Jurnal Teknologi

Full Paper

ANTIBACTERIAL ACTIVITY OF *Zingiber officinale* AND *Zingiber zerumbet* ESSENTIAL OILS EXTRACTED BY USING TURBO EXTRACTOR DISTILLATOR (TED)

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Article history
Received:
23 March 2015
Received in revised form:
7 July 2015
Accepted:
20 August 2015

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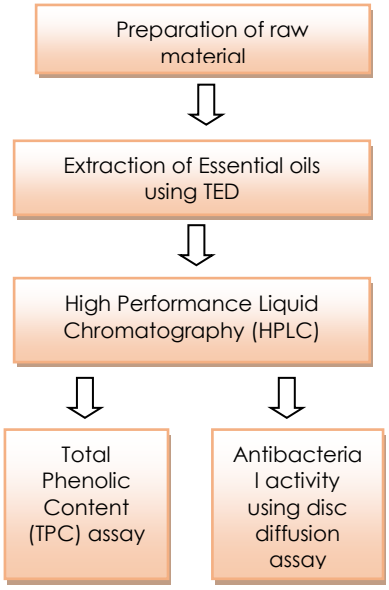
Graphical abstract



Abstract

Plants *Zingiber officinale* (ginger) and *Zingiber zerumbet* (lempoyang) of Zingiberaceae family have been traditionally used as treatment for stomach problems, nausea, vomiting, epilepsy, sore throat, muscular pains and several other disorders. In this study, essential oils from both plants were investigated for their efficacy on antibacterial activity against two Gram positive (*Staphylococcus aureus*, and *Bacillus cereus*) and two Gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria species using the disc diffusion assay. A zone of inhibition was compared with the standard antibiotic chloramphenicol, whilst a blank disc impregnated with the methanol was used as negative control. At concentration 20 µL/disc, *Z. officinale* essential oil produced zone of inhibition ranging from 16 to 36 mm, while *Z. zerumbet* essential oil produced zone inhibition ranging from 11 to 14 mm. These findings showed *Z. officinale* essential oil inhibited the growth of all tested bacteria with large zone of inhibition. The most susceptible bacteria was *B. cereus* while the lowest was *P. aeruginosa*. It can be concluded that, *Z. officinale* and *Z. zerumbet* essential oils might provide potential therapeutic agents against bacterial infection.

Keywords: *Zingiber officinale, Zingiber zerumbet,* antibacterial, essential oils, Disc diffusion

Abstrak

Tumbuhan *Zingiber officinale* (halia) dan *Zingiber zerumbet* (lempoyang) daripada keluarga Zingiberaceae telah digunakan secara tradisional sebagai rawatan untuk masalah perut, rasa loya, sawan, sakit tekak, sakit otot dan beberapa gangguan lain. Dalam kajian ini, minyak pati daripada kedua-dua tumbuhan ini telah diselidiki untuk keberkesanannya terhadap aktiviti antibakteria terhadap dua Gram positif (*Staphylococcus aureus* dan *Bacillus cereus*) dan dua Gram negatif (*Pseudomonas aeruginosa* dan *Escherichia coli*) bakteria dengan menggunakan asai cakera resapan. Zon perencatan telah dibandingkan dengan antibiotik kloramfenikol, manakala cakera kosong dengan metanol telah digunakan sebagai kawalan negatif. Pada kepekatan 20 µL/cakera, minyak pati *Z. officinale* menghasilkan zon perencatan dari 16 ke 36 mm, manakala minyak pati *Z. zerumbet* menghasilkan zon perencatan dari 11 ke 14 mm. Penemuan ini menunjukkan minyak pati *Z. officinale* menghalang pertumbuhan semua bakteria yang diuji dengan zon perencatan yang besar. Bakteria yang paling mudah direncatkan adalah *B. cereus* manakala yang sukar direncat ialah *P. aeruginosa*. Kesimpulannya, minyak pati *Z. officinale* dan *Z. zerumbet* mungkin mengandungi agen terapeutik berpotensi terhadap jangkitan bakteria.

Kata kunci: *Zingiber officinale*, *Zingiber zerumbet*, antibakteria, minyak pati, cakera resapan

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1.0 INTRODUCTION

Herbal remedies have played an enormous important role in infectious disease treatment throughout the history of mankind. Therefore, 30% to 40% of today's drugs are sourced from various plants extracts and employed as supplements and nutraceuticals [1]. The nutrient contents of different types of herbs vary considerably and they are not only a major source of carbohydrates but also contain vitamins, essential amino acids as well as minerals and antioxidants [2]. Furthermore, herbal medicines are also included in meals mainly for their nutritional values and some are reserved for sick and convalescing because of their medicinal properties.

Zingiber officinale, commonly known as ginger belongs to Zingiberaceae family is cultivated commercially in India, China, South East Asia, West Indies, Mexico and other parts of the world [3]. It is consumed worldwide as a spice and flavouring agent and is attributed to have many medicinal properties. The British Herbal Compendium reported its action as carminative, anti-emetic, spasmolytic, peripheral circulatory stimulant and anti-inflammatory [4]. The most abundant constituents in ginger essential oil is 6-gingerol (40% to 50%) and it has antioxidant properties which are very effective therapeutic agent for skin disorders and it also has protective role to toxicity and lethality against some agent like carbon-tetra chloride, cisplatin [5].

Zingiber zerumbet also called as Pinecone ginger or traditionally known as 'lempoyang' in Malaysia belong to Zingiberaceae family is native to Southeast Asia but has been widely cultivated in tropical and subtropical areas around the world [6]. *Z. zerumbet* plant is reported to contain sesquiterpenoids, flavonoids, aromatic compounds, vanillin, kaempferol derivatives and other polyphenolic compounds. Zerumbone has been identified as the most active ingredient as it accounts for the greatest percentage of total substance in *Zingiber zerumbet* [7]. Zerumbone has been found to suppress tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA)–induced Epstein-Barr virus activation in a potent manner [8].

Plant oils and extracts have been used for a wide variety of purposes for many thousands of years. The aim of this study was to test the antibacterial activity of essential oils produce by using Turbo Extractor Distillator (TED) against a diverse range of organisms comprising Gram-positive and Gram-negative bacteria. The purpose of this study was to create directly comparable, quantitative, antimicrobial data and to generate data for oils which little data exist.

2.0 EXPERIMENTAL

2.1 Plant Materials

Ginger (*Zingiber officinale*) was obtained from local farm in Bentong, Pahang, Malaysia while pinecone ginger (*Zingiber zerumbet*) was obtained from local farm in Batu Pahat, Johor, Malaysia. Rhizomes were cleaned and inspected to remove any damage, diseased or pest infested samples.

2.2 Extraction of Essential Oils

The essential oil from these plants was extracted using Turbo Extractor Distillator (TED) located at Institute Bioproduct and Development (IBD), Universiti Teknologi Malaysia (UTM). TED is accelerated hydrodistillation that allows increasing the input quantity and reducing the distillation time. The result is a very fresh product which makes an ideal base for the production of natural extracts for use in flavours and nutraceuticals. The extraction was done using 100% water. The raw material to solvent ratio used was 1:5 and time was from 1 hour to 6 hours.

2.3 Total Phenolic Content (TPC)

Total phenolic were determined using Folin-Ciocalteu reagent. Samples (1 mg/mL) were used for total phenolics assay. 50 µL of sample was mixed with 100 µL of Folin-Ciocalteu reagent (previously diluted with distilled water) and allowed to stand at 22°C for 5 min; 80 µL of sodium carbonate (70 g/L) solution was added to the mixture. After 120 min at 22°C, absorbance was measured at 760 nm. TPC was standardized against gallic acid and expressed as milligrams per liter of gallic acid equivalents (GAE).

2.4 High Performance Liquid Chromatography (HPLC)

The analytical High Performance Liquid Chromatography (HPLC) used in this experiment was Waters apparatus (2487 Dual λ Absorbance and 2690 Separation Module). The system was equipped with online degasser, binary HPLC pump, PDA detector, Auto sampler and Column heater and a Luna 5u C18 (2) 100 A column (4.6 mm x 150 mm), with 5 µm particle size or equivalent. The mobile phase for 6-gingerol consists of 1% acetic acid (solvent A) and acetonitrile (solvent B) while for zerumbone consists of 0.01M of potassium dihydrogen phosphate (solvent A), acetonitrile (solvent B) and methanol (solvent C). The mobile phases were prepared daily, filtered through a 0.45 µm membrane and sonicated before use. Total running time for 6-gingerol was 7 min and the separation was carried out in isocratic elution with 35 % and 65 % of solvent A and B, respectively. The total running time for zerumbone was 9 min and the separation was carried out in isocratic elution with 20 %, 25 % and 55 % of solvent A, B and C, respectively

[9]. PDA detector is set at 230 nm due to the highest sensitivity and best wavelength obtained for both compounds.

2.5 Microorganism

Essential oils from both plants were investigated for their efficacy on antibacterial activity against two Gram positive (*Staphylococcus aureus*, ATCC 25923 and *Bacillus cereus*, ATCC 11778) and two Gram negative (*Pseudomonas aeruginosa*, ATCC 27853 and *Escherichia coli*, ATCC 35218).

2.6 Disc Diffusion Assay

The disc diffusion method was applied for the determination of antibacterial activities of the essential oil from *Z. officinale* and *Z. zerumbet*. The bacteria culture was diluted with sterile physiological saline solution with reference to the 0.5 McFarland standard to achieve an inoculum of approximately 1.5 x 10^8 CFU/mL. A 5 mL portion of this inoculum was placed onto the surface of Nutrient Agar plates and allowed to remain in contact for 1 min. Excess inoculum was removed using a sterile syringe and the plates were allowed to dry for 20 min at room temperature. Sterile 6 mm discs were placed on the plates and immediately 20 µL of the essential oils were added. Then they were incubated at 37°C for 24 h. The diameters of the inhibition zones were measured in millimeters. A zone of inhibition was compared with the standard antibiotic chloramphenicol (10 µg/disc), whilst a blank disc impregnated with the methanol was used as negative control.

3.0 RESULTS AND DISCUSSION

The extraction yield by Turbo Extractor Distillator (TED)

Figure 1 shows the yield of essential oils produced by TED. The total yield of essential oil for *Z. officinale* was 0.17% while for *Z. zerumbet* was 0.35%. First 60 minutes from the extraction showed the highest essential oils produced for both plants. During minutes 300 to 360, the essential oils produced decreases until no more essential oil being produced during the extraction process.

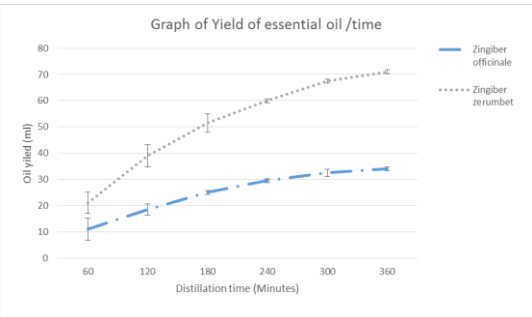

Figure 1 Cumulative graph of essential oil yield

Total Phenolic Content

Phenolic compounds are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals. The amount of total phenol was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g Gallic acid equivalent using the standard curve equation: y = 0.0032x + 0.699, R^2 = 0.9747, where y is absorbance at 760 nm and x is total phenolic content in the extracts. Figure 2 shows the standard curve of gallic acid.

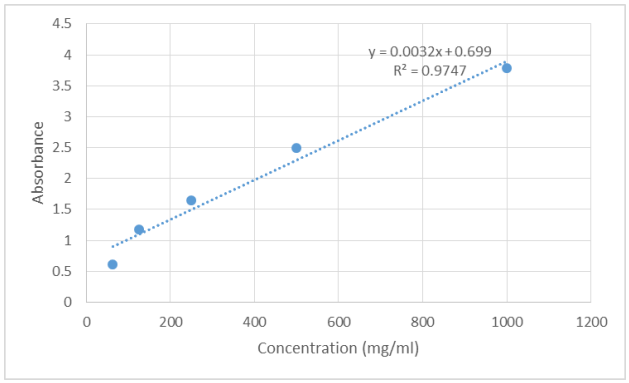

Figure 2 Standard curve of Gallic acid

The higher gallic acid was found in *Z. officinal* oil in comparison to the *Z. zerumbet* oil. The TPC value shows significantly different between both plants with p value less than 0.05 using independent t-test by SPSS version 21. Figure 3 shows that both essential oils have high phenolic compounds. This result is in agreement with other study that revealed the moderate level of phenolic content were found in the Zingiberaceae family, such as dried ginger, villous amomum fruit, and tsaoko amomum fruit [10, 11].

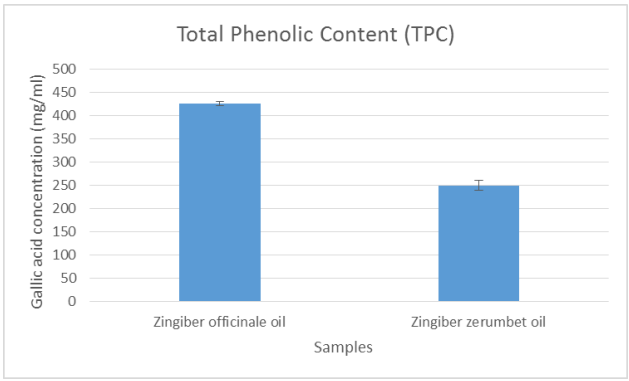

Figure 3 TPC of *Z. officinale* and *Z. zerumbet* extract

High Performance Liquid Chromatography (HPLC)

The HPLC analysis indicated that the major chemical compound in *Z. officinale* and *Z. zerumbet* essential oil extracts were 6-gingerol (Figure 4) and zerumbone (Figure 5), respectively. The qualitative study was done by comparing the peak area with external standard at retention time of 6.92 and 8.89 minute for 6-gingerol and zerumbone, respectively. The concentration of 6-gingerol in *Z. officinale* was 15.99 μg/L while the concentration for zerumbone in *Z. zerumbet* was 126.54 mg/mL.

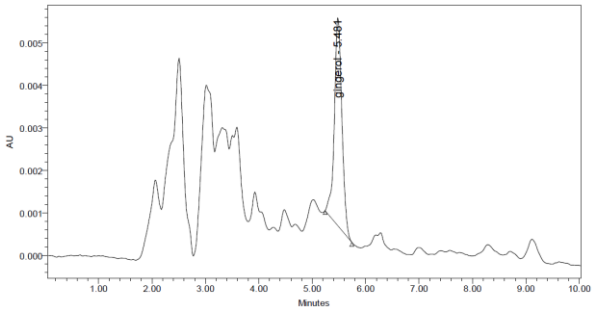

Figure 4 The chromatogram of essential oil from *Z. officinale*

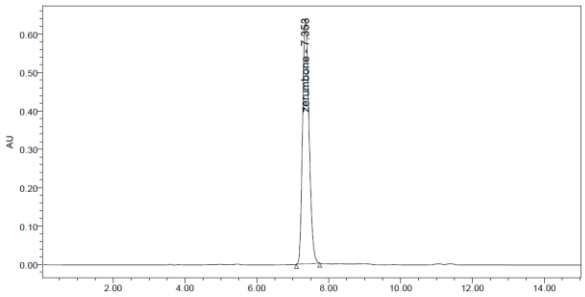

Figure 5 The chromatogram of essential oil from *Z. zerumbet*

Antibacterial Activity

The growth inhibition zones measured by disc diffusion method are presented in Table 1. At concentration of 20 μL/disc, *Z. officinale* essential oil) produced zone of inhibition ranging from 16 to 36 mm, while *Z. zerumbet* essential oil produced zone inhibition ranging from 11 to 14 mm. From these findings, *Z. officinale* essential oil inhibited the growth of all tested bacteria with larger zone of inhibition compared to that of *Z. zerumbet* essential oil. The most susceptible bacteria was *B. cereus* while the lowest inhibition was *P. aeruginosa*.

In the present study, *Z. officinale* and *Z. zerumbet* essential oils were found to exhibit antibacterial properties by using disc diffusion assay. The antibacterial activity of *Z. officinale* and *Z. zerumbet* extract could attributed to their chemical compounds [12-13,21]. Singh *et al*. [21] reported that the ginger essential oil and oleoresin contain considerable amounts of phenolic compounds (eugenol, shogaols, zingerone, gingerdiols, gingerols, etc.), which might be responsible for the observed antimicrobial potency. In addition, the use of Turbo extractor distillator allows a good extraction of phenol (95.86 %) and flavonoid (55.94 %) contents [23]. Several studies suggest that that the efficacy of plant extracts is contributed by the synergistic action of constituent phytochemicals [21]. However, the isolation of the single constituent is favoured when the bulk of activity resides in a single ingredient.

Table 1 Antibacterial properties of *Z. officinale* and *Z. zerumbet* essential oils using disc diffusion method The diameter of the zone of inhibition includes the paper disc (6 mm)

Bacterial Strain	Inhibition zone (mm)			
	ZOEO	***ZZEO***	**+ve**	**-ve**
S. aureus	30 ± 1.0	13 ± 1.0	26 ± 4.3	0
B. cereus	34 ± 2.6	11.3 ± 0.5	21 ± 0.6	0
E. coli	32 ± 2.5	12 ± 0.0	28 ± 1.0	0
P. aeruginosa	17 ± 1.0	11.3 ± 0.5	15.3 ± 3.8	0

ZOEO = *Z. officinale* essential oil; ZZEO = *Z. zerumbet* essential oil, +ve = (Chloramphenicol 10 μg/disc), -ve = Methanol, Values are mean ± SD of three parallel measurements, 0=no activity

The results of this study reflect the potent antibacterial phytochemicals present in the essential oils of *Z. officinale* and *Z. zerumbet*. These findings are in agreement with other reports that revealed the antibacterial activity in other species of Zingiberaceae family such as *Zingiber cassumunar, Alpinia galanga, Z. officinale* var. *rubrum Theilade, Curcuma mangga* and *Zingiber nimmonii* which exhibit antibacterial activity [14-16,22].

These results also show that the content of phenolic compund might besignificant to the antibacterial activity as most phenolic compund provide high inhibition of antibacterial activity.

4.0 CONCLUSION

In conclusion, *Z. officinale* and *Z. zerumbet* essential oils might provide potential therapeutic agents against bacterial infection. Further investigation on the phytochemical compounds of both plants can be conducted in order to investigate the specific antibacterial agent that contributes to the remedies of disease of extracts from this plant.

Acknowledgement

The authors acknowledge Ministry of Higher Education (MOHE) for financial support under NKEA Research

Grant Scheme (NRGS) with vote number 4H019. Appreciation went to Institute Bioproduct andDevelopment (IBD), UTM for the bioactivity facilities.

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