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Anticancer and Antimicrobial Activities of Zerumbone from the Rhizomes of *Zingiber zerumbut*

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Abstract: The aim of this study is to evaluate the anticancer and antimicrobial activities of zerumbone (ZER) from the rhizomes of *Zingiber zerumbut*. ZER is a crystalline sesquiterpene from the wild ginger, *Z. zerumbet*. This bioactive component has its unique structure, with a cross-conjugated ketone in an 11-membered ring, as well as remarkable biological activity. Thus, this compound has been isolated from the fresh rhizomes of *Z. zerumbet* using steam distillation and evaluated for its antimicrobial and anticancer activities. The antimicrobial effects were examined using disc diffusion method and group of microorganism, namely known as Methicilin resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella choleraesuis*, *Bacillus subtilis*, *Candida albicans*, *Aspergillus ochraceaus* and *Sacchoromyces cerevisiae*. However, MTT assay was performed to determine the anti-cancer properties of zerumbone on human cervical cancer cells (HeLa) compared to cisplatin as positive control. Zerumbone has shown a dose dependent ($p<0.05$) anti-bacterial effect on *S. choleraesuis*, while no antifungal activity were observed. Zerumbone was also able to exert an antiproliferative effect towards cervical cancer cell line (HeLa) in time-dependent manner ($p<0.05$) (24, 48 and 72 h). It could be concluded that, zerumbone with its unique chemical structure and versatile pharmacological activities might be a potential primer to develop new curative agents for possible various ailments.

Key words: Zerumbone, biological activities, structure activity relationship

INTRODUCTION

Zingiber zerumbet (L.) Sm., known as *lempoyang* among the Malays, is a member of the family Zingiberaceae and used in the traditional medicine as a cure for swelling, sores, loss of appetite, worm infestation in children (Somchit and Nur-Shakirah, 2003). This plant has shown anti-tumor (Sakinah *et al*., 2007), anti-inflammatory (Murakami *et al*., 2002) and suppressant of cyclooxygenase-2 properties (Tanaka *et al*., 2001). Zerumbone is a crystalline monocyclic sesquiterpene derived from this plant. This bioactive component has its unique structure, with cross-conjugated ketone in an 11-membered ring, as well as remarkable biological activities. It has been reported that zerumbone constitute about 37% of *Z. zerumbet* (Matthes *et al*., 1980; Sakinah *et al*., 2007). Moreover, this compound showed a potential candidate for the development of anticancer treatment. Therefore, screening of this compound the biologically is needed. The objective of this research is to investigate antimicrobial and cytotoxic properties of zerumbone.

MATERIALS AND METHODS

Zerumbone and sample preparation: ZER was isolated using steam distillation method (Kitayama *et al*., 1999). Briefly, the fresh plant of *Z. zerumbet* was sliced and placed in flask and heated using Mentel heater. This flask was connected with a special glass ware (Dienstag), to collect the volatile essential oil of the boiled plant material. The collected volatile oil was crystallized spontaneously using circulating cool water during the extraction procedure. To obtain a pure material of zerumbone, recrystallization was performed using hexane for three times followed with thin layer chromatography to check the purity. Then the crystals of zerumbone were obtained and kept at -4°C for further biological activities.

Cell culture and MTT cytotoxicity assay: Human Cervical cancer cell line (HeLa) was obtained from American Type Culture Collection (ATCC), Maryland, USA. ATCC protocol recommended that RPMI 1640 can used for HeLa cell cells which was purchased from Culture Labs (Australia). The disposable items (Tissue culture

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flask, Filter system, 96-well plates) were purchased from NUNC™, Denmark. Trypsin, EDTA, Feotal Calf Serum, Amphotericin B and Penicillin Streptomycin were ordered from FlowLab (Australia). The MTT (Microtetrazolium) powder was purchased from Amresco and the DMSO (Dimethylsulphoxide) was purchased from Sigma Aldrich. MTT assay reading was performed (n = 3) using ELISA plate reader (Universal Micro plate reader).

Antimicrobial assay: Minimum inhibitory concentration method was utilized to test the antimicrobial properties of zerumbone against fungi and bacteria compared to standard antimicrobial agents using disc diffusion method. The diameter of the clear zone was measured in millimeter, A group of microorganism was utilized, namely known as MRSA = Methicilin resistant *Staphylococcus arureus*; PA = *Pseudomonas aeruginos*, SC = *Salmonella choleraesuis*, BS = *Bacillus subtilis*, CA = *Candida albicans*, AO = *Aspergillus ochraceaus* and SCE = *Sacchoromyces cerevisiae*. The bacterial and fungal stock cultures were maintained on nutrient agar (Oxoid, UK) and potato dextrose agar (Oxoid, UK) slants, respectively. For the purpose of antimicrobial assay, the microorganisms were cultured in nutrient broth for bacteria and potato dextrose broth for fungi at 30°C overnight. The concentrations of the cultures were adjusted turbidometrically at wavelength of 600 nm to 500,000-1000,000 colony forming units (CFU) per mL.

Statistical analysis: Data was presented as Mean±SD as a measure of descriptive statistics. Dependency of antimicrobial treatment of zerumbone on dose was tested statistically using Pearson Correlation Coefficient. A p-value of <0.05 was considered statistically significant. All statistical analysis was performed SPSS statistical package software version 16.0 (USA).

RESULTS

Cytotoxicity assay: As shown in Fig. 1, ZER was able to exert the antiproliferative effects towards cervical cancer cell line, HeLa, tested in time-dependent manner ($p<0.05$) (24, 48 and 72 h). The IC_{50} value which is the concentration required for 50% growth inhibition of zerumbone towards HeLa cell viability is 20.30±1.2 μM mL^{-1}. Comparatively, cisplatin, a commercial drug with anti-neoplastic activity was used as a positive control in this study. Cisplatin revealed an inhibitory effect on HeLa cells with an IC_{50} value of 5.45±0.44 μM mL^{-1}.

Antimicrobial activity of zerumbone: Zerumbone was tested for its ability to inhibit the growth of certain bacteria and fungi using disc diffusion method. Table 1 showed the results of the antimicrobial experiment. Whereby, the compound did not show any antifungal properties in *C. albicans*, *A. ochraceaus* and *S. cerevisiae*

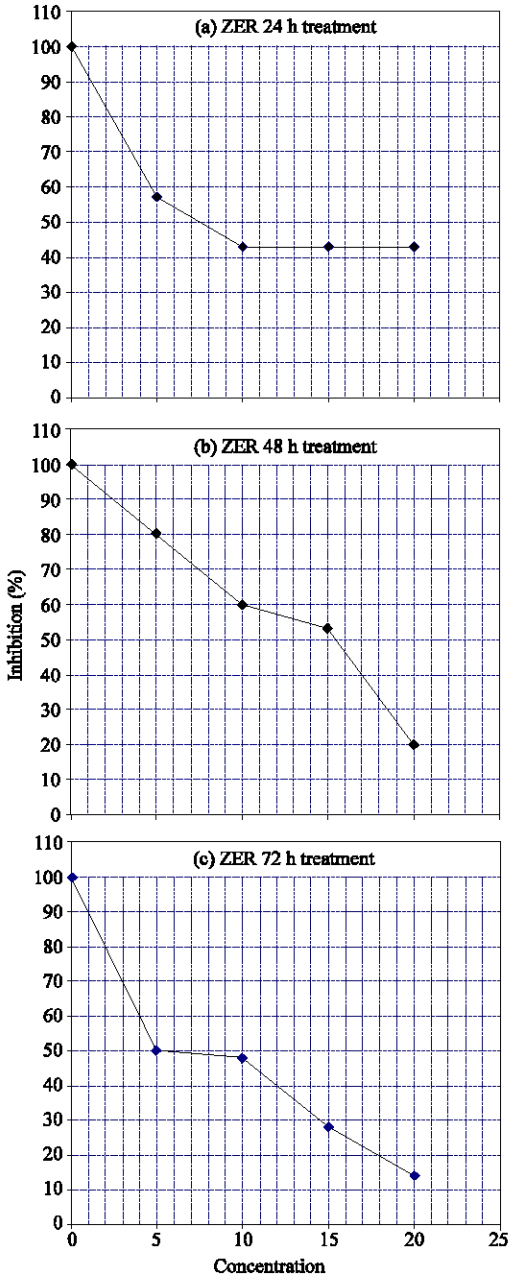

Fig. 1: Effects of zerumbone on cell viability of HeLa cell lines. Treatment of zerumbone on HeLa cell lines significantly decreases the number of viable cells with IC_{50} values of 20.30±1.2 μM mL^{-1} (A 24 h, B 48 h and C 72 h). Y-axis is the percentage of inhibition; X-axis is the concentration of zerumbone (μM mL^{-1})

Table 1: Antimicrobial activity of zerumbone on selected bacteria and fungi based on disc diffusion method (n = 3)

	Inhibition zone diameter (mm)			
	Zerumbone			
	0.13	1.3	13	
Microorganism	($mg\ mL^{-1}$)			Standards
Methicillin resistant bacteria				Streptomycin (10 μg)
S. aureus	-	-	-	30±1.2
P. aeruginosa 60690	-	-	-	12±1.3
S. choleræsuis	7±0.12	8±0.09	11±0.13	17±2.4
B. subtilis B29	-	-	-	30±3.1
Fungi				Nystatin (0.5 μg)
C. albicans CA	-	-	-	17±1.8
A. ochraceaus 398	-	-	-	25±3.7
S. cerevisiæ 20341	-	-	-	18±3.4

compared to the standard drug, Nystatin. However, zerumbone has shown a dose dependent ($p<0.05$) anti-bacterial effect on *S. choleraesuis*. In the anti-bacterial screening test, streptomycin was functioned as standard drug (positive control).

DISCUSSION

Natural products as alternative form of health care and the development of microbial resistance to available antibiotics have led researchers to investigate the antimicrobial activity of medicinal plants (Marjorie, 1999). These medicinal plants have also studied for their anticancer properties (Gordaliza, 2007). One of these potential biologically active plants is *Z. zerumbet* which shown a versatile pharmacological properties such as anti-atherosclerosis (Eguchi *et al*., 2007), anti-inflammatory, insulin-like grow factor-1 and induced Waf-1 gene expression, glutathione S-transferase activity and heat shock protein. Zerumbone was also found to exert induction of differentiation and cytoprotective activity (Rodriguez *et al*., 1997). Thus, the present study was proposed to investigate the antimicrobial activity of zerumbone using gram-positive and negative bacteria and fungi. In addition, the anticancer properties of this compound were also evaluated using human cervical cancer cell line (HeLa). The IC_{50} value which is the concentration required for 50% growth inhibition of zerumbone towards HeLa cell viability is 20.30± 1.2 μM mL^{-1}. This value could be accepted among the recommended clinical range of new anti-cancer drugs. Comparatively, cisplatin, a commercial drug with anti-neoplastic activity was used as a positive control in this study. Cisplatin is used widely in the treatment of ovarian, bladder, cervical and testicular cancer (Teni and Maria, 2004; Janson *et al*., 2008). The results of this study are supported with the previous data showed that zerumbone has antiproliferative effects towards human hepatic, leukemic (Xian *et al*., 2007) and colonic adenocarcinoma cells (Sakinah *et al*., 2007). Zerumbone is bioactive crystalline sesquiterpene and has unique structure, with a cross-conjugated ketone in an 11-membered ring (Kitayama *et al*., 2003). This unique structure could be used as a primer to develop new antimicrobial agents that have the ability to overcome the current microbial resistance. Obtained results of this research paper revealed that zerumbone showed no anti-fungal effects against *C. albicans*, *A. ochraceaus* and *S. cerevisiae*. However; this compound has shown a dose dependent anti-bacterial effect on *S. choleraesuis* but not on methicillin resistant *S. aureus*, *P. aeruginosa*, *S. choleraesuis* and *B. subtilis*. Moreover, it has been reported that this compound did not show any antibacterial activity against *B. subtilis* but its derivative has shown antibacterial agents that inhibit histidine protein kinase YycG of *B. subtilis* (Yamamoto *et al*., 2001; Kitayama *et al*., 2007). Moreover, it further suggest the potentiation of ZER as a preface to identify new synthesized antimicrobial agents.

In conclusion, zerumbone has shown remarkable anti-proliferative properties against human cervical cancer cells and antibacterial effects against *S. choleraesui*. Moreover, these versatile biological activities might be a corner stone to obtain new anticancer and antimicrobial agents using zerumbone.

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