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

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**ARTICLE INFO**

**Article History:**
Received: 22 September 2011; 15 October 2011; Accepted: 9 November 2011;

**Keywords**

**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.

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**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 (Priya et al., 2010), hepatoprotective activity (Nevin and Vijayammal, 2005), anti-inflammatory 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 antiandrogenic 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.
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 extract powder was dissolved in distilled sterilized 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*, *Penicillum 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. 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 bored 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 24 hours. Experiment was performed in triplicates.

Determinant 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)

\[
\text{Relative percentage inhibition of the test extract} = \left( \frac{100 \times (x-y)}{y} \right)
\]

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 \( \pi r^2 \) 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 37°C 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 threat 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.*
Fatidial 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.
Extraction 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 
(CF), 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.

Acknowledgement
The authors wish to thank the Management and Staff of VIT 
University, Vellore, TN, India for providing necessary facilities 
to carry out this study. Authors are thankful to Professor Hemen 
Chandra Majumdar, Assistant Professor, Department of Botany, 
B. Borooah College, Guwahati, Assam, India for the 
identification of the plant.

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

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Zone of inhibition (mm)</th>
<th>E. ganitrus</th>
<th>PC</th>
<th>NC</th>
<th>PC</th>
<th>NC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>11.0±1.73 18.3±1.15 0±0</td>
<td>11.0±1.73 18.3±1.15 0±0</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>15.6±1.15 14.0±1.0 0±0</td>
<td>15.6±1.15 14.0±1.0 0±0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>12.3±1.52 34.3±1.32 0±0</td>
<td>12.3±1.52 34.3±1.32 0±0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Micrococcus luteus</em></td>
<td>0±0 34.6±0.57 0±0</td>
<td>0±0 34.6±0.57 0±0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>12.0±1.0 15.3±0.57 0±0</td>
<td>12.0±1.0 15.3±0.57 0±0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>10.6±1.52 17.6±0.57 0±0</td>
<td>10.6±1.52 17.6±0.57 0±0</td>
<td></td>
<td></td>
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</tbody>
</table>

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*

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Zone of inhibition (mm)</th>
<th>E. ganitrus</th>
<th>PC</th>
<th>NC</th>
<th>PC</th>
<th>NC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>5.3±3.21 14.6±1.52 0±0</td>
<td>5.3±3.21 14.6±1.52 0±0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
<td>17.6±0.57 23.6±1.52 0±0</td>
<td>17.6±0.57 23.6±1.52 0±0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Penicillium sp.</em></td>
<td>23.3±1.52 24.8±1.52 0±0</td>
<td>23.3±1.52 24.8±1.52 0±0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>0±0 17.3±0.57 0±0</td>
<td>0±0 17.3±0.57 0±0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>15.3±1.15 19.5±0.57 0±0</td>
<td>15.3±1.15 19.5±0.57 0±0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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*

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Aqueous extract of E. ganitrus</th>
<th>RPI (%)</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus cereus</em></td>
<td>124.16 125</td>
<td>124.16 125</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>12.85 500</td>
<td>12.85 500</td>
<td></td>
</tr>
<tr>
<td><em>Micrococcus luteus</em></td>
<td>- -</td>
<td>- -</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>61.27 500</td>
<td>61.27 500</td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>36.27 250</td>
<td>36.27 250</td>
<td></td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>13.07 1000</td>
<td>13.07 1000</td>
<td></td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
<td>55.61 500</td>
<td>55.61 500</td>
<td></td>
</tr>
<tr>
<td><em>Penicillium sp.</em></td>
<td>88.26 125</td>
<td>88.26 125</td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>- -</td>
<td>- -</td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>78.21 125</td>
<td>78.21 125</td>
<td></td>
</tr>
</tbody>
</table>

RPI: Relative percentage inhibition, MIC: Minimum inhibitory concentration