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Antimicrobial Properties and Phytochemical Studies of Lemongrass (Cymbopogon Citratus) Leaves Essential Oil

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ABSTRACT
The phytochemicals detected in lemongrass leaves essential oil were flavonoids, tannins, saponins, steroids, terpenoids andcoumarins. The antibacterial activity of Lemongrass leaves essential oil was tested against five potential pathogens by agarwell diffusion method and the results depicted that lemongrass essential oil generated the inhibition zones of 33.0±0.74, 47.0±1.04 andagainst all twogram positive pathogens viz. Staphylococcus aureus, Bacillus subtilis respectively, whereas and no inhibition zone was observed for Pseudomonas aeruginosa as well as Escherichia coli and Salmonella typhi. The zone of inhibition produced by lemongrass oil against Bacillus subtilis was significantly ($P<0.05$) higher, followed by Staphylococcus aureus. Moreover, it was observed that the zone of inhibition produced by lemongrass oil against Bacillus subtilis, Staphylococcus aureus and were significantly ($P<0.05$) higher than the corresponding inhibition zones produced by the antibiotic i.e., Azithromycin (200 mg/ 5 ml) suspension (positive control).
Keywords: Phytochemical, essential oil, antibacterial, lemongrass, Cymbopogon citratus

I. INTRODUCTION

Cymbopogon citratus, commonly known as West Indian lemon grass or simply lemon grass(plants.usda.gov). is a tropical plant native to Maritime Southeast Asia and introduced to many tropical regions. [Plants of the world online)Cymbopogon citratus is often sold in stem form. While it can be grown in warmer temperate regions, such as the UK, it is not hardy to frost.Cymbopogon citratus is part of the grass family, Poaceae. They contain simple, bluish-green leaves with entire margins and are linear in shape. The blades tend to be 18–36 inches long. Like other grasses, the leaves also have parallel venation. (Shah et al., 2011)

Cymbopogon citratus is native to Island Southeast Asia (Malesia). It has been introduced extensively to South Asia since precolonial times. After the World War I, lemongrass was introduced to Madagascar, South America, and Central America. It has now been naturalized throughout the tropics and subtropics worldwide.(Oyen et al., 2019)

In its native range, Cymbopogon citratus is known as sereh, serai, or serai dapur in Indonesia and MalaysiaCymbopogon citratus is abundant in the Philippines and Indonesia where it is known as tanglad or sereh. Its fragrant leaves are traditionally used in cooking, particularly for lechon and roasted chicken.(Market Manila. 2006). The dried leaves can also be brewed into a tea, either alone or as a flavouring

in other teas, imparting a flavour reminiscent of lemon juice but with a mild sweetness without significant sourness or tartness.In Sri Lanka, lemongrass is known as sera. It is used as an herb in cooking, in addition to its use for the essential oils. (www.srilankanspices.com. 2011)

Lemongrass in Thailand is called takhrai. It is the essential ingredient of tom yam and tom kha kai. Fresh thin slices of lemongrass stem also used in miangpla, as a snack food.The leaves of Cymbopogon citratus have been used in traditional medicine and are often found in herbal supplements and teas. Evidence of effective Cymbopogon citratus essential oil anti-protozoa activity against Leishmania amazonensis.(Santinet al., 2009)

Lemon grass oil contains 65–85% citral in addition to myrcene, citronellal, citronellol, linalool and geraniol.(*Shaikh,et al., 2019, Baby et al., 2007)*Hydrosteam distillation, condensation, and cooling can be used to separate the oil from the water. The hydrosol, as a by-product of the distillation process, is used for the production of skin care products such as lotions, creams, and facial cleansers. The main ingredients in these products are lemon grass oil and "negros oil" (mixture of lemon grass oil with virgin coconut oil) used in aromatherapy.(Inquirer.net 2008)

Citronellol is an essential oil constituent from Cymbopogon citratus, Cymbopogon winterianus, and Lippia alba. Citronellol has been shown to lower blood pressure in rats by a direct effect on the vascular smooth muscle leading to vasodilation.(Bastos et al., 2010) In a small, randomized, controlled trial, an infusion made from C. citratus was used as an inexpensive remedy for the treatment of oral thrush in HIV/AIDS patients.(Wright et al., 2008)

Laboratory studies have shown cytoprotective, antioxidant, and anti-inflammatory properties in vitro.(Figueirinha et al., 2009, Lee et al., 2008, Tiwari et al., 2010)

In the folk medicine of the Krahô people of Brazil, it is believed to have anxiolytic, hypnotic, and anticonvulsant properties. (Blanco et al., 2009,Rodrigues et al., 2006)

In traditional medicine of India, the leaves of the plant are used as stimulant, sudorific, antiperiodic, and anticatarrhal, while the essential oil is used as carminative, depressant, analgesic, antipyretic, antibacterial, and antifungal agent.

Beekeepers sometimes use lemon grass oil in swarm traps to attract swarms. Lemon grass oil has also been tested for its ability to repel the pestilent stable fly,which bite domestic animals. (Baldacchino et al., 2013)which bite domestic animals.

II. MATERIAL AND METHODS

Materials

Lemongrass (Cymbopogon citratus):

Grown in the Botanical Garden of Dr. Babasaheb Ambedkar Marathwada University was used for the study. Phyto-chemical screening of lemongrass leaves essential oil: The various screening tests to detect the presence of Phyto-chemicals (i.e. flavonoids, alkaloids, tannins, phlobatannins, saponins, steroids, terpenoids, glycosides, cardiac-glycosides, proteins and amino acids, carbohydrates, reducing sugars, quinones, anthraquinones, anthocyanins, leucoanthocyanins and coumarins) through qualitative analysis were

performed using procedures described by Kokate et al. (2008) and Evans (2009) with slight modifications. Freeze dried cultures of pathogenic bacteria: The freeze-dried cultures of two-gram positive pathogenic bacteria viz. Staphylococcus aureus, Bacillus subtilis, and three-gram negative pathogenic bacteria viz. Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi.

Methods Preparation of lemongrass essential oil

The essential oil was extracted from lemongrass leaves by steam distillation process using vertical steam distillation unit, consisting of a hot plate, boiling flask, biomass flask, still head, condenser and receiver. The lemongrass leaves were chopped into small pieces of size 1-2 cm and transferred into biomass flask whereas distilled water was added to the boiling flask. Biomass flask was set over the top of boiling flask and the distilled water in boiling flask was heated with the help of hot plate. The steam thus produced in the boiling flask travelled upward into the biomass flask where essential oil and water-soluble compounds were extracted into the vapour stream. The vapours passed through the still head and condenser was collected in the receiver as condensatecomprising two separate layers i.e., essential oil and water from which the essential oil layer was carefully transferred into a clean dry beaker.

III. RESULTS AND DISCUSSION

Phyto-chemical screening of lemongrass leaves essential oil The Phyto-chemical screening tests results are given in Table 1. The phytochemicals detectedin lemongrass essential oil were flavonoids, tannins, saponins, steroids, terpenoids and coumarins.The results obtained in present research are supported by the studies conducted by different scientists regarding Phyto-chemicals screening of lemongrass (C. citratus) leaves.(Balakrishnan etal., (2015) performed phytochemical analysis of lemongrass oil and confirmed the presence of tannins, saponins, flavonoids and phenols whereas terpenoids, cardiac glycosides, steroids and phlobatannins were reported to be absent, however results (Table 1) of present study revealed the presence of flavonoids, tannins, saponins, steroids, terpenoids and coumarins in lemongrass essential oil. The above variations in phytochemicals are due to a number of environmental factors e.g., climate, altitude and rainfall (Refaat and Balbaa, 2001; Mirza et al., 2003; Assous et al., 2013; Gazwi, 2020).

Table 1: Phyto-chemical screening of lemongrass leaves essential oil

Phyto-constituents	Name of the test	Observation
Flavonoids	Alcohol-acid test	+
Tannins	Braymer's test	+
Phlobatannins	Precipitation test	-
Saponins	Emulsion Formation	+
	Foam Formation	+
Steroids	Salkowski test	+
Terpenoid	Salkowski test	+

Cardiac-glycosides	Keller-Kiliani test	-
Coumarins	Alkaline solution	+
Representations: + = Present, – = Absent or not detectable		

Antibacterial activity of lemongrass leaves essential oil

Antibacterial activity of lemongrass leaves essential oil was tested against five potential pathogens by agar well diffusion method. The in-vitro antibacterial activity was evaluated against two-gram positive pathogenic bacteria viz. Staphylococcus aureus, Bacillus subtilis and three-gram negative pathogenic bacteria viz. Pseudomonas aeruginosa,Escherichia coli andSalmonella typhiby measuring the diameter (mm) of zone of inhibition (i.e., no microbial growth produced by the sample) against the test organisms. The results are presented in Table 2.

Table 2: Antibacterial activity of lemongrass leaves essential oil

Sr. No	Microorganism	Zone of inhibition (mm)*		
		Lemongrass leaves essential oi	Positive control	Negative control
1	Bacillus subtilis	47.0± 1.06dB	22.0± 0.91cA	-
4	Staphylococcus aureus	31.0 ± 0.76cB	28.0± 0.87dA	-
2	Escherichia coli	-	20.0± 1.03b	-
3	Pseudomonas aeruginosa	-	18.0± 0.82a	-
5	Salmonella typhi	-	21.0± 0.99abA	-

The results given in Table 2 depicted that lemongrass essential oil has generated the inhibition zones of 47.0 ± 1.06and31.0 ± 0.76 mm against all two-gram positive pathogens viz.Bacillus subtilis, Staphylococcus aureusrespectively, whereas no inhibition zone was observed for Escherichia coli, Pseudomonas aeruginosa as wellas Salmonella typhi. The zone of inhibition produced by lemongrass oil against Bacillus subtilis was significantly ($P<0.05$) higher, followed by Staphylococcus aureus. Moreover, it was observed that the zone of inhibition produced by lemongrass oil against Bacillus subtilis, Staphylococcus aureus were significantly ($P<0.05$) higher than the corresponding inhibition zones produced by the antibiotic i.e., Azithromycin (200 mg/ 5 ml) suspension (positive control). The results of Table 2 revealed that lemongrass leaves essential oil sample possess antibacterial potential as indicated by the formation of zone of inhibition. Manyscientists had reported the antibacterial activity of lemongrass oil against a diverse range of microorganisms comprising gram positive and gram-negative microorganism, yeast and fungi (Helal et al., 2006, Bassole et al., 2011; Singh et al., 2011, Falcao et al.,2012). In literature, it has been cited that lemongrass essential oil exhibits antibacterial properties and inhibits a host of microorganisms including Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Acinetobacter baumanii, Klebsiella pneumoniae, Aeromonas veronii, Candida albicans, Salmonella enteric serotype typhimurium, Enterobacter aerogenes,Serratia

marcesens, Corynebacterium equii and Proteus vulgaris (Onawunmi et al., 1984; Ogunlana et al., 1987; Baratta et al., 1998; Cimanga et al., 2002; Pereira et al., 2004)and shows antifungal effects against Epidermophyton floccosum, Trichophyton rubrum, T. mentagrophytes, and Microsporun gypseum, ringworm fungi (Shadab et al.,1992). Many other studies have reported the antimicrobial activity of essential oil of lemongrass plant against pathogenic bacterial strains and found that Enterococcus fecalis was the most sensitive microorganism, while P. aeruginosawas most resistant (Yazdani et al., 2003; Olivero-Verbel et al., 2010; Bassole et al., 2011). In another study, Kumar et al., (2017) tested the antimicrobial potential of lemongrass, clove and cinnamon essential oils against nine common food spoilage and pathogenic microorganisms by using zone inhibition assay and revealed maximum zone diameter (mm) of lemongrass oil for Staphylococcus aureus followed by Listeria monocytogenes, Vibrio parahaemolyticus and Klebsiella pneumonia showing strong activity against gram positive bacteria. Similarly, Srivastava et al., (2015)investigated the antibacterial activity of essential oils extracted from leaves of 16 aromatic plants (including Cymbopogon citratus) by discdiffusion method and stated that highest zone of inhibition was formed by C.citratus essential oil which showed complete inhibition of B. subtilis and 35.67, 40.33, 32.33 mm zone of inhibition was recorded against E.coli, S.aureus, S.flexneri,respectively, theseresults are in partial agreement with the results of present investigation wherein highest zone was observed against B. subtilis (48 mm) followed by S. aureus (32 mm) but no zone was observed against E. coli. Further, the authors reported that lemongrass oil was effective against both gram positive and gram-negative bacterial strains but gram-positive strains were found more susceptible which supports the results of present study. (Aiemsaard et al., (2011) investigated the antibacterial activity of lemongrass oil and its major components (citral, geraniol and myrcene) against four strains of clinically isolated bovine mastitis pathogens and demonstrated that Streptococcus agalactiae and Bacillus cereus were more susceptible to lemongrass oil, citral and geraniol than Staphylococcus aureus and Escherichia coli, concluding that citral and geraniol to be major antibacterial compounds in lemongrass oil and thus confirms the findings of present research. Additionally, the observations of present investigation are in concurrence with the results obtained by Naik et al. (2010) [27] who reported that except P. aeruginosa, the lemongrass (C. citratus) essential oil was effective against all other tested organisms (B. subtilis, B. cereus, S. aureus, K. pneumoniae) and they also mentionedthat gram positive organisms were more susceptible to oil than gram negative organisms.

IV. CONCLUSION

It could be inferred that the antimicrobial properties demonstrated by lemongrass (C. citratus) samples in present study were because of the presence of phytochemicals in the leaves since the antibacterial activity of lemongrass is allegedly because the leaves have bioactive compounds such as alkaloids, flavonoids, tannins and phenolic compounds. From the presentstudy, it is clear that lemongrass leaves essential oil possess a promising antibacterial activity against the test organisms and the comparative effects of lemongrass oil with the standard antibiotic (positive control) on various test pathogens are demonstrable indications of the lemongrass leaves essential oil as an antibacterial agent.

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