In Vitro Antimicrobial Activity Analysis Of Lemon Grass Leaf Extracts Against Pathogenic Bacteria

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Abstract

Lemongrass is used to treat fever, fatigue, convulsions, common cold, rheumatism, vomiting, convulsions, discomfort, and digestive system spasms. The present investigation was performed to investigate the antibacterial property of methanolic, acetone and benzene extract of lemon grass against pathogenic bacteria. The maximum inhibition zone for methanol extract was Pseudomonas aeruginosa and Staphylococcus aureus (18 mm) at a concentration of 100 μl/ml. Benzene extract also exhibited a 17 mm zone at 100 μl/ml against bacteria- Pseudomonas aeruginosa. Behind it, 50 μl/ml of methanol extract exhibited 16 mm of Pseudomonas aeruginosa and 100 μl/ml of benzene extract against Klebsella pneumoneae. As concentration increases against all bacteria, the anti-bacterial activity of extracts increases. Among all the extracts methanol extract have been found more potential extract for antibacterial as well as lowest MIC. So lemon grass can be used to treat many bacterial diseases.

Keywords: Antimicrobial potency, Lemon grass, Minimum Inhibitory Concentration, Pseudomonas aeruginosa, Staphylococcus aureus.

Introduction

Lemon grass (Cymbopogon citratus) is a tall shrub with broad, unevenly striped leaves. It has a distinctive aroma that is smokey, sweet, herbaceous, and lemony. The herb is frequently used to make soups, curries, and drinks. This plant has a relaxing quality. It is tall sedge native to the area, with a delicious scent. It belongs to the Poaceae family. It grows in many tropical and subtropical regions of South East Asia, Africa, and other continents. Cymbopogon citratus is indigenous to Sri Lanka, Pakistan, and India. Other than the foothills of Sikkim and Arunachal Pradesh, the Western Ghats (Kerala, Maharashtra), Karnataka, and Tamil Nadu states are rich in this vegetation in India (Tzortzakis and Economakis, 2007; Manzoor et al., 2013). In traditional medicine, Cymbopogon citratus is typically used to treat gastrointestinal disorders and has analgesic, antispasmodic, antipyretic, anti-inflammatory, sedative, and diuretic properties (Santin et al., 2009). Cymbopogon citratus leaf extracts are shown to have antibacterial, antifungal, and antioxidant properties (Hanaa et al., 2012). The essential oil content of its leaf is high. It contains -oxobisabolene, mycrene, genariol, citronellol (a blend of cymbopogonol and cymbopogone), and citral (a mixture of terpenoids and geranial). Depending on the type of plant and the region, they can contain anything from 12 to 15% lemongrass in the West Indies to 10 to 13 percent in the East. Citral is crucial for the development of a plant's taste (Piarua et al., 2012; Costa et al., 2013; Ranitha et al., 2014). Onawunmi and Ogunlana, 1986; Syed and Khalid, 1990; Tiziana et al., 1998; Hammer et al., 1999; Shigeharu et al., 2001; Cimango et al., 2002; Nguefack et al., 2004; Pereira et al., 2004; Naik et al., 2010 had noted that lemongrass oil exhibited antibacterial properties against a wide variety of species, including
gram positive and gram negative bacteria, yeast, and fungi. The present investigation was performed to investigate the antibacterial property of lemon grass extract against pathogenic bacteria.

Materials and Methods
The plant material was collected from local area of Shahjahanpur district and has been identified by Department of Botany, G. F. College, Shahjahanpur and the reference samples were deposited in the department for future references.

Preparation of extracts
The extracts were prepared from the samples by using different solvent systems via Soxhlet apparatus. The surface area for solvent extraction was improved by finely grinding the material for better extraction. We put 20 g of finely ground plant material in a round-bottom flask with a capacity of 200 ml. The flask was filled with 200 ml of solvent (between 40 and 60°C). Material was filtered once the procedure was finished. The solvent was then extracted from the filtrate by distillation at low pressure. Finally, the extractive solvent was eliminated in an air current at ambient temperature. The samples of essential oils that were subsequently extracted using various techniques from various plant sections and the entire plant of the two distinct species were kept in the refrigerator in glass vials with screw-top lids and labelling for later analysis. The stock of the extract was prepared in 5% DMSO (Dimethyl sulfoxide) in 50 mg/ml concentration and working solution prepared according to the test.

Anti-bacterial assay
The anti-bacterial sensitivity of all samples against all test bacteria was carried out by agar well diffusion method. The test sample of extracts for the antibacterial activity, stock was prepared by dissolving 50 mg extracts in 1 ml 5% DMSO. The test was performed by diluting stock extract to the test concentration i.e. 5, 10 and 20 mg/ml. Therefore, according test concentration the dose was determined as 0.25, 0.5 and 1.0 mg/well. During the test analysis 5% DMSO was used as a negative control however Amoxycillin (0.1 mg/well) was used as positive control. Freshly prepared microbial broth cultures were standardized according to the Mcfarland 0.5 standard solution (1x10^8 cfu/ml) and about 0.1 ml culture broth was spread over the Nutrient agar media using L-shaped sterilized spreader separately. The wells were made with the help of cork borer of 8 mm diameter. In these wells, about 30 μl of each extract was loaded individually. After loading the extracts test plates were further incubated at 37ºC for 24 hours. For the negative and positive controls 30 μl DMSO and Amoxycillin was loaded in the wells, respectively. After incubation, clear zone of inhibition was measured.

Estimation of minimum inhibitory concentration
Potential plant extracts showed effective anti-bacterial inhibition by well diffusion method was further tested for Minimum inhibitory concentration (MIC assay) determination. To perform this test crude extracts and standard Amoxycillin was dissolved in 5% DMSO to prepare a stock of 10 mg/ml concentration. Final working concentration was maintained from 0.1 to 5 mg/ml concentration. From each concentration 100μl of test sample was loaded into the microtiter (96 well) plate, the experiment was performed for each bacterium separately. After adding the samples, 100 μl of standard inoculum was loaded. The test was performed in triplicate and plates were incubated at 37ºC for 24 hours. After incubation 50 μl p-iodonitrotetrazoleum chloride (INT dye 2mg/ml) was added to the wells and again incubated at 37ºC for 30 minutes. The development of reddish or pink colour indicated the growth of bacteria whereas clear or transparent colour showed the inhibition potential of extracts. The lowest value of test sample at which no colour development occurred noted down as MIC value.

Results and Discussion
In present study anti-bacterial activity of Lemon grass was tested. The antimicrobial activity of spices derived from acetone, methanol and benzene solvents against Staphylococcus aureus, Klebsella pneumoneae, Escherichia coli and Pseudomonas aeruginosa was shown in Table-1. The maximum inhibition zone for methanol extract was Pseudomonas aeruginosa and Staphylococcus aureus (18 mm) at a concentration of 100 μl/ml. Benzene extract also
exhibited a 17 mm zone at 100 µl/ml against bacteria - Pseudomonas aeruginosa. Behind it, 50 µl/ml of methanol extract exhibited 16 mm of Pseudomonas aeruginosa and 100 µl/ml of benzene extract against Klebsella pneumoniae. As concentration increases against all bacteria, the anti-bacterial activity of extracts increases. Less activity against Escherichia coli bacteria was seen in all extracts All three extracts were also evaluated for the determination of Minimum Inhibitory Concentration ion (MIC). On the basis of observed results shown in the table 2, it is observed that methanol extract showed inhibitory properties at lowest dose that is 1.3 mg/ml against Pseudomonas aeruginosa bacteria followed by methanol extract at 1.6 mg/ml against Staphylococcus aureus. Among all the extracts methanol extract have been found more potential extract for antibacterial as well as lowest MIC. Onawunmi et al., 1986; Torris et al., 2002; Pereira et al., 2004; Marta War et al., 2004; Naik et al., 2010 and Ewansiha et al., 2012, investigated Lemon grass oil for activity against Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa, using Agar Well Diffusion Method and Broth Dilution Method and results showed that Lemon grass was effective against all the test organisms except P. aeruginosa. Gram positive organisms were found more sensitive to lemon grass oil as compared to gram negative organisms. Lemongrass essential oil and its bioactive component citral were previously demonstrated to possess strong antimicrobial efficacy against pathogenic bacteria and fungi. So lemon grass can be used to treat many bacterial diseases.

Table 1: Zone of inhibition of Lemon grass extract against pathogenic bacteria

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Benzene (µl/ml)</th>
<th>Acetone (µl/ml)</th>
<th>Methanol (µl/ml)</th>
<th>Negative Control</th>
<th>Amoxycillin (Positive control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
<td>50</td>
<td>100</td>
<td>25</td>
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<tr>
<td>Staphylococcus aureus</td>
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</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>12</td>
<td>15</td>
<td></td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsella pneumoniae</td>
<td></td>
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</tr>
</tbody>
</table>

Table 2: Minimum inhibitory concentration (MIC) of Lemon grass extracts against pathogenic bacteria in mg/ml

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Pathogenic Bacteria</th>
<th>Benzene</th>
<th>Methanol</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Staphylococcus aureus</td>
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<td>1.6</td>
<td>2.1</td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas aeruginosa</td>
<td>2.1</td>
<td>1.3</td>
<td>2.2</td>
</tr>
<tr>
<td>3</td>
<td>Escherichia coli</td>
<td>2.3</td>
<td>2.0</td>
<td>3.1</td>
</tr>
<tr>
<td>4</td>
<td>Klebsella pneumoniae</td>
<td>2.6</td>
<td>1.8</td>
<td>2.9</td>
</tr>
</tbody>
</table>

References