



ANTIBACTERIAL STUDIES OF ESSENTIAL OIL FROM THE FRESH LEAF OF LEMON GRASS *CYMBOPOGON CITRATUS*

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ABSTRACT

Cymbopogon citratus, belongs to Gramineae family. This study used a microwave-assisted hydro-distillation process to extract lemongrass essential oil and examined its antimicrobial qualities. Physical characteristics of the essential oils included a yellow color, a yield percentage of 4.67%, solubility in trichloromethane, and a lemony aroma. The disc diffusion method was used to assess the oil's effectiveness. *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Staphylococcus aureus*, and *Escherichia coli* were all susceptible to the oil's concentration-dependent antibacterial qualities. 5.72 mmL/disc was the oil's most effective concentration against *E. coli*, while 1.43 mmL/disc was its least effective. The zone of inhibition shrank as the concentration of oil per disc dropped, indicating that the oil's activity against all species was concentration-dependent. At concentrations of 5.72 mmL/disc, 2.86 mmL/disc, and 1.43 mmL/disc, respectively, the zone of inhibition for *E. coli* was 24, 11.3, and 7.7 mm. For other creatures, the pattern is the same. *Pseudomonas aeruginosa* had the smallest zone of inhibition, measuring 7.0, 7.0, and 5.7 mm at concentrations of 5.72 mmL/disc, 2.86 mmL/disc, and 1.43 mmL/disc, respectively. *Staphylococcus aureus* was 13.3, 10.3, and 9.0 mm at concentrations of 5.72 mmL/disc, 2.86 mmL/disc, and 1.43 mmL/disc, while *Klebsiella oxytoca* was 11.3, 9.7, and 9.0 mm at concentrations of 5.72 mmL/disc, 2.86 mmL/disc, and 1.43 mmL/disc, respectively. These results imply that lemongrass essential oil may be a viable natural substitute for synthetic antibiotics, with potential uses in medical and food preservation. It is advised that additional bioassays be conducted and contrasted with the results obtained from alternative extraction techniques.

Keywords: Lemon grass, Essential oil, Organisms, Bacteria, Zone of inhibition

INTRODUCTION

Cymbopogon citratus, commonly known as lemongrass, as shown in fig. 1, is a member of the *Gramineae* family. The term "lemongrass" is derived from its distinctive lemon-like aroma, which is primarily attributed to citral, a cyclic monoterpene compound (Uraku, 2015). This herb is well known for its wide range of phyto-constituents, which include alkaloids, flavonoids, tannins, and several types of essential oils. The diverse pharmacological actions of the plant are mostly attributed to these secondary metabolites. The Greek words "kymbe," which means "boat," and "pogon," which means "beard," are the origin of the scientific name *Cymbopogon*, which reflects the look of the plant (Uraku, 2015). Originally native to South India and Sri Lanka, *Cymbopogon citratus* has now been extensively cultivated across tropical regions of America and Asia (Uraku, 2015). Extracts from the various plant parts (leaves, stem bark, and roots) of various higher plants are used in herbal medicine production (Sofowora *et al.*, 2013). Plant extracts are given ordinarily or as concoctions for the treatment of various ailments. In reality, more than 75% of the world's population depends on these various forms of concoctions and herbal decoctions for the treatment of various infections (Robenson and Zhang, 2011).

Even among the literate in urban areas, plants are becoming more and more accepted as natural sources of medicinal agents. This is likely because many modern medications used to treat infections like typhoid fever, gonorrhoea, and tuberculosis are becoming less effective, various bacteria are

becoming more resistant to antibiotics, and the cost of prescription medications for maintaining one's health is rising (Doughari *et al.*, 2009; Dutta *et al.*, 2013; Grant & Mierzejewski, 2023).

Masyita *et al.*, (2022) claimed that essential oils are volatile, concentrated liquids that are extracted from different plant components and are usually recognized by their pleasing scents. The plants from which they are harvested give them their names. Despite being frequently linked to their scent, they can also contain both non-aromatic and aromatic substances (Rios, 2016). Scott (2005) shows that essential oils were used for medicinal, cosmetic, religious, and embalming purposes in ancient Egypt, the first known use of essential oils. The Chinese were using herbal and aromatic plant extracts for therapeutic purposes at the same time, and Ayurvedic medicine in India eventually adopted similar methods. Monks continued to employ medicinal herbs during the Dark Ages, using essential oils' antibacterial and insecticidal qualities to treat wounds and control parasite diseases (Scott, 2005).

Studies have highlighted the biological activities of volatile oils, including their larvicidal effects. For instance, volatile oils extracted from plants like *Solenostemon monostachyus* (Atiko *et al.*, 2016) and *Hyptis spirigera* (Yohanna *et al.*, 2021) have shown activity against the *Anopheles gambiae* larvae, demonstrating effectiveness at relatively low concentrations (23.44 to 53.87 µg/mL) (Morais *et al.*, 2007). Essential oils' antimicrobial qualities are especially important in the food, pharmaceutical, and cosmetic industries. Essential

oils offer a good substitute as natural additions in light of the growing antibiotic resistance (Chavez-Gonzalez et al., 2016). Essential oils from thyme, oregano, lavender, and lemongrass have demonstrated strong antimicrobial activity against *Klebsiella pneumoniae*, *Escherichia coli*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* (Man et al., 2019). Similarly, essential oils from tea tree, rosemary, and cassia have shown broad-spectrum antimicrobial effects against a range of pathogens including *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Mycobacterium smegmatis*, *Streptococcus pyogenes*, methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bordetella bronchiseptica*, antibiotic-resistant *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Candida albicans* (Abers et al., 2021). *Cymbopogon citratus* is well known for its medicinal properties in traditional medicine as well as its uses as a flavoring and scent. Its anticonvulsant, antispasmodic, hypotensive, analgesic, antiemetic, antitussive, antirheumatic, and antiseptic qualities make it valuable. It is also used to treat fevers, gastrointestinal issues, and neurological conditions (Shah et al., 2011).

Recent studies have further elucidated the pharmacological potential of *Cymbopogon citratus*. Mukherjee et al. (2024) and Oladeji et al. (2019) have identified a significant array of bioactive compounds within the plant, including flavonoids, essential oils, and phenolic compounds. Numerous pharmacological properties, including anti-obesity, antibacterial, antifungal, antinociceptive, antioxidant, anti-diarrheal, and anti-inflammatory effects, are demonstrated by these phytochemicals, underscoring their potential to improve health and offer therapeutic advantages.

Lemongrass (*Cymbopogon citratus*) is a widely used aromatic plant known for its essential oil, which is utilized in various industries including pharmaceuticals, cosmetics, and food flavoring. The extraction of essential oils from lemongrass involves several methods, each influencing the yield and composition of the oil (Okpo and Edeh, 2023).

The purpose of this study is to better understand the potential therapeutic and industrial applications of *Cymbopogon citratus* by examining the antibacterial activity of essential oils extracted from fresh leaves against clinically relevant bacterial strains. The emergence of novel infections and antibiotic resistance presents serious obstacles to global health. Creating novel treatments is essential to resolving this problem. Commonly used in communities, medicinal plants may offer important sources of novel medications to assist address these new health risks.

The extraction and antibacterial properties of lemongrass oil have been extensively studied, but little is known about the extraction and antibacterial properties of essential oil from lemongrass using the microwave-assisted hydro-distillation process. Furthermore, there haven't been many reports on the antibacterial properties of lemongrass essential oil using the disc diffusion method, as far as we can tell.

MATERIALS AND METHODS

Collection, Identification, and Preparation of Plant Material

Lemon grass leaf was collected from Northern-Eastern university, Gombe state Nigeria and was identified by a botanist in the Department of Biological sciences, Northern-Eastern University. The plant was stored under cool conditions to prevent drying (Abdullahi et al., 2024a).

Extraction of Essential Oil by Microwave Assisted Hydro-Distillation

Utilizing a home microwave oven (600 W, Daewoo, China), the essential oils were extracted using a modified version of the Cardoso et al., (2013) method. A 500 mL flask with a flat bottom was filled with 150 g of the sample (dry leaves), and then distilled water was added as the solvent. To enable heating of the herb-water blend and the resulting vapor production, the microwave oven was turned on, and the desired parameters of time (10 minutes) and power (60%) were set. The flask containing the sample was then placed inside the microwave oven and adjusted to a condenser connected to a cold water recirculation system. Although the flask could not be rotated or stirred, the amount of water allowed for sufficient homogeneity and appropriate convection. When the extracted liquid entered a trap, the essential oil was recovered and its volume was calculated. Vapors started to ascend into the flask's neck until they reached the condenser, where they were cooled. The volume of extracted oil per weight of dry herb was used to determine yields as a percentage. Before being analyzed, the oil was refrigerated and put into screw-cap amber test tubes. The procedure was carried out twice more using new leaves, and the following formula was used to determine the yield percentage:

$$\% \text{ yield} = \frac{\text{mass of extract in grams}}{\text{mass of dry plant in grams}} \times 100\%$$

Preparation of Antibacterial Reagents and Apparatus

All the glassware and apparatus used for the antibacterial studies were sterilized for 15 minutes at 121°C in an autoclave and dried in a hot air oven. The dried glassware and apparatus were stored under UV light in a laminar flow chamber to ensure continuous sterility until application (Webber et al., 2022).

Preparation of Stock Solution

A clean and sterilized 10 mL test tube was used. 0.5mL of the essential oil from Lemongrass was measured and dissolved in 3.5 mL trichloromethane and serially diluted to obtain final concentrations 143 mmL/mL, 71.5 mmL/mL and 35.75 mmL/mL (Lucien et al., 2023; Abdullahi et al., 2024b).

Preparation of Discs

The discs were prepared by immersing 25 sterile discs made with filter paper (6 mm) into 0.5 mL of each of 143 mmL/mL, 71.5 mmL/mL and 35.75 mmL/mL resulting in 5.72 mmL, 2.86 mmL and 1.43 mmL per disc respectively. Commercially available antibiotic diffusion discs containing multiple standard antibiotics was used as positive reference standards for all bacterial strains. Negative control was prepared using discs impregnated with trichloromethane. The solutions and discs were prepared according to the method of (Dibala et al., 2014; Webber et al., 2022) with modification.

Source and Maintenance of Organism

The microbes used were both clinical isolates obtained and confirmed at the Research Laboratory of the Department of Medical Microbiology and Parasitology, Federal Teaching Hospital, Gombe, Nigeria. The Gram-negative bacteria isolates are *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella oxytoca*, while *Staphylococcus aureus*, as Gram-positive bacteria. They were maintained and sub-cultured on Nutrient agar (NA) to obtain pure colonies.

Preparation of McFarland Standard

Approximately 0.5 mL of a solution containing 0.0448 M of BaCl₂ (equivalent to 1.17% weight/volume of BaCl₂) was introduced into 99.5 mL of 0.18 M H₂SO₄ (equal to 1% v/v). It was done under continuous stirring. The resulting standard solution was divided into screw-capped bottles, tightly sealed to prevent evaporation, and stored away from light, as described by Andrews, (2001).

Preparation of Mueller-Hinton Agar (MHA)

The MHA was prepared according to the manufacturer's instructions. 38 g MHA was weighed into a sterile conical flask and 1000 cm³ of distilled water was added. The suspension was gently heated with stirring at intervals until the broth dissolves completely. The dissolved medium was tightly sealed with aluminum foil and sterilized in an autoclave for 15 minutes at 121 °C. The sterilized medium was allowed to cool in a laminar flow.

Preparation of the Inoculums

A loop full of the sub-cultured test organism on NA at 37 °C was taken and suspended in normal saline solution (0.85 % w/v) NaCl. The density of the organism suspension was adjusted against a black line until it matched the turbidity of

0.5 M McFarland standard which represents approximately 1.0 x 10⁶ cfu/mL of the bacteria (Yohanna et al., 2021).

Antibacterial Assay by Disc-Diffusion Method

Petri dishes (90 mm) were prepared with 20 ml of a base layer of molten Mueller Hinton agar. Each Petri dish was inoculated with 10 µl of each bacterial suspension (10⁶ CFU/mL). After drying in a sterile hood, 6 mm diameter discs with the essential oil were placed on the medium. Discs containing commercially available multiple standard antibiotics were used as positive control and discs impregnated with trichloromethane were used as a negative control. The plates were incubated for 24 hours at 37 °C. The diameters of the zones of inhibition were measured in millimeters. All tests were performed in triplicate and the bacterial activity was expressed as the mean of inhibition diameters (mm) produced (Dibala, et al., 2014).

RESULTS AND DISCUSSION

The present study involved carrying out the extraction of essential oil by microwave-assisted hydro distillation and determining the antibacterial activities of the oil. The colour, odour, and solubility of the oil were determined by the physical method as presented in Table 1. The percentage yield of the essential oil was also calculated using the formula

Table 1: Physical Parameters of the Essential Oil

Parameter	Characteristics
Colour	Yellow
Odour	Lemon scented
Solubility	Trichloromethane
Yield	4.67%

Antibacterial Activity of Lemongrass Essential Oils

The antibacterial assay for the lemongrass essential oil was carried out using a disc impregnated with different concentrations of the oil, following the method described by Dibala, et al. (2014) with little modification. The inhibition

zone was measured after 24 hours of incubation at 37°C as presented in Table 2. The control experiments were done using trichloromethane and standard commercially available antibiotic diffusion discs containing multiple standard antibiotics used as control as shown in Table 3.

Table 2: Antimicrobial Activity of Lemongrass Essential Oils

Sample	Concentration	Zones of inhibition of organisms in mm			
		Escherichia coli	Staphylococcus aureus	Klebsiella oxytoca	Pseudomonas aeruginosa
Lemongrass	5.72 mmL/disc	24.0	13.3	11.3	7.0
	2.86 mmL/disc	11.3	10.3	9.7	7.0
	1.43 mmL/disc	7.7	9.0	9.0	5.7

The essential oil of lemongrass shows activity against all tested organisms at all concentrations of oil per disc. The oil was most active against *E. coli* at a concentration of 5.72 mmL/disc with a zone of inhibition of 24 mm and the least activity was at 1.43 mmL/disc with a zone of inhibition of 5.7 mm. The activity of the oil against all organisms was concentration-dependent as the zone of inhibition decreased with a decrease in concentration of oil per disc. The zone of inhibition for *E. coli* was 24, 11.3, and 7.7 mm at concentrations of 5.72 mmL/disc, 2.86 mmL/disc and 1.43 mmL/disc respectively. The trend goes for other organisms.

Staphylococcus aureus was 13.3, 10.3 and 9.0 mm at concentrations of 5.72 mmL/disc, 2.86 mmL/disc and 1.43 mmL/disc respectively; *Klebsiella oxytoca* was 11.3, 9.7 and 9.0 mm at concentrations of 5.72 mmL/disc, 2.86 mmL/disc and 1.43 mmL/disc respectively, while *Pseudomonas aeruginosa* had the least zone of inhibition of 7.0, 7.0 and 5.7 mm at concentrations of 5.72 mmL/disc, 2.86 mmL/disc and 1.43 mmL/disc respectively. The activity shown by the oil against tested organisms was in accordance with that reported by Ali et al. (2017) and Shukr and Metwally, (2013).

Table 3: Antimicrobial Activity of Commercially Available Antibiotic Diffusion Discs Containing Multiple Standard Antibiotics Used and Trichloromethane as Control

Antibiotic disc	Concentration per disc µg	Zones of inhibition of organisms in mm			
		Escherichia coli	Staphylococcus aureus	Klebsiella oxytoca	Pseudomonas aeruginosa
AZ	12	16	18	18	19
OFX	10	15	Nd	19	20
PEF	30	17	Nd	20	21
CN	30	19	13	22	19
AU	10	20	Nd	17	18
AM	30	Nd	9	16	17
CPX	30	17	Nd	19	19
SP	10	18	Nd	20	20
CF	10	17	Nd	19	21
LEV	20	19	15	18	19
TCM	-	Nd	Nd	Nd	Nd

Table 3. shows the zone of inhibition of standard antibiotics and solvent used (TCM). The solvent showed no activity against all test organisms while different standard antibiotics showed varying activities. The highest activity shown by the standard disc against *E. coli* was 20 mm by AU which is less than that shown by the essential oil against the same organism. The lowest was 15 mm by OXF while AM showed no activity. The oil showed inhibition of 24 mm 5.72 mmL/disc implying more activity by the oil than the standard drug at this concentration. However, the activity shown by the oil at other concentrations were less than lowest shown by the disc. The highest activity shown by the standard disc against *Staphylococcus aureus*, *Klebsiella oxytoca* and *Pseudomonas aeruginosa* were 18 mm, 22 mm and 21 mm by AZ; CN; and PEF and CF respectively. In all cases the lowest activity exhibited by the standard disc were more than the lowest by the oil except for *Staphylococcus aureus* in which the standard disc and the oil at 1.43 mmL/disc were 9.0

CONCLUSION

Against the investigated bacterial pathogens, the essential oil of lemongrass has strong antibacterial action. Its mechanisms of action may be explained by the suppression of biofilm formation and the rupture of bacterial cell membranes. Its effectiveness and potential as a natural antibiotic substitute are confirmed by comparative research. Its uses in medicine and food preservation highlight its usefulness. However, further bioassays and safety precautions need to be taken.

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