

**Original Article**

**ANTI-MICROBIAL EFFECTIVENESS OF LEMON GRASS OIL (*CYMBOPOGAN CITRATE*) AGAINST AEROBIC AND ANAEROBIC ORGANISMS**

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**ABSTRACT**

**Objective:** To find the antibacterial effectiveness of lemon grass (*Cymbopogon citrate*) oil against aerobic and anaerobic bacteria.

**Methods:** This is an observational study conducted at Microbiology Clinical laboratory, Department of Microbiology, Saveetha Medical College and Hospital, Chennai. It was done over a period of 3 mo from January to March. The extracts of lemongrass leaves were investigated for its effectiveness against *Staphylococcus aureus* (*S.aureus*), *Escherichia coli* (*E.coli*) and *Clostridium perfringens* (*C.perfringens*) by Disc Diffusion assay.

**Results:** Our study indicates that the extract of lemongrass oil shows antibacterial activity. Among the tested organisms, aerobic organisms were sensitive.

**Conclusion:** This study thus provides insightful knowledge on antibacterial activity that would lead to further development of lemongrass oil for infectious diseases in the future.

**Keywords:** *Cymbopogon Citrate*, Antimicrobial activity, *Staphylococcus aureus*, *Escherichia coli*, *Clostridium perfringens*

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**INTRODUCTION**

The development of bacterial resistance due to misuse and overuse of antibiotics renders the common antimicrobial agents ineffective. This leads to the exploitation of other antimicrobial substances from other sources. Alternatives of developing novel antimicrobial agent from natural sources, such as traditional medicinal plants, spices and herbs need to be explored [1]. *Cymbopogon citrate* and *C. flexuosus* generally known as lemon grass, is an aromatic, evergreen, perennial herb cultivated in tropics and subtropics known for its medicinal purposes all over the world. It is known for its strong lemon taste. It is used widely as an essential ingredient in Asian cuisines. It has high efficacy in combating infection of stomach, prevents peptic ulcer disease, and stimulates digestion and excretion [2]. Lemongrass also holds antidepressant, antioxidant, antiseptic, antispasmodic, bactericidal, fungicidal, nervine and sedative properties. Abundance of citral and essential oil components i. e Geranial, Myrcene, 6-Methylhept-5-en-22-one contributes to the anti-microbial activity against a series of micro-organisms [3].

The present study was undertaken to find out the antimicrobial activity of lemongrass oil to treat infections caused by *Staphylococcus aureus*, *Escherichia coli* and *Clostridium perfringens*.

**MATERIALS AND METHODS**

This is an observational study conducted at Clinical Microbiology laboratory, Department of Microbiology, Saveetha Medical College Chennai. Test Strains were isolated from the patients over a period of 3 mo from January to March were evaluated and then was used in this study.

**Extraction procedure**

Lemongrass leaves were collected, washed using clean water and dried at room temperature for 4 d. The dried plants were kept in a sealed plastic bag and kept in ambient temperature in a dark room. The leaves were then milled to increase the extraction yield. The plant material was soaked in distilled water for 30min before the extraction procedure.

N hexane is used as analytical reagent and solvent extraction method was followed. 150g of the dry sample of lemongrass was placed in a 1 litre clean flat bottom flask. 500 ml of N-hexane solvent were poured into the flask. The flask and content were allowed to stand for 36 h. 200 ml of Ethanol was added to it and the mixture was then transferred to 500 ml separating funnel and separated by a process called liquid/liquid separation process. The content of the separating funnel was allowed to come to equilibrium, which separated into two layers depending on their different density. The lower Ethanol extract and the upper Hexane layer were collected into two separate 250 ml beaker and were placed in a water bath at 78 °C. This was done to remove the ethanol leaving only the natural essential oil [4].

**Isolation of *Staphylococcus aureus***

The samples collected are inoculated on mannitol salt agar and is incubated overnight. The *Staphylococcus* colonies identified by their bright yellow colonies were then subjected to coagulase test, gram staining and catalase test. They were then subjected to various morphological and biochemical tests, including oxidase test, Motility test, indole, ornithine, Methyl Red, Voges-Proskauer, lipid hydrolysis, starch hydrolysis and gelatinase test [5].

**Isolation of *Escherichia coli***

The sample suspension is inoculated into MacConkey agar and is incubated for 18-24 hour at 37 °C. *Escherichia coli* colonies are identified tentatively by their characteristic pink, round medium-sized colonies. A loop of the isolates was inoculated into nutrient broth for further investigation [6].

**Isolation of *Clostridium perfringens***

Samples were inoculated in cooked meat medium and incubated anaerobically at 37 °C for 24h in anaerobic jar. Samples were streaked on Sulphite polymyxinsulphadiazine SPS agar plates. They were incubated anaerobically and the colonies were gram stained and subcultured on Brain Heart Infusion (BHI) agar plates. This was done until they were free of contaminating bacteria. The pure colonies were streaked on 5% sheep blood agar and egg yolk agar plates. They were incubated anaerobically for 24 hour. *Clostridium perfringens* colonies were identified by their characteristic double

zone of hemolysis around them on blood agar and zone of opalescence around the colonies on egg yolk agar. For further use, they were then preserved at -80 °C as glycerol stock (25% glycerol in BHI broth [7].

#### Disc-diffusion assay

The agar diffusion assay was performed with Muller Hinton agar according to the Kirby-Bauer disc diffusion method as per the CLSI guidelines. The strains of *Staphylococcus aureus*, *Escherichia coli* and *Clostridium perfringens* were seeded in Muller Hinton agar and incubated in 37 °C shaking incubator overnight. Incubated strains

were mixed with warm Muller Hinton agar and poured into petri dish and cooled to room temperature. 1%, 5% and 10% of 60 µl of lemongrass was loaded on sterilised paper disc (8 mm). It was placed upside down on the surface of MHA and incited at 37 °C for 24 h. The diameter of the zone was measured after incubation [8].

#### RESULTS

The extract from the leaves of lemon grass shows the inhibitory effect against aerobic organisms like *Staphylococcus aureus* and *Escherichia coli* showing 24 mm of the zone of inhibition and no inhibitory effect against anaerobic organism *Clostridium perfringens* were observed.



Fig.1: *Clostridium perfringens* (anaerobic organism)



Fig.2: *Staphylococcus aureus* (aerobic organism)

#### DISCUSSION

Development of plant essential oils as natural antimicrobial agents has shown enormous positive potential. The main components of antimicrobial activity of essential oil that are volatile could be developed for agricultural applications. Natural products are usually less toxic when compared to synthetic chemicals. They are also natural products are easily bio-degraded and therefore harmful effects on the environment and public health are lower [9,10]. The results obtained indicated that aerobic organisms are more sensitive than anaerobic organisms. *Clostridium perfringens* were found resistant at all concentrations. For antibacterial treatment against antibiotic-resistant bacterial infections, lemongrass oil in combination with antibiotics could be of potential use. Also, most plant extracts have an inhibition effect on gram-positive organisms than gram-negative organisms. This inhibition effect has been related to active components [11-13].

#### CONCLUSION

Lemongrass essential oil has the potential to be used as an antimicrobial treatment against aerobic organisms. This study thus provides an insightful knowledge on antibacterial activity that would lead to further development of lemongrass oil for infectious diseases in future.

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Nil

#### AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

#### CONFLICT OF INTERESTS

Declared none

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