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Research article

**ANTIMICROBIAL ACTIVITY OF ZERUMBONE FROM ZINGIBER ZURUMBET AGAINST
STAPHYLOCOCCUS EPIDERMIDIS AND ASPERGILLUS SPP.**Vishwanatha. H. N¹, Niraguna Babu. P¹, Gowrishankar B. S² and Shridhar. S. B³¹Department of Chemical Engineering, Siddaganga Institute of Technology, Tumkur, Karnataka, India²Department of Biotechnology, Siddaganga Institute of Technology, Tumkur, Karnataka, India³Department of Chemical Engineering, Rural Engineering College, Hulkoti, Gadag, Karnataka, India

ABSTRACT: *Zingiber zerumbet* belongs to the family Zingiberaceae and is generally known as bitter ginger. Zerumbone one of the major compound from *Zingiber zerumbet* rhizomes. Zerumbone finds its usage in medicinal applications, food flavoring, condiments, fragrances etc. Bioactive compound zerumbone was isolated from the column chromatography eluting with hexane and ethyl acetate mixture varying the polarity. The compound zerumbone exhibited strong antibacterial activity against *Staphylococcus epidermidis*, moderate activity against *Escherichia coli*, moderate antifungal activity against *Aspergillus oryza* and *Aspergillus niger*.

Keywords: *Zingiber zerumbet*, Zerumbone, Antibacterial activity, Disc diffusion method, antifungal activity.

INTRODUCTION

Zingiber zerumbet belongs to the family Zingiberaceae and is generally known as bitter ginger. Zingiberaceae is one of the largest families of the plant kingdom most frequently used as raw material for making various traditional medicines (The wealth of India, 1976). Plants as source of potential chemo therapeutic agent, antimicrobial agent and their ethno medicinal use (Prashanth *et al.*, 1998). Anti-cancer activity (Abdul *et al.*, Sharifah Sakinah *et al.* and Mihye Kim *et al.* 2008, 2007 and 2009), anti-pyretic and analgesic activity (Somchit *et al.* 2005), anti-cholinesterase activity (Ahmad Bustamam *et al.* 2008), anti-inflammatory agent (Faizah *et al.* 2002), anti-proliferative activity (Rumiza *et al.* 2005), anti-allergic activity (Supupinya *et al.* 2006), anti-microbial activity (Abdul *et al.* 2008), anti-HIV-1 integrase activity (Tewtrakul *et al.* 2006).

MATERIALS AND METHODS

Zerumbone was isolated using steam distillation method. The fresh plant of *Z. zerumbet* was sliced and placed in flask and heated using Mantle 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 (Ahmad *et al.*, 2008).

Preparation of different concentration filter discs

The procedure of serial dilution method has been used to prepare the different concentrations discs. The dilutions of the test compound was completely dissolved in n-hexane, final concentrations are 12.5, 25, 50, 100 and 200 mg/ml. The resultant solution was thoroughly mixed and approximately 10 µl was dropped onto each sterile 6 mm Whatman No 1 filter discs. It was dried over night to remove n-hexane from the filter discs (Pongpaichit, *et al.*, 2005). Similarly 10 µl oil sample was taken for each of the filter disc.

Bacterial and fungal strains

Gram-positive bacteria *Staphylococcus epidermidis*, gram-negative bacteria *Escherichia coli*, fungal strains are *Aspergillus niger* and *Aspergillus Oryza* stock cultures were obtained from Siddaganga Institute of Technology, Tumkur.

Preparation of nutrient broth for seed culture

20 ml of nutrient broth (0.1 gm Beef extract, 0.2 gm Yeast extract, 0.5 gm Peptone, 0.5 gm Sodium chloride, 1.5 gm Agar, 100 ml distilled water) was transferred into a 50 ml conical flask. All flasks were inoculated with *Bacterial and fungal* strains and maintained at 37°C for 14 hours in rotary incubater.

Antimicrobial Activity

The antimicrobial properties of zerumbone against fungi and bacteria compared to standard antimicrobial agents was done using disc diffusion method (Mohamed, 1996).

RESULTS

Table.1 represents the results of anti bacterial activities of zerumbone against *Staphylococcus epidermidis* showing high activity at 20 and 10 mg/disc (19.0 and 16.5mm). 5 mg/disc (15 mm) was moderately active. At 2.5 and 1.25mg/disc (12 and 11 mm) of zerumbone did not show any antibacterial activity. The anti bacterial activity of zerumbone against *Escherichia coli* showed moderately active with only 20mg/disc (12.5 mm). At 10, 5, 2.5 and 1.25 mg/disc (10, 8.5, 7 and 5 mm) zerumbone did not exhibit any antibacterial activity.



Figure 1. Antibacterial inhibition zones of *Staphylococcus epidermidis* and *Escherichia coli*

Table 1: Antibacterial activities of different concentrations of zerumbone

S. No.	Organism	Concentration (mg/disc)	Diameter of inhibition zone (mm)
1	<i>Staphylococcus epidermidis</i>	20	19
2		10	16.5
3		5	15
4		2.5	12
5		1.25	11
6	<i>Escherichia coli</i>	20	12.5
7		10	10
8		5	8.5
9		2.5	7
10		1.25	5

Table. 2 represents the results of anti fungal activities of zerumbone against *Aspergillus oryza*, shows moderately active at 20 mg/disc (13.5 mm). Zerumbone did not show any antifungal activity at 10, 5 and 2.5 mg/disc (12, 10 mm and no zone). The anti fungal activity of zerumbone against *Aspergillus niger* showed moderate activity at 20 and 10 mg/disc (14 and 12.5 mm). Zerumbone showed no antifungal activity. At 5 and 2.5 mg/disc (8 mm and no zone).

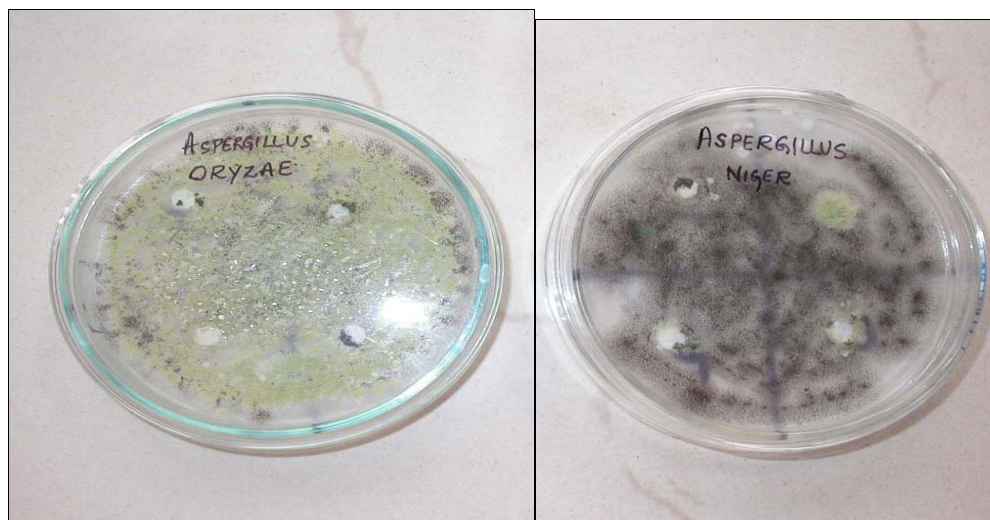


Figure 2. Antifungal inhibition zones of *Aspergillus oryza* and *Aspergillus niger*.

Table 2 Antifungal activity of different concentrations of zerumbone

S No	Organism	Concentration (mg/disc)	Diameter of inhibition zone (mm)
1	<i>Aspergillus oryza</i>	20	13
2		10	12
3		5	10
4		2.5	--
5	<i>Aspergillus niger</i>	20	14
6		10	12.5
7		5	8
8		2.5	-

DISCUSSION

Zinger zurember shows strong medical importance hence the present investigation showed that Zerumbone the major constituent has got strong antimicrobial activity against bacterial and fungal.

CONCLUSION

The effect of zerumbone on antimicrobial activity was studied by disc diffusion method. Zerumbone exhibited strong antibacterial activity against *Staphylococcus epidermidis* at 20 and 10 mg/disc and moderate activity against *Escherichia coli* at 20 mg/deisc. Zerumbone showed moderate antifungal activity against *Aspergillus oryza* at 13 mg/disc and *Aspergillus niger* at 14 and 12.5 mg/disc.

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