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# Phytochemical screening and antimicrobial activity of rhizome extract of *Zingiber Zerumbet* (l) smith

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**Abstract**
The present investigation was aimed to evaluate the phytochemical screening and antimicrobial activity of rhizome extract of *Zingiber is* which belongs to the family Zingiberaceae. The rhizome is mainly used to cure digestive problems. The plant samples were shade- dried and were subjected to different solvent extracts such as ethanol, ethyl acetate and acetone. The preliminary phytochemical screening revealed the presence of alkaloids, anthraquinones, glycosides, flavonoids, phenols, tannins, saponins, quionones, coumarins and triterpenoids. Subsequently, these solvent extracts were tested against gram positive bacteria such as *Bacillus subtilis, Streptococcus faecalis, Staphyllococcus aureus* and gram negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and a fungal strain *Candida albicans*. It is evident from the results that maximum zone of inhibition was noticed with ethanol extract of *Z. zerumbet* against *Staphylococcus aureus.* These results confirmed that the tested plants may be effective and potential sources of natural antimicrobials.

**Keywords:** *Zingiber zerumbet*, phytochemical compounds, antimicrobial activity

## 1. Introduction
*Zingiber zerumbet* (L) smith belongs to the family Zingiberaceae is widely in traditional system of medicine by Kanikkars in Western Ghats, Tirunelveli district. The important source of this plant rhizome has been regularly used as food flavoring agent. The other *Zingiber* genus like *Zingiber officinale* and *Zingiber spectabile* has high medicinal value. *Zingiber zerumbet* (Bitter zinger) is specifically well known as properties of traditional food and medicine (Job *et al*., 2011) [2]. Medicinal plants based traditional systems of medicines are playing important role in providing health care to large section of population, especially in developing countries. Interest in them and utilization of herbal products produced based on them is increasing in developed countries also (Ravishankar *et al*., 2007) [15]. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003) [13]. Numerous studies have identified compounds within herbal plants that are effective antibiotics (Basile *et al*., 2000) [14]. There is a need to screen medicinal plants for their promising biological activity. In the present study we studied the phytochemical screening and antimicrobial activity of *Zingiber zerumbet* plants against infected human pathogens.

## 2. Materials and Methods
### Plant material
*Zingiber zerumbet* plant was collected from the natural population growing in the western ghats, Tirunelveli District, Tamil nadu, India, during October 2015. The plant sample was taken to the Botany Research Laboratory and a Voucher specimen of the plant was deposited in the Botany research laboratory of V.H.N.S.N. College (Autonomous) for further references.

### Preparation of Rhizome extract
The dried rhizome extracts were treated to sequential extraction using three organic solvents on the basis of polarity of solvents (Acetone, Ethyl acetate and Ethanol). 30g of the rhizome and stems sample was taken in a separate conical flask and 200 ml of acetone was added. The conical flask was kept on mechanical shaker for 24 hours, after which the extract was filtered through Whatman filter paper 1. The pellet was allowed to dry and this pellet was used for the next solvent extraction (Ethyl acetate and Ethanol). The dried extract was recovered and stored in Refrigerator for further analysis.

### Phytochemical Screening
The collected plant extracts were subjected to qualitative phytochemical analysis for identification of various classes of active chemical constituents carried out using standard methods.

### Test for Tannins (Braymer's Test)
1ml of the rhizome extracts were mixed with 2ml of water. To this, 2 drops of 5% ferric chloride solution was added. Appearance of dirty green precipitate indicated the presence of tannins (Edeoga *et al*., 2005; Harbone, 1973) [12, 8].

### Test for phenols
1ml of the rhizome extracts were treated with 3% ferric chloride. The appearance of deep blue color shows the presence of phenols (Kokate, 2000; Harborne, 1999).

**Test for flavonoids**
1ml of the rhizome extracts were added to 1ml of sulphuric acid. Orange color formation confirmed the presence of flavonoids (Kokate, 2000; Harborne, 1999).

**Test for Quinones**
1ml of the rhizome extracts were treated with 5 ml of HCL. Formation of yellow color precipitate indicated the presence of quinones (Kokate, 2000; Harborne, 1999).

**Test for Coumarins**
2ml of the rhizome extract were taken and 3ml of 10% sodium hydroxide was added. Formation of yellow coloration indicated the presence of coumarins (Sofowara, 1993; Harborne, 1973; Ogbuewu, 2008) [9, 11].

**Test for alkaloids**
To 1 ml of rhizome extracts, 6 drops of Mayers reagent was added. The formation of yellowish creamish precipitate indicated the presence of alkaloids (Edeoga *et al*., 2005; Harbone, 1973) [12, 8].

**Test for Saponins (Foam Test)**
1ml of rhizome extracts were mixed with 5ml of distilled water. The contents were heated in a boiling water bath. Frothing indicated the presence of saponins (Edeoga *et al*., 2005; Harbone, 1973) [12, 8].

**Test for Catechins**
2ml of alcoholic rhizome extract solution were treated with few drops of Echrilich reagent and few drops of concentrated HCL. The pink color formation indicated the presence of catechins (Koperuncholan and John, 2011) [5].

**Antimicrobial activity**
In the present research work, the antimicrobial activity of three solvent extracts of *Z. zerumbet* was analyzed. Three gram positive bacteria *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis* and three gram negative bacteria *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli* and one of the fungus *Candida albicans* were used (Microorganisms were collected from the stock of Gandhigram Rural Institute, Dindugul). The test organisms were maintained on nutrient agar slopes and kept in a refrigerator at 4ºC. 100ml aliquots of nutrient broth were inoculated with the culture of test micro-organisms using a loop and then incubated at 37ºC for 24 hrs.
Antimicrobial activities of ethanol, acetone and ethyl acetate extract of *Z. zerumbet* were carried out using the agar well diffusion method (Irobi *et al*., 1994) [16]. The stock was maintained on nutrient agar slant and subculture in nutrient broth for incubation at 37ºC prior to each antimicrobial testing. Mueller Hinton agar medium (MHA) was used for antimicrobial susceptibility tests. The MHA medium was prepared by pouring 15ml of molten media into sterile petriplates. The plates were allowed to solidify after 0.1ml of an overnight broth culture of test microorganisms were added to 20ml of cooled molten agar was swabbed uniformly on the medium and allowed to dry for 5min. For agar well diffusion method, four equidistant wells (6mm in diameter) were cut from the agar with the help of a cork-borer. 40µl of each solvent extracts were loaded on 6mm of three well. The standard antibiotic gentamicin (10µg) was placed on the surface of the plates. The plates were kept for incubation for 8 hrs at 37ºC. The zone of inhibition was measured around the well containing samples and standard. The experiments were performed in triplicates.

## 3. Results and discussion
A preliminary phytochemical screening of fruit extracts of *Zingiber zerumbet* was carried out using three solvent extracts for the presence of active components (Table 1). All the three extracts of the organic solvents such as acetone, ethyl acetate and ethanolic fruits extracts of *Zingiber zerumbet* revealed the presence of saponins, quinones and coumarins. The ethanol extract reported to have the presence of all the phytoconstituents. The acetone leaf extract of *Zingiber zerumbet* revealed the presence of flavonoids, phenols, saponins, alkaloids, anthroquinines, quinones, coumarins and triterpenoids. Ethyl acetate extract also possess phytoconstituents like saponins, quinones and coumarins.

**Table 1:** Preliminary phytochemical screening of *Zingiber zerumbet* (L.) Sm. From different solvent

| Phytochemical constituents | Different solvent extracts | | |
|---|---|---|---|
| | Ethanol | Ethyl acetate | Acetone |
| Alkaloids | + | - | + |
| Anthraquinones | + | - | + |
| Glycosides | + | - | - |
| Flavonoids | + | - | + |
| Phenols | + | - | + |
| Tannins | + | - | - |
| Saponins | + | + | + |
| Quionones | + | + | + |
| Coumarins | + | + | + |
| Triterpenoids | + | - | + |

Antimicrobial efficacy of the various solvent extracts namely ethanol, ethyl acetate and acetone, has been investigated against few human pathogenic microbes (Table 2). It is evident that ethanol extract showed maximum zones of inhibition ranges between 7.83±0.47mm and 17.83±1.04mm. Acetone extract showed highest zone of inhibition against all tested cultures ranges between 9.16±0.76mm and 15.00±1.00 mm. Ethanol, ethyl acetate and acetone extracts showed leading zone of inhibition only against *B. Subtilis*, *S. aureus* and *K. pneumoniae*. Ethyl acetate extract showed minimum activity against all tested cultures compared with other solvents extracts. As a result, this plant extracts have high efficiency effect of antimicrobial activity compared to Gentamicin standard drugs.

**Table 2:** Antimicrobial activity of leaf extracts of *Zinziber zerumbet.*

| Microbial strains | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | **Ethanol** | **Ethyl acetate** | **Acetone** | **Gentamicin** |
| *Bacillus subtilis* | 16.00±1.00 | 8.83±0.76 | 14.50±1.50 | 14.00±1.00 |
| *Staphylococcus aureus* | 17.83±1.04 | 10.50±0.50 | 15.00±1.00 | 15.33±1.53 |
| *Streptococcus faecalis* | 8.33±1.04 | 8.50±0.50 | 10.16±1.04 | 27.67±1.15 |
| *Escherichia coli* | 8.00±0.00 | 10.50±0.50 | 12.16±1.04 | 15.00±1.00 |
| *Klebsiella pneumoniae* | 7.83±0.47 | 10.50±0.50 | 13.33±1.15 | 26.00±1.00 |
| *Pseudomonas aeruginosa* | 8.16±0.76 | 17.00±1.00 | 9.16±0.76 | 19.00±1.00 |
| *Candida albicans* | 8.66±0.57 | 8.83±1.04 | 14.33±2.08 | 12.00±1.00 |

Each value represents Mean ± Standard deviation of triplicates

Nowadays there is an increasing interest worldwide in herbal medicine accompanied by increased laboratory investigation into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases (Lobo *et al*., 2009) [3]. The presence of phytochemicals in the plant extracts highly correlated to the biological activity (Lawanya *et al*., 2016). Our results were supported previous studies which showed that the phytochemical screening and antimicrobial activity of ethanol, petroleum ether and chloroform extracts of stem bark of *Z. officinalae* was studied against gram positive, gram negative bacterial and fungal pathogens and the results exhibited that the ethanol extract of the plant was active on *Bacillus cerus, Bacillus megaerium, B. subtilis* and *staphylococcus aureus and Vibreo parahemolyticus*. The petroleum ether extract extract of the plant showed very high inhibitory activity on only *candida albicans, Salmonella typhii* and *Staphylococcus aureus*. The chlorofomrm extract showed lowest activity on the tested bacterial and fungal pathogens (Kader *et al.*, 2011). Also, Sharma *et al.*, 2016, reported that *Zingiber* leaves and seeds exhibited antimicrobial activity against a wide spectrum of Gram-Positive, Gram- Negative microorganisms and fungal pathogens.

The present study may offer a support to use of the plant in traditional medicine. Based on this, results, presence of phytochemical and antimicrobial investigations to isolate and identify chemical constituents in *Zingiber zerumbet* and to screen other potential bioactivities may be recommended.

## 4. Conclusions

In present study, crude leaf extract of *Z. zerumbet* plant material was tested with polar and non-polar organic solvent against seven bacterial strains. All the extracts have significant antimicrobial activity on most of the bacteria tested in this study. Ethanol extract had maximum inhibition activity as compared to ethyl acetate and acetone. The crude extract of the leaves are rich in phytochemicals and secondary metabolites such as steroids, alkaloids, terpenoids, flavonoids and tannins, these compounds may have direct interaction with the bacterial strains as antibacterial substances. Further studies are necessary to evaluate the safety of the herb for pharmaceutical applications.

## 5. Acknowledgment

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