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Original Research Article

Unlocking the bioactivity of lemongrass (*Cymbopogon flexuosus*): A dual assessment of antioxidant and antimicrobial efficacy

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Abstract

Background: *Cymbopogon* is a member of the family *Poaceae* and has been explored for its phytochemicals and bioactivities. Although the antimicrobial activities of *Cymbopogon flexuous* oil have been extensively studied, comprehensive analyses are required to identify promising compounds for the treatment of antimicrobial resistance. Therefore, this study investigated the antioxidant and antimicrobial properties of *Cymbopogon flexuous*.

Aim: To explore the phytochemical constituents of the oil extract of *Cymbopogon flexuous.*

Materials and Methods: In vitro analyses were used to evaluate the antioxidant and antimicrobial properties of the *Cymbopogon flexuous*. In addition, bioactivity was measured using cytotoxicity assays. Antioxidant assays were performed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis [3-ethylbenzothiazoline-6-sulfonic acid (ABTS) Finally, the antimicrobial activity of these extracts was evaluated against *Candida albicans, Bacillus subtilis, Staphylococcus aureus, and Escherichia coli* using a well diffusion assay.

Results: GC–MS analysis revealed 53 metabolites. Of these, 2,5-bis(1,1-dimethylethyl)- phenol (27.87%), alpha-cadinol (26.76%), and 1,2-dimethoxy-4-(1-propenyl)-benzene (20.56%) were the predominant compounds. C. *winterianus and C. nardus* exhibited the highest antioxidant activity against DPPH and ABTS, respectively. Oil extract exhibited the highest antimicrobial activity against *E. coli* and *S. aureus*, whereas *Cymbopogon flexuous* leaf extract showed the highest activity against microbes. Furthermore, computational pathway analysis predicted that antimicrobial activity mechanisms were related to antioxidant activity.

Conclusions: These findings demonstrate that the leaves had strong antioxidant activity, whereas oil showed great antimicrobial activity.

Keywords: Anti-oxidant assay GC.MS, DPPH, ABTS, Cymbopogon flexuous, Anti-microbial.

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1. Introduction

One major issue with global health is infections.[1] Infections account for 28.1% of all fatalities in Indonesia. Viruses, fungi, bacteria, and protozoa are among the microorganisms that can cause infection.[2] To boost research into new antibiotics that overcome drug resistance, the World Health Organization (WHO) has released a list of priority diseases.[3] The search for new antimicrobial medication sources, such as natural compounds originating from plants, is therefore urgently needed.[4,5] Natural substances derived from therapeutic plants are used by about 80% of the global population.[1] Unfortunately, research on many Indonesian plants that could have medical properties is lacking. Just 700–

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1000 of the 9,600 plant species known to have therapeutic qualities actually see any kind of usage in medicine. Serai (*Cymbopogon spp*.) is one such species; it has several purposes in Indonesian culture, such as a spice, a beverage, and an oil source. Seasoned lemongrass and fragrant lemongrass (*citronella*; *Cymbopogon nardus* (DC.) Spatf and *Cymbopogon winterianus* Jowitt) are both members of the family *Poaceae* and are species of *Cymbopogon flexuosus*.[6,7] Much research has focused on the bioactivity of Cymbopogon species. Research has examined the antibacterial properties of several species of Cultus, including C. flexuous,[8] *C. citratus, C. nardus, and C. Schoenanthus.* Researchers have also looked into *C. citratus* antioxidant capabilities and C. winterianus pesticidal effects. For certain lemongrass bioactivities, the essential oil of *Cymbopogon flexuosus* is crucial. In human trials, this essential oil has demonstrated great biocompatibility and little negative effect. Additionally, proteomic investigations have been used to assess the molecular processes of the bioactive components of *Cymbopogon flexuosus* against certain bacteria. The bioactivities and metabolite profiles of various plant organs were described in detail by *Wahyuni et al.* To fill a gap in the literature, this study also performed computational predictions of the mechanism pathway and antimicrobial and antioxidant activity assays of *Cymbopogon flexuosus* organs. Additionally, ethanol was utilized as the solvent in this investigation because of its structural capabilities that permit the dissolving of polar (water), non-polar, hydrophilic (hexane), and hydrophobic (other chemicals) substances. The nonpolar properties and low toxicity of ethanol make it a promising candidate for use as a medical solvent. Ethanol extract has the same advantage of being more soluble.[9,10] The purpose of this research was to examine the antioxidant and antibacterial properties, as well as the inhibitory mechanisms, of three different commercially available species of *Cymbopogon flexuosus* from Indonesia. Based on our research, these three species of Cymbopogon flexuosus may have medicinal uses in Indonesia.(**Figure 1**)

Figure 1: *Cymbopogon flexuosus plant parts*

2. Materials and Methods

2.1. Plant material collection and identification

Cymbopogon flexuosus employed in this investigation. The CSIR-Central Institute of Medicinal & Aromatic Plants in Lucknow is where the plants were acquired. The authors gathered and verified the samples at Maharana Pratap College of Pharmacy's Plant Systematics Laboratory, Department of Pharmacology, Kanpur, Uttar Pradesh. They placed a voucher specimen in the Plant Systematics Laboratory. (No. CIMAP-HA-1000420251).

2.2. Extraction of Cymbopogon flexuosus oil

Fresh *Cymbopogon flexuosus* leaves (chopped), distilled water, Clevenger-type apparatus, round-bottom flask, heating mantle. Wash fresh lemongrass leaves thoroughly with distilled water to remove dust. Chop into small pieces (~2–3 cm). Weigh about 200–300 g of the sample. Transfer the plant material into a 1 L round-bottom flask and add distilled water (enough to immerse the plant material, usually 3× the weight of the sample). Assemble the Clevenger apparatus and connect it to a condenser and a heating mantle. Heat the mixture gently to boiling and maintain steady distillation for 3–4 hours. The steam carries the volatile oil into the condenser where it is condensed and collected in the Clevenger trap. After distillation, allow the apparatus to cool. Carefully collect the separated essential oil layer from the Clevenger arm using a micropipette. Dry the oil over anhydrous sodium sulfate to remove moisture. Store the purified oil in amber glass vials at 4 °C until further use.[12]

2.3. In vitro antioxidant activity

2.3.1. DPPH scavenging assay

To evaluate antioxidant activity, 10 µL of different concentrations of test samples or standard (Ascorbic Acid, SRL Cat. No. 23006) were mixed with 200 µL of 0.1 mM DPPH solution (SRL Chem, Cat. No. SR-29128) in a 96-well plate. Reactions were prepared in quadruplicate; duplicate blanks (sample + methanol without DPPH), red blanks (without DPPH), and untreated wells served as controls. Plates were incubated in the dark for 30 min, and absorbance was read at 517 nm using a microplate reader (iMark, Bio-Rad). A combination of test solution with 20 µL deionized water served as control. Scavenging activity (%) was calculated relative to control. IC_{50} values were derived using GraphPad Prism 6 by plotting inhibition (%) vs. sample concentration.[11]

Calculations: % RSA= (ABS control-Abs Sample)/Abs control) x100

2.3.2. ABTS radical scavenging ability

ABTS radicals were generated by mixing 7 mM ABTS (SRL Chem, Cat. No. 28042) with APS and diluting the mixture 1:100 with ethanol. In a 96-well plate, 200 µL of ABTS solution was mixed with 10 µL of sample or standard (Ascorbic Acid, SRL Cat. No. 23006) at various concentrations. Plates were incubated at room temperature in the dark for 10 min. Absorbance was recorded at 734 nm using a microplate reader (iMark, Bio-Rad). Controls contained no treatment. IC_{50} was determined via GraphPad Prism 6, using % inhibition plotted against sample concentration, following the DPPH assay method.[15]

% RSA = ((Abs Control- Abs Sample/Test)/Abs Control) ×100

% RSA = ((Abs Control- Abs Sample/Test)/Abs Control) ×100

2.3.3. GC-MS analysis

GC-MS analysis of oil extracts was performed using a PerkinElmer Clarus 600 system equipped with an Rtx-5MS capillary column. Helium (99.99%) was used as the carrier gas at a constant flow rate of 1.0 mL/min. Injection volume: 1 µL, split ratio: 10:1, ionization energy: 70 eV, scan range: 40–650 m/z, scan time: 0.2 sec. Injector temperature: 260°C. Oven temp held at 50°C for 3 min, then ramped to 300°C at 10°C/min. Compounds were identified by comparing retention times and mass spectra with those in the NIST and Wiley-8 libraries.[11,14]

2.4. Antimicrobial activity

2.4.1. Antimicrobial activity test (Anti-microbial zone inhibition test (E. coli))

Antibacterial activity was evaluated using the Kirby-Bauer Zone Inhibition Method. We added 100 l of *E. coli* culture to MHA plates, adjusted the cell density to 0.5 McFarl and Unit-Unit-Approx (1.5 x108 CFU/mL), and added discs with 10 l of various concentrations (0-5%). To load the disk, we serially diluted 10% of the sample. We employed a Ciprofloxacin disc (10 g) as the positive control and filled one disk each plate with solvent as the vehicle control. The *E. Coli* plates were incubated for twenty-four hours at 37°C. We measured and noted the clear zone of the disk.

2.4.2. Anti-microbial zone inhibition test (S. aureus)

Antibacterial activity was evaluated using the Kirby-Bauer Zone Inhibition Method.We inoculated MHA plates with 100 liters of *S. aureus* (0.5 McFarland Unit—approximate cell density (1.5 x 108 CFU/mL)) bacterial culture. Next, we added 10 liters of different doses to the wells. To fill the well, we serially diluted 10% of the substance. Each plate had 10 g of ciprofloxacin for the positive control and a solvent-only well for the vehicle control. For twenty-four hours, we incubated *S. aureus* plates at 37 °C (Basil Scientific Corp., India). We kept an eye on and recorded the well's clear zones.

2.4.3. Anti-fungal zone inhibition test (C. albicans)

The Kirby-Bauer Zone Inhibition Method was used to assess its antifungal activity. The SDA plates were inoculated by dispersing 100 litters of C. albicans culture, adjusting it to 0.5 McFarland Unit-approximate cell density (1.5 x 108 CFU/mL), and then adding 10 liters of various doses to the wells indicated in the excel sheet. Each plate contained a solvent-only well for the vehicle control and a 50 g Amphotericin B well for the positive control. A Basil Scientific Corp. India-Incubator was used to incubate C. albicans plates for 24 hours at 37°C. The well's surrounding clean zones were measured and recorded.

2.4.4. Minimum inhibitory concentration activity (E. coli)

A standard microbe dilution and the *E. Coli* Minimum Inhibitory Concentration Activity: 0.5 McFall were utilized in the study. We placed 5 liters of the generated treatment dilutions in a microcentrifuge tube and incubated 100 liters of diluted log cultures of bacteria (E. coli, MTCC452) for 24 hours. Following incubation, turbidity measurements at 630 nm were obtained for each substance in the 96-well plate using the Elisa Plate Reader (iMark BioRad). As a positive control, we employed 10 g of ciprofloxacin.

2.4.5. Minimum inhibitory concentration activity (S. aureus)

The study made use of conventional microbe dilutions and the *S. aureus* Minimum Inhibitory Concentration Activity of 0.5 McFall. In a microcentrifuge tube, combine 5 liters of prepared treatment dilutions with 100 liters of diluted log cultures of S. aureus (MTCC 96) and incubate for 24 hours. Following incubation, turbidity measurements at 630 nm were obtained for each substance in the 96-well plate using the Elisa Plate Reader (iMark BioRad). As a positive control, we employed 100 g of Ciprofloxacin (SRL Chem-78079).[12,13]

3. Result and Discussion

3.1. Identification of components

Identification was done using the calculated fragments, molecular mass, and molecular structure. The NIST database and the Willy8-library, which has more than 62,000 pattern entries, were used to interpret the GC-MS spectra. Together with their molecular weights and chemical structures, the components of the test materials were determined. The percentage amounts discovered in the sample were contrasted with component spectra from the Willy8 collection and the NIST library. This is done in order to determine whether this plant species has any chemicals or a combination of them that could support its traditional and commercial therapeutic purposes. It also helps determine the most effective methods for removing harmful compounds. The test materials' constituent parts were determined (**Table 1**, and **Figure 2**).

3.2. Activities of antioxidants

Oxidative stress has been linked to age-related neurodegenerative diseases in a number of studies. Numerous studies have also examined the protective effects of antioxidants in preventing or reducing neuronal death that occurs in the pathophysiology of this disorder. Indicators of a compound's antioxidant potential also include its total antioxidant activity and ability to scavenge radicals. To determine the plant extracts' ability to act as antioxidants, two activities—DPPH and ABTS—were evaluated. The 1, 2-diphenyl-2-picryl hydroxyl radical (DPPH) was used to examine the antioxidant activity of many oils. **Table 2** presents the results.

Table 1: Compound of *Cymbopogon flexus* oil

Peak	R. Time	Area	Area%	Name
	9.671	2289371	2.27	β-Myrcene
	11.669	529208	0.53	γ-terpinene
	11.785	420151	0.42	Linalool oxide
	13.413	4504197	4.47	Camphor
	13.474	6514918	6.47	Spirojatamol
	14.180	960517	0.95	2-Bornanol, 2-Methyl-
	15.074	3482967	3.46	2,6 Bis (1,1-dimethylethyl)-4- [(4-chloro-6-(3,5, bis (1,1-dimethylethyl)-4- hydroxy anilino)-1,3,5 triazin-2-yl) amino] phenol -
	15.450	7028372	6.98	2,2,7,7-Tetramethyl-Tricyclo [6.2.1.0 1,6] Undec-4-En-3-One
	15.536	1744090	1.73	2-Propenal, 3-(2,4,5,6,7,7a-Hexahydro-3,7-Dimethyl-1h-Inden-4-Yl)-2-Methyl-, (4s-(4alpha(E),7beta,7aalphA))-
	15.828	12189437	12.10	1,3a-Ethano(1H) Inden-4-Ol, Octahydro-2,2,4,7a-Tetramethyl
	15.983	6275373	6.23	Valerianic Acid, Methyl Ester
	16.220	1175032	1.17	4a,5-Dimethyl-3-(Prop-1-En-2-Yl)-1,2,3,4,4a,5,6,7-Octahydronaphthalen-1-Ol
	17.061	782712	0.78	Hexadecenoic Acid, Methyl Ester
	18.702	1113918	1.11	9,12-Octadecadienoic Acid (Z, Z)-, Methyl Ester
	18.764	1457587	1.45	9-Octadecenoic Acid, Methyl Ester, (E)-
	23.692	2195767	2.18	Oleoyl Chloride
	24.043	1673673	1.66	1,4-Benzenedicarboxylic Acid, Bis(2-Ethylhexyl) Ester
		100709079	100.0	

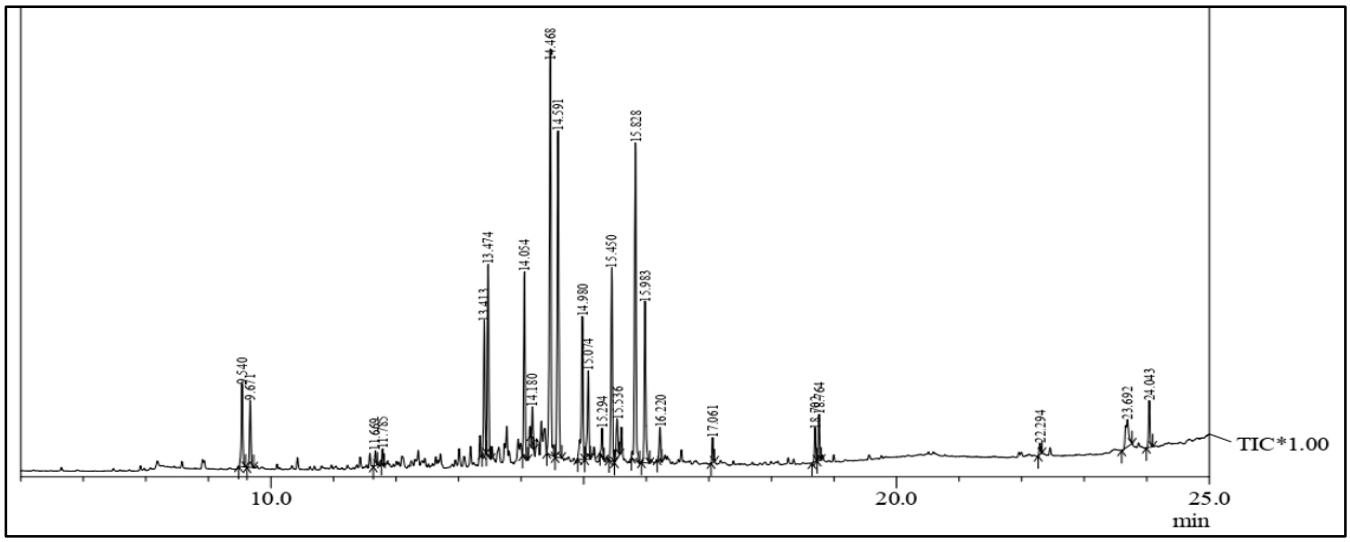

Figure 2: Gas chromatography- Mass Spectrometry (GC-MS) *Cymbopogon flexuous* oil

Table 2: DPPH scavenging assay

Sample code	IC_{50} value (µg/ml) (Mean ± SEM)
Ascorbic Acid	11.95 ± 0.02
Cymbopogon flexuous oil	575.2 ± 0.06

Table 2 lists the 50% inhibitory concentration (IC_{50}) and the antioxidant activity (DPPH Assay) determined in the sample based on the experimental results. In contrast to sample CFW, sample CFE was discovered to be extremely active. 11.95 µg of standard ascorbic acid was determined to be comparable to 575.2 µg and 252.6 µg of sample, respectively. Since the standard chemical is highly active and pure, it was evaluated utilizing a smaller concentration range of 0 to 50 µg/ml. However, because the potency of the sample is still unbound, a wider concentration range (0 to 1000 µg/ml) was used for the sample analysis. The anti-oxidant activity of many extracts was investigated utilising the ABTS radical scavenging ability. **Table 3** discusses the findings.

Table 3: ABTS radical scavenging ability

Sample code	*IC50* value (µg/ml) (Mean ± SEM)
Ascorbic Acid	27.36 ± 0.02
Cymbopogon flexuous oil	252.3 ± 0.03

Table 3 lists the 50% inhibitory concentration and the estimated antioxidant activity (ABTS Radical Scavenging Assay) in the samples based on the experimental results. The most active sample was discovered to be CFE in oil. It was discovered that sample, weighing 252.3 respectively, were comparable to 27.36 µg of normal ascorbic acid. Since the standard chemical is highly active and pure, it was evaluated utilizing a smaller concentration range of 0 to 50 µg/ml. However, because the potency of the sample is still unbound, a wider concentration range (0 to 1000 µg) was used for the sample analysis.

Table 4 Anti-microbial-zone inhibition test- *escherichia coli*

Table 4 summarize the microbial growth inhibition by Cymbopogon *flexuous* oil *on E. coli*, which showed good antibacterial activities.

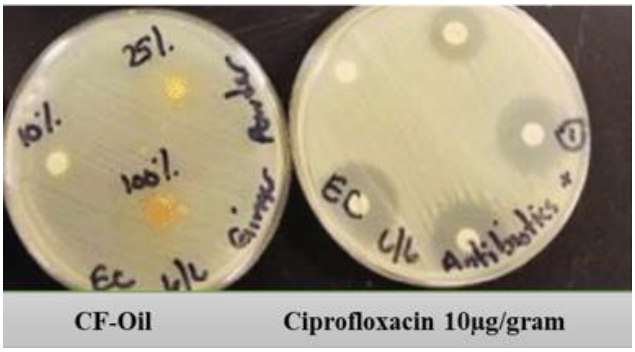

Figure 3: Zones of Inhibition Plates for *E. coli*

3.3. Anti-microbial zone inhibition test (S. aureus and C. albicans):

The microbial growth suppression of *S. aureus* and *C. albicans* by the *Cymbopogon flexuous oil*, which shown good antibacterial activity, is summarized in **Table 5**.

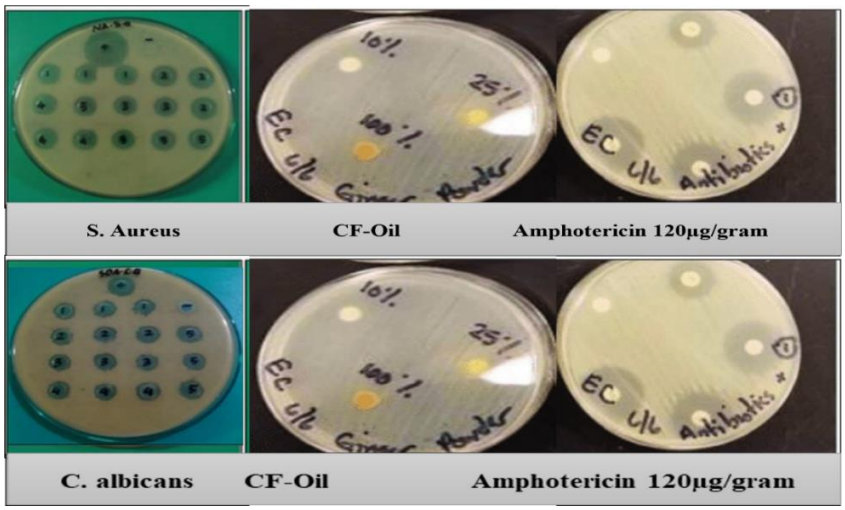

Figure 4: Zones of Inhibition Plates for *S. aureus, C. albican*

Table 4: Anti-bacterial activity of *E. coli*

S.No.	Sample Id	Effective Amount	Average Zone at Effective amount (in mm)
1	Ciprofloxacin (PC)	10µg	31
3	*Cymbopogon flexuous* oil	-	25

Table 5: Anti-bacterial activity of *S. aureus and C. albicans*

Amount (µg/disk)	Average, Plate ABC Positive control	Average, Plate ABC *Cymbopogon flexuous oil*	Standard Drug Amphotericin B 120µg *C. albicans* Average Zone at Effective Amount (in mm)	Standard Drug Amphotericin B 120µg *S. aureus* Average Zone at Effective Amount (in mm)
0	0	0	0	0
62.5	9	8	9	15
125	8	7	9	15
250	7	6	9	15
500	6	5	9	15
1000	5	8	9	15
0	0	0	9	15

4. Discussion

Cymbopogon flexuous oil extracts may have antibacterial and antioxidant qualities. It has a diverse phytochemical content that offers it biological capabilities and may be useful in food preservation, cosmetics, and pharmaceuticals. Further study is required to completely comprehend its safety profile and therapeutic potential. The researchers discovered that the growth of *S. aureus* and *E. coli* infections was suppressed when essential oil leaves were added to broth cultures. Gram-negative *E. coli* bacteria exhibited a higher rate of inhibition than gram-positive S. aureus bacteria. The size of the inoculum and the concentration of essential oil leaves typically dictated the organisms' growth and survival rates. Since the investigated microorganisms were significantly suppressed by high concentrations of ethanol and water extract of *Cymbopogon flexuous* of *C. albicans* leaves, it was assumed that they were oil sensitive. When tested against two types of clinically relevant bacteria, the essential oils from *Cymbopogon flexuous* water and ethanol extracts shown varying levels of antibacterial activity. According to the test results previously mentioned, both Gram-negative (*S. aureus*) and positive (*E. coli*) bacteria showed noticeably reduced growth in response to the extract. Additionally, the same study found that certain strains of Candida albicans are inhibited in their proliferation by essential oil. *Cymbopogon flexuous* oils from *C. albicans* significantly reduced the growth of the bacteria under study. Such suppression was shown based on the MIC values for *Escherichia coli*. The discovery might open the door for its possible use in clinical settings to treat bacterial infections. Strong antifungal activity was demonstrated by the ethanolic and water extracts *of Cymbopogon flexuous C. albicans*, which prevented the growth of test fungi such *Aspergillus flavus, Gillus niger, and Candida albicans*. This characteristic implies that *Cymbopogon flexuous* water and ethanol extracts may find use as an antifungal against *Candida albicans*. However, because *Aspergillus niger and Aspergillus flavus* are so commonly used as food preservatives, ethanolic and water extract of *Cymbopogon flexuous* of -*C. albicans* shows promise. DPPH is a stable free radical scavenger that links unpaired electrons by donating hydrogen protons. This study shows that plant extracts can scavenge DPPH radicals, enabling hydrogen protons to contribute radical lone pair electrons. Given that all of the solvent extracts showed greater levels of inhibition, it's probable that plant extracts contain substances that give free radicals protons. In vitro tests are used to evaluate plant medicines' capacity to stop the production of free radicals. Among other things, the free radical NO promotes inflammation. The Griess reagent is used in NO radical scavenging to measure the quantity of nitrite ions generated by a reaction between oxygen and sodium nitroprusside in a salt solution. The portions exhibit chromophore absorbance at 546 nm. All parts of the methanol extract reduced the amount of nitrite generated by the breakdown of sodium nitroprusside in vitro. By competing with oxygen for the reaction of NO, fractional antioxidant principles may prevent the production of nitrite. We reduced Fe^{3+} to Fe^{2+} in different percentages to assess their reduction capacity. Reductions (antioxidants) contribute a hydrogen atom to break the chain of free radicals, allowing compounds to decrease. The antioxidants in the methanol fractions changed the $Fe^{3+/}$ complex into ferrous, demonstrating its reducing ability. To examine the metal chelation potential of methanol extract fractions from various plants, a ferrousus ion-ferrozine complex was created. Light at 562 nm is absorbed by a red substance made of ferrous ions and ferrozine. Chelating agents that create σ bonds with metals have the potential to act as secondary antioxidants by stabilizing the oxidized metal ion and reducing the redox potential. According to our research, the fractions exhibit substantial iron binding capabilities, which raises the possibility that they have antioxidant qualities. The metal-chelating activity of the fractions reduced the catalytic transition metal in lipid peroxidation. The methanol extract of every plant included a significant quantity of total phenolic components, measuring 198.81±12.66 milligrams of gallic acid equivalents. When moderately consumed from a diet high in fruits and vegetables, polyphenolic compounds have been shown to lower mutagenesis and carcinogenesis in humans.[16] Plants contain phenolic chemicals, which are antioxidants. Synthetic antioxidants outperformed their natural counterparts in the majority of cases.[17] The phytochemical components of plants, particularly polyphenols like tannins, flavonoids, phenylpropanoids, and phenolic acids, are responsible for their antioxidant and free radical scavenging capabilities. The chemical structure of saponins, tannins, and some flavonoids determines their DPPH and ABTS radical activity, which in turn determines their ability to scavenge free radicals. Tanning agents chelate iron ions. There was a concentration-dependent inhibition of oxidative stress in many models by the previously isolated plant compounds luteolin, chlorogenic acid, and caffeic acid. Flavonoids are a powerful scavenger of NO radicals. Phenolics, flavonoids, tannins, and other compounds in plant extracts may be responsible for their antioxidant properties. Methanol extracts of many plants have potent anti-Microbial and antioxidant properties, supporting traditional folk remedies for anti-Microbial and antioxidant properties, etc. The extract and its active components' modes of action require more study.

5. Conclusion

Through scientific analysis, we evaluated and verified the antioxidant and antimicrobial properties of Cymbopogon flexuosus. GC–MS profiling of the plant’s essential oil indicated a high abundance of bioactive compounds with known antimicrobial potential. The oil extract also demonstrated strong antioxidant capacity, as evidenced by its significant activity against DPPH and ABTS free radicals.

Overall, these findings suggest that Cymbopogon flexuosus essential oil is highly effective against various pathogens and may serve as a promising candidate for both preclinical and clinical investigations. Its natural, plant-based composition supports the potential development of cost-effective therapeutic products with minimal side effects, offering an alternative option within herbal medicine

6. Ethical Approval

This study not involved in human and animals Participants.

7. Authors Contribution

All authors participate equally

8. Source of Funding

None.

9. Conflict of Interest

The authors declare that they have no conflict of interest.

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