

## Antibacterial Activity of *Zingiber officinale* and *Zingiber zerumbet* by using Turbo Extractor Distillator (TED)

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### Graphical abstract



*Zingiber Officinale*



*Zingiber zerumbet*

### Abstract

*Zingiber officinale* (ginger) and *Zingiber zerumbet* (lempoyang) are belong to Zingiberaceae family. These plants have been traditionally used as a treatment for stomach problems, nausea, vomiting, epilepsy, sore throat, muscular pains and several other disorders. 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) bacteria species using the disc diffusion assay. 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. At concentration 20µl/disc, *Zingiber officinale* essential oils produced zone of inhibition ranging from 16mm to 36mm, while *Zingiber zerumbet* essential oils produced zone inhibition ranging from 11mm to 14mm. From these findings, *Zingiber officinale* essential oil inhibited the growth of all tested bacteria with large zone of inhibition. The most susceptible bacteria was *Bacillus cereus* while the lowest was *Pseudomonas aeruginosa*. It can be concluded that, *Zingiber officinale* and *Zingiber zerumbet* essential oils might provide potential therapeutic agents against bacterial infection.

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

*Zingiber officinale* (halia) dan *Zingiber zerumbet* (lempoyang) adalah berasal daripada keluarga Zingiberaceae. Tumbuhan ini telah digunakan secara tradisional sebagai rawatan untuk masalah perut, rasa loya, sawan, sakit tekak, sakit otot dan beberapa gangguan lain. Minyak pati daripada kedua-dua tumbuhan ini telah dikaji untuk menguji keberkesannya terhadap aktiviti antibakteria terhadap dua Gram positif (*Staphylococcus aureus*, ATCC 25923 dan *Bacillus cereus*, ATCC 11778) dan dua Gram negatif (*Pseudomonas aeruginosa*, ATCC 27853 dan *Escherichia coli*, ATCC 35218) bakteria dengan menggunakan asai cakera resapan. Zon perencatan telah dibandingkan dengan antibiotik chloramphenicol (10 µg / cakera), manakala cakera kosong dengan metanol telah digunakan sebagai kawalan negatif. Pada kepekatan 20µl / cakera, minyak pati *Zingiber officinale* menghasilkan zon perencatan dari 16mm ke 36mm, manakala minyak pati *Zingiber zerumbet* menghasilkan zon perencatan dari 11mm ke 14mm. Daripada penemuan ini, minyak pati *Zingiber officinale* menghalang pertumbuhan semua bakteria yang diuji dengan zon perencatan yang besar. Bakteria yang paling mudah direncatkan adalah *Bacillus cereus* manakala *Pseudomonas aeruginosa* adalah yang paling susah. Kesimpulannya, minyak pati *Zingiber officinale* dan *Zingiber zerumbet* mungkin mengandungi ejen potensi terapeutik terhadap jangkitan bakteria.

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

## 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<sup>1</sup>. 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<sup>2</sup>. 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<sup>3</sup>. 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<sup>4</sup>. 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<sup>5</sup>.

*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<sup>6</sup>. Zerumbone has been identified as the most active ingredient as it accounts for the greatest percentage of total substance in *Zingiber zerumbet*<sup>7</sup>. Zerumbone has been found to suppress tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced Epstein-Barr virus activation in a potent manner<sup>8</sup>.

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 was to create directly comparable, quantitative, antimicrobial data and to generate data for oils for which little data exist.

## 2.0 EXPERIMENTAL

### 2.1 Plant materials

Ginger (*Zingiber officinale*) were obtained from local farm in Bentong, Pahang, Malaysia while pinecone ginger (*Zingiber zerumbet*) were 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 were extracted using Turbo Extractor Distillator (TED) located at Institute Bioproduct and Development (IBD), Universiti Teknologi Malaysia (UTM). TED is accelerated hydrodistillation that allows to increase the input quantity and reduce 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 (1mg/ml) were used for total phenolics assay. 50 microliters of sample was mixed with 100 microliters of Folin-Ciocalteu reagent (previously diluted with distilled water) and allowed to stand at 22 °C for 5 min; 80 microliters of sodium carbonate (70 g/L) solution was added to the mixture. After 120 min at 22 °C, absorbance was measured at 760 nm. TP content 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) that used in this experiment was Waters apparatus (2487 Dual  $\lambda$  Absorbance and 2690 Separation Module). 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 (4.6mm x 150mm), with 5 $\mu$ m 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 phase should be prepared daily, filtered through a 0.45  $\mu$ m and sonicated before use. Total running time for 6-gingerol is 7 min and the separation was carried out in isocratic elution with 35 % of A and 65 % of B respectively based on previous experiment and from other research done by Yodhnu et al. (2009) with slightly modification. The total running time for zerumbone is 9 min and the separation was carried out in isocratic elution with 20 % of A, 25 % of B and 55 % of C respectively<sup>9</sup>. PDA detector is set at 230 nm due to highest sensitivity and best wavelength obtained for both compound.

### 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).

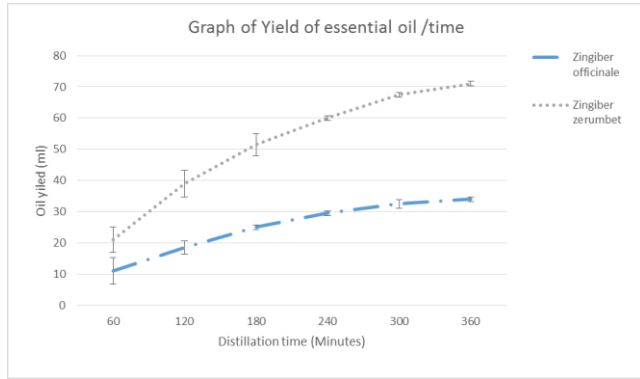
### 2.6 Disc Diffusion Assay

The disc diffusion method was applied for the determination of antibacterial activities of the essential oil from *Zingiber officinale* and *Zingiber 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<sup>8</sup> 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 $\mu$ l of the essential oils were added. Then they were incubated at 37 °C for 24 h for

bacteria. 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)*

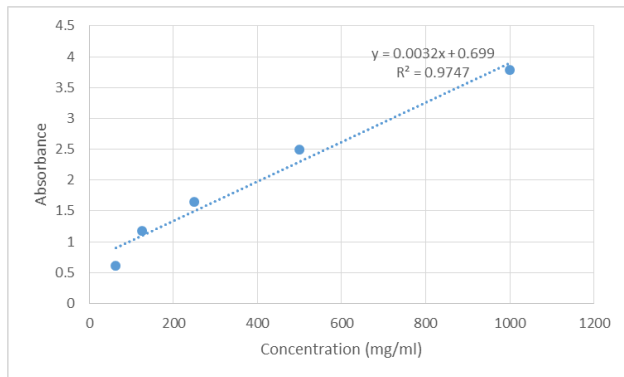


**Figure 1:** Cumulative graph of essential oils yield

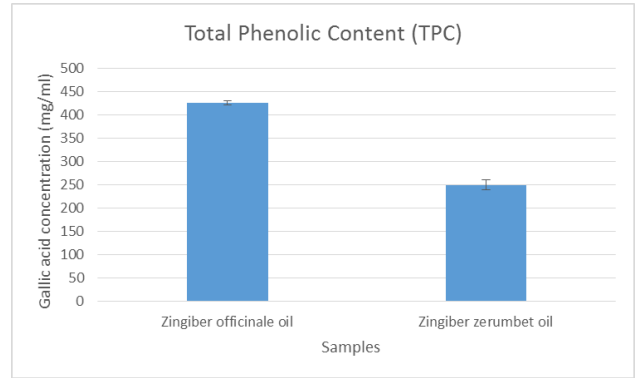
The graph shows the yield of essential oils produced from TED. The total essential oil for *Zingiber officinale* is 0.17% while for *Zingiber zerumbet* is 0.35%. The first 60 minutes of extraction show the highest essential oil production for both types of plants. During minutes 300 to 360, the essential oil production decreases until no more essential oil is produced during the extraction process.

#### 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<sup>10</sup>.



**Figure 2:** Standard curve of Gallic acid

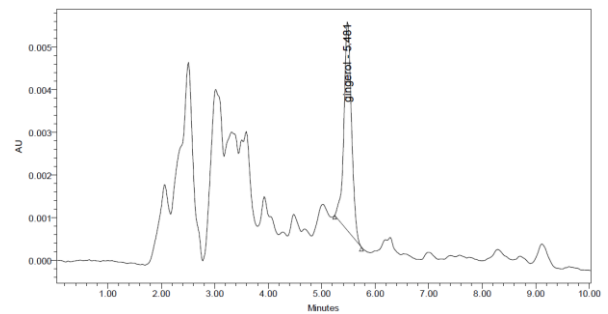


**Figure 3:** TPC of *Zingiber officinale* and *Zingiber zerumbet* extract

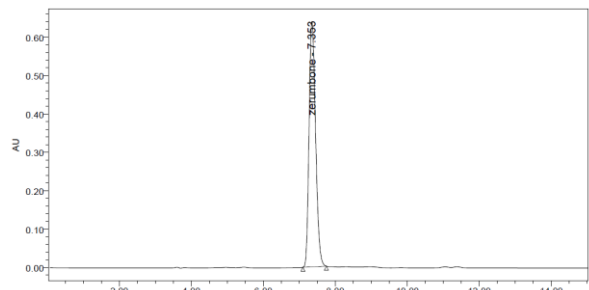
The maximum Gallic acid shown in *Zingiber officinale* oil. The TPC value shows significant differences for both plants with a  $p$  value less than 0.05 using an independent t-test by SPSS version 21. Figure 3 shows that both essential oils for both plants have high phenolic compounds. This result is supported by other studies that show a moderate level of phenolic content in the Zingiberaceae family such as dried ginger, villosus amomum fruit, and tsaoko amomum fruit, while the Lauraceae (with two tested spices) and Rutaceae (with three tested spices) contained very high levels of phenolics<sup>10,11</sup>.

#### High Performance Liquid Chromatography (HPLC)

Based on the HPLC analysis, the results indicated that the selected compounds in *Zingiber officinale* and *Zingiber zerumbet* essential oil extracts were 6-gingerol and zerumbone respectively. Comparison with external standards and the retention times of 6-gingerol is 6.923 minutes while for zerumbone is 8.8923 minutes. The concentration of 6-gingerol in *Zingiber officinale* is 15.9982 mg/L while the concentration for zerumbone in *Zingiber zerumbet* is 126,542.5608 µg/ml.



**Figure 4:** The chromatograms of essential oil from *Zingiber officinale*



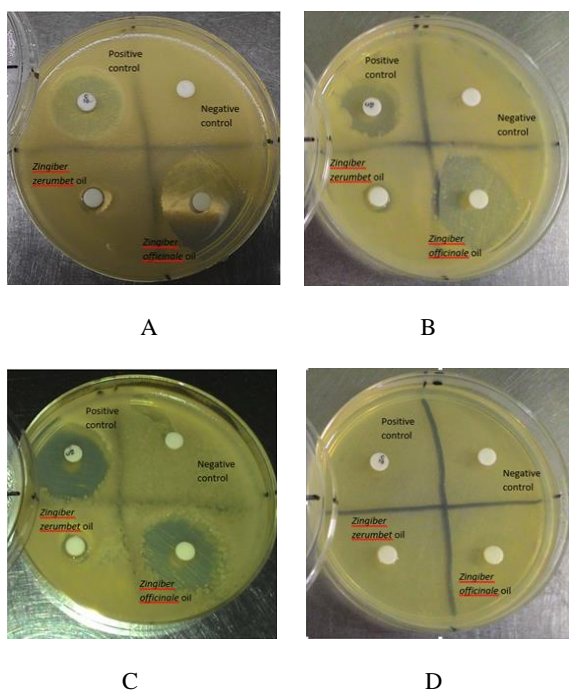
**Figure 5:** The chromatograms of essential oil from *Zingiber zerumbet*

## Antibacterial activity

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

Bacterial Strain	Inhibition zone (mm)			
	<i>Zingiber officinale</i> essential oil	<i>Zingiber zerumbet</i> essential oil	Positive control (Chloramphenicol 10µg/disc)	Negative control (Methanol)
<i>Staphylococcus aureus</i>	30 ± 1.0	13 ± 1.0	26 ± 4.3	-
<i>Bacillus cereus</i>	34 ± 2.6	11.3 ± 0.5	21 ± 0.6	-
<i>Escherichia coli</i>	32 ± 2.5	12 ± 0.0	28 ± 1.0	-
<i>Pseudomonas aeruginosa</i>	17 ± 1.0	11.3 ± 0.5	15.3 ± 3.8	-

The growth inhibition zones measured by disc diffusion method are presented in Table 1. At concentration 20µl/disc, *Zingiber officinale* essential oils produced zone of inhibition ranging from 16mm to 36mm, while *Zingiber zerumbet* essential oils produced zone inhibition ranging from 11mm to 14mm. From these findings, *Zingiber officinale* essential oil inhibited the growth of all tested bacteria with large zone of inhibition. The most susceptible bacteria was *Bacillus cereus* while the lowest was *Pseudomonas aeruginosa*.



**Figure 6:** Zone of inhibition for different type of bacteria; **A** (*Escherichia coli*); **B** (*Bacillus cereus*); **C** (*Staphylococcus aureus*); **D** (*Pseudomonas aeruginosa*)

*Zingiber officinale* and *Zingiber zerumbet* essential oils were found to exhibit stronger antibacterial properties extracted by TED using disc diffusion assay. The antibacterial activity and inhibition activity of *Zingiber officinale* and *Zingiber zerumbet* extract could attributed to the chemical compounds<sup>12,13</sup>. The results for the antibacterial screening have shown that the entire extracts have antibacterial activity.

The results of this study reflect that potent antibacterial phytochemicals are present in essential oils of both plants. These findings are supported by the reported result of species of Zingiberaceae plants such as *Zingiber cassumunar* and *Alpinia galanga*, *Boesenbergia rotunda*, *Piper betel*, *Barleria lupulina*, *Curcuma mangga* and *Zingiber nimmonii* which exhibit antibacterial activity<sup>14-16</sup>.

These result also show that the content of phenolic compound might be significant to the antibacterial activity as most phenolic compound provide high inhibition of antibacterial activity.

## 4.0 CONCLUSION

In conclusion, *Zingiber officinale* and *Zingiber zerumbet* essential oils that extracted from TED might provide potential therapeutic agents against bacterial infection. Further investigation on the phytochemical compound of both plant can be conducted in order to find the specific antibacterial agent that contribute to the remedies of disease of extracts from this plant.

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