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Phytochemical screening and antibacterial activity 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 and coumarins. The antibacterial activity of lemongrass leaves essential oil was tested against six potential pathogens by agar well diffusion method and the results depicted that lemongrass essential oil generated the inhibition zones of 32.0±0.75, 48.0±1.05 and 21.0±0.64 mm against all three gram positive pathogens *viz. Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus cereus*, respectively, whereas *Proteus vulgaris* was the only gram negative pathogenic bacteria against which a zone of inhibition of 23.0±0.73 mm was reported and no inhibition zone was observed for *Pseudomonas aeruginosa* as well as *Escherichia coli*. The zone of inhibition produced by lemongrass oil against *Bacillus subtilis* was significantly (P<0.05) higher, followed by *Staphylococcus aureus*, *Proteus vulgaris* and *Bacillus cereus*. Moreover, it was observed that the zone of inhibition produced by lemongrass oil against *Bacillus subtilis*, *Staphylococcus aureus* and *Proteus vulgaris* 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) while non-significant differences were observed in case of *Bacillus cereus*.

Keywords: Phytochemical, essential oil, antibacterial, lemongrass, *Cymbopogon citratus*

Introduction
Cymbopogon citratus (*C. citratus*) is commonly known as lemongrass, barbed wire grass, citronella grass, fever grass and tanglad but due to its broad distribution (Karpagam *et al*., 2016; Oladeji *et al*., 2019) [21, 30]. *C. citratus* flourishes in sunny, warm, humid conditions of the tropics and grown in a wide variety of soil ranging from rich loam to poor laterite, but calcareous and water-logged soils are unsuitable for its cultivation (Farooqi and Sreeramu, 2001) [13] while plants growing in sandy soils have higher leaf oil yield and citral content (Balakrishnan *et al*., 2014) [5].

C. citratus is rich in bioactive compounds and the isolated and identified phytochemicals from its leaves mainly includes flavonoids, alkaloids, saponin, tannins and phenolic compounds, which consist of quercetin, luteolin, apiginin, isoorientin 2'-O-rhamnoside and kaempferol that are known to have many benefits, especially in the fields of pharmacy, food, health and agriculture (Negrelle and Gomes, 2007; Hasim *et al*., 2015; Erminawati *et al*., 2019) [28, 17, 11]. The other compounds identified in *C. citratus* are mainly alcohols, aldehyde, ketones, esters and terpenes (Hasim *et al*., 2015) [17].

The plant also contains 1-2 per cent essential oil on a dry basis with wide variation of chemical composition as a function of habitat, genetic diversity and agronomic treatment of culture. The volatile oil from the roots contains longifolene -(V4) (56.67%) and selina-6-en-4-ol (20.03%) (Ademuyiwa and Grace, 2015) [1]. The main chemical component of lemongrass essential oil is citral whereas many other compounds like neral, geranial, geraniol, β-myrcene, limonene, geranyl acetate, borneol, estragole, methyleugenol, citronellal, pinene, careen-2, farnesol, alpha-terpineol, (+)-cymbodiacetal, proximadiol, methyl heptenone, terpinolene, linalool, linalyl acetate and β-caryophyllene have also been reported (Carlson *et al*., 2001; Huynh, 2008; Shah *et al*., 2011; Ademuyiwa and Grace, 2015) [8, 20, 37, 1].

Citral (3, 7-dimethyl-2, 6-octadien-2-al) is the name given to a natural mixture of two isomeric acyclic monoterpene aldehydes i.e. geranial (citral A or *trans* citral) and neral (citral B or *cis*-citral) (Carlson *et al*., 2001; Huynh, 2008) [8, 20] which have same molecular formula ($C_{10}H_{16}O$), but different structures (Mirghani *et al*., 2012; Manvitha and Bidya, 2014; Hartatie *et al*., 2018) [25, 24, 16].

However, the chemical composition is influenced by several factors, such as seasonal, climatic, local and experimental conditions (Perry *et al*., 1999; Daferera *et al*., 2000) [34, 10]. Citral changes enzyme's activity of metabolism of drugs and decreases oxidative stress (Li *et al*., 2018) [23]. The bioactivity of lemongrass have been extensively studied, especially as antioxidant, antimicrobial, antifungal, antibacterial, insecticidal and insect repellent activities (Helal *et al*., 2006a; Ganjewala, 2009; Mirghani *et al*., 2012) [18, 14, 25].

Material and Methods

Materials

Lemongrass (*Cymbopogon citratus*): grown in the Centre for Agro-Forestry, Forage Crops and Green Belt, SDAU 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 three gram positive pathogenic bacteria *viz*. *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 2414), *Bacillus cereus* (MTCC 9017) and three gram negative pathogenic bacteria *viz*. *Pseudomonas aeruginosa* (MTCC 7602), *Escherichia coli* (MTCC 739), *Proteus vulgaris* (MTCC 744) were procured from Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India.

All glasswares used in the study were standard quality supplied by authorized dealers. Glasswares used in the experiments were washed with detergents, rinsed with distilled water and dried before use. All chemicals used during the investigation were of analytical grade (AR) and obtained from standard suppliers.

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 condensate comprising two separate layers i.e. essential oil and water from which the essential oil layer was carefully transferred into a clean dry beaker.

Results and Discussion

Phyto-chemical screening of lemongrass leaves essential oil

The phyto-chemical screening tests results are given in Table 1. The phytochemicals detected in 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 *et al*. (2015) [4] performed phytochemical analysis of lemongrass oil and confirmed the presence of tannins, saponins, flavonoids and phenols whereas terpenoids, cardiac glycosides, steriods 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) [35, 26, 3, 15].

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	+
Terpenoids	Salkowski test	+
Cardiac-glycosides	Keller-Kiliani test	-
Coumarins	Alkaline solution	+

* - All tests were performed thrice
Representations: + = Present, – = Absent or not detectable

Antibacterial activity of lemongrass leaves essential oil

Antibacterial activity of lemongrass leaves essential oil was tested against six potential pathogens by agar well diffusion method. The *in-vitro* antibacterial activity was evaluated against three gram positive pathogenic bacteria (*viz*. *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 2414) and *Bacillus cereus* (MTCC 9017)) and three gram negative pathogenic bacteria (*viz*. *Pseudomonas aeruginosa* (MTCC 7602), *Escherichia coli* (MTCC 739) and *Proteus vulgaris* (MTCC 744)) by measuring the diameter (mm) of zone of inhibition (i.e. no microbial growth produced by the sample) against the test organisms using graduated scale. All experiments were carried out in triplicate and 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 oil	Positive control$	Negative control#
1	*Staphylococcus aureus*	32.0 ± 0.75^{cB}	29.0 ± 0.88^{dA}	-
2	*Bacillus subtilis*	48.0 ± 1.05^{dB}	23.0 ± 0.92^{cA}	-
3	*Bacillus cereus*	21.0 ± 0.64^{aA}	21.0 ± 0.95^{bA}	-
4	*Pseudomonas aeruginosa*	-	19.0 ± 0.81^{a}	-
5	*Escherichia coli*	-	21.0 ± 1.02^{b}	-
6	*Proteus vulgaris*	23.0 ± 0.73^{bB}	20.0 ± 0.98^{abA}	-

Means with different superscripts in each column (a, b, c, d) and in row (A, B) differ significantly (LSD test, $P < 0.05$) from each other. Data presented as means ± SEm (n = 3).
* - well size of 8 mm (diameter) included
$ - 150 µl of antibiotic i.e. Azithromycin (200 mg/ 5 ml) suspension (positive control)
# - sterile distilled water (150 µl) (negative control)

The results given in Table 2 depicted that lemongrass essential oil has generated the inhibition zones of 32.0±0.75, 48.0±1.05 and 21.0±0.64 mm against all three gram positive pathogens *viz*. *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus cereus*, respectively, whereas *Proteus vulgaris* was the only gram negative pathogenic bacteria against which a zone of inhibition of 23.0±0.73 mm was reported and no inhibition zone was observed for *Pseudomonas aeruginosa* as well as *Escherichia coli*. The zone of inhibition produced by lemongrass oil against *Bacillus subtilis* was significantly ($P<0.05$) higher, followed by *Staphylococcus aureus*, *Proteus vulgaris* and *Bacillus cereus*. Moreover, it was observed that the zone of inhibition produced by lemongrass oil against *Bacillus subtilis*, *Staphylococcus aureus* and *Proteus vulgaris* 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) while non-significant differences were observed in case of *Bacillus cereus*. The results of Table 2 revealed that lemongrass leaves essential oil sample possess antibacterial potential as indicated by the formation of zone of inhibition.

Many scientists 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[a,b]; Bassole *et al*., 2011; Singh *et al*., 2011, Falcao *et al*., 2012) [18, 19, 7, 38, 12]. 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) [32, 29, 6, 9, 33] and shows antifungal effects against *Epidermophyton floccosum*, *Trichophyton rubrum*, *T. mentagrophytes*, and *Microsporun gypseum*, ringworm fungi (Shadab *et al*., 1992) [36]. 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. aeruginosa* was most resistant (Yazdani *et al*., 2003; Olivero-Verbel *et al*., 2010; Bassole *et al*., 2011) [40, 31, 7]. In another study, Kumar *et al*. (2017) [22] 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) [39] investigated the antibacterial activity of essential oils extracted from leaves of 16 aromatic plants (including *Cymbopogon citratus*) by disc diffusion 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, these results 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) [2] 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 mentioned that gram positive organisms were more susceptible to oil than gram negative organisms.

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 present study, 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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