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In vitro Antibacterial Activity of Traditionally Used Medicinal Plant: *Cymbopogon citrates* Extract Against Clinical Isolates

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Abstract
In the face of increasing bacterial resistance to various antibiotics and continuous efforts to look for new and safer antibacterial substance. Lemon grass a well known herb that was widely used as a remedy for various ailments in traditional medicine. The Lemon grass had antimicrobial properties. The objective of this study was to assess the Lemon grass's antimicrobial potential. The extract showed antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* except *Pseudomonas aeruginosa* and *Proteus vulgaris*. Thus, Lemon grass can be one of the substitutes over antibiotics against tested pathogens. Present research will be a guiding path formulation of potent drug against life threatening pathogens. Identification of phyto-constituent will encourage drug designing with novel mechanism of action.

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1. Introduction

Medicinal plants have long been utilized as a source of therapeutic agents worldwide. While resistant bacteria have become commonplace in healthcare institutions, inadequate empirical therapy resulted in increased mortality rate due to resistant bacteria. (Alade et al., 1993). In phytomedicine research, phytochemicals have become key area of interest because many diseases possess a multicasual agents and complex pathophysiology requiring treatment with well chosen target potential drugs rather than random application.(Perez et al., 1990) Some plants have been employed in the treatment of various ailments, and some are employed as poisons to kill since pre-historic times. (Abioye et al., 2004; Abed et al., 2007)

The crude extracts from different parts of the plant have been used in the folk medicine in the treatment of various ailments. It is used either singly or in combination with other herbs in the traditional herbal preparations by different communities to treat various diseases. (Stadtman et al., 1996) The crushed leaves juice is applied on the tongue as a treatment for thrush in children; the latex is applied as an antibacterial agent in eardrops, and as chewing stick (Adekunle et al., 2009 and Ayandele et al., 2007).

Medicinal use of lemon grass *Cymbopogon citrullus* is known to mankind since antiquity. Lemon grass belongs to the section of *Andropogan* called *Cymbopogam* of the family Germineae. A very large genus of the family, including about 500 described species out of

which eight species occur in Iraq. Due to the production of lemon grass oil as major component, two of the species i.e. *Cymbopogan citrates* and *C. flexuosus* are generally called Lemon grass (Alade et al., 1993). Its oil has been used to cure various ailments like cough, cold, spitting of blood, rheumatism, lumbago, digestive problems, bladder problems, leprosy, and as mouth wash for the toothache and swollen gums. It is also been claimed to be stimulating, diuretic, anti purgative and reduce fever. To cure cholera, colic and obstinate vomiting only 3-6 drops of the oil is effective medicine of choice. It is most often used as a flavoring agent for its aroma and taste. (Bhoj Raj Singh et al, 2011; Jae-Young Choi et al., 2012)

Lemon grass is most abundantly found herb which also can be cultivated easily in farms making it cheaper raw source. Lemon grass have been used since past for its medicinal purpose. Because of its edible and no toxicity value making the plant most desired one for the study.

The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents. Hence the present study was carried out to find out the antibacterial activity of lemongrass oil against the selected pathogenic bacteria.

1.1 Objective of Research

Continuous alarming need of new antimicrobial therapy to deal with pathogens, because of emergence new diverse and novel drug resistance mechanisms making the treatment more tedious. Current study laid an emphasis on investigation over exploring Lemon grass for the extermination of pathogens as the most antibiotic have encountered for the allergic reactions and side effects. Study over herbal medicine would be a potent choice for formulation of drug.

2. Experimental

2.1 Collection of Plant Material

The leaves of lemongrass were collected in August 2010 from the Botanical garden of Nagpur and were authentified as *Cymbopogon citrates.*

2.2 Plant storage

Lemon grass were separated from stems and roots, washed in clean water, and dried at room temperature. The dried plants were milled to a fine powder, and stored in the dark at room temperature in closed containers until required. (Chamber et al., 2001; Cheesbrough et al., 2000)

2.3 Extraction procedure

The essential oils are present in the oil glands, oil sacks and glandular hairs of the plant (Chamber et al., 1979). Therefore, before extraction, the plant material is cut into small pieces enable them to expose directly as many oil glands as is possible (Chamber et al., 2001). Once the plant material has been reduced in size, it must be extracted immediately to avoid oil loss. Dried plant leaves were extracted by weighing samples of 1 g of finely ground plant material and extracting with 10 ml of boiled water in polyester centrifuge tubes. Tubes were vigorously shaken for 3 to 5 min in shaking machine at high speed. After centrifuging at 3500 rpm for 10 min the supernatant was decanted into pre-weighed, labeled containers. The process was repeated three times for separate to exhaustively extract the plant material and all the extracts were combined. (Chopra et al., 2005; Elastal et al., 2005; Johnson et al., 1995)

So, the final extract will contain extract of 3g of lemon grass powder in 30ml of distilled water.

2.4 Microorganisms

The clinical isolates of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris* and *Salmonella typhi* were used in the study were obtained from microbiological laboratory. These isolates were identified on the basis of morphological, cultural and biochemical characteristics (Collee and Marr et al., 1996) and results were compared with Bergey's Manual of Determinative Bacteriology, 9th edition.

2.5 Preparation of Concentrations of Lemon grass Extract

The different concentrations (v/v) of lemongrass extract viz., 5%, 10%, 15%, 20%, 25%, 30% were prepared aseptically in sterile tween-80.

2.6 Antimicrobial Activity of Lemon Grass Extract

The testing of the bacterial cultures for the inhibitory effect of essential oil of lemon grass for different concentration (5 %, 10 %, 15%, 20%, 25% and 30 %) were performed by using agar well diffusion method as described by Southwell et al (Chopra et al., 2005 and SouthWell et al., 1993) .

The Nutrient agar media containing 0.5% tween-80 was melted and 20 mL of media was added to individual sterilized petriplates separately on a level plate form and allowed to solidify. 1 mL of active cell suspension of test organisms was spread with the help of sterilized swabs on the agar surface uniformly. Wells of 5 mm diameter each were made in agar petriplates of the solidified agar medium using sterilized hollow stainless steel gel cutter. The measure quantity of 25 µL of each concentration was pipetted out with a sterilized pipette and filled in the wells aseptically. In the control plate only Tween-80 was added into the well. The oil was allowed to defuse in the well for a period of one hour and plates were incubated at 37^{0}C for 24-48 hours. The zone of inhibition (mm) was measured with graduated scale after the period of incubation.

3. Results and Discussion

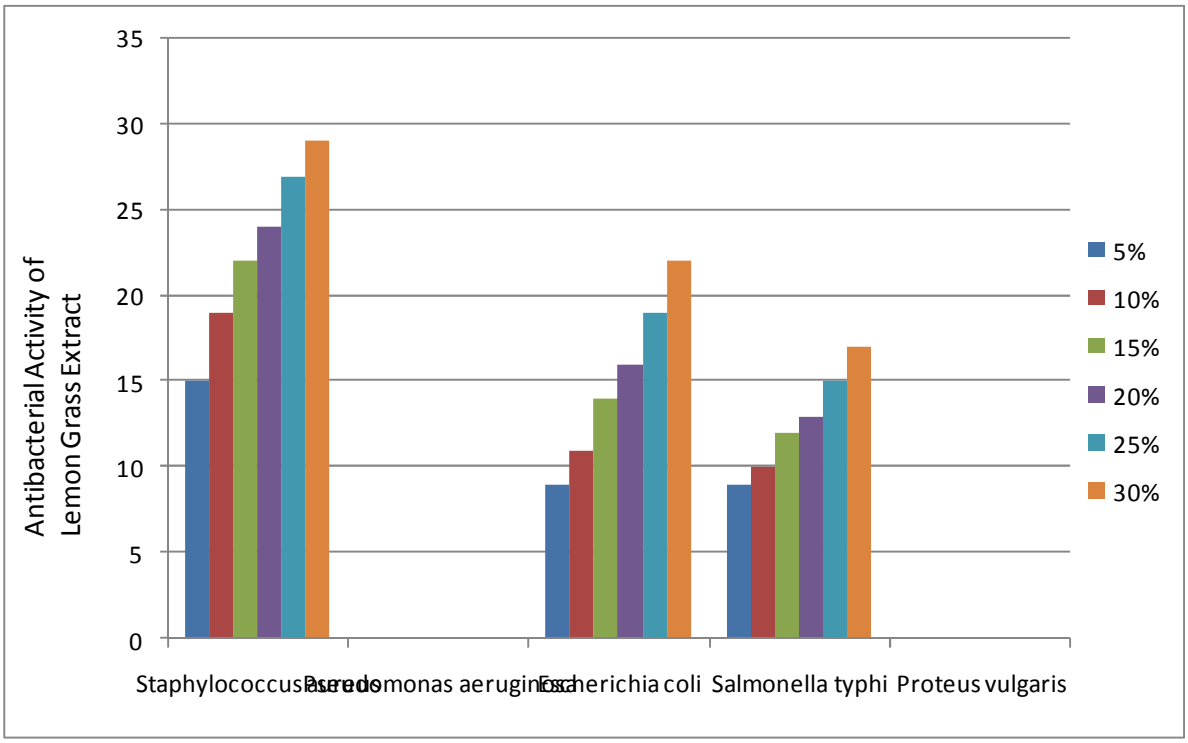
In the present investigation, antimicrobial activity of hot water extract of dried Lemon grass were analyzed against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris* and *Salmonella typhi.* Zone of inhibition was interpreted with reference to protocol of Johnson *et al.* (1995) (Table1) (Johnson et al., 1995). Lemon grass extract was found to be effective against *Staphylococcus aureus,* followed by *Escherichia coli* and *Salmonella*

Table 1: *Antibacterial Activity of Lemon Grass Extract against Various Selected Pathogenic Bacteria*

Bacterial Pathogens	Dilutions of Lemon Grass Extract					
	5%	10%	15%	20%	25%	30%
Staphylococcus aureus	15mm	19mm	22mm	24mm	27mm	29mm
Pseudomonas aeruginosa	NZ	NZ	NZ	NZ	NZ	NZ
Escherichia coli	9mm	11mm	14mm	16mm	19mm	22mm
Salmonella typhi	9mm	10mm	12mm	13mm	15mm	17mm
Proteus vulgaris	NZ	NZ	NZ	NZ	NZ	NZ

Where, NZ= No Zone of Inhibition

Figure 1: *Antibacterial Activity of Lemon Grass Extract against Various Selected Pathogenic Bacteria*



typhi except *Pseudomonas aeruginosa* and *Proteus vulgaris*. From the present study it is clear that lemongrass oil possess a promising antibacterial activity against the test organisms. Singh *et al.* (2011) studied effect of Lemon grass on number of bacteria and fungi and found that they were sensitive for Lemon grass extract (Bhoj Raj Singh et al., 2011). The antibacterial activity was found progressively increasing with the increase in

concentration of extract. The maximum effect was found at 30% concentration and minimum effect was observed at 5% concentration of extract (Table 1). The results obtained from the Agar diffusion assay method supported the general indication that gram positive organisms were more sensitive to the oil than gram negative bacteria. Similar observations were made by Onawunmi and Ongulana. 1986 and Behboud Jafari et al., 2012; Cimanga et al). Hindumathy (2011); Behboud et al. (2012); Jae-Young Choi *et al.* (2012) investigated the antibacterial activity of lemongrass and reported most effective against *Staphylococcus aureus.* P. aeruginosa were found resistant at all the concentration of lemongrass oil. Similar results were reported by Pereira et al, Marta War et al., Torris et al, Alam et al, and Onawunmi et al, (Bhoj Raj Singh., 2011; Diallo D et al., 1999; Elastal, Z. Y. et al., 2005; Johnson et al.1995; Cheesbrough et al., 2000).

Conclusion

Extracts of *Lemon grass* in this study demonstrated a broad-spectrum of activity against both gram-positive and gram-negative bacteria. The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents. Thus, we conclude that in present era of emerging multidrug resistance among gram positive and gram negative organisms lemongrass extract will be helpful in treating such infections.

Research Highlights

(1) Dealt with common and abundantly found herb.
(2) Evaluated the anti bacterial activity of edible fraction of phyto chemical extract to avoid toxicity value (factor).
(3) The most commonly found pathogens studied for antimicrobial activity which have been previously demonstrated to have high resistance profile.

Limitations

Study of actual mechanism of action and responsible target phyto-constituent was beyond the scope of our study.

Recommendations

Modern biophysical techniques need to be studied to encounter potent phyto constituent and method of genetic engineering and cytology to be applied to study mechanism of action.

Funding and Policy Aspects

Grants and funding for establishment of modern instrumentation and genetic analysis lab need to be provided. Establishment of government/ privet lab at institution level or as an individual body can bring quality research in life sciences.

Authors' Contribution and Competing Interests

Potential activity of lemon grass essential oil was evaluated against pathogens which provide faithful data for formulation and drug designing with herbal medicine. Responsible target mechanisms need to be studied for strong scientific evidence.

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