	References
Anti-inflammatory	RAW 264.7 macrophages		Suppressed the LPS- and IFN-γ-induced protein expressions of iNOS synthase and COX-2 along with the release of TNF-α while the α-humulene remained inactive.	48
	RAW 264.7 macrophages		Zerumbone found to suppress the LPS-stimulated COX-2 gene expression	49
	RAW 264.7 macrophage/ Male ICR mice		Inhibited the PGE_2 production at high doses (20 μM) while COX-2 was found unaffected. In carrageenan-induced paw edema, markedly reduced in paw edema as compared to non-treated controls.	39
	Adult male ICR mice (25–35 g)	5, 10, 50 and 100 mg/kg	Marked inhibition of paw edema in carrageenan-induced mice and also on the granulomatous tissue formation on implanted cotton pellets.	38
	Female Sprague–Dawley rats	10 and 20 mg/kg, i.p.	Produced significant anti-inflammatory effects with maximum inhibition comparable to piroxicam	46
	Female balb/c mice	50 mg/kg	Zerumbone and NLC-zerumbone decreased the inflammatory mediators to be exact iNOS, NF-kB, COX-2 and VEGF	52
	RAW 264.7 macrophages		Zerumbone inhibited NO production, iNOS, COX-2 expression and production of PGE2 in a dose-dependent manner. It enhanced HO-1 expression.	53
	RAW 264.7 macrophages		Zerumbone inhibited expression of iNOS, IL-1β	54
Anti-nociceptive	Adult male *Balb C* mice	10, 50 and 100 mg/kg, i.p.	Marked antinociceptive activity was observed in acetic acid-induced abdominal writhing test and hot plate test.	55
	Male SD rats	1 mg/5 mg, p.o.	Zerumbone lessen the volume of paw edema and pain response	53
Immunomodulatory	Mice thymocytes, splenocytes and PBMC		Exhibited ability to activate mice thymocytes, splenocytes, and human PBMCs	56
	Female balb/c mice	50 mg/kg	Zerumbone alone and NLC-encapsulated elevated the serum IL-2 and IFN-g level and suppressed IL-1β and IL-6 level. NLC-zerumbone increased cytotoxic T cells, natural killer T cells and helper T cells in spleens.	52
	Female BALB/c and C57BL/6 mice	0.1, 1 and 10 mg/kg bodyweight, i.p.	Zerumbone downregulated eotaxin, IL-4, IL-5, IL-10, and IL-13, KC production and enhance production of Th1 cytokine IFN-γ. It enhanced the T cell proliferation in LPS activated dendritic cells.	57
NO production	RAW 264.7 macrophage		Inhibited LPS-induced NO production with IC_{50} values of 14.1 and 23.5 μM, respectively.	58
	RAW 264.7 macrophages	50 μM	Zerumbone can suppress more than 90% NO generation in inflammation.	59
	RAW 264.7 macrophage		Significant inhibition of NO production along with suppression of iNOS expression	39
	RAW 264.7 macrophage		5-hydroxyzerumbone inhibited the NO production in a dose-dependent manner.	60
	Female balb/c mice	50 mg/kg	Zerumbone alone and NLC-encapsulated suppressed the	52

View Article Online
DOI: 10.1039/C7FO00595D

		production of NO in animal serum	
Male Wistar rats	20 mg/kg and 40 mg/kg	Zerumbone blocked the p38 MAPK phosphorylation and NF-kB signaling pathways in the retina of diseased rats.	61

Studies on the effects of zerumbone on NO production postulated that the inhibition of NFκB might be responsible for the suppressed NO production activity. In a recent study, zerumbone along with Nanostructured Lipid Carriers (NCL) encapsulated zerumbone were investigated on female Balb/c mice. ELISA was used to measure the serum cytokine levels. It was evident that the productions of IL-2 and IFN-γ were augmented and were statistically significant ($p<0.05$) in NLC-zerumbone-treated mice rather than NLC-treated and zerumbone-treated mice. On the contrary, the productions of IL-1β and IL-6 were significantly lower in NLC–zerumbone-treated mice rather than the NLC-treated and zerumbone-treated mice. Moreover, reduced NO production was also noted in NLC-zerumbone treated mice serum. Besides these, NLC-zerumbone showed an increment of cytotoxic T cells, natural killer T cells, and helper T cells in the spleen. The authors further reported that zerumbone and NLC-zerumbone could decrease the inflammatory mediators at genomic (iNOS, NF-kB) and proteomic (COX-2 and VEGF) levels.[52] Recently, the antihyperlipidemic and antihyperglycemic effects of zerumbone have been reported by one study. The oral administration of the compound at doses of 20 and 40 mg/kg has been shown to reduce the severity of retinal inflammation and angiogenesis in streptozotocin-induced diabetic rats. Mechanistically, these effects were found to be associated with blocking of p38 MAPK phosphorylation and NF-κB signaling pathways in the retina of diseased rats.[61]

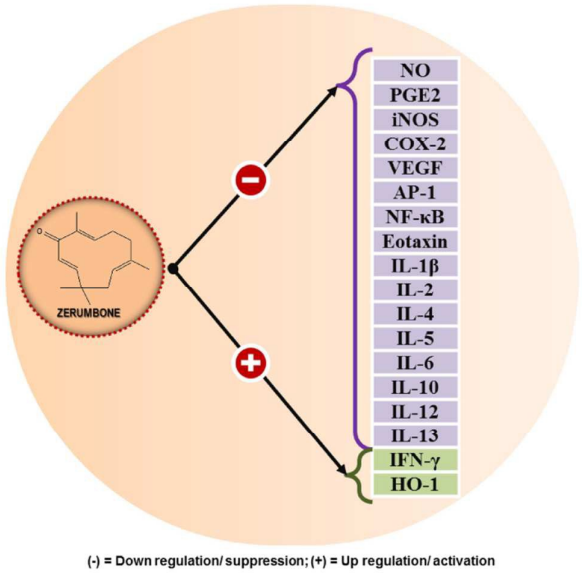


Figure 2 Signaling events modulated by zerumbone in cellular model

Likewise, Shieh et al. through a model of ovalbumin (OVA) induced T helper 2 (Th2)-mediated asthma evaluated the immunomodulatory role of zerumbone in mice. The investigations revealed that zerumbone treatment at doses of 0.1, 1 and 10 mg/kg bodyweight (i.p.) exerted antiallergic effects and mechanistically these effects were due to the modulation of Th1/Th2 cytokines. Zerumbone downregulated the production of eotaxin, interleukin (IL)-4, IL-5, IL-10, and IL-13, keratinocyte-derived chemokine (KC) and enhanced production of Th1 cytokine IFN-γ in asthmatic mice. In addition to *in vivo* studies, *in vitro* experiment was also executed and it was observed that zerumbone enhanced the T cell proliferation in LPS-activated dendritic cells along with Th1 cell polarization in allogeneic mixed lymphocyte reaction.[57] Contemporary, one of the studies narrated the mode of action by which zerumbone exhibited its anti-inflammatory effects. The findings indicate that zerumbone induced proteo stress in RAW 264.7 macrophages that ultimately activated heat shock factor 1 (HSF-1). This factor further enhanced the inhibitory effects of zerumbone on the expression of several pro-inflammatory genes like iNOS, IL-1β, ultimately producing anti-inflammatory effects of zerumbone.[54] Taken together these investigations, it can be hypothesized that, zerumbone can activate the cellular signaling by triggering the NFκB and MAPK signaling pathways, which can be responsible for potential immunomodulating properties of zerumbone.

4.2. Effects of zerumbone as a potent antinociceptive and anti-inflammatory agent

The anti-inflammatory effects of zerumbone were mostly assessed through *in vivo* models. As in a model of acute and chronic inflammation in mice, it was seen that the compound exhibited a notable reduction in paw edema stimulated by carrageenan with 33.3, 66.7, 83.3, and 83.3% inhibition, respectively, and hence exhibiting a dose-dependent response.[38] Analogously, in cotton pellet induced carcinoma model, intraperitoneal injection of zerumbone exhibited a concentration-dependent inhibition (34.8, 60.6, and 70.6%) on the granulomatous tissue formation in implanted cotton pellets. Another *in vivo* study reported the anti-inflammatory activity of zerumbone observed in Sprague–Dawley rats (200 to 250 g body weight) using the carrageenan-induced paw edema and PGE2-induced paw edema tests.[46] The authors concluded that zerumbone produced substantial ($P<0.05$) anti-inflammatory effects with maximum inhibition of 45.67% at 10 mg/kg and 70.37% at 20 mg/kg. Moreover, the results were found parallel to that of piroxicam's anti-inflammatory effects as zerumbone suppressed the rat paw edema by 87.80%, whereas piroxicam inhibited the inflammation by 92.68%. Thus, based on these studies zerumbone can be proposed to possess strong anti-inflammatory effects as it showed remarkable inhibition in carrageenan- and PGE2-induced rat paw edema.

In another investigation, the antinociceptive effects of zerumbone extracted from the rhizome *Z. zerumbet* were

View Article Online
DOI: 10.1039/C7FO00595D

investigated through two *in vivo* models explicitly acetic acid-induced abdominal writhing test and hot plate test using adult male Balb/c mice (25–35 g).[55] Hence, it was deduced that the compound showed pronounced antinociceptive activity in a concentration-dependent manner. Consequently, the observations recorded revealed the inhibition of acetic acid-induced visceral nociceptive response of 19.3%, LPS 40.4% and 64.8% inhibition for 10, 50, 100 mg/kg doses, respectively, as compared to control. However, the positive control group treated with acetylsalicylic acid (100 mg/kg) inhibited a remarkable number of writhes (51.4%). Interestingly, it was also seen that pre-treatment with naloxone greatly overturned the antinociceptive activity of zerumbone in the hot plate test. In another report, the anti-inflammatory and antinociceptive effects of zerumbone have been studied by using both *in-vitro* and *in vivo* models. The results demonstrated that in a model of activated RAW 264.7 macrophages, zerumbone significantly inhibited NO production, iNOS, COX-2 expression and production of PGE2 in a dose-dependent manner. These outcomes can give justification for its potent effects against inflammation. However, the expression of heme oxygenase (HO-1) was stimulated. While in mono-iodoacetate (MIA)-rat osteoarthritis model, zerumbone (1 mg/ 5 mg oral administration) lessen the volume of paw edema and pain response along with reduced writhing response in acetic acid-induced writhing test in mice at a dose of 10 or 50 mg.[53]

4.3. Effects of zerumbone on nitric oxide production

A couple of studies have also illustrated the inhibitory effects of zerumbone and its derivatives on NO production. As evident by one study, in LPS-stimulated mouse macrophage cells specifically RAW 264.7, 5-hydroxyzerumbone, a novel humulene derivative and zerumbone oxide from *Z. zerumbet* showed significant inhibition of NO production.[58] The results exhibited the inhibition of LPS-induced NO production in RAW 264.7 macrophage with IC_{50} values of 14.1 and 23.5 µM, respectively, for the aforesaid compounds while the positive control, N-monomethyl-L-arginine possessed an IC_{50} value of 21.3 µM. Further mechanistic elucidation demonstrated that the compound inhibited LPS-treated NO production in macrophage cell lines through downregulation of iNOs and mRNA expressions. Syahida et al. reported that zerumbone could suppress NO generation during inflammation in RAW 264.7 macrophages. It was noted there was more than 90% inhibition at a dose of 50 uM of zerumbone.[59] Correspondingly, another study reported LPS-induced NO production in RAW 264.7 macrophages of four compounds, i.e. zerumbone, 3-O-methyl kaempferol, kaempferol-3-O-(2, 4-di-O-acetyl-α-l-rhamnopyranoside) and kaempferol-3-O-(3,4-di-O-acetyl-α-l-rhamnopyranoside) isolated from *Z. zerumbet* rhizome.[39] The data revealed that zerumbone and 3-O-methyl kaempferol exhibited significant suppression of NO production with IC_{50} values of 4.37 and 24.35 µM, respectively. Moreover, the NO scavenging activity was considered associated with the significant suppression of iNOS expression in a concentration-dependent manner. Another derivative of zerumbone namely, 5-hydroxyzerumbone has also been found to inhibit NO production in RAW 264.7 macrophages activated by LPS in a dose-dependent manner.[60] The IC_{50} value was 19 µM, which was comparable to that of the positive control L-NMMA (non-selective inhibitor of NOS).

4.4. Other immunomodulatory effects

Besides aforementioned investigations, the ability of zerumbone to exhibit immunomodulatory effects was evaluated by Keong et al. employing lymphocyte proliferation assay, cell cycle progression, and cytokine induction. The authors reported that zerumbone showed the potential to activate mice thymocytes, splenocytes and human peripheral blood mononuclear cells (PBMC) in a dose-dependent manner and the most active dose was considered as 7.5 µg/mL. Zerumbone-treated lymphocytes was found prominently up-regulated at 24 h and down-regulated from 48 to 72 h in the production of human IL-2 and human IL-12 cytokines. In addition, zerumbone has also been investigated on Human Immunodeficiency Virus (HIV). To determine the anti-HIV effect of zerumbone from 1991 to till date, several researchers have performed a number of studies.[25, 62, 63] One study suggested that zerumbone exerted HIV inhibitory effects along with cytotoxic properties in cell lines.[21] The EC_{50} was recorded as 0.04 µg/mL. However, the scientific evidences of anti-HIV effects of zerumbone are not sufficient and still yet to be investigated for proper justification of existing reports.

5. Anticancer activity

Zerumbone had been extensively explored for its anticancer activities using various cell-signaling pathways and different cancer cell lines. Zerumbone with a ketone group in its structure may play a pivotal role for its enormous chemotherapeutic potential. Numerous *in vivo* and *in-vitro* attempts have been undertaken by researchers to prove zerumbone as a potent anticancer agent.[64]

Murakami and co-workers studied zerumbone for its suppressive effects in Epstein–Barr virus (EPV) in Raji cells.[36] Zerumbone suppressed tumor promoter 12-O-tetradecanoylphorbol-13-acetate-induced EPV activation. The IC_{50} value was 0.14 µM, which suggested that zerumbone was a potent chemopreventive agent. Huang and co-workers investigated the effects of zerumbone on human promyelocytic leukemia (HL-60, CCL-240) and murine lymphoid neoplasm (P-388D1, CCL-46) cells *in vitro* as well as P-388D1-bearing CDF1 mice *in vivo* model where P-388D1 cells (1×10^6 cells/mouse) had been intraperitoneally transplanted into CDF1male mice (DBA male × BALB/c female). Zerumbone was found to suppress the P-388D1 cells growth and induced DNA fragmentation in the culture as well as remarkably enhanced life of P-388D1-bearing mice where the ILS% = 120.5 at 2 mg/kg dose. It was also found to suppress the HL-60 cells growth in a dose-dependent manner with IC_{50} values of 22.29, 9.12 and 2.27 µg/mL, respectively, for 6, 12, and 18 h. Moreover, it was found to induce G2/M cell cycle arrest in HL-

View Article Online
DOI: 10.1039/C7FO00595D

60 cells in a dose-dependent manner and found to reduce the cyclin B1/cdk 1 protein level.[65] Subsequently, zerumbone and NLC-encapsulated zerumbone showed a promising effect in controlling tumor growth and metastasis by apoptosis and delaying cancer cell cycle progression in 4T1 challenged female Balb/c mice at a dose of 50 mg/kg. The authors reported that treatment with NLC–zerumbone in mice could decrease the size and weight of tumor as compared to that of NLC-control mice and zerumbone-treated mice. Moreover, NLC–zerumbone treated mice also showed decreased numbers of metastatic lung nodules as compared to that of NLC-control and zerumbone-treated mice. The metastatic lung nodules numbers in the zerumbone-treated mice were also found remarkably lower than the NLC control mice.[52]

5.1. Effects of zerumbone on apoptotic pathways and cell proliferation

A number of studies have been reported on the effects of zerumbone on the mechanistic signaling pathways involved in cancer. The effect of zerumbone on human promyelocytic leukemia cells (HL-60) was reported to be due to the inhibition of 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced superoxide anion generation from NADPH oxidase. Enhanced arrest of G2/M cell cycle in a time and dose-dependent manner was also observed with reduced cyclin B1/CDK1 protein level.[48] In another investigation, zerumbone showed inhibitory effects on NF-κB activation in human squamous cell carcinoma, explicitly FaDu and LICR-LON-NH5 tumor cell lines. Interestingly, it inhibited TNF-induced phospho-IkBα and phospho-p65 protein. Moreover, zerumbone inhibited the NF-κB-dependent reporter gene expression activated by TNF, TNFR1, TRAF2, NIK, TRADD, and IKK. The authors also found that zerumbone downregulated the NF-κB-regulated gene products related to cell proliferation, cyclin D1 and c-Myc; anti-apoptosis, inhibitor of apoptosis protein (IAP)-1, (IAP)-2, survivin, X-linked inhibitor of apoptosis (XIAP); B-cell lymphoma 2 (Bcl-2), Bcl-2-related protein (A1), B-cell lymphoma-extra-large (Bcl-xL), TRAF1, cellular FLICE-like inhibitory protein (c-FLIP); and invasion of COX-2, ICAM-1 and matrix metalloproteinase-9 (MMP-9). This downregulation resulted in the potentiation of apoptosis induced by cytokines and chemotherapeutic agents. The authors suggested that the zerumbone mediates its effect by modulating NF-κB activation and this inhibition may provide a molecular basis for the prevention and treatment of cancer by zerumbone.[66]

Likewise, Alwi et al. reported the antiproliferative activity of zerumbone on human liver cancer HepG2 cell line, human breast cancer MCF-7 cell line, human ovarian cancer Caov-3 cell line and human cervix cancer HeLa cell line. Zerumbone showed antiproliferative activities towards the HepG2 cells with an IC_{50} of 3.45 ± 0.026 µg/ml; MCF-7 cells with an IC_{50} of 3.73 ± 0.085 µg/ml; Caov-3 cells with an IC_{50} of 4.73 ± 0.052 µg/ml and HeLa cells with an IC_{50} of 5.43 ± 0.033 µg/ml. A significant increment in apoptosis of HepG2 cells was observed in a time-dependent manner and that was found to be more potent than the control drug, cisplatin.[67] Furthermore, another study reported significant antiproliferative activity of zerumbone upon HepG2 cells with an IC_{50} of 3.45 ± 0.026 µg/ml as well as inhibition of proliferation of non-malignant Chang Liver and MDBK cells have also been reported. In addition, significant enhancements of apoptosis in HepG2 cells were observed in a time-dependent manner via up/down regulation of pro-apoptotic Bax protein and anti-apoptotic Bcl-2 protein expression.[68] However, it is yet to be studied whether these outcomes are cell-type independent or cell-type specific. Zerumbone was also found active on promyelocytic leukemia cells (NB4) at a dose of 10 µM where zerumbone induced arrest of G2/M cell cycle and apoptosis via Fas/CD95- and mitochondria-mediated signaling pathway.[63] Additionally, zerumbone induced the apoptosis and morphological changes in several types of leukemic cells.[69] Abdelwahab et al. reported the antiproliferative activity of zerumbone to cervical cancer cell lines (HeLa cells) which was found to exhibit time-dependently at 24, 48 and 72 h.[40]

Zerumbone has also been investigated on human cancer cell lines of the ovary (Coav-3) and breast (MCF-7) along with (HeLa) as well as Chinese hamster ovary normal cells. Enhanced cellular level of caspase-3 was noted in HeLa cells and zerumbone furthermore produced distinctive morphological features of cell death, which corresponded to apoptosis.[41] Yodkeeree et al. reported the increased TNF–related apoptosis-inducing ligand (TRAIL)-induced apoptosis in human cell lines HCT116 (colon adenocarcinoma).[70] Zerumbone at 20 µmol/L was found to activate caspase-3, caspase-8, caspase-9 and poly (ADP-ribose) polymerase (PARP) in combination with TRAIL. It exhibited production of TRAIL death receptors that is the DR4 and DR5 expression. Pre-treatment with zerumbone in HCT116 cells followed by ROS scavengers N-acetylcysteine (NAC) and glutathione (GSH) suppressed the zerumbone-treated activation of DR4 and DR5 in a concentration-dependent manner. Moreover, the activation of DR4 and DR5 was also found to be facilitated followed by activation of MAPK pathway. Zerumbone was further found to inhibit anti-apoptotic protein c-FLIP expression and activate ERK time-dependently. These investigations summarized that zerumbone might enhance the TRAIL-induced apoptosis followed by the ROS-mediated up-regulation of ERK through the activation of DR4 and DR5. Figure 3 represents the impact of zerumbone on NFkB-, TRAIL- and receptor activator of nuclear factor kappa-B ligand (RANKL)-induced signaling events in tumor cell lines.

View Article Online
DOI: 10.1039/C7FO00595D

Food & Function

ARTICLE

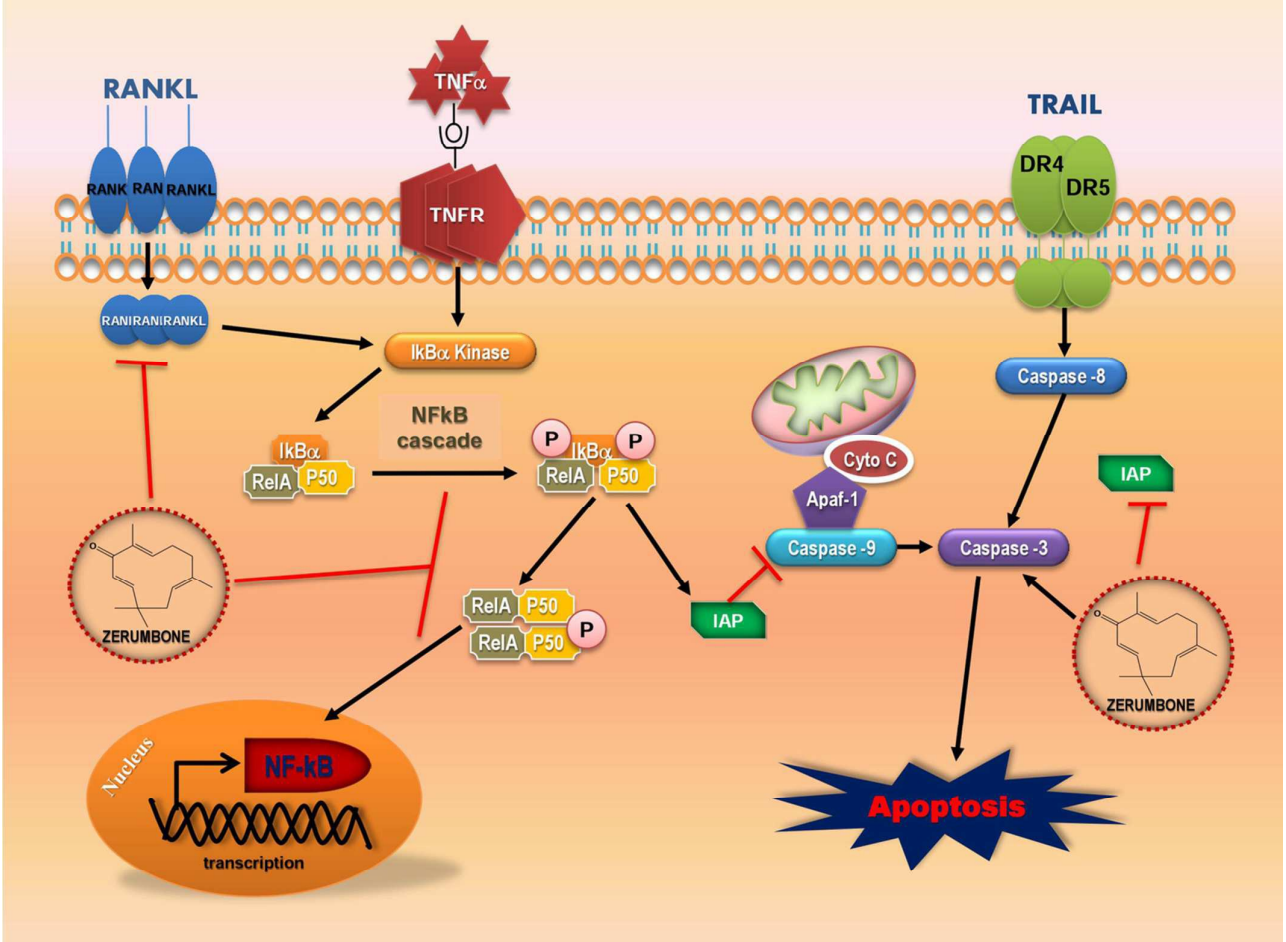


Figure 3 The impact of zerumbone on NFκB-, TRAIL- and RANKL-induced signaling events in tumor cell lines

Further studies on zerumbone reported that at concentrations of 10-50 µM, it induced human glioblastoma multiforme (GBM8401) cells death in a dose-dependent order and enhanced the percentage of apoptotic cells.[71] Also, significant inhibition of IKKα phosphorylation level was noted in a time-dependent manner. It has also been found to activate caspase-3 and poly (ADP-ribose) polymerase (PARP) production and remarkably diminished the Akt phosphorylation level in a time-dependent manner. Zerumbone may activate apoptosis of GBM cell line through an alternative pathway such as IKK-FOXO cascade. FOXO members are a group of tumor suppressor proteins; those can arrest the cell cycle and have the ability to induce apoptosis in tumor cells. Akt has the ability to phosphorylate FOXO members, resulting in nuclear export, cytoplasmic retention, and inhibition of transcriptional activity of FOXOs. This investigation revealed that zerumbone-induced decrease in Akt and FOXO1 phosphorylation was mediated through IKKα. The authors further suggested that zerumbone might reduce FOXO1 phosphorylation through two different possible mechanisms notably, through IKKα-Akt signaling and another one through IKKα directly. Figure 4 summarizes the zerumbone-induced apoptosis in human glioblastoma multiforme (GBM8401) cells.

An induced apoptosis via high and low expression of respective Bax and Bcl2 proteins in human hepatocellular liver carcinoma (HepG2) cells was noticed by Kamalidehghan et al. while treatment was carried out with zerumbone at a

View Article Online
DOI: 10.1039/C7FO00595D

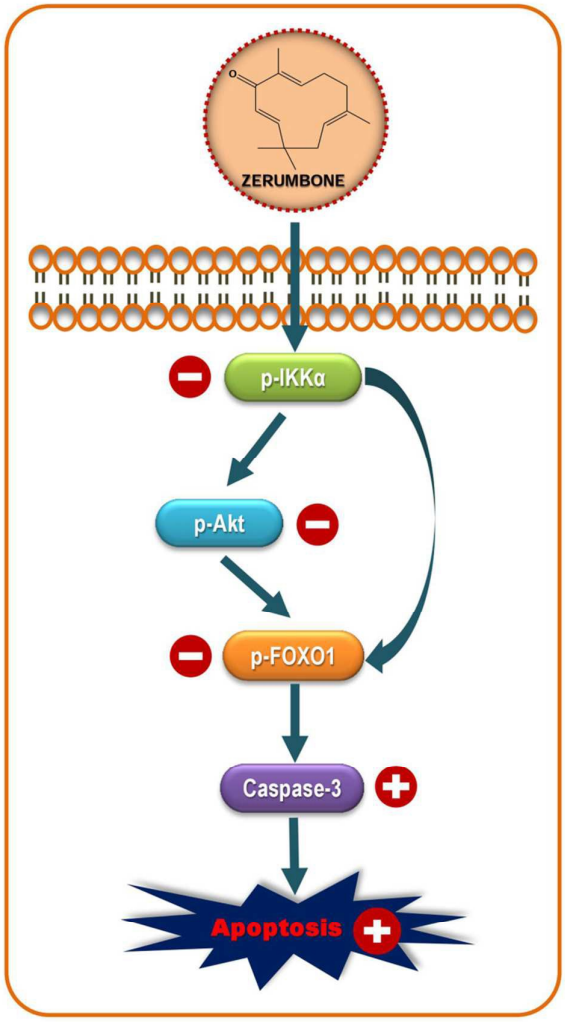


Figure 4 Zerumbone induced apoptotic events in human glioblastoma multiforme cells

concentration ranging from 2 to 8 µg/mL.[72] Analogously, Muhammad Nadzri and co-workers also reported treating HepG2 cells with zerumbone at a concentration of 1.563, 3.125, 6.25, 12.5, 25, and 50 µg/mL. Encapsulated zerumbone with hydroxypropyl-β-cyclodextrin (HPβCD) was found to induce apoptosis in HepG2 cells following caspase-8/BID cleavage switch and the modulating ratio of Bcl2/Bax.[73] Interestingly, an investigation was conducted in the same year where 50 µM zerumbone had been applied in human umbilical cord blood endothelial progenitor cells, human mammary epithelial cells and human smooth muscle cells as well as colon cancer cell lines HCT116 and HCT116Bax KO. It was observed mitochondrial permeabilization and the cytochrome c-dependent caspase activation overlooked in zerumbone-treated cancer cell death. The endothelial progenitor cells, mammary epithelial cells, and smooth muscle cells had shown resistance to zerumbone-treated cell death with lower ROS accumulation than cancer cell lines.[74] Recently, the anticancer effect of zerumbone on human hormone-refractory prostate cancer (HRPC) cell lines has been reported. Zerumbone inhibited tubulin assembly and induced a crosstalk between endoplasmic reticulum (ER) stress and mitochondrial insult followed by autophagy and apoptosis in HRPCs.[75] All these reports prove zerumbone as a wonderful therapeutic potential in the prevention of cancer. In one study, the anticancer effect of zerumbone was examined by using RCC cell lines and xenograft mouse model[76]. The study reported that in RCC lines, zerumbone dose-dependently inhibited the activation of STAT 3 signaling cascade. The same pharmacological effect was observed when this natural product was administered intraperitoneally to athymic mice. Furthermore, it was explored that STAT 3 inhibition was due to the suppression of upstream kinases c-Src, Janus-activated kinase 1, and Janus-activated kinase 2 and it also caused the suppression of the gene products responsible for proliferation, survival, and angiogenesis.

Zerumbone at 5-100 µg/mL treated in T-acute lymphoblastic leukemia (CEM-ss cells) was found to suppress the cell proliferation followed by inducing apoptosis. Additionally, zerumbone exhibited DNA internucleosomal degradation and enhanced cellular level of caspase-3.[77] In line with these investigations, it can be concluded that, the anti-proliferative activities of zerumbone might be due to its potent inducing capabilities of apoptosis in various cancer cell lines. The effects zerumbone on the proliferation, migration, and mitochondrial function in human melanoma cell line CHL-1 have been studied recently[78]. The results indicated that zerumbone significantly inhibited the cell proliferation and migration of CHL-1 cells. Additionally, zerumbone significantly enhanced cellular ROS levels and inhibited the matrix membrane potential, ATP as well as mitochondrial DNA levels. The authors suggested that zerumbone possessed anticancer activities on human melanoma cells by altering the mitochondrial function.

Other than previously mentioned antiproliferative, antitumor, and apoptotic effects of zerumbone, it has been reported to have potent effects on various cancer models. As revealed, mainly all the studies carried out were *in vivo* and *in-vitro,* taking into account animal and cellular models including, liver cancer, breast cancer, colon cancer, pancreas cancer, skin cancer, leukemia, colorectal cancer, lung cancer and so on. All the updated anticancer studies of zerumbone have been summarized in Table 3, as for *in vivo* and Table 4, as for *in vitro* investigations.

View Article Online
DOI: 10.1039/C7FO00595D

Food & Function

ARTICLE

Table 3 Oncogenic activity of zerumbone *in vivo*

Type of activities	Subjects	Dose	Key effects	References
Antitumor	P-388D_1-bearing CDF_1 mice	0.5, 1.0, 2.0 mg/kg, i.p.	Prolonged the life of P-388D_1-bearing CDF_1 mice significantly (ILS% = 120.5) at 2 mg/kg.	65
	Female balb/c mice	50 mg/kg	Controlled effectively the tumor growth and metastasis by apoptosis and delaying cancer cell cycle progression in 4T1 challenged mice.	52
Breast cancer	Female severe combined immune deficient (SCID) mice	7.5 and 15.7 mg/kg, i.p.	Significant retardation of orthotopic growth of MDA-MB-231 xenografts	79
Cervical cancer	Inbred Balb/c mice	16 mg/kg, i.p.	Regressed the Cervical Intraepithelial Neoplasia (CIN). The combination of zerumbone and cisplatin modulated IL 6 serum level	80
	Female Balb/c mice	4, 8 and 16 mg/kg, i.p.	Suppressed CIN exposed prenatally to Diethylstilbestrol (DES). Inhibited the cell proliferation marker PCNA expression. Exerted over-expression of Bcl-2-associated X protein (Bax) Inhibited B-cell lymphoma 2 (Bcl-2) specific mRNA expression	81
Colitis in large intestine	Female ICR mice	1000 ppm each	Inhibited DSS-induced acute colitis Reduced the expression of inflammatory biomarkers (PGE2, IL-1β and TNF-α) in colonic mucosa	82
Colon cancer	Male ICR mice	100, 250 or 500 ppm, orally	Significant inhibition of multiplicity of colonic adenocarcinomas Inhibition of colonic inflammation	83
Cancer-associated bone loss	Female BALB/c *nu/nu* mice (Harlan)	20 or 100 mg/kg body weight, i.p.	Suppressed osteolysis concentration-dependently in MDA-MB-231 breast cancer tumor-bearing athymic nude mice	84
Lung cancer	Female A/J mice	100, 250 or 500 ppm, orally	Significant inhibition in the multiplicity of lung adenomas at 250 and 500 ppm, which was dose-dependently	83
Liver cancer	Male Sprague-Dawley rats	15, 30 or 60 mg/kg body weight, i.p.	Protected liver of experimental animals from carcinogenesis of diethylnitrosamine and 2-acetylaminofluorene Reduced the serum levels of ALT, AST, AP and AFP Reduced GSH concentration in liver tissue Reduced proliferating cell nuclear antigen (PCNA) expression in liver sections of animals Observed increment in Bax and reduction in Bcl-2 protein expression in rat liver	85
Skin cancer	Female ICR mice	Topical application of zerumbone (0, 0.2 or 2 µmol/ 0, 1.6 or 16 nmol in 100 µL of acetone)	Significantly inhibited tumor growth by 60% and number of tumors by 80%/mouse during single topical administration to mouse skin (2 µmol) one day before applying dimethylbenz[*a*]anthracene (0.2 µmol) Repeated administration of 16 nmol twice a week during post-initiation phase found to suppress the number of 12-*O*-	86

View Article Online
DOI: 10.1039/C7FO00595D

			tetradecanoylphorbol-13-acetate (TPA) (1.6 nmol)-induced tumors by 83% and their diameter by 57%. At 2 µmol, zerumbone induced the expression of mRNA level of manganese superoxide dismutase, glutathione peroxidase-1, glutathione S-transferase-P1 and NAD(P)H-quinone oxidoreductase in the epidermis. Suppressed TPA-induced COX-2 expression and phosphorylation of ERK ½ Inhibited leukocyte infiltration and suppressed proliferating cell nuclear antigen labeling indices.	
	Female HR-1 hairless mice	10 µmol, topically	Activated the NF-E2-related factor 2 (Nrf2) and heme oxygenase- 1 (HO-1) expression	87

Table 4 Oncogenic activity of zerumbone *in vitro*

Type of activities	Subjects	Dose	Key effects	References
Antitumor	Raji cells	10 µM	Inhibited the tumor promoter, TPA-induced activation of Epstein–Barr virus in Raji cells	36
	HL-60, CCL-240, P-388D_1, CCL-46 cell		Suppressed growth of P-388D_1 cells Induced the DNA fragmentation in culture	65
Antiproliferative and apoptotic	HL-60 cells	50 µM	Inhibited 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced superoxide anion generation from NADPH oxidase Enhanced arrest of G2/M cell cycle in a time- and dose-dependent manner Reduced cyclin B1/CDK1 protein level	48
	H1299, KBM-5, A293, FaDu and LICR-LON-HN5		Zerumbone showed inhibitory effects on NF-kB activation in FaDu and LICR-LON-NH5 cells. It inhibited TNF-induced p-IkBa and p-p65 protein. Downregulated cyclin D1 and c-Myc, IAP-1, IAP-2, survivin, XIAP, Bcl-2), A1, Bcl-xL, TRAF1, c-FLIP, COX-2, ICAM-1, and MMP-9. It inhibited the NF-kB-dependent reporter gene expression activated by TNF, TNFR1, TRAF2, NIK, TRADD, and IKK.	66
	HepG2, MCF-7, Caov-3 and HeLa cell line.		Showed antiproliferative activities towards The HepG2 cells (IC50of 3.45 ±0.026 µg/ml), MCF-7 cells (IC50of 3.73 ±0.085 µg/ml), Caov-3 cells (IC50of 4.73 ±0.052µg/ml) and HeLa cells (IC50 of 5.43±0.033 µg/ml). A significant increment in apoptosis of HepG2 cells in a time-dependent manner and that was found more potent than control drug, cisplatin.	67
	Leukemic cells		Induced apoptosis and morphological changes in several types of leukemic cell	69
	HepG2, non-malignant cells of Chang's Liver, MDBK, and Vero	3.45 µg/mL	Showed significant antiproliferative activity upon HepG2 cells with an IC50 of 3.45 ± 0.026 µg/ml Inhibited proliferation of non-malignant Chang Liver and MDBK cells Significant enhancement of apoptosis in HepG2 cells in a time-dependent manner via up/downregulation of pro-apoptotic Bax protein and anti-apoptotic Bcl-2 protein expression.	68
	NB4 cells	10 µM	Induced arrest of G2/M cell cycle and apoptosis via Fas/CD95- and mitochondria-mediated signaling pathway	63
	HeLa cell line		Exhibited antiproliferative activity to HeLa cells time-dependently at 24, 48 and 72 h	40
	HeLa, Coav-3 and MCF-7 and Chinese Hamster ovary normal cells		Enhanced the cellular level of caspase-3 on HeLa cells Produced distinctive morphological features of cell death that correspond to apoptosis	41
	HCT116 cell line	20 µmol/L	Increased TNF–related apoptosis-inducing ligand (TRAIL)-	70

View Article Online
DOI: 10.1039/C7FO00595D

			induced apoptosis Activated the caspase-3, caspase-8, caspase-9 and PARP in combination with TRAIL Produced TRAIL receptors specifically DR4 and DR5 expression Inhibited anti-apoptotic protein c-FLIP expression activated ERK time-dependently	
	GBM8401 cells	10-50 μM	Induced cell death in a dose-dependent order Enhanced percentage of apoptotic cells Activated caspase-3 and poly (ADP-ribose) polymerase (PARP) production Significant inhibition of IKKα phosphorylation level in a time-dependent order Remarkably diminished Akt phosphorylation level in a time-dependent manner	71
	HepG2 cells	2, 4, 6 and 8 μg/mL	Induced apoptosis via high and low expression of respective Bax and Bcl2 proteins	72
	Human umbilical cord blood endothelial progenitor cells, human mammary epithelial cells, and human smooth muscle cells as well as HCT116 and HCT116BaxKO	50 μM	Mitochondrial permeabilization and cytochrome c-dependent caspase activation overlooked in zerumbone-treated cancer cell death. Endothelial progenitor cells, mammary epithelial cells, and smooth muscle cells had shown resistance to zerumbone-treated cell death with lower reactive oxygen species (ROS) accumulation than cancer cell lines.	74
	HepG2 Cells	1.563, 3.125, 6.25, 12.5, 25, and 50 μg/mL	Encapsulated Zerumbone with HPβCD induced apoptosis in HepG2 cells following caspase-8/BID cleavage switch and modulating ratio of Bcl2/Bax	73
	PC-3, DU-145, HRPC cell lines		Inhibited tubulin assembly Induced a crosstalk between endoplasmic reticulum (ER) stress and mitochondrial insult followed by autophagy and apoptosis in HRPCs.	75
Blood cancer	4T1 cells		NLC encapsulated zerumbone remarkably accelerated the splenocyte cytotoxic activity against 4T1 cells	52
	CEM-ss cells	5-100 μg/mL	Suppressed the cell proliferation followed by inducing apoptosis Exhibited DNA internucleosomal degradation Enhanced cellular level of caspase-3	77
Breast cancer	Cancer cells		Inhibits IKKβ kinase, an activator of NF-κB Induce anti-proliferation and apoptosis	88
	MDA-MB-231, MCF-7, and MCF-10A cells	20 μM	Decreased in viability of MCF-7 and MDA-MB-231 cells in a dose-dependent manner together with G_2/M phase cell cycle arrest and apoptosis induction Downregulation of Bcl-2 in MDA-MB-231 and MCF-7 cells Showed a robust activation of both Bax and Bak in MDA-MB-231 and MCF-7 cells	79
	MCF-7 and MDA-MB-231 cells		Decreased cleaved Notch1 and Notch4 proteins level Increased cleavage of Notch2 protein together with induction of Presenilin-1 protein and transcriptional activation of Notch Inhibited cell migration and induced apoptosis by knockdown of Notch2 protein. Markedly attenuated of cleavage of Notch2 protein in MDA-MB-231 cells	89
	Hs578T and MDA-MB231 cells		Zerumbone suppressed IL-1β-induced cell migration and invasion.	90
Cholangiocarcinoma	CCA cell lines; KKU-100, KKU-M139, KKU-M156, KKU-M213 and KKU-M214	0.025, 0.16, 0.8, 4 and 20 μg/well	Zerumbone derivatives (7) exhibited significant antiproliferative effect against KKU-100 cell line.	91
Gastric cancer	MKN1, MKN28, MKN45, MKN74,		Significantly inhibited the cell proliferation, VEGF expression and NF-κB activity in AGS cells	92

View Article Online
DOI: 10.1039/C7FO00595D

	NUGC4 and AGS cells		Inhibited the enhanced tube formation of human umbilical vein endothelial cells (HUVECs) by co-culture with AGS cells	
Lung cancer	A549 cells	10/20 μM	Inhibited TGF-β-induced EMT *via* enhancement of E-cadherin Inhibited TGF-β1-mediated (metastatic) migration, invasion, and anchorage-independent growth Induced autophagy and apoptosis in A549 cells	93
Laryngeal carcinoma	Hep-2 cells	0.01-100 μM	Produced death of Hep-2 cells in a dose-dependent manner Significantly suppressed the proliferation of Hep-2 cells Induced the arrest of Hep-2 proliferation at S and G2/M phases	94
Other chemo preventive activities	HeLa, MCF7, MDA-MB-231 and CEM-SS cell lines	4-120 μg/mL	Proved HPβCD to be suitable encapsulant that capable of forming thermodynamically stable complex with zerumbone for safer delivery as an anticancer drug in the future	95
	RL34 cells		Significantly induced glutathione *S*-transferase Induced nuclear localization of transcription factor Nrf2 which binds to antioxidant response element (ARE) of the phase II enzyme genes Potentiated expression of several Nrf2/ARE-dependent phase II enzyme genes, for instance, γ-glutamylcysteine synthetase, glutathione peroxidase, and hemeoxygenase-1.	96
	MCF7/HER2, KBM-5, U266, H1299, SCC4, PANC-1, PANC-28, MIA PaCa-2, AsPC-1, and A293 cell lines	25 μmol/L	Down-regulated the CXC chemokine receptor 4 (CXCR4) expressions on HER2-overexpressing breast cancer cell lines in a time- and dose-dependent manner. Down-regulation of expression of mRNA Inhibited NF-κB activity Suppressed chromatin immunoprecipitation effect	97
	U937 cell line		Zerumbone antagonized the effect of DDT and TCDD in upregulation of COX-2 and VEGF mRNA expressions.	98
Pancreatic cancer	AsPC-1, PANC-1, and SW1990	3, 10, 30, 100 μM	Inhibition of cell viability of PANC-1 cells in a time and dose-dependent manner. Produced apoptosis of PANC-1 cells Significantly upregulated the expression of p53 and p21 level in PANC-1 cells Enhanced ROS production by about 149% in PANC-1 cells	99
	BxPC-3 and MIA PaCa-2	0–50 μM	Inhibited expression of mRNA and protein secretion of angiogenic factors and NF-[kappa]B properties. Inhibited the enhanced tube formation in the HUVECs by co-culture with the PaCa cells	100
Prostate cancer	DU145 and PC3 cell lines		Inhibited JAK2 selectively in both cells Inhibited IL-6/JAK2/STAT3 in DU145 cells Blocked expression of prostate cancer-associated genes, for example, cyclin D1, IL-6, COX2, and ETV1 Enhanced the sensitivity to paclitaxel (PTX) synergistically	101

5.2. Effects of zerumbone on breast cancer

A couple of studies have been carried out to examine the effects of zerumbone on breast cancer. Sehrawat et al. reported the effect of zerumbone (7.5 and 15.7 mg/kg, i.p.) on female severe combined immune deficient (SCID) mice (6–8 weeks old) and observed significant retardation of orthotopic growth of MDA-MB-231 xenografts. *In vitro* effect of zerumbone on various breast cancer cell lines specifically MDA-MB-231, MCF-7, and MCF-10A cells at a dose of 20 μM has been reported. Zerumbone decreased the viability of MCF-7 and MDA-MB-231 cells in a dose-dependent manner together with G2/M phase cell cycle arrest and apoptosis induction. Furthermore, down-regulation of Bcl-2 in MDA-MB-231 and MCF-7 cells were observed and additionally zerumbone showed a robust activation of both Bax and Bak in MDA-MB-231 and MCF-7 cells.[79] In another investigation of zerumbone on MCF-7 and MDA-MB-231 cells, zerumbone was found to decrease cleaved Notch1 and Notch4 proteins level as well as increase cleavage of Notch2 protein together with induction of Presenilin-1 protein and transcriptional activation of Notch. In addition, zerumbone inhibited cell migration and induced apoptosis by knockdown of Notch2 protein. Zerumbone also markedly attenuated cleavage of Notch2 protein in MDA-MB-231 cells.[89] Zerumbone was also reported as a suppressor of IL-1β-induced, IL-8 and MMP-3 expression in breast cancer cell lines. The authors reported that zerumbone was able to decrease IL-1β-induced cell migration and invasion in Hs578T and MDA-MB231 cells. Additionally, zerumbone inhibited the IL-1β-induced cell migration and invasion by suppressing IL-8 and MMP-3 expression in TNBC cells. Taking into account these outcomes, the authors speculated that zerumbone could be a potent therapeutic agent for treating patients with triple-negative breast cancer.[90] In a recent study, zerumbone was found to inhibit IKKβ kinase,

View Article Online
DOI: 10.1039/C7FO00595D

an activator of NF-κB as well as induce anti-proliferation and anti-apoptosis in cancer cells.[88] In another study, NLC encapsulated zerumbone was found to enhance the splenocytes cytotoxic activity against the breast cancer 4T1 cells. The authors reported that splenocytes harvested from the NLC–zerumbone treated mice showed promising cytotoxic effects against 4T1 cells in a 5:1 and a 2:1 ratio of co-culture.[52] These investigations clearly indicate the potentials of zerumbone to be further developed into an agent for the treatment of breast cancer. Kim et al. (2015) studied the effects of zerumbone on TGF-β1 signaling cascade and tumorigenecity of triple-negative breast cancer (TNBC) and HCC1806 cells[102]. The results demonstrated that, zerumbone significantly inhibited TGF-β1-induced FN, MMP-2, and MMP-9 expression in HCC1806 cells. Zerumbone was also found to decrease TGF-β1-induced phosphorylation of smad3. Moreover, zerumbone inhibited the tumorigenecity e.g. tumor volume, weight, Ki67 expression, and metastasis in TNBC cells xenograft models. Thus, the authors suggested that zerumbone might be a potent drug target for the treatment of TNBC. Another study inspected the inhibitory effects of zerumbone on triple negative breast cancer cells and reported that zerumbone by inhibiting the NF-κB activity, suppressed the elevated expression of IL-1β, which ultimately inhibited cell invasiveness of triple negative breast cancer cells.[103]

5.3. Effects of zerumbone on bile ducts cancer

Cholangiocarcinoma is an aggressive form of cancer of bile duct epithelial cell with high mortality and morbidity. In one study, 17 zerumbone derivatives were synthesized and investigated for anti-cancer activity along with zerumbone in cholangiocarcinoma cell lines explicitly KKU-100, KKU-M139, KKU-M156, KKU-M213 and KKU-M214.[91] One of the zerumbone derivatives exhibited a potent antiproliferative effect in KKU-100 cell line. The IC_{50} value was noted as 16.44 mM, which indicated that the zerumbone derivative was a good candidate for chemoprevention.

5.4. Effects of zerumbone on cervical cancer

Correspondingly, by the use of different cancer cell lines, the effects of zerumbone were observed on cervical cancer. Treatment with zerumbone on human HeLa cervical cancer cells exhibited an IC_{50} value of 11.3 μM (2.5 μg/mL) while the IC_{50} value of control, cisplatin was of 7.5 μM (1.6 μg/mL). It significantly retarded the growth of HeLa cancer cells and increased the caspase-3 level.[43] Furthermore, effects of zerumbone on cervical cancer have been further justified by Abdul et al., where zerumbone was administered intraperitoneally to inbred Balb/c mice at a dose of 16 mg/kg. Zerumbone regressed the Cervical Intraepithelial Neoplasia (CIN) and the combination of zerumbone and cisplatin modulated the IL 6 serum level. Additionally, 100 μmol/L of zerumbone showed antitumor activity in human HeLa cervical cancer cells. The down-regulation of the immunoexpression of proliferating cellular nuclear antigen ($P<0.05$) was also observed.[80] In another investigation, female Balb/c mice were treated with zerumbone at a dose of 4, 8 and 16 mg/kg and were found to suppress CIN in the experimental mice exposed prenatally to diethylstilbestrol (DES). Zerumbone also inhibited the cell proliferation marker PCNA expression in a concentration-dependent manner and exerted over-expression of Bcl-2-associated X protein (Bax). Additionally, it inhibited B-cell lymphoma 2 (Bcl-2) specific mRNA expressions.[81] These effects of zerumbone on various cervical cancer cell lines indicate zerumbone as a potential therapeutic candidate in the prevention of cervical cancer both in initiation and propagation stages.

5.5. Effects of zerumbone on colon cancer

Zerumbone was also investigated for its activities on pro-inflammatory genes expression in human colon adenocarcinoma cell lines, Caco-2, Colo320DM, and HT-29 using RT-PCR assays.[104] It was concluded that the production of IL-6, IL-1α, IL-1β, and TNF-α was notably stimulated in a time and dose-dependent manner in each cell line. As well as, Kim et al. investigated the effects of zerumbone on 85 male ICR mice to assess colon carcinogenesis. 100, 250, or 500 ppm oral doses were followed and significant inhibitions of a multiplicity of colonic adenocarcinomas have been observed. Moreover, it was also found to inhibit colonic inflammation.[83]

5.6. Effects of zerumbone on gastric cancer

An investigation on zerumbone on six gastric cancer cell lines including MKN1, MKN28, MKN45, MKN74, NUGC4 and AGS cells have been carried out by Tsuboi et al. Zerumbone significantly inhibited the cell proliferation, vascular endothelial growth factor (VEGF) expression, and NF-κB activity in AGS cells. It also inhibited the enhanced tube formation of human umbilical vein endothelial cells (HUVECs) by co-culture with AGS cells.[92] Recently, in one study, the anticancer effects of zerumbone have been studied on SGC-7901, one of human gastric cancer cell line. Here the results also indicated that zerumbone by exerting apoptosis remarkably inhibited the progression of human gastric cancer cells dose-dependently. Mechanistically, it was revealed that the inhibition of gastric cancer cell growth was the result of downregulation of Cyp A and Bcl-2 while upregulating Bax levels, which ultimately cause the cytochrome c (Cyt-C) release, and activation of caspase-3.[105]

5.7. Effects of zerumbone on lung cancer

Significant dose-dependent inhibitions in the multiplicity of lung adenomas have been observed when zerumbone was administered orally at 250 and 500 ppm on female A/J mice.[83] In a recent study, 10/20 μM of zerumbone administered on human lung cancer (A549) cells revealed inhibition of TGF-β-induced epithelial-mesenchymal transition via enhancement of E-cadherin. Additionally, it was found to inhibit TGF-β1-mediated (metastatic) migration, invasion, and anchorage-independent growth as well as induced autophagy and apoptosis in A549 cells.[93] Recently, Kang et al. (2016) reported that zerumbone caused significant inhibition of osteopontin-

View Article Online
DOI: 10.1039/C7FO00595D

induced cell invasion via suppression of the FAK/AKT/ROCK pathway in A549 cells[106].

5.8. Effects of zerumbone on liver cancer

In a study, the antitumorigenic activity of zerumbone was investigated in liver cancer-induced male Sprague–Dawley rats. The animals were injected zerumbone intraperitoneally at doses of 15, 30, or 60 mg/kg body weight, twice per week for 11 weeks. Zerumbone protected the liver of experimental animals from carcinogenesis of diethylnitrosamine and 2-acetylaminofluorene. Furthermore, it reduced the serum levels of ALT, AST, AP, and AFP as well as GSH concentration in liver tissue. Additionally, it also reduced proliferating cell nuclear antigen (PCNA) expression in liver sections of animals. Furthermore, the increment in Bax and reduction in Bcl-2 protein expression in rat liver was observed.[85]

5.9. Effects of zerumbone on laryngeal cancer

Very recently, zerumbone was found to be effective on laryngeal cancer cell lines. Zerumbone at 0.01-100 μM produced death of laryngeal carcinoma cells (Hep-2) in a dose-dependent manner and significantly suppressed the proliferation with an IC_{50} value of 15 μM. It was also observed to induce the arrest of Hep-2 proliferation at S and G2/M phases.[94]

5.10. Effects of zerumbone on prostate cancer

Jorvig & Chakraborty have reported activity of zerumbone on the prostate cancer cell lines DU145 and PC3. Zerumbone inhibited JAK2 selectively in both cells and inhibited IL-6/JAK2/STAT3 in DU145 cells. In addition, this compound blocked expression of prostate cancer-associated genes, for example, cyclin D1, IL-6, COX2, and ETV1. Furthermore, it enhanced the sensitivity to paclitaxel (PTX) synergistically.[101] The properties of zerumbone on radiation sensitivity and its protective effects contrary to ionizing radiation–induced double-strand breaks (DSB) in human prostate cancer cell lines have been investigated. The authors revealed that zerumbone sensitized DU145 and PC3 prostatic cancer cells to ionizing radiation through modulating the radiation-induced ataxia telangiectasia-mutated (ATM) activation during repair of DNA DSBs.[107]

5.11. Effects of zerumbone on pancreatic cancer

Zhang et al. reported the effects of zerumbone (3, 10, 30, 100 μM) on human pancreatic carcinoma cell lines AsPC-1, PANC-1 and SW1990. It was observed that zerumbone inhibited cell viability of PANC-1 cells in a time and dose-dependent manner. Moreover, zerumbone produced apoptosis of PANC-1 cells as well as significantly up-regulated the expression of p53 and p21 level in PANC-1 cells. It was also found to enhance ROS production by about 149% in PANC-1 cells.[99] In another study, treatment with zerumbone (0–50 μM) on human PaCa cell lines, BxPC-3 and MIA PaCa-2 inhibited expression of mRNA and protein secretion of angiogenic factors and NF-κB properties as well as inhibited the enhanced tube formation in the HUVECs by co-culture with the PaCa cells.[100]

5.12. Effects of zerumbone on skin cancer

Zerumbone topically applied to the skin of female ICR mice showed significant inhibition of tumor growth (60%) during single topical administration to mouse skin (2 μM) one day before applying dimethylbenz[a]anthracene (0.2 μM). Repeated administration of 16 nmol of zerumbone, twice a week during post-initiation phase was found to suppress the number of 12-O-tetradecanoylphorbol-13-acetate (TPA) (1.6 nM)-induced tumors by 83% and their diameter by 57%. Additionally, at 2 μmol, zerumbone induced the expression of mRNA level of manganese superoxide dismutase, glutathione peroxidase-1, glutathione S-transferase-P1 and NAD(P)H-quinone oxidoreductase in the epidermis. Furthermore, zerumbone suppressed TPA-induced COX-2 expression and phosphorylation of ERK 1/2. It also inhibited leukocyte infiltration and suppressed proliferating cell nuclear antigen labeling indices.[104] Consistent with this investigation, topical administration of zerumbone (10 μmol) on the dorsal skin of female HR-1 hairless mice (6–7 weeks of age) have found to activate the NF-E2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) expression which is considered to be a basis of preventing skin carcinogenesis.[87] However, the exact mechanisms by which zerumbone employs its effects on Nrf2 are yet to be investigated.

5.13. Other chemo preventive effects of zerumbone

Apart from the previously mentioned chemopreventive actions of zerumbone, it has also been investigated in other arrays of molecular targets. Ulcerative colitis is an inflammatory disorder and zerumbone was found to inhibit dextran sodium sulfate (DSS)-induced acute colitis in female ICR mice. The expression of inflammatory biomarkers (PGE2, IL-1β, and TNF-α) in colonic mucosa have also been reduced by zerumbone treatment.[82]

Treatment with zerumbone at 20 or 100 mg/kg body weight, i.p. doses on female BALB/c nu/nu mice (Harlan) could suppress osteolysis concentration-dependently in MDA-MB-231 breast cancer tumor-bearing athymic nude mice that further indicated zerumbone as a potent blocker of RANKL-induced NF-kB activation (Fig. 3). Zerumbone suppressed RANKL-induced p-IkBα, activation of IKK and osteoclastogenesis. Based on these result it has been suggested as a potent agent for osteoporosis as well as the cancer-associated bone loss.[84]

In accordance with a study conducted by Nakamura et al., zerumbone could significantly induce GSTP1, a pi-class glutathione S-transferase that is known to be responsible for detoxification of carcinogens and pollutants. Moreover, zerumbone could also induce nuclear localization of transcription factor Nrf2 which binds to antioxidant response element (ARE) of the phase II enzyme genes in rat liver epithelial cells (RL34 cells). Furthermore, it was found to

View Article Online
DOI: 10.1039/C7FO00595D

potentiate expression of several Nrf2/ARE-dependent phase II enzyme genes, for instance, γ-glutamylcysteine synthetase, glutathione peroxidase, and hemeoxygenase-1.[96] Notably, two structural analogs of zerumbone, 8-hydroxy-α-humulene and α-humulene were found inactive in this study. It may be due to their lacking electrophilic carbonyl moiety of the parent compound, which further reminding the potential role of zerumbone as an alpha-beta carbonyl-based component. Later, the anticancer activity on several cancer cell lines including MCF7/HER2, KBM-5 (human chronic myeloid leukemia), U266 (multiple myeloma), H1299 (lung adenocarcinoma), SCC4 (squamous cell carcinoma), PANC-1 (pancreatic duct cell carcinoma), PANC-28 (pancreatic carcinoma), MIA PaCa-2 (pancreatic carcinoma), AsPC-1 (pancreatic adenocarcinoma), and A293 (embryonic kidney carcinoma) was investigated.[97] Zerumbone at 25 μmol/L downregulated the human epidermal growth factor receptor 2 (HER2)-induced CXC chemokine receptor 4 (CXCR4) expression in breast cancer cell lines (MCF7) in a time- and dose-dependent manner. Moreover, zerumbone-treated suppression of CXCR4 did not occur through lysosomal and proteasomal degradation. Furthermore, zerumbone downregulated the expression of CXCR4 mRNA, which proved that zerumbone could modulate the expression of CXCR4 at the transcription level. In addition, zerumbone also inhibited the NF-κB activity and CXCL12-induced invasion of pancreatic and breast cancer cell lines. In another investigation, zerumbone antagonized the effect of p,p0-dichlorodiphenyltrichloroethane (DDT) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in upregulation of COX-2 and VEGF mRNA expressions in U937 cell line.[98] Another study displayed the inclusion complexation between zerumbone and hydroxypropyl-β-cyclodextrin (HPβCD) and investigated the anticancer potentials of zerumbone alone and zerumbone-HPβCD with human cervical cancer (HeLa), breast cancer (MCF7 and MDA-MB-231) and human leukemic (CEM-SS) cell lines. The data of cytotoxic assay demonstrated the anticancer efficacy of zerumbone-loaded HPβCD inclusion complex was higher than that of zerumbone alone which further encouraged to suggest HPβCD to be a suitable encapsulant capable of forming a thermodynamically stable complex with zerumbone for safer delivery as an anticancer drug in the future.[95]

All the *in vitro* and *in vivo* evidences discussed in this review prove zerumbone as a potent phytochemical that might play key roles in treatment and prevention of innumerable cancers. Although zerumbone has shown much therapeutic potentials, in comparison to the existing natural immunomodulators and anti-cancer agents like curcumin, celastrol, genistein, resveratrol, etc. that are being studied extensively in human to justify their safety, efficacy, and potency, much more planned and extensive research in terms of both pre-clinical and clinical studies are required to rationalise the existing anticancer potential of this potent sesquiterpene.

6. Other biological activities of zerumbone

Along with the immunomodulatory and anticancer effect, zerumbone has been comprehensively studied for other biological activities. Tzeng et al. reported that zerumbone could be a therapeutic potential in the treatment of diabetes.[108, 109] Additionally, the antihyperlipidemic effect of zerumbone also been reported by the same research group.[110] Analogously, significant anti-atherosclerotic effect has also been reported in a study where zerumbone inhibited the LOX-1 in THP1 cell lines, which is a fundamental event in atherosclerosis.[50] In another investigation, zerumbone possessed prominent antioxidative activity where it was subjected to FRAP assay to determine the free radical scavenging effect where the outcome found significant as paralleled to the control drug, ascorbic acid.[111] In addition, same research group has observed gastroprotective, antisecretory, and anti-*Helicobacter pylori* effects.[111] Correspondingly, zerumbone was also found to possess hepatoprotective[112] and nephroprotective[113] effects indistinct *in vivo* animal model explicitly in Sprague-Dawley rats. However, all the outcomes represent zerumbone as a multi-targeting agent even though, all the reports are at the primary stage. Intended research should be carried out before the final appraisal.

7. Toxicological investigations

Zerumbone have been investigated for toxicity studies in order to measure the safe concentration to the subjects. These studies had been conducted through three perspectives mainly acute toxicity, genotoxicity, and cytotoxicity examinations. All the studies performed so far have been briefly explained and summarized in Table 5.

Table 5 Toxicity investigations carried out with zerumbone

Type of Toxicity Studies	Subjects	Dose	Key Effects	References
Acute toxicity	Female rats (*Sprague Dawley*)	100-3000 mg/kg, i.p.	The death of all experimental animals reported at high doses of 2500, 3000 mg/kg, and 20 and 40% animals died at doses of 1500 and 2000 mg/kg, respectively. No death was observed at the doses of 100, 200, 500 and 1000 mg/kg and no side effect was seen towards the renal and liver tissues of the animals.	114

View Article Online
DOI: 10.1039/C7FO00595D

Genotoxicity	Chinese hamster ovary (CHO) cell lines	2.5 to 80 µM/mL	No mutational changes were recorded in *Salmonella typhimurium* strain TA100 either in presence/absence of S-9 liver metabolic activation system during bacterial reverse mutagenicity assay (Ames test). Induction of micronucleus (MN) and frequency of chromosome aberrations in zerumbone-treated cultures enhanced remarkably. Moreover, exhibited toxicity at higher concentration.	115
Cytotoxicity	Hepatoma tissue culture (HTC), a neoplastic rat liver cell and normal mouse fibroblast (3T3)		Zerumbone exhibited significant cytotoxic effects.	116
	CEM-SS, HL-60, WEHI-3B, 3T3 and PBMC cells	0.46, 0.93, 1.87, 3.75, 7.5, 15 and 30 µg/mL	Exhibited marked inhibition towards leukemia cell lines. It showed strong toxicity towards CEM-SS, associated with WEHI-3B and HL-60.	117
	HL60 and V79 cell line	0 to 200 µM	Showed the cytotoxic effect on HL60 cells	118
	KB, BC, NCI-H187 and Vero cells		Exhibited potent cytotoxic effects against all the cancer cells and also found strongly toxic to Vero cells (IC50= 19.9 µM)	119
	INS-1 rat pancreatic β cells	10-60 µM	The inhibition of viability of cells induced by high glucose was prevented	120

7.1. Acute toxicity studies

Ibrahim and co-workers reported the acute toxicity study of zerumbone for the first time in 2010.[114] The experiment was executed by using the female Sprague-Dawley rats of 180 - 200 g weight. The animals were injected intraperitoneally with several doses of zerumbone ranging from 100 to 3000 mg/kg. During the study, it was recognized that all experimental animals died at the high doses of 2500, 3000 mg/kg, and 20 and 40% animals died at doses of 1500 and 2000 mg/kg, respectively. However, no death was reported at the doses of 100, 200, 500 and 1000 mg/kg. The median acute toxicity (LD_{50}) of zerumbone was calculated as 1.84 g/kg. Further, it was also revealed from the findings that single injected doses of zerumbone at the concentration 100 - 200 mg/kg showed no adverse effects towards the renal and liver tissues of the experimental animals.

7.2. Genotoxicity studies

The genotoxicity of zerumbone was illustrated by using the bacterial mutagenicity assay in Chinese hamster ovary (CHO) cell lines (Ames test).[115] The concentrations followed for the genotoxicity study ranged from 5-80 uM/mL. No mutational changes were recorded in Salmonella typhimurium strain TA100 in the presence or absence of S-9 liver metabolic activation system during bacterial reverse mutagenicity assay (Ames test). Contrary to this, induction of micronucleus (MN) and frequency of chromosome aberrations in zerumbone-treated cultures showed an increased stimulation along with a dose-dependent manner. The aberrations induced by zerumbone include mainly, chromatid and whole chromosome breaks/gaps, as well as dicentrics, interchanges, endoreduplication and ring chromosomes. However, when observed at higher concentrations, it exhibited toxicity.

7.3. Cytotoxicity studies

Numerous lines of evidence suggest that the cytotoxicity study of zerumbone have been accomplished most likely since the 1980s. In one study, the author and coworkers isolated five bioactive components namely 3",4"-O-diacetyl afzelin, zerumbone epoxide, diferuloylmethane, feruloyl-p-coumaroyl methane, di-p-coumaroyl methane and zerumbone from pentane and ether extract from *Z. zerumbet* that were investigated for cytotoxicity studies against hepatoma tissue culture (HTC), a neoplastic rat liver cell and normal mouse fibroblast (3T3). The results manifested remarkable cytotoxic effects for the above-mentioned compounds along with zerumbone.[116] In the same manner, Dai et al. also depicted the cytotoxic effects of zerumbone.[21] Subsequently, a number of cytotoxic studies have been carried out by several scientists taking into account various cell lines and models.[21, 41, 43, 68, 91, 121, 122] For the leukemic cell lines, it was seen that zerumbone exhibited considerable inhibition with the IC_{50} value less than 8 µg/mL. Moreover, it also demonstrated strong toxicity towards CEM-SS with IC_{50} value of 1.6 ± 0.8 µg/mL, associated with WEHI-3B and HL-60 with respective IC_{50} values of 4.4 ± 0.2 and 7.4 ± 0.8 µg/mL.[117] Furthermore, zerumbone was also found to exhibit potent cytotoxic effects against all the cancer cells including human epidermoid carcinoma (KB), breast cancer cell (BC), and human small cell lung cancer (NCI-H187) and also presented strong toxicity to normal cells, i.e. Vero cells (IC_{50} of 19.9 µM).[119] Recently, Wang and co-workers conducted a cytotoxic study and reported that the inhibition of viability of INS-1 rat pancreatic β cells induced by high glucose was significantly prevented ($P<0.05$) by zerumbone and also found to be in a dose-dependent manner up to 60 uM.[120]

View Article Online
DOI: 10.1039/C7FO00595D

8. Conclusions & future prospects

In a nutshell, zerumbone is a key natural marker in the management of cancer and disorders related to immunity. Contemporary scientific reports from both *in vitro* and *in vivo* data prove that zerumbone can modulate immune system via several signaling events mainly, inflammatory mediated signaling pathways and play pivotal role in chronic inflammation and carcinogenesis. Still, there is much research gap as no complete studies have been reported yet correlating all the signaling events associated with immunomodulation on specific cellular models. Consequently, more detailed studies and extensive research work are required to justify the existing reports and hence unfold the knowledge in this area of work. Future research can be focused on key immune cells like macrophages and should cover all imperative signaling events associated with immune responses including TLR4, MyD88, ATF2, proteomic and genomic level research on MAPK and NF-κB pathways as well as kinase activity of Syk, Src, and IRAK1. In addition, research on promoter activity of AP1, NFkB, and CREB can give additional support to estimate zerumbone as a persuasive immunomodulatory lead molecule. Conversely, numerous *in vivo* and in-vitro molecular investigations of zerumbone against cancer treatment also revealed zerumbone as a potent chemotherapeutic agent in healing against various carcinogens. It is preliminarily estimated that the anticancer properties of the compound may be due to the presence of ketone functional group in its structure. Even though, detailed analysis of structure-activity relationship (SAR) studies is important to support this preliminary report. Additionally, the toxicological reports of this marker provide safety and toxicity profile against various *in vivo* and in-vitro living beings. However, more cellular and molecular level investigations are required to elucidate its exact mode of actions towards the targeted syndromes. According to our literature survey, although numerous investigations have been carried out for anticancer potential, the research on several cancers like blood cancer, bile duct cancer, gastric cancer, laryngeal carcinoma, lung cancer, and prostate cancer are still at the premature stage. More research on these cancers is required to explain the existing outcomes. To the best of our knowledge, no data has been reported for its pharmacokinetic and pharmacodynamics profiles in humans. Hence, prospective clinical trials, as well as pharmacokinetic and pharmacodynamic evaluation and detailed toxicological and clinical studies can give strong support for using zerumbone as a natural immunomodulator and chemopreventive therapeutic potential either alone and/or in conjunction with approved targeted therapies for the treatment of cancers in future.

Acknowledgements

The authors thanked the Ministry of Agriculture and Agro-based Industries for providing grant under the NKEA Research Grant Scheme (NRGS) (Grant no. NH1015D075).

References

1. M. Ilangkovan, I. Jantan, M. A. Mesaik and S. N. A. Bukhari, Immunosuppressive effects of the standardized extract of *Phyllanthus amarus* on cellular immune responses in Wistar-Kyoto rats, *Drug Des., Dev. Ther.,* 2015, **9**, 4917.
2. U. Patil, A. Jaydeokar and D. Bandawane, Immunomodulators: A pharmacological review, *Int. J. Pharm. Pharm. Sci.*, 2012, **4**, 30-36.
3. M. K. Shanmugam, J. H. Lee, E. Z. P. Chai, M. M. Kanchi, S. Kar, F. Arfuso, A. Dharmarajan, A. P. Kumar, P. S. Ramar and C. Y. Looi, Cancer prevention and therapy through the modulation of transcription factors by bioactive natural compounds. *Semin. Cancer Biol.,* 2016, **40**, 35-47.
4. A. Bishayee and G. Sethi, Bioactive natural products in cancer prevention and therapy: Progress and promise. *Semin. Cancer Biol.,* 2016, **40**, 1-3.
5. Y.-J. Surh, J. K. Kundu, H.-K. Na and J.-S. Lee, Redox-sensitive transcription factors as prime targets for chemoprevention with anti-inflammatory and antioxidative phytochemicals, *J. Nut.*, 2005, **135**, 2993S-3001S.
6. J. K. Kundu and Y.-J. Surh, Breaking the relay in deregulated cellular signal transduction as a rationale for chemoprevention with anti-inflammatory phytochemicals, *Mutat. Res. Fund. Mol. Mech. Mut.*, 2005, **591**, 123-146.
7. H. Kuper, H. O. Adami and D. Trichopoulos, Infections as a major preventable cause of human cancer, *J. Intern. Med.*, 2000, **248**, 171-183.
8. L. M. Wahl and H. K. Kleinman, Tumor-associated macrophages as targets for cancer therapy, *J. Natl. Cancer Inst.,* 1998, **90**, 1583-1584
9. K. E. De Visser, A. Eichten and L. M. Coussens, Paradoxical roles of the immune system during cancer development, *Nat. Rev. Cancer*, 2006, **6**, 24-37.
10. W.-W. Lin and M. Karin, A cytokine-mediated link between innate immunity, inflammation, and cancer, *J. Clin. Invest*., 2007, **117**, 1175-1183.
11. M. J. Smyth, G. P. Dunn and R. D. Schreiber, Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity, *Adv. Immunol.*, 2006, **90**, 1-50.
12. I. Jantan, W. Ahmad and S. N. A. Bukhari, Plant-derived immunomodulators: an insight on their preclinical evaluation and clinical trials, *Front. Plant. Sci.*, 2015, **6**.
13. L. Arshad, M. A. Haque, S. N. A. Bukhari and I. Jantan, An overview of structure–activity relationship studies of curcumin analogs as antioxidant and anti-inflammatory agents, *Fut. Med. Chem.*, 2017, **9**, 605-626.
14. H. Raina, G. Soni, N. Jauhari, N. Sharma and N. Bharadvaja, Phytochemical importance of medicinal plants as potential sources of anticancer agents, *Turk. J. Bot.*, 2014, **38**, 1027-1035.
15. R. Kaur, K. Kapoor and H. Kaur, Plants as a source of anticancer agents, *J. Nat. Prod. Plant Resour.*, 2011, **1**, 119-124.
16. G. C.-F. Chan, K.-W. Cheung and D. M.-Y. Sze, The immunomodulatory and anticancer properties of propolis, *Clin. Rev. Allerg. Immunol.*, 2013, **44**, 262-273.

View Article Online
DOI: 10.1039/C7FO00595D

17. M. A. Haque, I. Jantan and S. N. A. Bukhari, *Tinospora* species: an overview of their modulating effects on the immune system, *J. Ethnopharmacol.*, 2017. https://doi.org/10.1016/j.jep.2017.06.013
18. T. Kitayama, T. Okamoto, R. K. Hill, Y. Kawai, S. Takahashi, S. Yonemori, Y. Yamamoto, K. Ohe, S. Uemura and S. Sawada, Chemistry of zerumbone. 1. Simplified isolation, conjugate addition reactions, and a unique ring contracting transannular reaction of its dibromide, *J. Org. Chem.*, 1999, **64**, 2667-2672.
19. T. Kitayama, Attractive reactivity of a natural product, zerumbone, *Biosci. Biotechnol. Biochem.*, 2011, **75**, 199-207.
20. V. V. Truong, P. D. Nam, N. D. Thanh, V. N. Huong, T. T. Thuy Lien and L. V. Chinh, Study on transformation of zerumbone with fatty acid hydrazides, *Vietnam. J. Chem.*, 2015, **53**, 194-199.
21. J.-R. Dai, J. H. Cardellina, J. B. M. Mahon and M. R. Boyd, Zerumbone, an HIV-inhibitory and cytotoxic sesquiterpene of *Zingiber aromaticum* and *Z. zerumbet*, *Nat. Prod. Lett.*, 1997, **10**, 115-118.
22. S. K. Sadhu, A. Khatun, T. Ohtsuki and M. Ishibashi, First isolation of sesquiterpenes and flavonoids from *Zingiber spectabile* and identification of zerumbone as the major cell growth inhibitory component, *Nat. Prod. Res.*, 2007, **21**, 1242-1247.
23. V. Gupta, J. Kataky and R. Mathur, Zerumbone from *Zingiber-rerumbei SM., Council Scientific Industrial Research Publ & Info Directorate, New Delhi 110012, India*, 1979, **18**, 290-291.
24. H. M. Sirat, Study on the terpenoids of *Zingiber ottensii*, *Planta Med.*, 1994, **60**, 497-497.
25. Y. Ozaki, N. Kawahara and M. Harada, Anti-inflammatory effect of *Zingiber cassumunar* Roxb. and its active principles, *Chem. Pharm. Bull.*, 1991, **39**, 2353-2356.
26. N. Kishore and R. Dwivedi, Zerumbone: a potential fungitoxic agent isolated from *Zingiber cassumunar* Roxb, *Mycopathologia*, 1992, **120**, 155-159.
27. K. Firman, T. Kinoshita, A. Itai and U. Sankawa, Terpenoids from *Curcuma heyneana*, *Phytochemistry*, 1988, **27**, 3887-3891.
28. M. Aspollah Sukari, T. S. Wah, S. M. Saad, N. Y. Rashid, M. Rahmani, N. H. Lajis and T.-Y. Y. Hin, Bioactive sesquiterpenes from *Curcuma ochrorhiza* and *Curcuma heyneana*, *Nat. Prod. Res.*, 2010, **24**, 838-845.
29. D. Kaushik, J. Yadav, P. Kaushik, D. Sacher and R. Rani, Current pharmacological and phytochemical studies of the plant *Alpinia galanga*, *Zhong Xi Yi Jie He Xue Bao*, 2011, **9**, 1061-1065.
30. I. A. Ogunwande, N. O. Olawore and K. A. Adeleke, Contribution to the study of essential oil of Xylopia Aethiopica (DUNAL) A. RICH: isolation and Characterization of Zerumbone, *J. Essen. Oil. Bear. Plant.*, 2005, **8**, 159-164.
31. G. Zhang, L. Liu, M. Wang and Y.-z. Chen, Chemical constituents of volatile oils from *Syringa pinnatifolia*, *Chin. Pharm. J. -Beijing*, 1994, **29**, 271-271.
32. S. K. Yeap, M. Abd Rahman, M. Alitheen, N. Banu, W. Y. Ho, A. R. Omar, B. K. Beh and H. Ky, Evaluation of immunomodulatory effect: selection of the correct targets for immunostimulation study, *Am. J. Immunol.*, 2011, **7**, 17-23.
33. M. Sirsi, Pharmacology of zerumbone, a sesquiterpene ketone isolated from *Zingiber zerumbet*, *Ind. J. Pharm.*, 1962, **24**, 83-85.
34. M. Kodama, Y. Shiobara, H. Sumitomo, K. Mitani and K. Ueno, Synthesis of macrocyclic terpenoids by intramolecular cyclization. XI. Total synthesis of zerumbone, *Chem. Pharm. Bull.*, 1987, **35**, 4039-4042.
35. Y. Fujimoto, K. Maruno and S. Made, Antitumor sesquiterpene extraction from ginger roots, *Patent-Japan Kokai Tokkyo Koho-01*, 1989, **221**, 6.
36. A. Murakami, M. Takahashi, S. JIWAJINDA, K. Koshimizu and H. Ohigashi, Identification of zerumbone in *Zingiber zerumbet* Smith as a potent inhibitor of 12-O-tetradecanoylphorbol-13-acetate-induced Epstein-Barr virus activation, *Biosci. Biotechnol. Biochem.*, 1999, **63**, 1811-1812.
37. T. Tanaka, M. Shimizu, H. Kohno, S.-i. Yoshitani, Y. Tsukio, A. Murakami, R. Safitri, D. Takahashi, K. Yamamoto and K. Koshimizu, Chemoprevention of azoxymethane-induced rat aberrant crypt foci by dietary zerumbone isolated from *Zingiber zerumbet*, *Life Sci.*, 2001, **69**, 1935-1945.
38. M. R. Sulaiman, E. K. Perimal, M. N. Akhtar, A. Mohamad, M. H. Khalid, N. A. Tasrip, F. Mokhtar, Z. A. Zakaria, N. Lajis and D. Israf, Anti-inflammatory effect of zerumbone on acute and chronic inflammation models in mice, *Fitoterapia*, 2010, **81**, 855-858.
39. T. Chien, L. Chen, C.-J. Lee, F. Lee and C.-C. Wang, Anti-inflammatory constituents of *Zingiber zerumbet*, *Food Chem.* 2008, **110**, 584-589.
40. A. B. Abdul, A. Wahab, S. Ibrahim, A. S. Al-Zubairi, M. M. Elhassan Taha, M. Mohan and S. Mohan, Anticancer and antimicrobial activities of zerumbone from the rhizomes of Zingiber zerumbut, *Int. J. Pharmacol.*, 2008, **4**, 301-304.
41. A. Wahab, S. Ibrahim, A. B. Abdul, A. S. Alzubairi, M. Mohamed Elhassan and S. Mohan, In vitro ultramorphological assessment of apoptosis induced by zerumbone on (HeLa), *BioMed Res. Int.*, 2009, **2009**.
42. I.-M. Chung, M.-Y. Kim, W.-H. Park and H.-I. Moon, Histone deacetylase inhibitors from the rhizomes of *Zingiber zerumbet*, *Die Pharmazie- Int. J. Pharm. Sci.*, 2008, **63**, 774-776.
43. A. B. Abdul, A. S. Al-Zubairi, N. D. Tailan, A. Wahab, S. Ibrahim, Z. N. Mohd Zain, S. Ruslay, M. Mohan and S. Mohan, Anticancer activity of natural compound (Zerumbone) extracted from *Zingiber zerumbet* in human HeLa cervical cancer cells, *Int. J. Pharmacol.*, 2008, **4**, 160-168.
44. M. Phan, N. Van and T. Phan, Chemical composition of the extract from rhizomes of Curcuma zedoaria (Berg.) ROSC. Study of sesquiterpenoids, *Hoa Hoc Cong Nghiep Hoa Chat*, 1997, **26**, 9-13.
45. M. Phan, Antibacterial activity of sesquiterpene constituents from some Curcuma species in Vietnam, *Tap Chi Hoa Hoc*, 2000, **38**, 91-94.
46. M. Somchit, J. Mak, A. A. Bustamam, A. Zuraini, A. Arifah, Y. Adam and Z. Zakaria, Zerumbone isolated from *Zingiber zerumbet* inhibits inflammation and pain in rats, *J. Med. Plants Res.*, 2012, **6**, 177-180.
47. L. Arshad, I. Jantan, S. N. A. Bukhari and M. A. Haque, Immunosuppressive Effects of Natural α, β-Unsaturated Carbonyl-Based Compounds, and Their Analogs and

View Article Online
DOI: 10.1039/C7FO00595D

Derivatives, on Immune Cells: A Review, *Front. Pharmacol.*, 2017, **8**.

48. A. Murakami, D. Takahashi, T. Kinoshita, K. Koshimizu, H. W. Kim, A. Yoshihiro, Y. Nakamura, S. Jiwajinda, J. Terao and H. Ohigashi, Zerumbone, a Southeast Asian ginger sesquiterpene, markedly suppresses free radical generation, proinflammatory protein production, and cancer cell proliferation accompanied by apoptosis: the α, β-unsaturated carbonyl group is a prerequisite, *Carcinogenesis*, 2002, **23**, 795-802.
49. A. Murakami, T. Shigemori and H. Ohigashi, Zingiberaceous and citrus constituents, 1′-acetoxychavicol acetate, zerumbone, auraptene, and nobiletin, suppress lipopolysaccharide-induced cyclooxygenase-2 expression in RAW264. 7 murine macrophages through different modes of action, *J. Nut.*, 2005, **135**, 2987S-2992S.
50. A. Eguchi, Y. Kaneko, A. Murakami and H. Ohigashi, Zerumbone suppresses phorbol ester-induced expression of multiple scavenger receptor genes in THP-1 human monocytic cells, *Biosci. Biotechnol. Biochem.*, 2007, **71**, 935-945.
51. K. Ohnishi, K. Irie and A. Murakami, In vitro covalent binding proteins of zerumbone, a chemopreventive food factor, *Biosci. Biotechnol. Biochem.*, 2009, **73**, 1905-1907.
52. N. E. Mohamad, N. Abu, H. S. Rahman, H. Ky, W. Y. Ho, K. L. Lim, C. W. How, A. Rasedee, N. B. Alitheen and S. K. Yeap, Nanostructured lipid carrier improved in vivo anti-tumor and immunomodulatory effect of Zerumbone in 4T1 challenged mice, *RSC Adv.*, 2015, **5**, 22066-22074.
53. T.-Y. Chien, S. K.-H. Huang, C.-J. Lee, P.-W. Tsai and C.-C. Wang, Antinociceptive and anti-inflammatory effects of zerumbone against mono-iodoacetate-induced arthritis, *Int. J. Mol. Sci.*, 2016, **17**, 249.
54. Y. Igarashi, K. Ohnishi, K. Irie and A. Murakami, Possible Contribution of Zerumbone-Induced Proteo-Stress to Its Anti-Inflammatory Functions via the Activation of Heat Shock Factor 1, *PLoS One*, 2016, **11**, e0161282.
55. M. R. Sulaiman, E. K. Perimal, Z. A. Zakaria, F. Mokhtar, M. N. Akhtar, N. Lajis and D. Israf, Preliminary analysis of the antinociceptive activity of zerumbone, *Fitoterapia*, 2009, **80**, 230-232.
56. Y. S. Keong, N. B. Alitheen, S. Mustafa, S. A. Aziz, M. A. Rahman and A. M. Ali, Immunomodulatory effects of zerumbone isolated from roots of *Zingiber zerumbet*, *Pak. J. Pharm. Sci.*, 2010, **23**, 75-82.
57. Y.-H. Shieh, H.-M. Huang, C.-C. Wang, C.-C. Lee, C.-K. Fan and Y.-L. Lee, Zerumbone enhances the Th1 response and ameliorates ovalbumin-induced Th2 responses and airway inflammation in mice, *Int. Immunopharmacol.*, 2015, **24**, 383-391.
58. D. S. Jang, H.-Y. Min, M.-S. Kim, A.-R. Han, T. Windono, G.-H. Jeohn, S. S. Kang, S. K. Lee and E.-K. Seo, Humulene derivatives from *Zingiber zerumbet* with the inhibitory effects on lipopolysaccharide-induced nitric oxide production, *Chem. Pharm. Bull.*, 2005, **53**, 829-831.
59. A. Syahida, D. Israf, N. Lajis, S. Khozirah, M. Habsah, Jasril, D. Permana and I. Norhadiani, Effect of Compounds Isolated from Natural Products on IFN-γ/LPS–Induced Nitric Oxide Production in RAW 264.7 Macrophages, *Pharm. Biol.*, 2006, **44**, 50-59.
60. H.-Y. Min, M. S. Kim, D. S. Jang, E.-J. Park, E.-K. Seo and S. K. Lee, Suppression of lipopolysaccharide-stimulated inducible nitric oxide synthase (iNOS) expression by a novel humulene derivative in macrophage cells, *Int. Immunopharmacol.*, 2009, **9**, 844-849.
61. W. Y. Liu, T.-F. Tzeng and I.-M. Liu, Zerumbone, a Bioactive Sesquiterpene, Ameliorates Diabetes-Induced Retinal Microvascular Damage through Inhibition of Phospho-p38 Mitogen-Activated Protein Kinase and Nuclear Factor-κB Pathways, *Molecules*, 2016, **21**, 1708.
62. C. Kirana, G. H. McIntosh, I. R. Record and G. P. Jones, Antitumor activity of extract of *Zingiber aromaticum* and its bioactive sesquiterpenoid zerumbone, *Nut. Cancer*, 2003, **45**, 218-225.
63. M. Xian, K. Ito, T. Nakazato, T. Shimizu, C. K. Chen, K. Yamato, A. Murakami, H. Ohigashi, Y. Ikeda and M. Kizaki, Zerumbone, a bioactive sesquiterpene, induces G2/M cell cycle arrest and apoptosis in leukemia cells via a Fas-and mitochondria-mediated pathway, *Cancer Sci.*, 2007, **98**, 118-126.
64. R. Prasannan, K. A. Kalesh, M. K. Shanmugam, A. Nachiyappan, L. Ramachandran, A. H. Nguyen, A. P. Kumar, M. Lakshmanan, K. S. Ahn and G. Sethi, Key cell signaling pathways modulated by zerumbone: role in the prevention and treatment of cancer, *Biochem. Pharmacol.*, 2012, **84**, 1268-1276.
65. G.-C. Huang, T.-Y. Chien, L.-G. Chen and C.-C. Wang, Antitumor effects of zerumbone from *Zingiber zerumbet* in P-388D1 cells in vitro and in vivo, *Planta Med.*, 2005, **71**, 219-224.
66. Y. Takada, A. Murakami and B. B. Aggarwal, Zerumbone abolishes NF-kappaB and IkappaBalpha kinase activation leading to suppression of antiapoptotic and metastatic gene expression, upregulation of apoptosis, and downregulation of invasion, *Oncogene*, 2005, **24**, 6957-6969.
67. S. Alwi, S. Sakinah, M. Nallappan, L. Pihie and A. Hawariah, Zerumbone exerts antiproliferative activity via apoptosis on HepG2 cells, *Malays. J. Biochem. Mol. Biol.*, 2007, **15**, 19-23.
68. S. S. Sakinah, S. T. Handayani and L. A. Hawariah, Zerumbone induced apoptosis in liver cancer cells via modulation of Bax/Bcl-2 ratio, *Cancer Cell Int.*, 2007, **7**, 4.
69. A. Hamid, A. Ali, N. Rajab, N. Alitheen, H. Sirat and A. Bakar, Zerumbone causes morphological changes on different types of leukemic cell lines. In: Proceeding of the 24th Annual Conference of the Microscopy Society of Thailand, Kasetsart University, 2007.
70. S. Yodkeeree, B. Sung, P. Limtrakul and B. B. Aggarwal, Zerumbone enhances TRAIL-induced apoptosis through the induction of death receptors in human colon cancer cells: evidence for an essential role of reactive oxygen species, *Cancer Res.*, 2009, **69**, 6581-6589.
71. H.-Y. Weng, M.-J. Hsu, C.-C. Wang, B.-C. Chen, C.-Y. Hong, M.-C. Chen, W.-T. Chiu and C.-H. Lin, Zerumbone suppresses IKKα, Akt, and FOXO1 activation, resulting in apoptosis of GBM 8401 cells, *J. Biomed. Sci.*, 2012, **19**, 86.
72. B. Kamalidehghan, F. Ahmadipour and M. I. Noordin, DNA fragmentation is not associated with apoptosis in zerumbone-induced HepG2 cells, *Iran. Biomed. J.*, 2012, **16**, 226.
73. N. Muhammad Nadzri, A. B. Abdul, M. A. Sukari, S. I. Abdelwahab, E. E. Eid, S. Mohan, B. Kamalidehghan, T. Anasamy, K. B. Ng and S. Syam, Inclusion complex of

View Article Online
DOI: 10.1039/C7FO00595D

zerumbone with hydroxypropyl-β-cyclodextrin induces apoptosis in liver hepatocellular HepG2 cells via caspase 8/bid cleavage switch and modulating Bcl2/Bax ratio, *Evid. Based Complement. Alternat. Med.*, 2013, **2013**.

74. P. K. Sobhan, M. Seervi, L. Deb, S. Varghese, A. Soman, J. Joseph, K. A. Mathew, G. Raghu, G. Thomas and E. Sreekumar, Calpain and reactive oxygen species targets Bax for mitochondrial permeabilisation and caspase activation in zerumbone induced apoptosis, *PloS one*, 2013, **8**, e59350.
75. M.-L. Chan, J.-W. Liang, L.-C. Hsu, W.-L. Chang, S.-S. Lee and J.-H. Guh, Zerumbone, a ginger sesquiterpene, induces apoptosis and autophagy in human hormone-refractory prostate cancers through tubulin binding and crosstalk between endoplasmic reticulum stress and mitochondrial insult, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 2015, **388**, 1223-1236.
76. M. K. Shanmugam, P. Rajendran, F. Li, C. Kim, S. Sikka, K. S. Siveen, A. P. Kumar, K. S. Ahn and G. Sethi, Abrogation of STAT3 signaling cascade by zerumbone inhibits proliferation and induces apoptosis in renal cell carcinoma xenograft mouse model, *Mol. Carcinogen.,* 2015, **54**, 971-985.
77. S. I. Abdelwahab, A. B. Abdul, S. Mohan, M. M. E. Taha, S. Syam, M. Y. Ibrahim and A. A. Mariod, Zerumbone induces apoptosis in T-acute lymphoblastic leukemia cells, *Leukemia Res.*, 2011, **35**, 268-271.
78. H. Yan, M. Y. Ren, Z. X. Wang, S. J. Feng, S. Li, Y. Cheng, C. X. Hu, S. Q. Gao and G. Q. Zhang, Zerumbone inhibits melanoma cell proliferation and migration by altering mitochondrial functions, *Oncol. Lett.*, 2017, **13**, 2397-2402.
79. A. Sehrawat, J. A. Arlotti, A. Murakami and S. V. Singh, Zerumbone causes Bax-and Bak-mediated apoptosis in human breast cancer cells and inhibits orthotopic xenograft growth in vivo, *Breast Cancer Res. Treat.*, 2012, **136**, 429-441.
80. A. B. Abdul, S. I. Abdelwahab, J. B. Jalinas, A. S. Al-Zubairi and M. M. E. Taha, Combination of zerumbone and cisplatin to treat cervical intraepithelial neoplasia in female BALB/c mice, *Int. J. Gynecol. Cancer*, 2009, **19**, 1004-1010.
81. S. I. Abdelwahab, A. B. Abdul, N. Devi, M. M. E. Taha, A. S. Al-Zubairi, S. Mohan and A. A. Mariod, Regression of cervical intraepithelial neoplasia by zerumbone in female Balb/c mice prenatally exposed to diethylstilboestrol: involvement of mitochondria-regulated apoptosis, *Exp. Toxicol. Pathol.*, 2010, **62**, 461-469.
82. A. Murakami, R. Hayashi, T. Takana, K. H. Kwon, H. Ohigashi and R. Safitri, Suppression of dextran sodium sulfate-induced colitis in mice by zerumbone, a subtropical ginger sesquiterpene, and nimesulide: separately and in combination, *Biochem. Pharmacol.*, 2003, **66**, 1253-1261.
83. M. Kim, S. Miyamoto, Y. Yasui, T. Oyama, A. Murakami and T. Tanaka, Zerumbone, a tropical ginger sesquiterpene, inhibits colon and lung carcinogenesis in mice, *Int. J. Cancer*, 2009, **124**, 264-271.
84. B. Sung, A. Murakami, B. O. Oyajobi and B. B. Aggarwal, Zerumbone abolishes RANKL-induced NF-κB activation, inhibits osteoclastogenesis, and suppresses human breast cancer–induced bone loss in athymic nude mice, *Cancer Res.*, 2009, **69**, 1477-1484.
85. M. M. E. Taha, A. B. Abdul, R. Abdullah, T. A. T. Ibrahim, S. I. Abdelwahab and S. Mohan, Potential chemoprevention of diethylnitrosamine-initiated and 2-acetylaminofluorene-promoted hepatocarcinogenesis by zerumbone from the rhizomes of the subtropical ginger (*Zingiber zerumbet*), *Chem.-Biol. Interact.*, 2010, **186**, 295-305.
86. A. Murakami, T. Tanaka, J. Y. Lee, Y. J. Surh, H. W. Kim, K. Kawabata, Y. Nakamura, S. Jiwajinda and H. Ohigashi, Zerumbone, a sesquiterpene in subtropical ginger, suppresses skin tumor initiation and promotion stages in ICR mice, *Int. J. Cancer*, 2004, **110**, 481-490.
87. J.-W. Shin, K. Ohnishi, A. Murakami, J.-S. Lee, J. K. Kundu, H.-K. Na, H. Ohigashi and Y.-J. Surh, Zerumbone induces heme oxygenase-1 expression in mouse skin and cultured murine epidermal cells through activation of Nrf2, *Cancer Prev. Res.*, 2011, **4**, 860-870.
88. A. Fatima, A. B. H. Abdul, R. Abdullah, R. A. Karjiban and V. S. Lee, Binding mode analysis of zerumbone to key signal proteins in the tumor necrosis factor pathway, *Int. J. Mol. Sci.*, 2015, **16**, 2747-2766.
89. A. Sehrawat, K. Sakao and S. V. Singh, Notch2 activation is protective against anticancer effects of zerumbone in human breast cancer cells, *Breast Cancer Res. Treat.*, 2014, **146**, 543-555.
90. J. Han, S. Y. Bae, S. J. Oh, J. Lee, J. H. Lee, H. c. Lee, S. K. Lee, W. H. Kil, S. W. Kim and S. J. Nam, Zerumbone Suppresses IL-1β-induced Cell Migration and Invasion by Inhibiting IL-8 and MMP-3 Expression in Human Triple-negative Breast Cancer Cells, *Phytother. Res.*, 2014, **28**, 1654-1660.
91. U. Songsiang, S. Pitchuanchom, C. Boonyarat, C. Hahnvajanawong and C. Yenjai, Cytotoxicity against cholangiocarcinoma cell lines of zerumbone derivatives, *Eur. J. Med. Chem.*, 2010, **45**, 3794-3802.
92. K. Tsuboi, Y. Matsuo, T. Shamoto, T. Shibata, S. Koide, M. Morimoto, S. Guha, B. Sung, B. B. Aggarwal and H. Takahashi, Zerumbone inhibits tumor angiogenesis via NF-κB in gastric cancer, *Oncol. Rep.*, 2014, **31**, 57-64.
93. Y.-C. Hseu, Y.-C. Huang, M. Korivi, J.-J. Wu, T.-D. Way, T.-T. Ou, L.-W. Chiu, C.-C. Lee, M.-L. Lin and H.-L. Yang, Zerumbone attenuates TGF-β1-mediated epithelial–mesenchymal transition via upregulated E-cadherin expression and downregulated Smad2 signalling pathways in non-small cell lung cancer (A549) cells, *J. Funct. Foods*, 2015, **18**, 58-72.
94. S. D. Jegannathan, S. Arul and H. Dayalan, Zerumbone, a Sesquiterpene, Controls Proliferation and Induces Cell Cycle Arrest in Human Laryngeal Carcinoma Cell Line Hep-2, *Nut. Cancer*, 2016, **68**, 865-872.
95. E. E. Eid, A. B. Abdul, F. E. O. Suliman, M. A. Sukari, A. Rasedee and S. S. Fatah, Characterization of the inclusion complex of zerumbone with hydroxypropyl-β-cyclodextrin, *Carbohydr. Polym.*, 2011, **83**, 1707-1714.
96. Y. Nakamura, C. Yoshida, A. Murakami, H. Ohigashi, T. Osawa and K. Uchida, Zerumbone, a tropical ginger sesquiterpene, activates phase II drug metabolizing enzymes, *FEBS letters*, 2004, **572**, 245-250.
97. B. Sung, S. Jhurani, K. S. Ahn, Y. Mastuo, T. Yi, S. Guha, M. Liu and B. B. Aggarwal, Zerumbone down-regulates

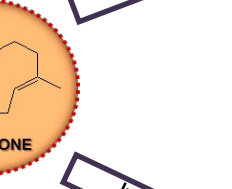
View Article Online
DOI: 10.1039/C7FO00595D

chemokine receptor CXCR4 expression leading to inhibition of CXCL12-induced invasion of breast and pancreatic tumor cells, *Cancer Res.*, 2008, **68**, 8938-8944.

98. E. M. Sciullo, C. F. Vogel, D. Wu, A. Murakami, H. Ohigashi and F. Matsumura, Effects of selected food phytochemicals in reducing the toxic actions of TCDD and p, p'-DDT in U937 macrophages, *Arch. Toxicol.*, 2010, **84**, 957-966.
99. S. Zhang, Q. Liu, Y. Liu, H. Qiao and Y. Liu, Zerumbone, a Southeast Asian ginger sesquiterpene, induced apoptosis of pancreatic carcinoma cells through p53 signaling pathway, *Evid. Based Complement. Alternat. Med.*, 2012, **2012**.
100. T. Shamoto, Y. Matsuo, T. Shibata, K. Tsuboi, T. Nagasaki, H. Takahashi, H. Funahashi, Y. Okada and H. Takeyama, Zerumbone inhibits angiogenesis by blocking NF-κB activity in pancreatic cancer, *Pancreas*, 2014, **43**, 396-404.
101. J. E. Jorvig and A. Chakraborty, Zerumbone inhibits growth of hormone refractory prostate cancer cells by inhibiting JAK2/STAT3 pathway and increases paclitaxel sensitivity, *Anti-cancer drugs*, 2015, **26**, 160-166.
102. S. Kim, J. Lee, M. Jeon, J. E. Lee and S. J. Nam, Zerumbone suppresses the motility and tumorigenecity of triple negative breast cancer cells via the inhibition of TGF-β1 signaling pathway, *Oncotarget*, 2016, **7**, 1544.
103. D. Wang, Y. Li, P. Cui, Q. Zhao, B.-b. Tan, Z.-d. Zhang, Y. Liu and N. Jia, Zerumbone induces gastric cancer cells apoptosis: Involving cyclophilin A, *Biomed. Pharmacother.*, 2016, **83**, 740-745.
104. A. Murakami, M. Miyamoto and H. Ohigashi, Zerumbone, an anti-inflammatory phytochemical, induces expression of proinflammatory cytokine genes in human colon adenocarcinoma cell lines, *Biofactors*, 2004, **21**, 95-101.
105. M. Jeon, J. Han, S. J. Nam, J. E. Lee and S. Kim, Elevated IL-1β expression induces invasiveness of triple negative breast cancer cells and is suppressed by zerumbone, *Chem. Biol. Interact.*, 2016, **258**, 126-133.
106. C. G. Kang, H.-J. Lee, S.-H. Kim and E.-O. Lee, Zerumbone suppresses osteopontin-induced cell invasion through inhibiting the FAK/AKT/ROCK pathway in human non-small cell lung cancer A549 cells, *J. Nat. Prod.*, 2015, **79**, 156-160.
107. P.-K. Chiang, W.-K. Tsai, M. Chen, W.-R. Lin, Y.-C. Chow, C.-C. Lee, J.-M. Hsu and Y.-J. Chen, Zerumbone Regulates DNA Repair Responding to Ionizing Radiation and Enhances Radiosensitivity of Human Prostatic Cancer Cells, *Integr. Cancer Ther.*, 2017, 1534735417712008.
108. T.-F. Tzeng, S.-S. Liou, C. J. Chang and I.-M. Liu, Zerumbone, a tropical ginger sesquiterpene, ameliorates streptozotocin-induced diabetic nephropathy in rats by reducing the hyperglycemia-induced inflammatory response, *Nut. Metabol.*, 2013, **10**, 64.
109. T.-F. Tzeng, S.-S. Liou, Y.-C. Tzeng and I.-M. Liu, Zerumbone, a phytochemical of subtropical ginger, protects against hyperglycemia-induced retinal damage in experimental diabetic rats, *Nutrients*, 2016, **8**, 449.
110. T.-F. Tzeng, H.-J. Lu, S.-S. Liou, C. J. Chang and I.-M. Liu, Lipid-lowering effects of zerumbone, a natural cyclic sesquiterpene of *Zingiber zerumbet* Smith, in high-fat diet-induced hyperlipidemic hamsters, *Food Chem. Toxicol.*, 2014, **69**, 132-139.
111. H. M. A. Sidahmed, N. M. Hashim, M. A. Abdulla, H. M. Ali, S. Mohan, S. I. Abdelwahab, M. M. E. Taha, L. M. Fai and J. Vadivelu, Antisecretory, gastroprotective, antioxidant and anti-helicobcter pylori activity of zerumbone from *Zingiber Zerumbet* (L.) Smith, *PloS one*, 2015, **10**, e0121060.
112. S. Fakurazi, H. Ithnin and S. Ganabadi, The effect of pretreatment of zerumbone on fatty liver following ethanol induced hepatotoxicity, *J. Biol. Sci.*, 2008, **8**, 1348-1351.
113. Z. A. Hamid, S. B. Budin, N. W. Jie, A. Hamid, K. Husain and J. Mohamed, Nephroprotective effects of *Zingiber zerumbet* Smith ethyl acetate extract against paracetamol-induced nephrotoxicity and oxidative stress in rats, *J. Zhejiang Univ. Sci. B*, 2012, **13**, 176-185.
114. M. Y. Ibrahim, A. B. Abdul, T. A. T. Ibrahim, S. I. Abdelwahab, M. M. Elhassan and M. Syam, Evaluation of acute toxicity and the effect of single injected doses of zerumbone on the kidney and liver functions in Sprague Dawley rats, *Afr. J. Biotechnol.*, 2010, **9**, 4442-4450.
115. A. S. Al-Zubairi, Genotoxicity assessment of a natural anticancer compound zerumbone in CHO cell lines, *Int. J. Cancer Res.*, 2012, **8**, 119-129.
116. H. Matthes, B. Luu and G. Ourisson, Cytotoxic components of *Zingiber zerumbet*, Curcuma zedoaria and C. domestica, *Phytochemistry*, 1980, **19**, 2643-2650.
117. M. Alitheen, N. Banu, M. Abd Rahman, S. K. Yeap, S. Mustafa, A. M. Ali and N. Lajis, Cytotoxic effect of damnacanthal, nordamnacanthal, zerumbone and betulinic acid isolated from Malaysian plant sources, *Int. Food Res. J.*, 2010, **17**, 711-719.
118. M. Norfazlina, M. F. Zuraina, N. Rajab, S. M. Nazip, A. Rumiza, C. S. Zaila, L. L. Mun, N. Nurshahirah and L. Florinsiah, Cytotoxicity study of *Nigella sativa* and *Zingiber zerumbet* extracts, thymoquinone and zerumbone isolated on human myeloid leukemia (HL60) cell. In: The Open Conference Proceedings Journal, 2014, **2**, 99-107.
119. U. Sriphanaa, S. Pitchuanchoma, P. Kongsaereeb and C. Yenjaia, Antimalarial activity and cytotoxicity of zerumbone derivatives, *ScienceAsia*, 2013, **39**, 95-99.
120. C. Wang, S. Zou, Z. Cui, P. Guo, Q. Meng, X. Shi, Y. Gao, G. Yang and Z. Han, Zerumbone protects INS-1 rat pancreatic beta cells from high glucose-induced apoptosis through generation of reactive oxygen species, *Biochem. Biophys. Res. Commun.*, 2015, **460**, 205-209.
121. S. Kushwaha, S. Roy, R. Maity, A. Mallick, V. K. Soni, P. K. Singh, N. D. Chaurasiya, R. S. Sangwan, S. Misra-Bhattacharya and C. Mandal, Chemotypical variations in Withania somnifera lead to differentially modulated immune response in BALB/c mice, *Vaccine*, 2012, **30**, 1083-1093.
122. M. Elhassan and M. Syam, Anti-tumor activities of analogues derived from the bioactive compound of *Zingiber zerumbet*, *Int. J. Cancer Res.*, 2008, **4**, 154-159.

Zingiber zerumbet

Isolation



Anticancer effects

Immunomodulating effects