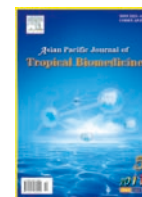




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Antimicrobial activities of the rhizome extract of *Zingiber zerumbet* LinnGolam Kader<sup>1</sup>, Farjana Nikkon<sup>1</sup>, Mohammad Abdur Rashid<sup>2</sup>, Tanzima Yeasmin<sup>1\*</sup><sup>1</sup>Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh<sup>2</sup>Department of Pharmaceutical Chemistry, University of Dhaka, Dhaka -1000, Bangladesh

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## ABSTRACT

**Objective:** To investigate antimicrobial effects of ethanolic extract of *Zingiber zerumbet* (*Z. zerumbet*) (L.) Smith and its chloroform and petroleum ether soluble fractions against pathogenic bacteria and fungi. **Methods:** The fresh rhizomes of *Zingiber zerumbet* were extracted in cold with ethanol (4.0 L) after concentration. The crude ethanol extract was fractionated by petroleum ether and chloroform to form a suspension of ethanol extract (15.0 g), petroleum ether fraction (6.6 g) and chloroform soluble fraction (5.0 g). The crude ethanol extract and its petroleum ether and chloroform fractions were evaluated for antibacterial and antifungal activity against thirteen pathogenic bacteria and three fungi by the disc diffusion method. Commercially available kanamycin (30 µg/disc) was used as standard disc and blank discs impregnated with the respective solvents were used as negative control. **Results:** At a concentration of 400 µg/disc, all the samples showed mild to moderate antibacterial and antifungal activity and produced the zone of inhibition ranging from 6 mm to 10 mm. Among the tested samples, the crude ethanol extract showed the highest activity against *Vibrio parahaemolyticus* (*V. parahaemolyticus*). The minimum inhibitory concentration (MIC) of the crude ethanol extract and its fractions were within the value of 128–256 µg/mL against two Gram positive and four Gram negative bacteria and all the samples showed the lowest MIC value against *V. parahaemolyticus* (128 µg/mL). **Conclusions:** It can be concluded that, potent antibacterial and antifungal phytochemicals are present in ethanol extract of *Z. zerumbet* (L.).

## 1. Introduction

Many plants are used as folk medicines to infectious diseases such as urinary tract infections, diarrhea, cutaneous abscesses, bronchitis and parasitic diseases[1–4]. Due to the indiscriminate use of antibacterial drugs, the microorganisms have developed resistance to many commercial antibiotics. Therefore, investigation of the chemical compounds within medicinal plants has become desirable[5]. Even though certain plants have been demonstrated for their effects on pathogenic bacteria[6], a number of them have not yet been investigated for their antimicrobial activities. Hence, it is essential to establish the scientific basis for their therapeutic actions as these may serve as the source for the development of effective drugs.

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The present study was undertaken to investigate the potential of medicinal plant, *Zingiber zerumbet* (*Z. zerumbet*) (L.) Smith as antimicrobial agent against some pathogenic Gram positive and Gram negative bacteria and some pathogenic fungi. *Z. zerumbet* (L.), known by various names, for example, “Bon adha” (Bangladesh), “Ghatian” and “Yaiimu” (India), “Lempoyang” (Malaysia and Indonesia), “Awapuhi” (Hawaii), “Zurunbah” (Arab), “Hong qiu jiang” (China), and “Haeo dam” or “Hiao dam” (Northern Thailand) [2,3,7,8], is a member of the family Zingiberaceae and widely cultivated throughout the tropics including Southeast Asia, Korea, India and Bangladesh for its medicinal properties[9–11]. Its rhizomes are used in the traditional medicine as a cure for swelling, loss of appetite, lumbago, diabetes, inflammation, chest pain, rheumatic pains, bronchitis, dyspepsia and sore throat[2,3,7,12]. The juice of the boiled rhizomes has also been used in indigenous medicine for worm infestation in children[13,14]. From the pharmacological point of view, *Z. zerumbet* has been reported to inhibit colon and lung carcinogenesis in mice[15] and CXCL12-induced invasion of breast and pancreatic

tumor cells<sup>[16]</sup>, apoptosis in liver cancer<sup>[17]</sup>, suppress phorbol ester–induced expression of multiple scavenger receptor genes in THP–1 human monocytic cells and Epstein–Barr Virus activation<sup>[18,19]</sup>. Bioactive compound(s) isolated and identified from various types of extracts of *Z. zerumbet*, only zerumbone has been studied extensively. Zerumbone has been demonstrated to possess *in vivo* antinociceptive<sup>[20]</sup> and anti–inflammatory<sup>[21–23]</sup> activities. While in the *in vitro* studies, zerumbone has been reported to exhibit antiproliferative<sup>[20]</sup> and antiplatelet aggregation<sup>[24]</sup> activities.

In our laboratory we have also found the anti–staphylococcal activity of zederone, isolated from the ethanolic extract of *Z. zerumbet* (L.) Smith against a series of multi–drug resistant (MDR) and methicillin resistant *Staphylococcus aureus* strains: SA1199B, ATCC25923, XU212, RN4220 and EMRSA15<sup>[25]</sup>. These lead us to evaluate the antibacterial, MIC and antifungal activities of the ethanol extract and its different fractions of the rhizomes of *Z. zerumbet* (L.) Smith against thirteen pathogenic bacteria and three fungi.

## 2. Materials and methods

### 2.1. Plant materials

Fresh rhizomes of *Z. zerumbet* were collected from the hilly areas of Chittagong, Bangladesh and identified by a taxonomist, Dr. Mohammed Yusuf, BCSIR Laboratory, Chittagong, Bangladesh where a voucher specimen (No. 1061) of this collection was deposited.

### 2.2. Extraction and fractionation

Fresh rhizomes of *Z. zerumbet* were sun dried for 7 days and finally autoclaved in an electric oven below 60 °C for 23 hours. The dried powdered plant materials (800 g) of *Z. zerumbet* were extracted in room temperature with ethanol (4.0 L) in an aspirator bottle for a week and then filtered. The filtrate was then concentrated by using a rotary evaporator at 45 °C under reduced pressure. The crude ethanol extract (15.0 g) was fractionated by solvent–solvent partitioning with petroleum ether (40–60 °C) and chloroform, yielding petroleum ether fraction (6.6 g) and chloroform soluble fraction (5.0 g).

### 2.3. Antimicrobial activity

The crude ethanol extract and their fractions were tested for their antibacterial activity against five Gram positive and eight Gram negative bacteria and three pathogenic fungi by disc diffusion method<sup>[26]</sup>. Thirteen pathogenic bacteria

[*Bacillus cereus* (*B. cereus*) QL 29, *Bacillus megaterium* (*B. megaterium*) QL 38, *Bacillus subtilis* (*B. subtilis*) QL 40, *Staphylococcus aureus* (*S. aureus*) ATCC25923, *Sarcina lutea* (*S. lutea*) QL 166, *Escherichia coli* (*E. coli*) ATCC 25922, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 27853, *Salmonella paratyphi* (*S. paratyphi*) A AM16590, *Salmonella typhi* (*S. typhi*) AM 16406, *Shigella boydii* (*S. boydii*) ATCC13147, *Shigella dysenteriae* (*S. dysenteriae*) ATCC 26131, *Vibrio mimicus* (*V. mimicus*) N 1967, *Vibrio parahemolyticus* (*V. parahemolyticus*) AM 16362] and three fungi [*Candida albicans* (*C. albicans*), *Aspergillus niger* (*A. niger*), *Sacharomyces cerevaceae* (*S. cerevaceae*)] were collected as pure culture from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. The sample solution of the plant materials (extract and fractions) were prepared by dissolving definite amounts of materials in appropriate solvent to attain the desired concentration and then applied to sterile disc (5 mm diameter, filter paper) followed by drying off the solvent in an aseptic hood. To compare the activity with standard antibiotics, kanamycin discs (30 µg/disc) and blank discs impregnated with the respective solvents were used as positive control and negative control, respectively.

### 2.4. Minimum inhibitory concentration (MIC)

MIC of crude ethanol extract and their fractions were determined by serial dilution technique<sup>[27]</sup> against *B. cereus* QL 29, *S. lutea* QL 166, *V. parahemolyticus* AM 16362, *E. coli* ATCC 25922, *S. typhi* AM 16406 and *P. aeruginosa* ATCC 27853.

## 3. Results

The results of antibacterial and antifungal activities of crude ethanol extract and its petroleum ether and chloroform fractions of the rhizome of *Z. zerumbet* (L.) were presented in Table 1. The zones of inhibition produced by the crude ethanol extract, petroleum ether and chloroform fraction were ranged from 9–10 mm, 8–9 mm and 6–8 mm, respectively at a concentration of 400 µg/disc. The petroleum ether and chloroform fractions showed mild to moderate activity against all the bacteria and fungi. But the crude ethanol extract showed the highest antibacterial activity (10 mm) against *V. parahemolyticus* and also showed good antifungal activity (9 mm) against all pathogenic fungi. The MIC of the crude ethanol extract and petroleum ether and chloroform fractions of the rhizome of *Z. zerumbet* (L.) were within the values of 128–256 µg/mL against 2 Gram positive (*B. cereus* and *S. lutea*) and 4 Gram negative (*V. parahemolyticus*, *E. coli*, *S. typhi* and *P. aeruginosa*) pathogenic bacteria and all the samples showed the lowest

**Table 1**  
In vitro antimicrobial activity of crude ethanol extract and its different fractions.

Test Bacteria	Zone of inhibition (Diameter in mm)			
	Crude ethanol extract (400 $\mu$ g/disc)	Petroleum ether fraction (400 $\mu$ g/disc)	Chloroform fraction (400 $\mu$ g/disc)	Kanamycin (30 $\mu$ g/disc)
<i>Gram positive bacteria</i>				
<i>B. cereus</i>	9	8	7	30
<i>B. megaterium</i>	9	8	7	31
<i>B. subtilis</i>	9	8	7	30
<i>S. aureus</i>	8	9	6	30
<i>S. lutea</i>	9	9	7	30
<i>Gram negative bacteria</i>				
<i>E. coli</i>	9	8	6	30
<i>S. paratyphi</i>	9	8	7	30
<i>S. typhi</i>	9	9	7	30
<i>V. parahemolyticus</i>	10	9	7	30
<i>V. mimicus</i>	9	9	8	30
<i>S. dysenteriae</i>	9	8	6	30
<i>S. boydii</i>	9	9	6	31
<i>P. aeruginosa</i>	9	8	7	30
<i>Fungi</i>				
<i>C. albicans</i>	9	9	6	31
<i>A. niger</i>	9	8	7	31
<i>S. cereviceae</i>	9	8	7	31

**Table 2**  
MIC values of crude ethanol extract and its different fractions ( $\mu$ g/mL).

Test bacteria	<i>Z. zerumbet</i> Linn.		
	Crude ethanol extract	Petroleum ether fraction	Chloroform fraction
<i>B. cereus</i>	256	128	256
<i>S. lutea</i>	128	256	256
<i>V. parahemolyticus</i>	128	128	128
<i>E. coli</i>	128	128	256
<i>S. typhi</i>	256	128	128
<i>P. aeruginosa</i>	128	256	256

MIC value (128  $\mu$ g/mL) against *V. parahemolyticus* (Table 2).

#### 4. Discussion

Presently there is an increasing interest worldwide in herbal medicines accompanied by increased laboratory investigation into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases<sup>[28]</sup>. Zingiberaceae plants have been received much attention, since they can produce many complex compounds that are useful in food as herbs and spices, flavoring and seasoning, and in the cosmetics and medicinal industries as antioxidant and antimicrobial agents<sup>[29]</sup>.

The antibacterial activity and inhibition activity of *Z. zerumbet* extracts could be attributed to the chemical compounds. Phytochemical investigations demonstrated the presence of zerumbone, zerumbone epoxide, diferuloylmethane, feruloyl-p-coumaroyl-methane, di-p-coumaroyl-methane sesquiterpenoids, flavonoids, aromatic compounds (e.g., hydroxybenzaldehyde), vanillin. Recent study has revealed the presence of a sesquiterpene, zederone, phenolic, saponins, and terpenoids in ethanol extract of *Z. zerumbet*<sup>[25,30]</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 and antifungal phytochemicals are present in ethanol extract of *Z. zerumbet* (L). These findings are supported by the reported results of species of Zingiberaceae plants such as *Zingiber cassumunar* and *Alpinia galanga*, *Boesenbergia rotunda*, *Piper betel*, *Barleria lupulina*, *Curcuma mangga* and *Zingiber nimmonii* which exhibited antibacterial activity<sup>[31–34]</sup>.

Several medicinal plants exhibiting antimycobacterial activity, including *Z. zerumbet*, are used as self-medication by AIDS patients in Thailand<sup>[35]</sup>. So, the crude ethanol extract of *Z. zerumbet* was biologically active and can be considered as a good candidate for further investigation.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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