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***In vitro* comparison of antimicrobial activity of methanol and aqueous extracts of *Cymbopogon flexuosus* (Lemongrass)**

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Abstract

Lemongrass, or *Cymbopogon flexuosus*, is a perennial grass of the Poaceae family's genus *Cymbopogon*. The antimicrobial activity of lemon grass leaf methanol and aqueous extracts was evaluated using the Microbroth dilution technique against a variety of bacteria at varying concentrations, including *Salmonella* Typhimurium, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Bacillus subtilis* and *Streptococcus pyogenes*. Apart from *Pseudomonas aeruginosa* and *Proteus mirabilis*, the outcomes showed that the lemongrass methanol extract exhibited effective antibacterial activity against all test species. At a dose of 20.48 mg/ml, the plant's methanol extract had inhibitory effects on *Salmonella* Typhimurium, *Escherichia coli*, and *Staphylococcus aureus* organisms. For *Bacillus subtilis* and *Streptococcus pyogenes*, the lemongrass methanol extract's minimum inhibitory concentration (MIC) was 1.28 mg/ml and 0.32 mg/ml, respectively. The findings indicate that the extract from the leaves of *Cymbopogon flexuosus* (lemongrass) has robust antibacterial properties in opposition to each gram-effective and gram-negative bacteria. Extracts from the leaves of *Cymbopogon flexuosus* have the capability to assist the plant's use as a natural antibacterial agent.

Keywords: Antibacterial, *Cymbopogon flexuosus*, Lemongrass, Methanol extract and Aqueous extract

1. Introduction

Antibiotics are biomolecule chemicals which work by either killing or suppressing the growth of bacteria without harming nearby healthy tissues. One well-known dangerous illness is infectious diseases. The antibacterial compound has been applied in many different industries, including the textile, pharmaceutical, food packaging, and water disinfection sectors (Variyathody *et al.*, 2023) ^[1]. According to Ajijolakewu *et al.*, (2021) ^[2], the main factors driving antibiotic pressures are misuse and overuse, which eventually speeds up the formation of novel resistant strains and/or the mechanisms of resistance in previously vulnerable bacteria. It has been determined that the emergence of antibacterial resistance to widely used antibiotic medications poses a substantial threat to researcher and the pharmaceutical sector. The effects of antibiotic resistance and the pressing need for a remedy make it imperative to look for synthetic antibacterial medicine alternatives and fight the global issue of antibiotic resistance. Today, a wide variety of plant species are employed as traditional medicines to prevent, treat, and cure illnesses as well as to preserve overall health (Mehrotra, 2021) ^[3]. Because of their low toxicity and natural origin, medicinal plants are becoming a more preferred alternative to synthetic antibiotics (Khan *et al.*, 2023) ^[4]. Therefore, compared to synthetic pharmaceuticals, the development of plant-based medications based on ethnomedical leads is comparatively easier and supports the healthy growth of humans, animals and fowl. The World Health Organization (WHO) estimates that over 80% of individuals in underdeveloped nations receive their main medical care from traditional medicine practitioners. (Sharma and Pandey, 2009; Subramoniam, 2014; Biradar, 2015; Rajeshwer, 2015; Ansari, 2016) ^[5, 6, 7, 8, 9]. As a result, scientists from all over the world have focused a great deal of effort on searching medicinal plants for phytochemical compounds with strong antibacterial activity that may be used as an emerging source of resistance against multidrug-resistant bacteria.

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Lemongrass is a well-known medicinal plant that possesses a multitude of active phytoconstituents that have demonstrated beneficial therapeutic effects on both human and animal health. This herb contains a variety of essential oils, flavanoids, tannins, and alkaloids, among other phytoconstituents. According to studies, the oil exhibits bactericidal, anti-bacterial, and anti-fungal qualities that are similar to Penicillin's efficacy (Lutterodt *et al.*, 1999) [10]. *Cymbopogon citrates*, a West Indian plant, and *Cymbopogon flexuosus*, an East Indian plant, are the two species that are identified as lemon lawn, a perennial plant that is often planted in the subtropics and tropics. This herb has a variety of medical uses and is a member of the Poaceae/Gramineae family. *Cymbopogon flexuosus*, also known as the Cochin or Malabar grass, and its sister species, *Cymbopogon citratus*, sometimes known as the West Indian lemon grass, are native to Sri Lanka, India, Thailand, and Burma. These days, both of these species are raised all over (Wannissorn *et al.*, 1996) [11]. Lemongrass oil showed antibacterial effects against a broad range of species, including gram positive and negative bacteria, yeast, and fungus, as reported by Pratap and Khan in 2022 [12]. Therefore, as far as we are aware, there hasn't been any discussion in the literature on using *Cymbopogon flexuosus* extract to combat different harmful bacteria. Therefore, it is necessary to conduct a study on the *in vitro* antibacterial activity of various extracts of *Cymbopogon flexuosus* (lemongrass), since this will be helpful for future investigations into the plant's potential medicinal uses.

2. Materials and Methods

2.1 Reagents

The supplier of iodinitrotetrazolium chloride (INT) was Sigma-Aldrich in St. Louis, USA. Dimethylsulfoxide (DMSO) was acquired from Merck Specialties Private Limited in Mumbai, while enrofloxacin was acquired from Himedia Laboratories Private Limited in Mumbai. From the National Collection of Industrial Microorganisms in Pune, bacterial cultures were acquired.

2.2 Collection of plant material and preparation of extracts

Cymbopogon flexuosus (Lemongrass) leaves were collected from surrounding area of Navsari, Gujarat. The collected plant leaves were properly washed in tap water followed by distilled water. For seven days, the clean plant leaves were air-shaded and kept at room temperature. Using a mixer grinder, the dried plant leaves were ground into a fine powder and put in an airtight container for storage. Methanol and aqueous solvents were used for plant leaves extraction. 100 g of powdered sample was separately soaked in conical flask and each containing methanol and distilled water for 24 hours at room temperature. In order to prepare a methanol extract, the extract was concentrated using a rotary vacuum evaporator after being filtered with sterilized Whatman No. 1 filter paper to produce a particle-free extract. To create a paste-like substance, the extract was finally dried in an oven at 45 to 50 °C to remove all solvents. For the preparation of aqueous extract, the filtrate was dried in a hot air oven at 45-50 °C to obtain a paste material. The methanolic and aqueous extracts were preserved in the refrigerator at -20 °C, and the concentrated extracts were used for the evaluation of antibacterial activity.

2.3 Evaluation of *in vitro* antibacterial activity

The method of micro broth dilution was adapted from the works of Modi *et al.* (2018) [13] and Wiegand *et al.* (2008) [14]. A range of gram positive and gram negative organisms, such as *Bacillus subtilis* (ATCC9372), *Salmonella Typhimurium* (ATCC23564), *Pseudomonas aeruginosa* (ATCC27853), *Proteus mirabilis* (NCIM2241), *Streptococcus pyogenes* (ATCC8668), and *Staphylococcus aureus* (ATCC25923), were tested for the antibacterial properties of methanol and aqueous extracts of lemon grass leaves and detected Minimal Inhibitory Concentration (MIC) value of plant extract against these selected organisms. All bacterial cultures were produced in accordance with the McFarland 0.5 standard, which is equivalent to 1.0×10^8 cfu/ml, after being incubated for single night. The last inoculums to be dispensed were prepared as 1×10^6 cfu/ml diluted in sterile broth. The fresh stock solution (20.48 mg/ml) of methanol and aqueous extracts were prepared in a 10% DMSO and D.W., respectively and Standard drug Enrofloxacin (1mg/ml) was prepared in sterile water. Final working concentration of methanol and aqueous extracts were maintained from 20.48 to 0.16 mg/ml. 100µl of each test sample from each concentration range were put into the 96-well micro titer plates. Following the addition of the test samples, individual loads of 100 µl standard inoculums containing seven chosen pathogens were made. The plates underwent a 24-hour incubation period at 37°C as part of the triplicate execution of this technique. After an overnight incubation period, each well received 30 µl of p-Iodonitrotetrazoleum chloride (INT dye, 2 mg/ml), and the mixture was incubated for an additional 30 minutes at 37°C. While a clear or transparent color demonstrated the plant extract's effective antibacterial action, a reddish or pink color suggested the proliferation of bacteria. The test sample's lowest result, known as the MIC value, indicated that it had inhibited bacterial growth on 96-well microtiter plates, since no color development had occurred.

3. Results

Methanol and water were used in an extraction procedure on the plant material. The Nature of *Cymbopogon flexuosus* plant leaves extracts are narrated in table 1. The antibacterial efficacy of methanol and aqueous extracts of *Cymbopogon flexuosus* leaves were evaluated in this study using the microbroth dilution technique against seven selected species of organisms: *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC25922), *Salmonella Typhimurium* (ATCC23564), *Pseudomonas aeruginosa* (ATCC27853), *Proteus mirabilis* (NCIM2241), *Bacillus subtilis* (ATCC9372) and *Streptococcus pyogenes* (ATCC8668). The outcomes were analyzed based on the test sample's lowest inhibitory concentration against the organisms. The lowest inhibitory concentration is considered as minimum inhibitory concentration (MIC), is the amount of the antibiotic required to prevent at least 99% of the germs from growing visibly. In this investigation, the MIC values of the two test plant leaf extracts were shown and noted. The various extracts' MIC values are displayed in Table 2 and Figure 1. These values relied on the species of the organisms and the solvent extraction process. The methanol extract of *Cymbopogon flexuosus* has very good antibacterial activity of 0.32 mg/ml against *Streptococcus pyogenes* (ATCC8668) then come methanol extract at 1.28 mg/ml against *Bacillus subtilis*

(ATCC9372). Nevertheless MIC of methanolic extract of *Cymbopogon flexuosus* for *Salmonella Typhimurium* (ATCC23564), *Escherichia coli* (ATCC25922) and *Staphylococcus aureus* (ATCC25923) was 20.48 mg/ml. However, *Cymbopogon flexuosus* aqueous extract's bacterial growth inhibitory qualities were found to be 5.12 mg/ml against *Proteus mirabilis* and 10.24 mg/ml against *Bacillus subtilis* (ATCC9372). On the other hand, *Streptococcus pyogenes* (ATCC8668) and *Escherichia coli* (ATCC25922) have MIC values of 20.48 mg/ml. The methanol and aqueous leaf extracts had no effect on *Pseudomonas aeruginosa* (ATCC27853). Aqueous leaf extracts shown inactivity against *Staphylococcus aureus* (ATCC25923) and *Salmonella Typhimurium* (ATCC23564) whereas, methanol leaf extracts demonstrated inactivity against *Proteus mirabilis* (NCIM2241).

Table 1: Nature of Lemon Grass (*Cymbopogon flexuosus*) plant leaves extract

Type of Extract	Colour	Consistency	Extractability
Methanol	Dark brown	Viscous	12%
Aqueous	Dark brown	Viscous	32.5%

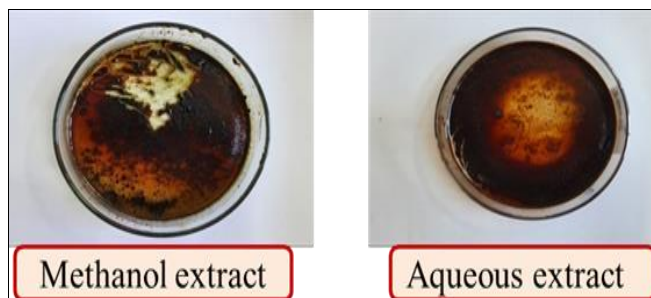


Table 2: Minimum inhibitory concentrations (MIC) of methanol and aqueous extracts of lemon grass (*Cymbopogon flexuosus*) against various organisms

Bacterial Pathogens	MIC (mg/ml) of Lemon Grass (<i>Cymbopogon flexuosus</i>) extracts	
	LGME	LGAE
<i>Staphylococcus aureus</i> (ATCC25923)	20.48	No effect
<i>Escherichia coli</i> (ATCC25922)	20.48	20.48
<i>Salmonella Typhimurium</i> (ATCC23564)	20.48	No effect
<i>Pseudomonas aeruginosa</i> (ATCC27853)	No effect	No effect
<i>Proteus mirabilis</i> (NCIM2241)	No effect	5.12
<i>Bacillus subtilis</i> (ATCC9372)	1.28	10.24
<i>Streptococcus pyogenes</i> (ATCC8668)	0.32	20.48

LGME: Lemongrass Methanol Extract; LGAE: Lemongrass Aqueous Extract

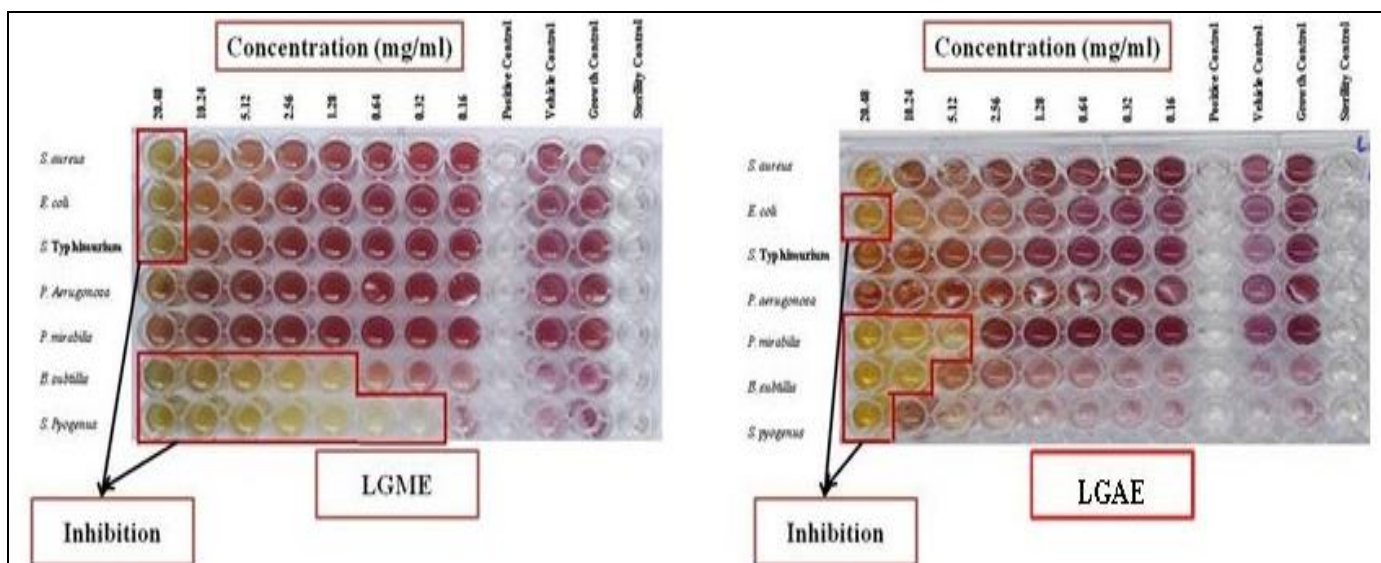


Fig 1: Minimum Inhibitory Concentration (MIC mg/ml) for the Methanol and Aqueous Extracts of Lemon Grass (*Cymbopogon flexuosus*) by microbroth dilution technique against various organisms

4. Discussion

Antibiotic abuse and misuse in humans and animals has led to an increasing danger of multidrug resistance in bacteria. Veterinary medicine's antimicrobial resistance (AMR-V) can result in treatment failure, which can have a detrimental impact on both the financial performance of farms and the physical condition, wellbeing, and production of animals (Bengtsson and Greko, 2014) [15]. Finding new substances that can counteract antibiotic resistance is therefore essential. Herbal remedies are becoming more and more well-liked these days in veterinary care for the treatment of different ailments. High citral content has been linked to lemongrass's potent antibacterial action (Marongiu *et al.*, 2006; Adukwu *et al.*, 2012; Kumar *et al.*, 2013; Kpoviessi *et al.*, 2014) [16, 17, 18,

19]. Specifically, the antibacterial properties of plant oils and extracts have served as the foundation for a wide range of uses, such as natural remedies, medicines, alternative medicine, and the preservation of both uncooked and prepared food (Singh *et al.*, 2004) [20]. Lemongrass extracts have been shown by numerous researchers to possess antimicrobial properties against a broad range of microorganisms, such as, viruses, yeast, fungi, and gram positive and gram negative bacteria (Nyamath and Karthikeyan, 2018; Gao *et al.*, 2020, Ajijolakewu *et al.*, 2021; Gangavarapu and Palwai, 2021; Ghanayem, 2022) [21, 22, 2, 23, 24]. The outcomes of earlier studies on these species using various lemon grass extracts have varied. Numerous other studies that have looked at the bacterial inhibitory activity of lemongrass plant essential oil

against pathogenic bacterial strains have found that *P. aeruginosa* was the most resistant and *Enterococcus faecalis* the most sensitive (Yazdani *et al.*, 2003; Olivero-Verbel *et al.*, 2010; Bassole *et al.*, 2011) [25, 26, 27]. By applying the agar well diffusion method, the antibacterial activity of both fresh and dried lemongrass leaves extract were investigated by Nyamath and Karthikeyan (2018) [21] against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. The investigation was carried out using ethanol and methanol as well as cold and hot water. They found that at 1000 ppm concentration in the ethanol dried leaves extract of lemon grass, *Staphylococcus aureus* recorded a greater zone of inhibition (12.50 mm) in comparison to other species. The least level of inhibition was shown by *Pseudomonas aeruginosa* (2.0 mm) in hot water fresh leaves extract at 250 ppm, suggesting that the lemongrass leaves extract possesses potent antibacterial activity against bacteria that are resistant to antibiotics. Furthermore, Naik *et al.*, (2010) [28] found that *C. citratus* essential oil was effective against all other tested organisms like, *B. subtilis*, *B. cereus*, *S. aureus* and *K. pneumoniae* with the exception of *P. aeruginosa*. These findings corroborate the findings of the current investigation. With the agar-well diffusion and broth-dilution techniques, Ajijolakewu *et al.*, 2021 [2] conducted a study to ascertain the antimicrobial activity of lemon grass (*Cymbopogon citratus*) extracts, both aqueous and ethanolic, against a selection of clinical isolates, including, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The MIC value of the ethanolic extract against *S. aureus* and *P. aeruginosa* were found to be 2.0 and 12.5 mg/ml, respectively. The aqueous extract's minimum inhibitory concentration (MIC) for *S. aureus*, *P. aeruginosa* and *E. coli* was 2.0, 12.5 and 25 mg/ml, respectively. The antibacterial efficacy of lemongrass methanol extracts against *Pseudomonas aeruginosa*, *Klebsella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus* was assessed by Pratap and Khan in 2022 [12]. The lowest inhibitory concentration (MIC) was found. It was demonstrated that bacteria from *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were inhibited by methanol extract at concentrations of 2.0, 1.6 and 1.3 mg/ml, respectively. The current study's results are entirely at odds with those of this one. Joshi *et al.*, (2023) [29] evaluated the antibacterial activity of conventional medication Cloxacillin against *S. aureus* at various concentrations of 100%, 75%, 50% and 25% as well as the essential oil derived from *Cymbopogon nardus*. The zone of inhibition for *S. aureus* was measured at 24 mm for cloxacillin (standard drug) and 25 mm for 100%, 11 mm for 75%, 9 mm for 50%, and 0 mm for 25%. These findings unequivocally showed that the essential oil isolated from *Cymbopogon nardus* was more sensitive to *S. aureus* at greater concentrations. In a different investigation, Shendurse *et al.*, 2021 [30] tested the antibacterial potential of lemongrass leaves essential oil against six putative pathogens: *Escherichia coli*, *Bacillus subtilis*, *Bacillus cereus*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*. They did this by applying the agar well diffusion method. Based on the findings, the inhibition zones for *Bacillus subtilis*, *Bacillus cereus*, and *Staphylococcus aureus* were measured and found to be 48.0±1.05, 21.0±0.64 and 32.0±0.75, mm, respectively. Regarding *Proteus vulgaris*, the inhibition zone measured was 23.0±0.73 mm, but *Pseudomonas aeruginosa* and *Escherichia coli* did not exhibit any inhibition zone. Using the agar well diffusion method, the antibacterial activity of essential oils extracted from *Cymbopogon citratus* was demonstrated by Kolawole *et al.*,

2023 [31]. They found that the zones of inhibition for *Proteus mirabilis* and *Staphylococcus aureus* were 29.5 ± 2.1 mm and 62.0 ± 2.8 mm, respectively. As opposed to this, *Pseudomonas aeruginosa*'s antibacterial activity was only moderate (8.5 ± 0.7 mm). According to Lambert (2002) [32], the differences in sensitivity between the two groups to the extract are probably due to structural and compositional changes in the membranes of gram-positive and gram-negative bacteria. The results of the data analysis for this investigation showed that *Cymbopogon flexuosus* plant leaf extracts had potent antibacterial activity against gram-positive and gram-negative pathogens, with no appreciable preference for any particular gram response.

5. Conclusions

Based on the aforementioned findings, the present study has shown that methanol and aqueous extracts of *Cymbopogon flexuosus* are powerful sources of potent antibacterial. Furthermore, additional study is needed to determine the possible inhibitory modes of action of the active principle of the extract. Toxicological studies as well as investigations into the other components of lemon grass would be conducted to examine the overall impact of using lemon grass in medication development.

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