In vitro antimicrobial activity of Lemon grass oil by MIC and agar well diffusion method

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Abstract-Food preservatives are used to increase the shelf life of food and to maintain the quality for longer time. In the last scenario no herbal ayurvedic preservative had been considered with respect to the use of chemical preservative. Due to increasing demands for natural and preservative free compounds promoted an idea of the replacement of synthetic preservatives with essential oils of antimicrobial properties.

Essential oils from medicinal plants are potential source of novel antimicrobial compounds especially against food spoilage pathogens. The aim of this project was to study antimicrobial activity of essential oil from Lemongrass (*Cymbopogan citratus)* oil against food spoilage organisms *Escherichia coli*, *Micrococcus luteus, Staphylococcus aureus* and *Bacillus cereus* and fungus *Aspergillus brasilliensis, Candida albicans, Chaetomium globosum* and *Penicillium funiculosum* using agar well diffusion method. The antimicrobial activity was evaluated by measuring the zone of inhibition. The oil at 30% concentration completely/partially inhibited the growth of food spoilage pathogens. The successful effectiveness of Lemon grass oil could also play a major role in replacing the chemical preservative.

Keywords: Lemon grass oil, antimicrobial activity, Agar well diffusion assay

Introduction

For the past two decades, there has been an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agent . Essential oils are potential sources of novel antimicrobial compounds especially against bacterial pathogens .

The use of natural antibiotics agents are the best alternative to synthetic or chemical antibiotics. It prevents development of antimicrobial resistance in bacteria and fungus and also devoid side defects. The medical world is on an immense requirement to discover novel antibiotics due to wide spread emergence of resistance among microbial pathogens against

currently available antibiotics. However, traditional plants have been proved to be better source for novel antimicrobial drugs. Among the medicinal plants, aromatic herbs are a rich source of biologically active compounds useful both in agriculture and medicine. Edible, medicinal and herbal plants and spices such as oregano, rosemary, thyme, sage, basil, turmeric, ginger, garlic, nutmeg, clove, mace, savoury and fennel have been successfully used either alone or in combination with other preservation methods .

Lemon grass belongs to the section of *Andropogan* called *Cymbopogon* 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. *Cymbopogon citratus* commonly known as lemongrass is an herb which belongs to the grass family of *Poaceae*. It is utilized for its distinct lemon flavour and citrusy aroma. It is a tall, perennial grass which is native to India and tropical regions of Asia. It is a coarse and tufted plant with linear leaves that grows in thick bunches, emerging from a strong base and standing for about 3 meters in height with a meter-wide stretch.

The genus *Cymbopogon* comprises of 55 species of grasses, two of which are referred to as lemongrass. These are *Cymbopogon citratus,* which is famously preferred for culinary use and *Cymbopogon flexuous,* which is used in the manufacturing of fragrances because of its extended shelf life, owing to the low amount of myrcene in that variety. Lemongrass is widely used as an essential ingredient in Asian cuisines because of its sharp lemon flavour. Lemongrass oil, used as a pesticide and preservative, is put on the ancient palm-leaf manuscripts found in India as a preservative. One of the main constituents of the many different species of lemongrass (genus *Cymbopogon*) is citral (3,7-dimethyl-2,6-octadien-1-al). The volatile oil from the roots contains 56.67% longifolene-(V4) and 20.03% selina-6-en-4-ol.

Fig.1 Leaf of lemongrass

Fig.2 Lemongrass oil

Cymbopogon citratus has been cultivated over many years for medicinal purposes in different countries through-out the world. Lemongrass is an aromatic storehouse of essential nutrients providing an array of health benefits. It is a source of essential vitamins such as vitamin A, vitamin B1(thiamine),vitamin B2 (riboflavin), vitamin B3(niacin), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), folate and vitamin C. It also provides essential minerals such aspotassium,calcium, magnesium, phosphorous,manganese, copper, zinc and iron, which are required for the healthy functioning of the human body. It offers no harmful cholesterol or fats. 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. It can be used in cleaning wounds and treatment of skin diseases such as ringworm. It can also be used in food poisoning, staphylococcal infections, and other common infections.

The oil has been found to posses bactericidal and anti fungal properties, which is comparable to penicillin in its effectiveness .

Antimicrobial activity of the Cymbopogon citratus (lemongrass) essential oil against food-borne pathogens was determined to investigate its potential for reducing microbial population of food products. Previous reports suggest that lemongrass essential oil is a safe natural flavour complex, preservative, and food spoilage inhibitor capable of reducing the risk of diseases associated with contaminated products.

The aim and objectives of this work is to determine therapeutic potentials of the plant extract on some pathogenic microorganisms. *Escherichia coli and* three strain of Gram-positive bacteria namely; *Micrococcus luteus, Bacillus cereus Staphylococcus aureus and against some fungus namely; Candida albicans* (ATCC No-3471), *Aspergillus brasilliensis, Penicillium funiculosum* and *Chaetomium globosum*. 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 Lemon grass oil.

Materials and Method

Procurement of lemongrass oil

The essential oil of lemongrass was procured from SIGMAALDRICH, India.

Test organisms

The test organisms used in this study was taken from National Institute of Chemical Laboratory (NCIM), Pune. The organisms used in the study were- *Escherichia coli, Micrococcus luteus, Staphylococcus aureus, Bacillus cereus, Aspergillus brasilliensis, Candida albicans, Chaetomium globosum* and *Penicillium funiculosum*

Propagation and maintenance of test organisms

The bacterial test organisms were streaked on the Nutrient Agar slants and were incubated overnight at 37˚C and fungus test organisms were streaked on the Chloramphenicol Yeast Glucose Agar slants and were incubated for 5 days at 22˚C.

Preparation of concentrations of lemongrass oil

The 30% concentrations (v/v) of lemongrass oil were prepared aseptically in sterile tween-80.

Antimicrobial activity

The testing of the bacterial and fungal cultures for the inhibitory effect of essential oil of lemon grass for 30% concentration was performed by using agar well diffusion method.

Agar Well Diffusion Assay (Zone of Inhibition Evaluation)

Antibiotic susceptibility and resistance were evaluated by agar well diffusion assay. 0.5 McFarland density of bacterial and fungal culture was adjusted using normal saline (0.85% NaCl) using densitometer to get bacterial and fungal population of 1.0 x 10^8 cfu/ml. 100µl of each of the adjusted cultures were mixed into separate 100 ml of sterile, molten, cool MHA (Mueller- Hinton agar), mixed well and poured into sterile Petri plates. These were allowed to solidify and then individual plates were marked for each individual isolates. Each plate was punched to make wells of 6 mm diameter with the help of sterile cork borer at different sites of the plates. 100 µl of respective essential oil were pipette out into the well in assay plates. Bacterial plates were incubated overnight at 37°C and fungal plates were incubated for 5 days at 22˚C. Following incubation, petri-plates were observed for the inhibition zones, diameters of which were measured by using Vernier Callipers.

The determination of MIC of the essential oil of lemongrass on the test bacterial strain was done using broth dilution method as explained by Hammer *et al.* with different concentrations of oil . The range of MIC taken was 1 µg/mL to 128 µg/mL. The cultures of the test strains were prepared by inoculating the test strain in sterilized test tube containing 5 mL nutrient broth. The tubes were incubated 24 hours for bacteria and 48 hours for fungus at 37°Cand 25°C respectively. The MIC was defined as the lowest concentration of the test compound to inhibit the growth of microorganisms and the MBC was defined as the lowest concentration of the test compound to kill the microorganisms. The test tubes containing 10 mL of sterilized tryptic soy broth (TSB) with 0.5% (v/v) tween-80 were inoculated with different concentration of lemon grass oil ranging from 1-256 µ mL). TSB with 0.5% (v/v) tween 80 without oil was used as positive growth control. An aliquot of bacterial suspension (30µL) to each tube was added uniformly. The tubes were incubated for 24 hours (bacteria) 48 hours (fungus). The tubes were observed for turbidity after the period of incubation. The lowest concentration at which no visible growth occurs in either culture tubes was taken as MIC. Then the tubes showing no increased in the turbidity at each time interval hours were streaked on nutrient agar plates

Results and Discussion

Lemon grass oil possesses a promising antimicrobial activity against the test organisms. The results obtained from the Agar diffusion assay and broth dilution method support the general indication that test organisms used are sensitive to the oil. Similar observations were made by Onawunmi and Ongulana and Cimanga *et al*. *P. aeruginosa* were found resistant at all the concentration of lemongrass oil including neat. Similar results were reported by Pereira et al, Marta War *et al* Torris *et al* , Alam *et al*, and Onawunmi *et al*. Lemongrass oil at concentration of 30% was found the most effective essential oil against all tested microorganisms.

All the selected microorganisms showed difference in their sensitivity at three different concentrations (5%, 15% and 30%). The strongest inhibition activity was observed in Lemongrass essential oil against *staphylococcus* and *Candida albicans*.

The activity of lemongrass oil (at 30%) was found in the series of *C. globosum* > *C. albicans* > *A. brasilliensis* > *S. aureus* > *B. cereus* > *E. coli* > *M. luteus* > *P. funiculosum*.

Table-1 Antimicrobial activity of lemon grass oil against various selected microorganisms

Test Organisms	Zone of Inhibition (mm)- Lemongrass oil		
	5%	15%	30%
Escherichia coli	13.76	19.20	23.14
Micrococcus luteus	13.44	17.45	20.47
Staphylococcus aureus	16.52	20.21	28.15
Bacillus cereus	14.56	20.48	26.20
Aspergillus brasilliensis	17.49	21.25	30.12
Candida albicans	18.88	23.45	31.25
Chaetomium globosum	19.25	24.60	34.25
Penicillium funiculosum	9.68	12.22	15.17

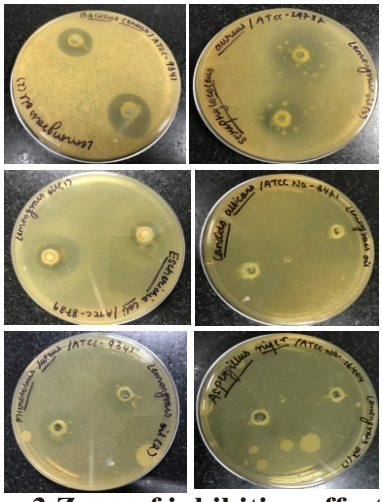

Fig. 3 Zone of inhibition effects of lemongrass essential oil

Table-2 MIC (Minimum Inhibitory Concentration) of lemongrass oil against various selected microorganisms

Test Organisms	MIC-Lemongrass oil (µg/mL)		
	5%	15%	30%
Escherichia coli	128	32	16
Micrococcus luteus	128	64	16
Staphylococcus aureus	64	16	8
Bacillus cereus	64	16	8
Aspergillus brasilliensis	64	16	8
Candida albicans	64	32	16
Chaetomium globosum	32	16	8
Penicillium funiculosum	16	8	4

Conclusion

The medical world is on an immense requirement to discover novel antibiotics due to wide spread emergence of resistance among microbial pathogens against currently available antibiotics. However, traditional plants have been proved to be better source for novel antimicrobial drugs.

The major concern is extensive uses of chemical food preservatives. The present scenario shows overuse of chemical preservatives in food products, this may not only lose the natural property of food but also affect the consumer's health.

Due to increasing demands for natural and preservative free compounds promoted an idea of the replacement of synthetic preservatives with essential oils of antimicrobial properties

The remarkable effect of Lemon grass oil on various test organisms are demonstrable indications of the oil as an antimicrobial agent. Thus, a study had been carried out to show that an herbal product Lemongrass oil is much potential against food spoilage organisms.

The study also recommends a new innovation and a challenging target in the food sector, vanishing the chemical substitutes, also protecting the naturality of the food product.

References

Bonjar, G. and Farrokhi, P. R. Antibacillus activity of some plant used in traditional medicine of Iran. *Niger J Nat Prod Med*. 2004. 8:34-9.

Prabuseenivasan, S., Jayakumar M.and Ignacimuthu, S. *In vitro* antibacterial activity of some plant essential oils. *BM Complementary Altern. Med*., 2006,6:39.

Mathela, C. S. "Allelochemicals in medicinal and aromatic plants," in *Allelopathy in Agriculture and Forestry*, eds Narwal S. S., Tauro P., editors. Jodhper: Scientific Publishers,1991; 213-228.

Cutler H. G. and Cutler, S. J. *Biologically Active Natural Products: Agrochemicals*. Boca Raton, FL: CRC Press, 1999

Tajkarimi, M.M.; Ibrahim, S.A. and Cliver, D.O. Antimicrobial herb and spice compounds in food. *Food Control*., 2010, 21:1199–2121.

McGuffin, M; Hobbs, C. and Upton, R. (American herbal products association botanical safety handbook). Boca Raton: CRC press,1997.

Lutterodt, G. D.; Ismail, A.; Basheer, R. H. and Baharudin, H. M. Antimicrobial effects of Psidium guajava extracts as one mechanism of its antidiarrhoeal action. Malay. *J. Med. Sci*., 1999, 6(2): 17-20.

Onawunmi, GO; Yesiak, WAB. and Ongulana EO. Antibacterial constituent in the essential oil of *Cymbopogon citratus*. *J Ethanopharmocol*., 1984, 12 (3): 279-86.

Cimanga, K.; Tona, L.; Apers, S.; Bruyne, Tde.; Hermans, N.; Totte, J, et al. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. *J Ethanopharmacol*., 2002, 79(2): 213-20.

Pereira, R.S.; Sumita, T.C.; Furlan, M.R.; Jorge, AOC. and Ueno, M. Antibacterial activity of essential oils on microorganisms isolated from urinary tract infections. *Revista de Saude Publica*, 2004,38(2): 326-8.

Marta, War ON.; Majra, Rajra Rodriguez, J.; Gaston Garcia, S. and Celia Lierene, R. Antimicrobial activity of the essential oil and cream of Cymbopogon citratus (DC.) stapf. *Revcubana Plt Med*., 2004,2: 44-7.

Torris, R.C.; Ontengco, D.C.; Balgos, N.S.; Villanuva, M.A.; Lanto, E.A.; Cruz, M.S. et al. (Antibacterial essential oils from some Philippine plants). Laguna: The Philippine society for Microbiol Inc; 2002, 219-20.

Alam, K.; Agua, T.; Maven, H.; Taie, R.; Rao, K.S. and Burrows, I.Preliminary screening of seaweeds, sea grass and lemongrass oil from Papua New Guinea for antimicrobial and antifungal activity. *Inter J Pharmacognosy*, 1994, 32 (4): 396-9.