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Antimicrobial activity of *Elaeocarpus ganitrus* Roxb (Elaeocarpaceae): An *in vitro* study

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ABSTRACT

Elaeocarpus ganitrus Roxb. (Elaeocarpaceae) is a large evergreen broad-leaved tree and found in Himalayan range in India and Nepal. Fruits and leaves are known for various medicinal properties and used in traditional medication system for the treatment of diseases. In this study, antimicrobial activity of the aqueous extract of leaves of *E. ganitrus* was tested against clinical isolates of bacteria and fungi. *In vitro* antimicrobial activity was performed by agar well diffusion method on Mueller Hinton agar and Sabouraud Dextrose agar for bacterial and fungal cultures respectively. The extract exhibited a broad spectrum of antimicrobial activity as it inhibited the growth of *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Penicillium* sp, *Aspergillus flavus*, *Candida albicans* and *C. tropicalis*. The extract showed maximum relative percentage inhibition against *B. cereus* (124.16%). Minimum inhibitory concentration test was performed by modified agar well diffusion method. Minimum inhibitory concentration values of the extract varied from 125-2000 µg/ml; however minimum value was reported against *B. cereus* and *A. flavus* (125 µg/ml). The results indicate the potential use of *E. ganitrus* leaves for the development of antimicrobial compounds.

Introduction

Microbial infections are the leading cause of health hazards and death around the globe. According to World Health Organization (WHO), microbial infections collectively resulted in 25% of death worldwide (WHO, 1999). In last few decades, reports of microbial drug resistance has been documented all around the world (Suller and Russell, 2000; Poole, 2005; Raghunath, 2008). Limited drug options for drug resistant organisms make them highly lethal and the severity is much higher in immunocompromised individuals, especially in patients suffering with Acquired Immuno Deficiency Syndrome (AIDS). A variety of antimicrobial compounds are known and being used to control microbial infection. However, upto certain extents it failed to control the infectious diseases especially in case of drug resistant pathogens. To counter this problem there is a continuous need of developing newer, safer and more potential antimicrobial drugs. Most of the synthetic antimicrobial drugs are potentially toxic and possess many side effects on the host body; therefore, there is a growing interest in the pharmacological evaluation of various plants used in different traditional system of medicine. As of natural origin, plants based antimicrobial compounds are less/non toxic, cheaper and ecofriendly.

Herbal medicines derived from plants are being utilised to cure variety of health problems since thousands of years. These plants are used in traditional Chinese, Ayurveda, Siddha, Unani and Tibetan medicines. Many medicines that dominate pharmaceutical market in the present days are the key from the ancient medicinal plant. Ancient literature such as Rigveda, Yajurveda, Atharvaveda, Charak Samhita and Sushrut Samhita also describes the use of plants gives the knowledge to prepare/manipulate the present drugs. Recently, many plants have been systematically studied for their medicinal values and reported for numerous pharmacological properties viz, anticancer activity (Rajkumar et al., 2009), antibacterial activity (Kumar et al., 2010a), anthelmintic activity (Adama et al., 2009), antifungal activity (Kumar et al., 2010b), antidiabetic activity (Pérez et al., 2000), antioxidant activity (Priya et al., 2010), hepatoprotective activity (Nevin and Vijayammal, 2005), haemolytic activity (Kalita et al., 2011), larvicidal activity (Pitasawat et al., 1998) and anti-inflammatory activity (Saha and Ahmed, 2009) etc.

Elaeocarpus ganitrus Roxb. is a large evergreen broad-leaved tree belonging to Elaeocarpaceae family. It is also known as *E. sphaericus*. *E. ganitrus* is a large and evergreen tree commonly known as Utrasum Bead tree. Leaves and seeds are known for various medicinal properties and traditionally used to cure stress, anxiety, depression, palpitation, nerve pain, epilepsy, migraine, lack of concentration, asthma, hypertension, arthritis and liver diseases (Dasgupta et al., 1984).

Scientific advancement brought a positive approach for the systemic exploration of *E. sphaericus* for its medicinal properties and in last two decades *E. sphaericus* has been reported to exhibit anti-inflammatory (Singh and Pandey, 1999), antimicrobial (Singh et al., 2010), antihypertensive effect (Sakat et al., 2009), anxiolytic effects (Shah et al., 2010) and anticonvulsant activity (Dasgupta et al., 1984). However, most of these studies were carried out on the seeds and very few efforts have been made on the leaves. The focus of this study was to determine the antimicrobial activity of the aqueous extract of *E. ganitrus* against a variety of pathogenic bacteria and fungi.

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Material and Methods

Chemicals and reagents

Nutrient agar, Mueller Hinton broth (MHB), Mueller Hinton agar (MHA), Potato Dextrose broth (PDB), Potato Dextrose agar (PDA), Amoxycillin disc, Penicillin G disc, Polymyxin-B disc and Fluconazole disc were purchased from Himedia Pvt Ltd, Mumbai, India.

Plant material

Mature and healthy leaves of *E. ganitrus* were collected from the Guwahati, Assam (26°11′ N 91°44′E) during January 2009. Plant was identified by Professor Hemen Chandra Majumdar, Assistant Professor, Department of Botany, B. Borooah College, Guwahati, Assam, India. The plant sample was brought to the Molecular and Microbiology Research Laboratory, VIT University, Vellore, TN, India. Voucher specimen was maintained in our laboratory (Accession number: EG/VIT/MMRL/13.01.2009-2).

Processing of the plant

Mature and healthy leaves of *E. ganitrus* were collected and washed properly with tap water followed by distilled water. The leaves were shade dried at room temperature. Dried leaves were uniformly grounded using mechanical grinder. The leaves powder was extracted in distilled water. Ten gram of plant powder was extracted in distilled water using a soxhlet extractor. The extract was concentrated using rotary evaporator and dried using lyophilizer. Dried extract was collect in air tight container and stored at 4°C.

The extracted powder was dissolved in sterilized distilled water as 10 mg/ml solution. This mixture was use to perform antimicrobial assay.

Test microorganism

The following clinical isolates of bacteria and fungi were used for the study: *Staphylococcus aureus*, *Bacillus cereus*, *Micrococcus luteus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Penicillium* sp., *Aspergillus niger*, *A. flavus*, *Candida albicans* and *C. tropicalis*. Microbial cultures were grown on nutrient agar and potato dextrose agar for bacteria and fungi respectively and maintained at 4°C in refrigerator.

Positive and negative control

Amoxycillin (10 µg/disc) was used as positive control for *B. cereus*; Erythromycin (10 µg/disc) for *M. luteus* and *E. coli*; Penicillin G disc (10 U/disc) for *S. aureus*; Polymyxin-B (300 U/disc) for *P. aeruginosa* and Chlorampinacol (30 µg/disc) for *K. pneumoniae*. Fluconazole disc (10µg/disc) was used for the fungal cultures. Sterilized distilled water was used as negative control.

Antibacterial assay

Antimicrobial activity of the crude extracts was determined by the agar well diffusion method (Kumar et al., 2010c). All test organisms were inoculated on MHB for 8 hours. Isolates were seeded on MHA plates by using sterilize cotton swabs. Agar surface was bored by using sterilized gel borer to make wells (7 mm diameter). 100 µl of the test extract and 100 µl of sterilized distilled water (negative control) were poured in to separate wells. The standard antibiotic disc was placed on the agar surface as positive control. Plates were incubated at 37°C for 48 hours. Experiment was performed in triplicates.

Antifungal assay

Antifungal activity of the crude extracts was determined by the agar well diffusion method (Kumar et al., 2010b). All test organisms were inoculated in PDB for 12 hours. Isolates were seeded on PDA plates by using sterilize cotton swabs. Agar surface was bored by using sterilized gel borer to make wells (7 mm diameter). 100 µl of the test extract and 100 µl of sterilized distilled water (negative control) were poured in to separate wells. The standard antibiotic disc was placed on the agar surface as positive control. Plates were incubated at 28°C for 72 hours. Experiment was performed in triplicates.

Determination of relative percentage inhibition

The relative percentage inhibition of the test extract with respect to positive control was calculated by using the following formula (Ajay et al., 2002)

$$\text{Relative percentage inhibition of the test extract} = \frac{100 \times (x-y)}{(z-y)}$$

Where,

x: total area of inhibition of the test extract

y: total area of inhibition of the solvent

z: total area of inhibition of the standard drug

The total area of the inhibition was calculated by using area = πr^2; where, r = radius of zone of inhibition.

Determination of minimum inhibitory concentration (MIC)

MIC of the plant extract was performed by modified agar well diffusion method. Two fold serial dilution of the stock solution was prepared in sterilized distilled water to make a concentration range from 0.01-10 mg/ml.

Test cultures were inoculated in MHB and PDB for bacteria and fungi respectively. Microbial suspensions were seeded on MHA and PDA for bacteria and fungi respectively plates using a sterilized cotton swab. In each of these plates four wells were cut out using a standard cork borer (7 mm). Using a micropipette, 100 µl of each dilution was added in to wells. Bacterial plates were incubated at 37ºC for 24 hours and fungal plates were incubated at 28°C for 72 hours. The minimum concentration of each extract showing a clear zone of inhibition was considered to be MIC (Rios et al., 1988; Okunji et al., 1990).

Statistical analysis

The values of antimicrobial activity of the aqueous leaves extract of *E. ganitrus* are expressed as mean ± standard deviation of the response of 3 replicates determinations per sample. Results were analyzed statically by using Microsoft Excel 2007 (Roselle, IL, USA).

Results and Discussion

Pathogenic microorganisms are one of the major causes of health problems in humans and animals and their contagious nature make it difficult to control. In pre antibiotic era, microbial infections were the major cause of untimely death in humans. Soon after the discovery of antibiotics, death rate of microbial infection has significantly decreased, even though, drug resistant microorganisms remain a major thereat for human beings. Therefore, newer antimicrobial compounds with low/no side effects are desirable for pharmaceutical applications. Higher trees synthesize a variety of phytochemicals compounds as secondary metabolites to protect themselves from the microbial infections and environmental stress conditions. These phytochemicals are the key compounds with many medicinal properties and can be exploited for the development of new pharmaceutical molecule.

In this study, aqueous extract of *E. ganitrus* leaves was screened for antibacterial activity against three Gram positive (*S. aureus*, *B. cereus* and *M. luteus*) and three Gram negative bacteria (*E. coli*, *P. aeruginosa* and *K. pneumoniae*), isolated from the clinical samples. The extract exhibited excellent antibacterial activity against all the bacterial cultures, except *M.*

luteus (Tables 1). Antifungal activity of the extract was evaluated against three molds (*Penicillium* sp, *A. niger* and *A. flavus*) and two yeast (*C. albicans* and *C. tropicalis*), isolated from clinical samples. Extract exhibited antifungal activity against all the fungal cultures except *A. niger* (Tables 2). However, the antimicrobial activity was lower than that of standard drugs. Results of antimicrobial activity are expressed as zone of inhibition and presented as mean ± standard deviation of the three replicates.

Antimicrobial activity of *E. ganitrus* leaves extract was compared with the antimicrobial activity of standard drugs for evaluating relative percentage inhibition (Table 3). The aqueous extract of *E. ganitrus* leaves exhibited maximum relative percentage inhibition against *B. cereus* (124.16 %) and *Penicillium* sp. (88.26%) for bacteria and fungi respectively. MIC values of the aqueous extract of *E. ganitrus* leaves against bacterial and fungal strains were range between 125-1000 µg/ml. Result of MIC are reported in Table 3.

Earlier, Petroleum ether (PE), benzene (BE), chloroform (CE), acetone (AE) and ethanol (EE) extracts of dried *E. sphaericus* fruit were reported to possess a broad spectrum of antimicrobial activity against a variety of Gram positive and Gram negative bacteria (Singh and Nath, 1999). Different organic solvent extracts of beads of *E. ganitrus* were screened for antifungal activity against five different fungal species. Chloroform and ethanol extracts inhibited *C. albicans* and *A. niger*, whereas water extract inhibited only *A. niger* (Singh, 2010).

We conclude that *E. ganitrus* is a less explored source of potentially useful antimicrobial compounds and it is worth for future clinical use. Further, the active principle can be isolated and the mechanism of antimicrobial activity can be studied using advance scientific techniques. In addition, urgent measures have to be taken to preserve the traditional knowledge about medicinal plants.

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Table 1: Antibacterial activity of aqueous extract of *Elaeocarpus ganitrus*

Test organisms	Zone of inhibition (mm)		
	E. ganitrus	PC	NC
Staphylococcus aureus	11.0±1.73	18.3±1.15	0±0
Bacillus cereus	15.6±1.15	14.0±1.0	0±0
Escherichia coli	12.3±1.52	34.3±1.52	0±0
Micrococcus luteus	0±0	34.6±0.57	0±0
Pseudomonas aeruginosa	12.0±1.0	15.3±0.57	0±0
Klebsiella pneumoniae	10.6±1.52	17.6±0.57	0±0

Here, PC: positive control, NC: negative control
Values are expressed as mean ± standard deviation of the three replicates,
Zone of inhibition not include the diameter of the well.

Table 2: Antifungal activity of aqueous extract of *Elaeocarpus ganitrus*

Test organisms	Zone of inhibition (mm)		
	E. ganitrus	PC	NC
Candida albicans	5.3±3.21	14.66±1.52	0±0
Candida tropicalis	17.6±0.57	23.6±1.52	0±0
Penicillium sp.	23.3±1.52	24.8±1.52	0±0
Aspergillus niger	0±0	17.3±0.57	0±0
Aspergillus flavus	15.3±1.15	19.5±57	0±0

Here, PC: positive control, NC: negative control
Values are expressed as mean ± standard deviation of the three replicates,
Zone of inhibition not include the diameter of the well.

Table 3: Relative percentage inhibition and Minimum inhibitory concentration of aqueous extract of *Elaeocarpus ganitrus*

Test organisms	Aqueous extract of *E. ganitrus*	
	RPI (%)	MIC (µg/ml)
Staphylococcus aureus	36.13	2000
Bacillus cereus	124.16	125
Escherichia coli	12.85	500
Micrococcus luteus	-	-
Pseudomonas aeruginosa	61.27	500
Klebsiella pneumoniae	36.27	250
Candida albicans	13.07	1000
Candida tropicalis	55.61	500
Penicillium sp.	88.26	125
Aspergillus niger	-	-
Aspergillus flavus	78.21	125

RPI: Relative percentage inhibition, MIC: Minimum inhibitory concentration