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In vitro antibacterial activity of lemongrass (*Cymbopogon citratus*) leaves extract by agar well method

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Abstract
Lemongrass is an herb which belongs to gramineae family. Scientifically it is called as *Cymbopogon citratus*. The prefix lemon owes to its typical lemon like odour, due to presence of citral a cyclic monoterpene. Lemongrass has phytoconstituents such as tannins, flavanoids, alkaloids, and various essential oils in this herb. Secondary active metabolites of a number of components have also been implicated in the varied pharmalogical effects of this plant. lemongrass possesses various antimicrobial properties. The extracts of lemon grass leaves (fresh and dried) with cold, hot and different solvents like ethanol and methanol were screened for its antimicrobial activity against various bacteria *Bacillus vallismortis, Lysinibacillus macroides, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, vibrio cholera*, at three different concentrations by agar well method. Lemon grass extract was found effective against all the test organisms Among the reported organisms, *staphylococcus aureus* recorded a greater zone of inhibition (12.50mm) at 1000 ppm concentration in ethanol dried leaves extract when compared to other organisms. The least zone of inhibition was observed in *Pseudomonas aeruginosa* (2.0mm) in hot water fresh leaves extract at 250 ppm this results pertainning that the lemongrass leaves extract possess a great antimicrobial activity against the antibiotic resistant microorganisms.

Keywords: lemongrass, cold water, hot water, ethanol, methanol antibacterial activity

Introduction
Lemon grass (*Cymbopogon Flexuosus*) and (*Cymbopogon Citraus)* is a native aromatic tall sedge/grass (Rangari vinod, 2009) [17]. Family (Poaceae / Gramineae) with diverse medicinal value and grown in many parts of tropical and subtropical south east Asia and Africa. It was grown in India a century back and now commercially cultivated in different parts of India. The oil has been found to posses bactericidal, anti-bacterial and anti-fungal properties, which is comparable to Penicillin in its effectiveness (Lutterodt *et al.*, 1999) [13]. The oil also contains male sex hormone agent (Gupta *et al*., 1993) [11]. It has analgesic and antipyretic properties. The juice extracted from the lemon grass contains inhibitor of the promotion stage of carcinogenesis induced by cotton oil. It is an oral anti tumor drug for the cancer and in combination with cyclodextrin and helps in increasing survival time (Parekh and Chanda, 2007; Oshiba *et al*., 1991) [16, 15]. Gallstone dissolving preparations have been made of oil (Elasta *et al*., 2005) [8]. *Cymbopogon citratus* is a great interest due to its commercially valuable essential oils and widely used in food technology as well as in traditional medicine. People now-a-days are more aware on health issue due to the emergence of new diseases. Treatment using plant-based medicine appears to be an alternative approach due to the adverse effects associated with the use of synthetic drugs (Mirghani *et al*., 2012) [14].
The ethanolic extracts of the leaves of Lemon grass showed potential antibacterial property against *Staphylococcus aureus*. Flavonoids and Tannins found in the extract are responsible for the activity (Danlmi *et al.*, 2011). Revathi *et al*., (2012) [19] said that the combined the antimicrobial activity of volatile oils obtained from *Cymbopogon citratus* and *Ocimum sanctum* against *Staphylococcus aureus* (bacteria) and *Aspergillus niger* (fungi) by agar disc diffusion method and turbidometry method. The results showed that the maximum antimicrobial activity was shown by lemon grass oil than the ocimum oil. The mixture of these two oils shows maximum antimicrobial activity than the individual oils. Chamdit and Siripermpool (2012) [3] stated that the antimicrobial activity of clove and lemongrass oil against planktonic cells and biofilms of *Staphylococcus aureus.* They stated that these two oils efficiently kills the *S.aureus* with in the biofilm and is therefore an alternative method for its eradication. Ewanishi *et al*., (2012) stated that the cold maceration and agar diffusion technique were employed to assess phytochemical properties and the antimicrobial potency of

Cymbopogon citratus (lemongrass) against selected microbial pathogens using hexane, chloroform and methanol as extracting solvents. Antimicrobial property of lemongrass (*Cymbopogon citratus*) oil against pathogenic bacteria isolated from pet turtles (De Silva *et al*., 2017) [7] lemongrass oil is used to control the turtle borne pathogens. The lemongrass oil and citral may change the activities of drug metabolizing enzymes and reduce oxidative stress in the liver (Chinen-chun *et al*., 2018) [4]

The antimicrobial activity of many plants have been reported by many researchers (Reddy *et al*., 2001; Atleb and Erdourul 2003) [18, 2]. However the studies of antimicrobial activities of lemongrass are very limited towards the pathogenic bacteria and fungi.

Materials and Methods

Location

The in *vitro* experiments were conducted out in the microbiology laboratory Department of microbiology Faculty of Agriculture, Annamalai University. Stalks and leaves are used the essential oil is extracted from fresh plant material by means of steam distillation.

Fresh leaves extract

Weigh 25 g of lemongrass and cut it into small pieces. The pieces were taken in 500 ml of round bottomed flask. Add 300 ml of distilled water to the flask containing the grass and set the apparatus for distillation. Boil the mixture vigorously and collect the distillate until no more oily drops can be seen passing over. More water should be added if necessary to avoid charring of flasks contents. Extract the distillate with hexane, dry them over sodium sulfate and remove the solvent on rotary evaporator with external heating at 45° C. Finally 2 ml of yellow to ochre colored oily liquid with fresh lemon like tone with a hint of rose was obtained. (Arputha bibiana *et al*., 2012) [1]

Dried plant extract

The dried samples were grinded and sieved. The powdery sample were then partitioned in two parts

a) Ethanol Extract

(12 & 16 gm) powdered samples were extracted with 100ml of ethanol. The powdered sample was soaked in the solvent for 24 hours and plant extract was prepared using reflux and steam distillation method. In this method, plant material is immersed in a solvent in the round- bottomed flask, which is connected to a condenser. The solvent is heated up to its boiling point. As the vapors are condensed, the solvent is recycled into the flask.

b) Methanol Extract

(12 & 16 gm) powdered sample were extracted with Methanol extract. The powdered sample was soaked in 100 ml water for 24 hours and plant extract was prepared using the same above mentioned method.

c) Cold Water Extract

(12 & 16 gm) powdered sample were extracted with cold water. The powdered sample was soaked in 100 ml water for 24 hours and plant extract was prepared using the same above mentioned method.

d) Hot Water Extract

(12 & 16 gm) powdered sample were extracted with hot water. The powdered sample was soaked in 100 ml water for 24 hours and plant extract was prepared using the same above mentioned method.

Preparation of bacterial inoculum:

The organisms to be used for the *in vitro* test were maintained and preserved on nutrient agar slopes by refrigeration at 4 ^{o}c subcultures are maintained at regular intervals.

Antibacterial activity of *Cymbopogon citratus*

Agar well diffusion method was followed to determine the antimicrobial activity of lemon grass of fresh leaves and dry leaves extract.

Each bacterial isolate was suspended in nutrient broth and diluted approximately to 10^5 colony forming units (CFU) per ml. they were flood inoculated onto the surface of nutrient agar and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 200µl of the sample solutions of different concentrations 250 ppm, 500ppm, and 1000ppm were delivered into the wells. The plates were incubated for 2 days at 37 ^{0}c antibacterial activity was evaluvated by measuring the zone of inhibition against the test organisms.

Results

The results of the experiments were carried out on the antimicrobial activity of fresh leaves and dried leaves extract of lemongrass with cold water, hot water and different solvents like ethanol and methanol against with different microorganisms.

Table 1: Inhibition effect of cold and hot water extract of lemon grass extract against *Bacillus vallismortis*, *Lysinibacillus macroides* and *Staphylococcus aureus.*

S.No	Lemongrass	Organism	Zone of Inhibition (Mm)		
			Concentration In Ppm		
			250	500	1000
1	Cold Water Fresh Leaves Extract	*Bacillus Vallismortis*	4.40	5.50	7.00
2	Cold Water Dried Leaves Extract		5.00	6.10	7.10
3	Hot Water Fresh Leaves Extract		4.20	5.00	6.00
4	Hot Water Dried Leaves Extract		4.70	5.40	6.50
5	Cold Water Fresh Leaves Extract	*Lysinibacillus Macroides*	4.60	6.40	7.00
6	Cold Water Dried Leaves Extract		4.90	6.50	7.40
7	Hot Water Fresh Leaves Extract		3.30	5.00	6.50
8	Hot Water Dried Leaves Extract		4.40	5.50	6.70
9	Cold Water Fresh Leaves Extract	*Staphylococcus Aureus*	8.30	9.40	10.90
10	Cold Water Dried Leaves Extract		8.60	9.90	11.50
11	Hot Water Fresh Leaves Extract		7.00	8.30	9.80
12	Hot Water Dried Leaves Extract		7.50	8.90	10.20

Table 2: Inhibition effect of cold and hot water extract of lemon grass extract against *Escherichia coli*, *Pseudomonas aeruginosa* and *vibrio cholera.*(Fig-2)

S. No	Lemongrass	Organism	Zone of Inhibition (Mm)		
			Concentration in Ppm		
			250	500	1000
1	Cold Water Fresh Leaves Extract	*Escherichia Coli*	3.50	4.00	5.10
2	Cold Water Dried Leaves Extract		3.60	4.50	5.50
3	Hot Water Fresh Leaves Extract		2.90	3.70	4.20
4	Hot Water Dried Leaves Extract		3.20	3.40	4.70
5	Cold Water Fresh Leaves Extract	*Pseudomonas Aeruginosa*	2.50	3.20	3.70
6	Cold Water Dried Leaves Extract		2.60	3.50	3.90
7	Hot Water Fresh Leaves Extract		2.00	3.00	3.20
8	Hot Water Dried Leaves Extract		2.30	2.50	3.60
9	Cold Water Fresh Leaves Extract	*Vibrio Cholera*	4.90	5,80	6.90
10	Cold Water Dried Leaves Extract		5.10	6.00	7.00
11	Hot Water Fresh Leaves Extract		4.50	5.20	6.00
12	Hot Water Dried Leaves Extract		4.70	5.70	6.50

Table 3: Inhibition effect of Ethanol and methanol extract of lemon grass extract against *Bacillus vallismortis, Lysinibacillus macroides* and *Staphylococcus aureus* (Fig-1)

S. No	Lemongrass	Organism	Zone of Inhibition (Mm)		
			Concentration in Ppm		
			250	500	1000
1	Ethanol Fresh Leaves Extract	*Bacillus Vallismortis*	*3.90*	*5.10*	*6.80*
2	Ethanol Dried Leaves Extract		5.10	6.30	7.40
3	Methanol Fresh Leaves Extract		5.30	6.20	7.10
4	Methanol Dried Leaves Extract		5.80	6.70	8.00
5	Ethanol Fresh Leaves Extract	*Lysinibacillus Macroides*	4.70	5.40	6.50
6	Ethanol Dried Leaves Extract		4.70	6.90	7.50
7	Methanol Fresh Leaves Extract		*3.10*	*5.20*	*6.30*
8	Methanol Dried Leaves Extract		6.00	6.80	7.40
9	Ethanol Fresh Leaves Extract	*Staphylococcus Aureus*	*8.00*	*9.40*	*11.80*
10	Ethanol Dried Leaves Extract		8.40	9.80	12.50
11	Methanol Fresh Leaves Extract		8.10	9.40	10.80
12	Methanol Dried Leaves Extract		9.50	10.30	11.00

Table 4: Inhibition effect of Ethanol and methanol extract of lemon grass extract against *Escherichia coli*, *Pseudomonas aeruginosa* and *vibrio cholera.*

S. No	Lemongrass	Organism	Zone of Inhibition (Mm)		
			Concentration in Ppm		
			250	500	1000
1	Ethanol Fresh Leaves Extract	*Escherichia Coli*	3.10	3.80	4.50
2	Ethanol Dried Leaves Extract		3.70	4.20	5.40
3	Methanol Fresh Leaves Extract		3.80	4.60	5.30
4	Methanol Dried Leaves Extract		4.00	4.90	6.30
5	Ethanol Fresh Leaves Extract	*Pseudomonas Aeruginosa*	3.60	4.10	4.80
6	Ethanol Dried Leaves Extract		3.40	4.60	5.10
7	Methanol Fresh Leaves Extract		2.20	2.70	3.70
8	Methanol Dried Leaves Extract		2.80	3.20	3.80
9	Ethanol Fresh Leaves Extract	*Vibrio Cholera*	5.80	6.80	7.60
10	Ethanol Dried Leaves Extract		6.00	6.90	8.10
11	Methanol Fresh Leaves Extract		4.40	5.40	6.10
12	Methanol Dried Leaves Extract		4.80	5.60	6.70

Among the six microorganisms tested *staphylococcus aureus* recorded a maximum zone of inhibition 12.50mm in Ethanol dried leaves extract followed by Ethanol fresh leaves extract and least zone of inhibition was observed in hot water fresh leaves extract of *Pseudomonas aeruginosa* 2.00mm (Table-1).

Fig 1: *Staphylococcus aureus* at three different concentrations

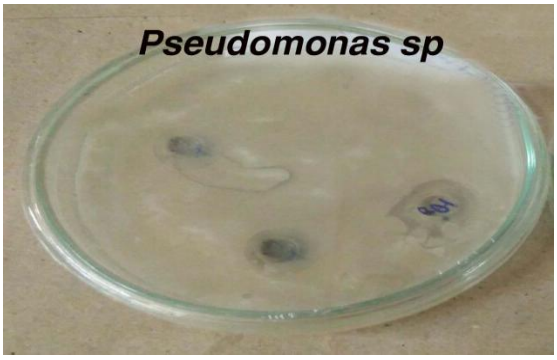

Fig 2: *Pseudomonas aeruginosa* at three different concentrations

Discussion

Inhibition effect of cold and hot water extract of lemongrass against different microorganisms

Among the six bacterial cultures were tested with cold water, hot water and different solvents like ethanol and methanol extracts of lemongrass the maximum zone of inhibition was observed in *staphylococcus aureus* followed by *Lysinibacillus macroides, Bacillus vallismortis, vibrio cholera, Escherichia coli,* and *pseudomonas aeruginosa* in cold water, hot water and different solvents like ethanol and methanol extracts of dried leaves followed by fresh leaves extract. In our investigation highest zone of inhibition were found in dried leaves cold water extract of in accordance with the results obtaining by Dave jyoti *et al.,* (2015) [6]. The dried leaves cold water extract of *Cymbopogon citrates* contains, alkaloids, acids, essential oils, steroids, tannins etc. and herbel activity depends upon the solubility of these comounds in the in various solvents (Sooad al-Daiham *et al*., 2013). The phyto chemical constituents like flavonoids, alkaloids, and tannins of plants have often considered as antimicrobial activities (Gopinath *et al*., 2013; Jayashree *et al*., 2013) [10]. This result is interesting because in the traditional method of treating a bacterial infection, decoction of the plant parts or boiling the plant water is employed. Whereas according to present study, the hot water fresh leaves extract also shown a better antimicrobial activity, in accordance with the results obtained by Sneh lata *et al*., (2014) [20]

Conclusion

On the basis of above results it can be stated lemongrass leaf extracts is highly effective in controlling different types of pathogenic microorganisms further studies is needed to develop and evaluate the other properties of lemon grass which can be used in the medicinal applications.

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