ANTIBACTERIAL ACTIVITY OF LEMONGRASS (CYMBOPOGON FLEXUOSUS) OIL AGAINST SOME SELECTED PATHOGENIC BACTERIAS.

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ABSTRACT

In this study the lemon grass oil was extracted from the plant sources Cymbopogon flexuosus antibacterial activity. Results of this study showed that most antibacterial effect of lemon grass and essence was against Gram positive bacteria. According to the considerable antibacterial effect of methanol extract of lemon grass leaves on pathogenic bacteria especially Gram positive bacteria that are involved in creating variety of nonsocial and malicious infections this extract can be considered as a natural antibacterial herbal product. Finally it is concluded that the mixture of lemon grass oil is used to treat bacterial infections. It is concluded that, in the present study, the plant contains potential antibacterial components that may be useful for evolution of pharmaceutical for the therapy of ailments. The antimicrobial activity was determined by well diffusion method. The activity was determined for different concentration of lemon grass oil against Escherichia coli, Proteus vulgaris, Bacillus subtilis and Staphylococcus aureus. Both Staphylococcus aureus and Bacillus subtalis had the highest microbial sensitivity and this inhibitory effect increased with increasing methanol extract concentration. Also results obtained showed that growth inhibitory effect of methanol extract of lemon grass oil on tested Gram negative bacteria was very low, so that no growth inhibition effect on the Proteus vulgaris and detected slight inhibitory effect against Escherichia coli at the highest extract concentration used (120 mg/ml concentration). These results indicated that among tested bacteria in term of sensitivity to methanolic dilution of lemon grass oil there was a significant difference.
KEYWORDS: Antibacterial activity, lemongrass oil, Gram positive and Gram negative bacteria.

INTRODUCTION

All over the world there is increasing tendency towards consuming natural products and thus living a natural life. *Cymbopogon* (lemongrass) is a genus of about 55 species of grasses, native to warm temperate and tropical regions of the Old World and Oceania. It is a tall perennial grass. Common names include lemon grass, lemongrass, barbed wire grass, silky heads, citronella grass, cha de Dartigalongue, fever grass, tanglad, hierba Luisa or gavati chaha amongst many others. Lemongrass is native to India and tropical Asia. It is widely used as a herb in Asian cuisine. It has a subtle citrus flavour and can be dried and powdered, or used fresh. Lemongrass is commonly used in teas, soups, and curries. The use of lemongrass was found in folk remedy for coughs, consumption, elephantiasis, malaria, ophthalmia, pneumonia and vascular disorders. Researchers have found that lemongrass holds antidepressant, antioxidant, antiseptic, astringent, bactericidal, fungicidal, nervine and sedative properties (McGuffin et al., 1997).

Lemongrass oil is used as a pesticide and a preservative. Research shows that lemongrass oil has fungal properties (Shadab et al., 1992). Three types of lemon grasses viz. East Indian lemongrass (*C. flexuosus*), West Indian lemon grass (*C. citrates*), and Jammu lemongrass (*C. pendulus*) are in cultivation in our country as the important sources of citrates. *C. flexuosus* grown commercially in Kerala and nearby adjacent state, its oil is popularly known as "Cochin oil" as it is shipped mainly through Cochin port. The chief constituent of the oil is the citral. It is the starting material for the preparation of important ionone viz. A - Ionone, used in flavours, cosmetics and perfume and P - Ionone- use in the manufacture of synthetic vitamin A. The plants are still a potential source of medical compounds. In the world plants traditionally are used oral health and to treat many diseases especially infectious including diarrhea, fever and cold (Mitscher et al. 1981) addition, many recreational compounds used in traditional medicine have plant root (Deans and Suboda, 1990). According to World Health Organization (WHO) definition a medicinal plant, is a plant that can be used for therapeutic purposes and or its compounds be used as a synthesis of semi-synthetic chemical drugs (World Health organization, 1979).

The aim of this study was to assess the antibacterial activity of lemon grass oil. The emergence of multiple antibiotic resistant bacteria has become a major challenge in the
treatment of infectious diseases. The use of plant extracts and phytochemicals both with known and unknown antimicrobial properties can be of great significance in therapeutic treatments. In the present study the extracts and oil of *Cymbopogan flexuosus*, an aromatic perennial grass is evaluated for its antibacterial activity. The extracts, oil collected were then screened for its antibacterial activity against various gram positive and gram negative by disc diffusion technique. The crude extracts, extracted oil from *C. flexuosus* was found to have significant broad spectrum antibacterial activity against all the tested microbes. The aim of this study was to investigate the antibacterial activity of volatile oils obtained from *Cymbopogon flexuosus*.

**MATERIALS AND METHODS**

The study was conducted under the objective *i.e. extraction of lemongrass oil & determination of its anti-microbial activity*. The *Cymbopogan flexuosus* plant collected from CAP (Centre for Aromatic Plants), Dehradun were used as material. The work was performed in plant tissue culture lab of Biotechnology Department of Dolphin (PG) Institute Manduwala, Dehradun.

**Extraction of lemongrass oil**

The Leaves of lemongrass were chopped into small 10cm long pieces, weighed and transferred into a 2 litre to which was added 200ml of demonized water, and distilled for 3½hrs using Clevenger Apparatus (AOAC, 1990). The total distillation time was approximately 4½ hrs (including about 1hr for oil to start distilling). Since the essential oil of lemongrass is less dense than water, the oil floated on top of the water.

**Preparation of Stock Solutions**

The stock solutions were made by dissolving 1mg/l of lemon grass oil in 1ml of methanol. From the stock solutions serial dilutions were made to obtain the test solution of different concentration of 15µl/1ml, 30µl/1ml, 60µl/1ml and 120µl/ml respectively.

**Antibacterial Assay**

Antibacterial activity was done by the disc diffusion method, which is normally used as a preliminary check and to select between efficient concentrations of the extract. It was performed using an 18 hr culture at 37°C. The plant extract disc was prepared from Whattman filter paper by punching with a cork borer of 6mm diameter. The disc was autoclaved at 121°C for 15mins. The plant extract disc was dried in an oven and stored in refrigerator until
required for use. The test organism \textit{i.e.} two gram-negative bacteria \textit{Escherichia coli} & \textit{Proteus vulgaris} and two gram positive bacteria \textit{Bacillus subtilis} and \textit{Staphylococcus aureus} collected from department of microbiology belongs to same institute, were cultured on Nutrient Agar plates prepared by dissolving 28g of Nutrient Agar in one liter of water. The media was autoclaved at 121°C for 15mins. 9ml of this media was poured in plates and left to solidify. The plant extract discs were placed in the triplicate cultured plates (disc-diffusion method) using a sterile forceps. The discs were placed far from each other to avoid overlap of zone of inhibition. The culture was incubated in an incubator for 24hrs at 37°C. After 24hrs, the zone of inhibition of plant extracts was observed and measured in millimeter. Antibacterial activity was recorded if the zone of inhibition was greater than 6mm, sterile water disc was used as negative control and antibiotic disc used as positive control. For each microbial species, negative control was maintained where 100μl of methanol and distilled water without the drug was used for methanol extract and aqueous extract. Also, conventional drugs were used for positive controls. The results were recorded by measuring the diameter of the zones of growth inhibition surrounding the wells (cylinders).

\textbf{RESULT AND DISCUSSION}

In the recent years much research has been conducted field of antimicrobial effects of different plants. The antimicrobial activity was determined by well diffusion method. The activity was determined for different concentration of lemon gas oil against \textit{Escherichia coli}, \textit{Proteus vulgaris}, \textit{Bacillus subtilis} and \textit{Staphylococcus aureus}.

The results of the antimicrobial activity of lemon grass Oil methanolic dilutions by well diffusion method were shown in Table 1. Both \textit{Staphylococcus aureus} and \textit{Bacillus subtilis} had the highest microbial sensitivity and this inhibitory effect increased with increasing methanol extract concentration. Also results obtained showed that growth inhibitory effect of methanol extract of lemon grass oil on tested Gram negative bacteria was very low, so that no growth inhibition effect on the \textit{Proteus vulgaris} and detected slight inhibitory effect against \textit{Escherichia coli} at the highest extract concentration used (120 mg/ml concentration). The results in Table 1 show that 15 mg/ml concentration of methanolic extract of lemon grass oil had a bactericidal effect on \textit{Staphylococcus aureus}. Lethal concentration of this oil against \textit{Bacillus subtilis} obtained at 120 mg/ml. These results indicated that among tested bacteria in term of sensitivity to methanolic dilution of lemon grass oil there was a significant difference. In the other words \textit{Bacillus subtilis} showed the highest sensitivity to methanolic dilution of
lemon grass oil and the least sensitivity was displayed by *Proteus vulgaris*. Experiments related to the 120μg/ml concentration effect of leaf essential oil against the tested pathogens showed that *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli* showed inhibitory effects and no inhibitory effect on *Proteus vulgaris* observed.

Table 1: Antimicrobial activity of Lemon Grass Oil

<table>
<thead>
<tr>
<th>Bacterial cultures used</th>
<th>Standard antibiotics (in mm)</th>
<th>Lemon Grass Oil</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Antibiotic</td>
<td>15mg/ml</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Ampicilline</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Gentamycin</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Oxythomycin</td>
<td>-</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>Ampicilline</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Gentamycin</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Oxythomycin</td>
<td>-</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>Ampicilline</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Gentamycin</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Oxythomycin</td>
<td>5</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Ampicilline</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Gentamycin</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Oxythomycin</td>
<td>11</td>
</tr>
</tbody>
</table>

Results of this study showed that most antibacterial effect of lemon grass and essence was against Gram positive bacteria. From the present study it is clear that lemongrass oil possess a promising antibacterial activity against the test organisms. The results obtained from the Agar diffusion assay and broth dilution method support the general indication that gram positive organisms are more sensitive to the oil than gram negative bacteria. Similar observations were made by Cimanga *et al.* (2002). *P. aeruginosa* were found resistant at all the concentration of lemongrass oil including neat. Similar results were reported by Pereira *et al.* (2004), Marta War *et al.* (2004), Torres *et al.* (2002) and Alam *et al.* (1994). The comparative effects of lemongrass oil and the standard antibiotic discs on the various test organisms are demonstrable indications of the oil as an antibacterial agent. Onawunmi and Ongulana (1986) had also reported the similar antibiotic susceptibility pattern and had suggested that the test organisms particularly gram negative were found to be more susceptible to lemongrass than standard antibiotics. Thus, we conclude that in present era of emerging multidrug resistance among gram positive and gram negative organisms lemongrass oil will be helpful in treating such infections.
In this study it was found that the methanol extract of lemon grass plant in concentrations around 15 mg/ml prevented the growth of tested Gram positive bacteria. While, inhibition of Gram negative bacteria needed higher concentrations. Its essential oil also has a significant inhibitory effect on *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*. Also the results showed that the methanol extract effect against Gram negative bacteria was much weaker than the Gram positive ones as 120 mg/ml concentration of methanol leaf extract showed weak inhibitory effect against *Proteus mirabilis*. Its probable cause is presence of cell wall polysaccharides which probably prevent reaching the active compounds, essential oil and extracts to the cytoplasmic membrane of Gram negative bacteria (Duraipandiya et al., 2006). In general, herbal products lead to granular cytoplasm and, cytoplasmic membrane rupture and inactivation or inhibition of intracellular and extra-cellular enzymes activity and being disintegrated into cell wall (Caccioni et al., 2000 and Kraft et al., 2004). Our results are consistent with findings of other researchers (Hindumathy, 2011 and Mothana et al., 2010) so that most plant extracts have inhibition effect on Gram positive bacteria and little effect on Gram negative bacteria. This inhibition effect can be related to its active compounds that include: steroids and terpenoids, alkaloids, citral, geraniol, flavonoids, eugenol, cytronolal, geranyl acetate, beta cariofiln, tannins, phenolic compounds, saponin and farnsul (Santin et al., 2009 and Adeleke et al., 2001). A study by Jeong et al. (2009) conducted on lemon grass plant has concluded that essential oil prepared from *Cymbopogon citrates* maybe a safe alternative environment inhibition of antimicrobial agents for various uses. It seems that generally antimicrobial properties of methanol extracts can be attributed to the presence of secondary metabolite especially flavonoids in first degree, in the second degree terpenes and in the third degree saponins (Eleyinmi, 2007). Results of this study showed that most antibacterial effect of lemon grass and essence was against Gram positive bacteria.

**CONCLUSION**

Medicinal plants containing essential oils in higher amounts shows antibacterial and antifungal activities. Natural essential oils from plant sources are potent and safe due their harmless nature and minimal or no side effects which are beneficial than the artificial ones. Volatile oils are widely used as analgesic, antibacterial, deodorizing, febrifuge, fungicidal, antiseptic, antidepressant, astringent, diuretic, galactogogue, insecticidal, antipyretic, antimicrobial and sedative properties. It finds utility in many areas due to these properties. The emergence of multiple antibiotic resistant bacteria has become a major challenge in the treatment of infectious diseases. The use of plant extracts and phytochemicals both
with known and unknown antimicrobial properties can be of great significance in therapeutic treatments. In the present study the extracts and oil of *Cymbopogan flexuosus*, an aromatic perennial grass is evaluated for its antimicrobial activity. In this study the lemon grass oil was extracted from the plant sources *Cymbopogan flexuosus* antimicrobial activity. According to the considerable antibacterial effect of methanol extract of lemon grass leaves on pathogenic bacteria especially Gram positive bacteria that are involved in creating variety of nosocomial and malicious infections this extract can be considered as a natural antibacterial herbal product. Finally it is concluded that the mixture of lemon grass oil is used to treat bacterial infections. It is concluded that, in the present study, the plant contains potential antibacterial components that may be useful for evolution of pharmaceutical for the therapy of ailments.

REFERENCES


