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Research article

ANTIMICROBIAL EFFICACY OF ESSENTIAL OILS EXTRACTED FROM SOME SPECIES OF ZINGIBERACEAE

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ABSTRACT: The Zingiberaceae is one of the largest families from the order Zingiberales, with approximately 50 genera and over 1,000 species. The family has noted antimicrobial effect. In this study the efficacy of essential oils extracted from three species of Zingiberaceae under genus *Curcuma* and *Zingiber* is tested on certain pathogenic bacteria and fungi. The oil samples did not show any bactericidal activity. They were active only under bacteriostatic condition. Antifungal assay was carried out for the *Curcuma amada* Roxb, *Zingiber officinale* Rosc. *var. moran, Z. officinale* Rosc., *Z. zerumbet* (L.) J. E. Smith, out of which *Z. offiicinale var. moran* showed greater inhibitory effect over the microbial strains.

Key words: Essential oil, Zingiberaceae, Antimicrobial efficacy, Fungi, Bacteria.

INTRODUCTION

In the recent years, the research works are now been focused on the higher plants for the search of antimicrobial substances (Trakranrungsie et al., 2004; Udomkusonsri et al. 2007). The earlier notion was that, the antimicrobial substances can be obtained only from microorganisms. But with the progressive research works in antibiotics and antifungal substances, plants as a potential source of such substances are receiving considerable attention. Various bacterial and fungal strains have developed immunity against antibiotics. So searching for new novel compound for infection fighting ways is of paramount importance. Also, the harmful side effects of the synthetic drugs can also be subsided by preparing the medicines of plant origin.

Research works in this field have shown that plants do possess certain active principles or compounds the concentration of which varies with the varying seasons, age of the plant and the plant parts used. The active principles, in the form of volatile oils are biochemical compounds that can be extracted from the plant parts by various methods. For centuries, plants have been used in herbal medicine for curing various diseases (Cowan, 1999). Recently, the acceptance of traditional medicine as an alternative form for health care and the development of microbial resistance to the available antibiotics (Srinivasan et al. 2001; Kumaraswamy et al. 2002) have led authors to investigate the antimicrobial activity of medicinal plants. The Zingiberaceae is one of the largest families from the order Zingiberales, with approximately 50 genera and over 1,000 species. The family is an important natural resource that provide many useful products for food, spices, medicines, dyes, perfumes and aesthetics to man. Zingiberaceae is distributed mainly in the tropical and subtropical areas. The centre of distribution is South East Asia (Burkill, 1966). The most studied genera are *Curcuma* sp. *Alpinia* sp. *Zingiber* sp. and *Kaempferia* sp. Various ginger rhizobia provide health-promoting effects and have been utilized to treat certain illnesses such as nausea, motion sickness, stomache, asthma, diarrhea, digestive disorder, vomiting, rheumatism, swelling, common cold, cough and other disorders from long time gonorrhea (Burkill, 1966; Grosvenor et. al., 1995). The biological properties of the extracts of several species of Zingiberaceae have been investigated by many workers. Some of the reported biological and pharmacological effects were antiemetic (Sharma et al., 1997), anticancer (Limtrakul et al., 1997), antiinflammation (Claeson et al., 1996), hypolipidemic (Babu & Srinivasan, 1997), antioxidant (Selvam et al., 1995), antibacterial (Pattnaik et al., 1997) and antifungal (Apisariyakul et al., 1995). These studies have emphasized the existence of marked chemical differences among oils extracted from different species or varieties.

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In North Eastern region of India, where the floral diversity is in abundance, it provides a valuable base for the search and collection of such plant and their further analysis to explore their hidden potentials. The climate of Assam is hot and very humid, with a high degree of moisture content all round the year. The high humidity of this region pampers the growth of the microorganisms which grow and multiply rapidly due to the hot humid climate.

The present study is concentrated on the screening of certain plant rhizome of the Zingiberaceae family for their anti-microbial activity. The pathogenic organisms were selected for the study on the basis of their clinical, pharmaceutical importance as well as for their potential to cause contamination of food and drugs. Among these were *Escherichia coli* ETEC, a causative organism of diarrhoea; *Salmonella paratyphi*, causing paratyphoid fever; *Listeria monocytogenes*, the causative agent of Listeriosis. In the fungus *Aspergillus niger* is the well known causative organism of respiratory diseases in human and post harvest diseases in plants.

So the present study aims at to test the efficacy of essential oils extracted from some species of Zingiberaceae family over some pathogenic bacterial and fungal strains.

MATERIALS AND METHODS

a. Plant collection: The plant specimens were collected from different parts of North-Eastern India mainly from the states of Assam, Arunachal Pradesh, Nagaland and Meghalaya.

The oil samples from the following species were extracted and tested during the work:

I. *Zingiber officinale* Rosc.var. *moran*
II. *Z. officinale* Rosc.
III. *Z. zerumbet* (L.) J. E. Smith.
IV. *Curcuma amada* Roxb.

b. Preparation of crude oil extract: The crude oil was extracted from the dried rhizomes of the plant specimen using Soxhlet apparatus. The oil was extracted using various polar and non-polar solvents. The solvents used were acetone, methamol, hexane, Isopropanol and petroleum ether.

c. Test micro-organism: The bacterial strains were revived from glycerol stock maintained at -80^0 C and the fungal strains were revived from glycerol stock (-20^0 C) and hard synthetic fungal media plates maintained at 4^{0}C.

Bacterial Strains-

1. *Escherichia coli* ETEC Migula
2. *Salmonella paratyphi.*
3. *Listeria monocytogene (*Murry *et al.)* Pirie

Fungal Strains-

1. *Aspergillus niger* van Tiegham

d. Revival and maintenance of micro-organisms: Nutrient broth (Hi-media chemicals) was used as the culture media for the growth of bacteria.

1. Revival of bacterial strains from glycerol stock:

2ml liquid Nutrient Broth (NB) media is taken in 5 test tubes and inoculated each with 50µl different bacterial strains. These were incubated in rotary shaker at 180 rpm and 37^{0}C for 12 hours. Subcultured each of these up to third subculture to obtain pure culture. From this third subculture serial dilution was done from 10^2 to 10^7 dilutions. 200µl of each of the dilutions for each bacterial strains was spreaded on different Petri plates. Incubated it at 37^{0}C and noted the number of colonies observed after 8hrs, 10hrs, 12hrs, 14hrs, 18hrs, 20hrs, 22hrs and 24hrs. A single colony from the 24hrs. grown culture plate was picked with a tooth pick and inoculated into a test tube with 2ml nutrient media. Incubated it at 37^{0}C, 180 rpm for 8-10hr. Then again serial dilutions were carried out (10^2, 10^3, 10^4, 10^5, 10^6, 10^7). Streaked the above dilution in different tubes and incubated it at 37^{0}C for 12hrs. Sub cultured from the above into liquid NB media and kept for incubation at 37^{0}C, 180 rpm for 8-10hr. The subcultures were maintained at 4^{0}C to be used for the anti-bacterial assay.

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2. **Systematic fungal media with the composition-**
   Glucose (50g/L), Potassium dihydrogen phosphate (2.0g/L), Potassium hydrogen phosphate (2.0g/L), Magnesium sulphate heptahydrate (1.0g/L), peptone (8.0g/L), yeast extract (2.0g/L) were used for the growth of fungal strain. The fungal strains were revived from the glycerol stock by inoculating (100µl) of the culture into a test tube with 2ml liquid media and those from hard agar plates were revived by taking a loopful of it and inoculating it into 2ml liquid media in a test tube. These were then incubated at 28^0C, 180 rpm. After 48hrs. each of these was sub cultured up to second subculture and incubated. Then 200µl from this was plated on Petri plates with hard agar media and incubated at 48^0C for 48 hrs. From here a single spore was picked and inoculated into 2 ml liquid media and incubated it at 28^0C, 180 rpm for 48 hrs. This was then used for anti fungal assay of oil samples.

3. **Anti-bacterial assay of oil samples:**
   Antibacterial activity of the above five different oil samples with different solvent extract was assayed separately using disc diffusion method. 100µl of 10 hrs. old culture of a selected bacterial strain, mixed with 9 ml soft agar media was poured into Petri plates containing 10 ml of hard agar media. Sterile filter paper disc (9mm in diameter) were placed on the surface of the medium. DMSO served as negative control. A standard disc containing 10µl of Ampicillin antibiotic drug was used as positive control with concentration 5µg/µl for *E. coli* ETEC and 1µg/µl for the rest of the strains. Using sterilized dropping pipettes, 10µl of extract with different dilutions (25%, 50%, 75% and crude extract) was carefully added into the filter paper discs and incubation was done 12 hrs at 37^0C. The assessment of antibacterial was based on the measurement of diameter of inhibition zone formed around the disc.
   Five independent sets of similar trials were carried out for different extracts of each oil samples. Another set of comparisons were made by adding different extracts of a particular oil samples with the most effective dilution on a plate. These set of experiments were conducted individually for each bacterial strain and the efficacy of the oil samples of the different bacterial strains were noted.
4. **Antifungal assay of oil samples:**
   Antifungal activity of the oil samples were tested by the disc diffusion technique, similar to that of antibacterial assay for each oil extracts separately. Here the positive control was taken as a Petri plate with octal disc placed over the media. Here negative control was taken as DMSO. Rest procedure was similar to that of antibacterial assay. Incubation of the plates was done at 28^0C.

RESULTS

The result of antimicrobial assay is presented in table 1. The oil samples did not show any bactericidal activity. They were active only under bacteriostatic condition. The zone of inhibition in antibacterial assay was observed up to 12 hrs. from the period of incubation. After that there was no considerable increase in the zone of inhibition. Whereas for antifungal assay, zone of inhibition was observed up to 24 hrs, from the period of incubation. DMSO taken as negative control do not show any zone of inhibition, providing that the solvent is inert and does not affect the antibacterial property of the oil samples. The dilute samples of oil were noted to be more effective then the crude samples, with some exceptions. Isopropanol extract of *Z. officinale* at 100% concentration showed inhibition zone against *Salmonella paratyphi*.

The oil samples did not show any inhibitory effect over the bacterial strain *E. coli* ETEC except the acetone extract of *Z. officinale* var. *moran* (50% conc.), which showed a moderate zone of inhibition. However *C. amada* showed a very narrow zone of inhibition. All the oil samples showed maximum inhibitory effect over the bacterial strain *Listeria monocytogenes.* Of all the oil samples, the oil extracted from *Z. officinale* var. *moran* was found to be most effective against the test organisms. For *Z. officinale* var. *moran* and *Z. zerumbet* 75% conc. (of all extracts) was found to be most effective against microbial strains tested. *Salmonella paratyphi* showed narrow zone of inhibition by 50% concentration of *Z. zerumbet* and isopropanol extract of *Z. officinale* (75% and 100% concentration). Isoprapanol extract and hexane extract of all the oil samples showed similar and comparatively greater inhibitory effect over the bacterial strains. Antifungal assay was carried out for the *C. amada, Z. officinale* var. *moran, Z. officinale* only, out of which *Z. officinale* var. *moran* showed greater inhibitory effect over the fungal strains.

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Table 1: Zone of inhibition in antimicrobial assay

S. No.	Plant species	Microbial species		Zone of inhibition in hours		Observation /Remarks	
		Bacteria	Fungus	Antibacterial assay	Antifungal Assay	Bacteria	Fungus
1	*Z. officinale* var. *moran*	*Escherichia coli*	*Aspergillus niger*	8	24	Acetone	Petrolium ether extract
		Salmonella paratyphii		6		All extracts	
		Listeria monocytogens		12		Isopropanol extract	
2	*Z. officinale*	*E. coli*	*A. niger*	-	16	All extracts	All extracts
		S. paratyphii		8		Isopropanol extract	
		L. monocytogens		8		All extracts	
3	*Z. zerumbet*	*E. coli*	-	-	-	All extracts	-
		S. paratyphii		10		All extracts	
		L. monocytogens		12		All extracts	
4	*Curcuma amada*	*E. coli*	*A. niger*	4	20	Isopropanol extract	All extracts
		S. paratyphii		4		All extracts	
		L. monocytogens		8		All extracts	

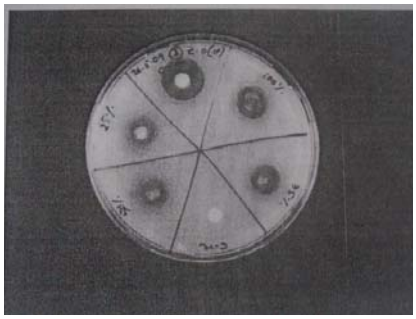

Figure 1: Plate seeded with *Salmonella paratyphi* showing zone of inhibition by different concentration of *Z. officinale* (Isopropanol extract)

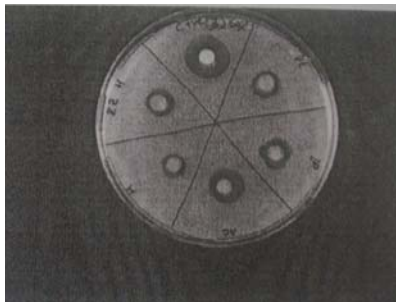

Figure 2: Plate seeded with *Salmonella paratyphi* showing zone of inhibition by 50% conc. of *Z. zerumbet* (all extracts).

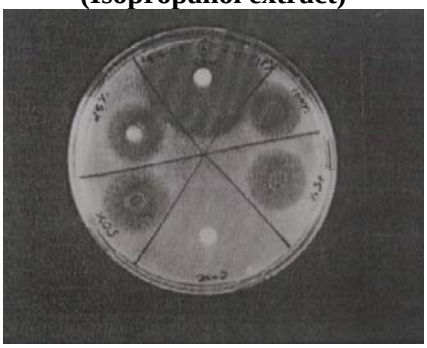

Figure 3: Plate seeded *with Listeria monocytogenes* showing zone of inhibition by different conc. of *Z. moran* (Isopropanol extract).

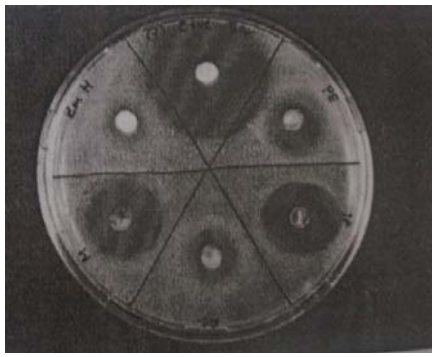

Figure 4: Plate seeded with *E. coli* ETEC enterotoxic showing zone of inhibition by 50% conc. of *Z. moran* (All extract).

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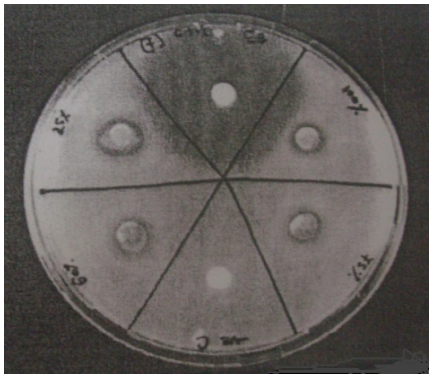

Figure 5: Plate seeded with E. coli enterotoxic showing zone of inhibition by different conc. of *C. amada* (Isopropanol extract).

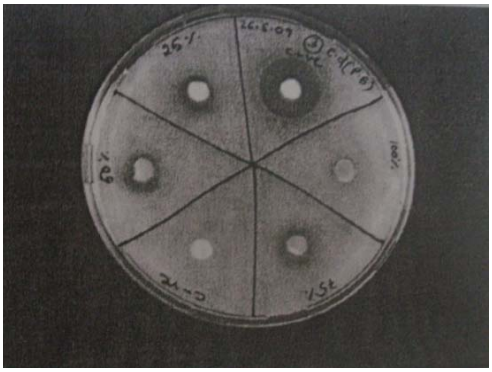

Figure 6: Plate seeded with *Aspergillus niger* showing zone of inhibition by different conc. of *Z. moran* (Petrolium ether extract)

Plate 1: Photo plate showing zone of inhibition by different concentrations of Zingiberaceae oil on test strains of bacteria and fungus.

DISCUSSION

Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated (Uniyal *et al.* 2006). Zingiberaceae species grow naturally in damp, shaded parts of the low-land or on hill slopes, as scattered plants or thickets. Most members of the family are easily recognized by the characteristic aromatic leaves and fleshy rhizome when both of them are crushed and also by the elliptic to elliptic-oblong leaves arranged in two ranks on the leaf-shoot. In Assam, members of Zingiberaceae grow luxuriantly due to very conducive climate. This study is a preliminary evaluation of antimicrobial activity of the selective species of Zingiberaceae of Northeast India. As it is an indigenous family found in the region, unrevealing its hidden potential would be a great achievement in cure of many harmful diseases. All the species tested here showed marked antimicrobial efficacy against the test organism. Through this work, it has been concluded that *Zingiber officinale* var *moran* has the maximum antimicrobial effect, among those studied here. The species can be utilized for further study to discover new class of antibiotics of plant origin that can be affective against some pathogenic strain of bacteria and fungi for maintenance of plant, animal and human health and provide biochemical tools for the study of infectious diseases.

CONCLUSION

The enhanced use of antibiotics against the microorganisms has attributed to the development of resistant strain and as a result the search for new and effective drugs has become the need of the hour. In this connection plant continue to be a rich source of therapeutic drugs. Further study of the major components of the studied species is to be done so that the active principles can be isolated and purified and undergo further testing in the way of drug designing.

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