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Potential Control of food spoilage Bacteria and Fungi by essential oil of Cymbopogan Citratus

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ABSTRACT: Food preservatives are used to accelerate stability of food and to establish the originality of food in terms of longer duration of 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 novel source of antimicrobial compounds especially against food spoilage isolates. The objective was to study inhibitory potential of essential oil from Cymbopogan citratus (lemongrass oil) oil against food spoilage organisms such as Escherichia coli ATCC 8739, Micrococcus luteus ATCC 9341, Staphylococcus aureus ATCC 6538 and Bacillus cereus ATCC 11778 and fungus Aspergillus niger ATCC 16404, Candida albicans ATCC 10231, Chaetomium globosum ATCC 6205 and Penicillium funiculosum ATCC 66829 using zone inhibition and MIC (minimum inhibitory concentration). The antimicrobial activity was determined, and zone of inhibition were observed. The oil at 30% concentration completely/partially inhibited the growth of food spoilage pathogens. The successful effectiveness of Lemongrass oil could also play a major role in replacing the chemical preservative.

Keywords: Lemongrass oil, Food spoilage Bacteria, Food spoilage Fungi, Antimicrobial activity, Cymbopogan Citratus

I. INTRODUCTION

For the past two decades, the interest of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agent has been enlighted[1]. Essential oils are potential sources of novel antimicrobial compounds especially against bacterial pathogens[2].

The use of natural antibiotics agents is the best alternative to synthetic or chemical antibiotics. It prevents development of antimicrobial resistance in bacteria and fungus and devoid side defects. The medical world is on an immense requirement to discover novel antibiotics due to widespread 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[3,4]. 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[5].

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.

Lemongrass, barbed wire grass, silky heads, Cochin grass, Malabar grass, oily heads or fever grass 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

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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. In ancient and even today lemongrass oil has been used as a pesticide and preservative, is put on the ancient palm-leaf manuscripts found in India as a preservative. The main constituents of 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.

Cymbopogon citratus has been cultivated over years and years for medicinal purposes in different countries of 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, thiamine (Vitamin B1), Riboflavin (Vitamin B2), Niacin (Vitamin B3),Pantothenic acid (Vitamin B5), Pyridoxine (Vitamin B6), Folate and Vitamin C. It also provides essential minerals such as Potassium, 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 to cure 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[5]. 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 possess bactericidal and antifungal properties, which is comparable to penicillin in its effectiveness[6].

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, Aspergillus brasilliensis, Penicillium funiculosum and Chaetomium globosum. Hence the present study was carried out to evaluate the antimicrobial activity of Lemon grass oil.

II. MATERIALS AND METHOD

Procurement of lemongrass oil

The essential oil of lemongrass was procured from SIGMA ALDRICH, India (CAS 8007-02-1- Sigma-Aldrich Chemical Pvt Limited) Table-1; Fig. 1.

Test organisms

The challenge microorganisms used in this study was procured from National Institute of Chemical Laboratory (NCIM), Pune. The organisms used in the study were- Escherichia coli ATCC 8739, Micrococcus luteus ATCC 9341, Staphylococcus aureus ATCC 6538, Bacillus cereus ATCC 11778, Aspergillus niger ATCC 16404, Candida albicans ATCC 10231, Chaetomium globosum ATCC 6205 and Penicillium funiculosum ATCC 66829.

Propagation and maintenance of test organisms:

The bacterial cultures were streaked on the NA (Nutrient Agar) slants and incubated for 24 hours at 37˚C. The fungal cultures were streaked on CYGA (Chloramphenicol Yeast Glucose Agar) slants and were incubated for 5 days at 22˚C.

Preparation of different lemon grass oil concentrations:

Three concentrations 5%, 15% and 30% (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 done by using agar well diffusion method.

Antimicrobial activity of the concentrations of lemongrass oil were evaluated zone of inhibition by agar well diffusion assay[7]. The bacterial and fungal cultures were set at 0.5 McFarland with the help of densitometer in normal saline (0.85% NaCl) using to achieve a concentration of 1.0×10^8 cfu/ml. A 100µl of each of the set culture concentration was mixed into individual 100 ml of sterile, molten, cool MHA (Mueller- Hinton agar), mixed well and poured into Petri plates. After solidification plates were marked

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with proper culture and concentration of lemongrass oil. With the help of sterile stainless-steel cork borer 6 mm diameter wells were made onto the MHA plates at marked places. A 100 µl of lemon grass oil concentration was were pipette out into the well in assay plates. For bacteria incubation for 24 hours at 37^{o}C and fungal for 5 days at 22˚C was done. After incubation, diameter of zone of inhibition was measured in mm by using Vernier Callipers.

Assessment of Minimum Inhibitory Concentration

The MIC (minimum inhibitory concentration) of the essential oil of lemongrass was determined in the range of 1 µg/mL to 256 µg/mL as per National Committee for Clinical Laboratory Standards (NCCLS). The test tubes containing 10 mL of sterilized tryptic soy broth (TSB) with 0.5% (v/v) polysorbate 20 were followed by inoculation of different concentration of lemon grass oil ranging from 1 µg/mL to 256 µg/mL). TSB with 0.5% (v/v) polysorbate 20 without oil was used as positive growth control. An aliquot of bacterial and fungal suspension (30µL) to each tube was added uniformly. The tubes were incubated for 24 hours (bacteria) 48 hours (fungus). The tubes were viewed for turbidity after the period of incubation. The minimum concentration of lemongrass oil at which no visible growth observed, those 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

III. RESULTS AND DICUSSION

Lemon grass oil possesses antimicrobial activity against the challenged organisms. The results obtained from the agar diffusion assay and broth dilution method support the study and showed that test organisms used are sensitive to the oil. Similar observations were made by Onawunmi and Ongulana[8] and Cimanga et al[9]. P. aeruginosa were found resistant at all the concentration of lemongrass oil including neat. Similar results were reported by Pereira et al[10], Marta War et al[11]Torris et al[12], Alam et al[13], and Onawunmi et al[14]. Lemongrass oil at concentration of 30% was found the most effective essential oil against all tested microorganisms.

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 Bacillus cereus and Candida albicans (Table-2 &3; Fig-4 & 7)

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

Fig. 1: Leaf of lemongrass (Cymbopogan Citratus)

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Fig 2: Essential oil of lemongrass (Cymbopogan Citratus)

Fig 3: Zone of inhibition effects of lemongrass essential oil against Staphylococcus aureus ATCC 6538

Fig 4: Zone of inhibition effects of lemongrass essential oil against Bacillus cereus ATCC 11778

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Fig 5: Zone of inhibition effects of lemongrass essential oil against Escherichia coli ATCC 8739

Fig 6: Zone of inhibition effects of lemongrass essential oil against Micrococcus luteus ATCC 9341

Fig 7: Zone of inhibition effects of lemongrass essential oil against Candida albicans ATCC 10231

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Fig 8: Zone of inhibition effects of lemongrass essential oil against Aspergillus niger ATCC 16404

Table-1 Chemical composition of essential oil of Lemongrass

Compounds	Concentration (%)
Geranial, Neral and Myrcene	20-30
Citral α and Citral β	70-80

Table-2 Potential Control of food spoilage Bacteria and Fungi by essential oil of Cymbopogan Citratus

Test Organisms	Strain No.	Inhibition Zone (In mm)		
		5%	15%	30 %
Escherichia coli	ATCC-8739	13.76	19.20	23.14
Micrococcus luteus	ATCC-9341	13.44	17.45	20.47
Staphylococcus aureus	ATCC-6538	16.52	20.21	28.15
Bacillus cereus	ATCC-11778	14.56	20.48	26.20
Aspergillus niger	ATCC-16404	17.49	21.25	30.12
Candida albicans	ATCC-10231	18.88	23.45	31.25
Chaetomium globosum	ATCC-6205	19.25	24.60	34.25
Penicillium funiculosum	ATCC-66829	9.68	12.22	15.17

Zone of inhibition measured in mm. with Vernier callipers (Diameter including well diameter of 6.0mm).

Table-3 MIC (Minimum Inhibitory Concentration) of lemongrass oil against food spoilage Bacteria and Fungi

Test Organisms	Strain No.	MIC- Lemongrass oil (µg/mL)		
		5%	15%	30 %
Escherichia coli	ATCC-8739	128	32	16
Micrococcus luteus	ATCC-9341	128	64	16
Staphylococcus	ATCC-6538	64	16	8

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aureus				
Bacillus cereus	ATCC-11778	64	16	8
Aspergillus niger	ATCC-16404	64	16	8
Candida albicans	ATCC-10231	64	32	16
Chaetomium globosum	ATCC-6205	32	16	8
Penicillium funiculosum	ATCC-66829	16	8	4

IV. CONCLUSION

With the emergence of mysterious and carcinogenic chemical antimicrobial compounds, the world is on threat with these slow poisoning compounds, leading to incurable diseases. India a land of traditional plants has been always been proved to be discovery of 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 an innovation add a challenging target in the food sector, vanishing the chemical substitutes, also protecting the naturality of the food product.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest

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