



Phytochemical Analysis and Antimicrobial Effect of Lemon Grass (*Cymbopogon citratus*) Obtained From Zaria, Kaduna State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author MU designed the study, wrote the protocol and wrote the first draft of the manuscript. Author IBM managed the literature searches, analyses of the study and performed the extraction analysis. Authors JOO and DYJ managed the experimental process. Authors AAA and IYT characterized and identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The research study aimed to extract *Cymbopogon citratus* leaf using various solvents with view to determine the phytochemical constituents and antimicrobial effect of the plant extracts on some selected microorganisms.

Methodology: The cold maceration and agar well diffusion technique were employed to assess phytochemical properties and the antimicrobial potency of *Cymbopogon citratus* (Lemongrass) leaf

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extracts against selected bacterial pathogens using different solvents; ethanol, chloroform, and acetone. All the extracts were subjected to standard phytochemical qualitative screening for the presence or absence of various primary or secondary metabolites. The susceptibility test of the plant extracts on *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* were done using the agar well diffusion method. Tetracycline, Ciprofloxacin and Erythromycin were used as controls. The Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) were determined in three concentrations; 100 mg/ml, 50 mg/ml and 25 mg/ml of each extract.

Mean zone of inhibition was used to measure the antimicrobial potential of leaf extracts against *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* respectively.

Results: Phytochemicals such as flavonoids, carbohydrates, tannins, alkaloids, steroids, and phytosteroids were detected except glycosides and phenol that were absent in the acetone and chloroform leaf extracts. The ethanol leaf extract showed antimicrobial activity at the concentration of 100 mg/ml, and intermediate antimicrobial activity at the concentration of 50 mg/ml against all isolates tested. Acetone and chloroform leaf extracts recorded inactivity against *E. coli* and *S. aureus* respectively.

Conclusions: The results of preliminary phytochemical screening of the leaf extracts revealed the presence of phytochemicals which could be used as medical regimens. Lemongrass has demonstrated antimicrobial properties which could be harnessed for the control of pathogens tested.

Keywords: Phytochemistry; antibacterial activity; bacterial pathogens; Cymbopogon citratus.

1. INTRODUCTION

Plants are very important sources of drugs used for centuries in the treatment of various microbial infections. Most of the plant-based drugs when used correctly, pose less or no toxic effect to the recipients compared to the chemically synthetic drugs. This necessitates the use of natural plants in the complementary medicine in order to showcase the long term adverse effects of some synthetic drugs.

Herbal drugs have increasingly been used worldwide during the last few decades as evidenced by rapidly growing global and national markets of herbal drugs. According to World Health Organization [1] estimates, the demand for medicinal plants is about US \$14 billion a year and by the year 2050 it would be about a trillion US dollars. Now people rely more on herbal drugs because of high price and harmful side effects of synthetic drugs, and this trend is growing not only in developing countries but in developed countries too [2]. A number of plants have been indicated to possess antimicrobial properties from traditional uses [3].

Medicinal plants have therefore been described as one in which one or more of its organs contain substance that can be used for therapeutic purposes [4]. It has been estimated that about one in four of all prescribed drugs, and almost 7,000 different medicaments contain compounds

of plants origin or their derivatives with their commercial value being put at about \$40 billion annually [5].

Studies indicated that about 33% of drugs produced in the developed countries are derived from plants. *Cymbopogon citratus* of the poaceae family is a tall, monocotyledonous aromatic perennial plant with slender sharp-edge green leaves, pointed apex that is native to tropical Asia. *C. citratus* is known as Guatemala in West Indian, or Madagascar Lemon grass. *Cymbopogon citratus* is cultivated in Africa, the West Indies, Central and South America, and Tropical regions. Lemongrass, *Cymbopogon citratus* is one of the important leaves among the species of grasses, and has various applications in traditional medicine. The grass is used in food or as culinary used i.e. it is eatable and also it can be used as cosmetic cream and even as herb. The linear leaves can grow up to 90 cm in height and 5m in width [6].

Lemon Grass (*Cymbopogon citratus*) and lemon (*Citrus limon*) can be used in treating HIV complications, especially secondary bacterial infections. Researchers from the Adelaide Tambo school of Nursing Science, Tshwan University of technology, Pretoria – West, South Africa have validated the use of lemongrass in treating thrush in people living with human immunodeficiency virus (HIV/Acquired immune deficiency syndrome (AIDS)). They concluded

that though the patients' population was small, the use of lemongrass for the treatment of oral candidiasis in an HIV population was validated by the randomized controlled trial [7,8].

Cymbopogon citratus belongs to the plant family Graminae to the French it is citronelle, citronella in Portuguese. In Nigeria, the Edos call it: *eti*, Efik: *Ikon eti*, Hausa call it: *tsauri*, Ibibio: *myobaka makara*, Igbo (Owerri) call it: *achara ehi* and Yoruba call it: *kooko oba* [8].

Lemongrass oil has analgesic, antimicrobial, antiseptic, carminative, astringent, fungicidal bactericidal and antidepressant properties, making it one of the most versatile and health promoting essential oils, which can help to kill both internal and external bacterial and fungal infections, such as ringworm and athlete's foot disease. Lemongrass ranked highest in inhibitory activity against methicillin-resistant *Staphylococcus aureus* (MRSA) infection. It is also helpful in relieving colitis indigestion and gastro-enteritis ailments. It helps relieve the symptoms of headache, body ache, nervous exhaustion and stress-related condition. Its infusions are often made useful in infections such as sore-throats, laryngitis, bronchitis etc [9].

Nigeria is covered with a large number of plant species, some of which have been used for centuries in folkloric medicines to diagnose, prevent and treat various ailments, but the scientific investigations and information on the therapeutic potentials of medicinal plants are limited. This lack of scientific knowledge has restricted the use of traditional herbs as remedies to be used in conjunction with or as an alternative to orthodox medical treatment [10]. Therefore, this research study aimed to qualitatively analyze the phytochemistry of lemongrass (*Cymbopogon citratus*), and to determine the antimicrobial effect of the leaf extracts on some selected clinical bacterial isolates, with view to determine the alternative or complementary therapy (using non synthetic drugs) against diseases caused by the test organisms.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh leaves of the plant *Cymbopogon citratus* (lemongrass) were collected from Zaria, Kaduna State in the month of January, 2016 and

identified and authenticated at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria. The plant leaves were washed in running water to remove adhesive contaminants. The plant leaves were dried in shade at room temperature for 5-7 days, and ground to obtain the dried powdered plant, which was kept in an airtight container before processing.

2.2 Procedure for the Extraction

A mass of 50 g dried powdered *Cymbopogon citratus* was suspended in 100 ml distilled water and filtered using a filter paper to obtain water soluble and water insoluble portions by maceration for 5 days at room temperature. The water insoluble fraction was partitioned with ethanol, chloroform, and acetone to give the ethanol, chloroform and acetone fractions respectively. The extract was concentrated using evaporation at reduced pressure. It was dried on an evaporating dish at a temperature of 50°C to 60°C to a semi-solid form. A gel semi-solid greenish substance was obtained for both samples. All the extracts were stored in a well corked universal bottle for further analysis.

2.3 Phytochemical Analysis (Qualitative Analysis)

The phytochemical screening of the crude *Cymbopogon citratus* extract was carried out using standard techniques for the plant secondary metabolites as described by Sofowora [11] and Silver et al. [12]. The tests carried out include: Test for Carbohydrates (Molisch's test); Test for Flavonoids (Shinoda Test); Detection of Glycosides: (Legal's test); Detection of Tannins; Detection for Phenols; Detection of Alkaloids; and Detection of Steroids.

2.4 Antibacterial Activity Study of Lemongrass

The susceptibility test of the plant extracts was carried out using the agar well diffusion method [13]. Nutrient agar was prepared according to manufacturer's specifications, sterilized, and poured into Petri dishes and allowed to set. Characterized clinical isolates of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* were collected on Nutrient agar from the Department of Medical Microbiology, Ahmadu Bello University, Zaria, Kaduna state, Nigeria. The isolates were sub-cultured and re-

characterized by microscopy and biochemical characterizations. A volume of 1 ml of a 0.5 McFarland preparation of each of the *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* was spread on the surface of the agar plates in triplicate using a sterile cotton swab, and the bacterium evenly spread over the entire surface of agar plate to obtain a uniform inoculum. Four wells were created using a sterile cork-borer on each plate that was previously seeded with the test organisms. A mass of 1 g of each extract was dissolved in 10 ml of distilled water to obtain a 100 mg/ml concentration. Serial dilution was employed to obtain the other concentrations of 50 mg/ml and 25 mg/ml. The volume of 1ml of different concentrations of each of the three concentrations of the ethanol, acetone and chloroform leaf extracts was dispensed into the corresponding wells and labelled accordingly. The fourth wells in each of the plates were filled with 1 ml preparation of Tetracycline (30 µg), Erythromycin (15 µg) and Ciprofloxacin (30 µg) which were used as control since these antibiotics are broad spectrum antibiotics, and are the common drugs prescribed against the selected bacterial species. The prepared cultured plates were incubated for 24 hours at 37°C. The plates were observed after incubation, and zones of growth inhibition were measured using metre rule and recorded. Oxoid [14] standard susceptibility range was used to classify zones of inhibition as either sensitive (> 10 mm) or resistant (≤ 10 mm).

2.5 Determination of Minimum Inhibitory Concentration (MIC) of Lemongrass Extract

The Minimum Inhibitory Concentration (MIC) was determined as the least concentration that showed an inhibitory effect on test organism using the tube method as described by Cheruiyot et al. [15]. The MICs were evaluated on plant extracts that showed antibacterial activity in the agar well diffusion assay. Two fold serial dilutions were made using nutrient broth up to the third dilution. Then 5 ml of a solution of the extracts (the concentrations of 100 mg/ml, 50 mg/ml and 25 mg/ml) was added aseptically to 5 ml of double strength medium and mixed by shaking. Using a fresh pipette, 5 ml of the mixture was transferred to test tube 2 which contained 5 ml of the single strength medium. This too was mixed by shaking and from it 5 ml was taken into test tube 3 aseptically and mixed by shaking. The 9th tube containing no test compound served as control. Finally, to each tube was

added 0.2 ml inoculums of the test organisms aseptically. The test tubes were covered with cotton wool and incubated at 37°C for 24hours and then observed for turbidity. The lowest concentration of the extract that inhibited growth of the test organism was noted as the MIC [16].

2.6 Evaluation of Minimum Bactericidal Concentration (MBC) of Lemongrass Extract

The MBCs of the extracts were determined using the method described by Adegboye et al. [17]. Samples were taken from tubes with no visible growth in the MIC assay and sub-cultured onto freshly prepared nutrient agar medium and later incubated at 37°C for 48 hours. The MBCs were taken as the lowest concentration of extract that did not allow any bacterial growth on the surface of the agar plates.

3. RESULTS AND DISCUSSION

3.1 Results

The results of this study showed that all the phytochemicals (flavonoids, carbohydrates, steroids, tannins, alkaloids, steroids, and phytosteroids) tested were all detected, except glycosides and phenols that were absent in the acetone and chloroform leaf extracts (Table 1).

The antibacterial activity of the leaf extracts is presented on Tables 2, 3 and 4. The ethanol leaf extract was active at the concentration of 100 mg/ml and showed intermediate antimicrobial activity at 50mg/ml against all the isolates tested. Whereas, acetone and chloroform leaf extracts showed inactivity against *E. coli* and *S. aureus* respectively (Tables 2, 3, and 4).

Table 5 shows the minimum inhibitory concentration and minimum bactericidal concentration of the *Cymbopogon citratus* plant extracts on the test organisms, with acetone extract recording least MIC of 25 mg/ml for *Salmonella typhi* and *Staphylococcus aureus*.

Table 6 shows the minimum bactericidal concentration and minimum bactericidal concentration of the *Cymbopogon citratus* plant extracts on the test organisms.

3.2 Discussion

The phytochemical analysis showed that various plant secondary metabolites are present in the *Cymbopogon citratus* leaf extracts. All the

phytochemicals tested for such as flavonoids, carbohydrates, steroids, tannins, alkaloids, steroids, glycosides, phenols and phytosteroids are present in chloroform and ethanol-leaf extract (Table 1). This agrees with the findings of Sofowora [11] and AOAC [18], who reported that many results of phytochemical composition of the

ethanol leaf extract of *Cymbopogon citratus* shows that it contains alkaloids, saponins, tannins, anthraquinones, steroids, phenols, and flavonoids. Each of these phytochemicals is known for various protective and therapeutic effects. For instance, phenol is known to be an erythrocyte membrane modifier.

Table 1. Phytochemical composition of *Cymbopogon citratus* (Lemongrass) leaf extract

Phytochemical constituent	Acetone extract	Ethanolic extract	Chloroform extract
Tannins	+	+	+
Carbohydrate	+	+	+
Flavonoids	+	+	+
Glycosides	-	+	-
Alkaloids	+	+	+
Steroids and phytosteroids	+	+	+
Phenols	-	+	-

Table 2. Antimicrobial activity of ethanol leaf extract of lemongrass in millimeter

Test organisms	Concentration				Control	
	100 mg/ml	50 mg/ml	25 mg/ml	TET(30 µg)	ERY(15 µg)	CIP(30 µg)
<i>Escherichia coli</i>	23 mm	17 mm	06 mm	15 mm	*	*
<i>Salmonella typhi</i>	19 mm	10 mm	07 mm	*	15 mm	*
<i>Staphylococcus aureus</i>	17 mm	13 mm	11 mm	*	*	14 mm

Key: (-) = No activity, (*) = Not used, (mm) = zone of inhibition in millimetre, TET= Tetracycline, ERY= Erythromycin, CIP= Ciprofloxacin

Table 3. Antimicrobial activity of acetone leaf extract of lemongrass in millimetre

Test organisms	Concentration				Control	
	100 mg/ml	50 mg/ml	25 mg/ml	TET(30 µg)	ERY(15 µg)	CIP(30 µg)
<i>Escherichia coli</i>	-	-	-	15 mm	*	*
<i>Salmonella typhi</i>	20 m	16 mm	14 mm	*	14 mm	*
<i>Staphylococcus aureus</i>	17 mm	15 mm	14 mm	*	*	14 mm

Key: (-) = No activity, (*) = Not used, (mm) = zone of inhibition in millimetre, TET= Tetracycline, ERY= Erythromycin, CIP= Ciprofloxacin

Table 4. Antimicrobial activity of chloroform leaf extract of lemongrass in millimetre

Test organisms	Concentration				Control	
	100 mg/ml	50 mg/ml	25 mg/ml	TET(30 µg)	ERY(15 µg)	CIP(30 µg)
<i>Escherichia coli</i>	15mm	10mm	06mm	16mm	*	*
<i>Salmonella typhi</i>	12mm	11mm	09mm	*	14mm	*
<i>Staphylococcus aureus</i>	-	-	-	*	*	14mm

Key: (-) = No activity, (*) = Not used, (mm) = zone of inhibition in millimetre, TET= Tetracycline, ERY= Erythromycin, CIP= Ciprofloxacin

Table 5. Minimum inhibitory concentration of lemongrass

Test organisms	MIC (mg/ml)		
	Ethanolic extract	Acetone extract	Chloroform extract
<i>Escherichia coli</i>	50	50	100
<i>Salmonella typhi</i>	100	25	100
<i>Staphylococcus aureus</i>	100	25	50

MIC= minimum inhibitory concentration

Table 6. Minimum bactericidal concentration of lemongrass

Test organisms	MIC (mg/ml)		
	Ethanol extract	Acetone extract	Chloroform extract
<i>Escherichia coli</i>	100	25	50
<i>Salmonella typhi</i>	50	25	100
<i>Staphylococcus aureus</i>	100	50	50

MBC= Minimum bactericidal concentration

The results of the study showed that all the tested phytochemicals (flavonoids, carbohydrates, steroids, tannins, alkaloids, steroids, and phytosteroids) were detected. This conformed to the work of Praditvarn and Samhandharaksa [19] who reported the presence of the aforementioned phytochemicals in the lemongrass extracts.

The results of the antibacterial activity of the extracts of *Cymbopogon citratus* against *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* showed that ethanol leaf extract was active against all the test organisms at the concentration of 100 mg/ml, 50 mg/ml and 25 mg/ml. The ethanol leaf extract recorded better antimicrobial activity when compared with acetone and chloroform leaf extracts. This may be as result of varying polarity and the ability of ethanol to extract more of the plant active components more than the other solvents used. The highest mean zone of inhibition (23 mm) of *Escherichia coli* by the ethanolic extract was recorded at 100 mg/ml while the lowest (06 mm) was at 25 mg/ml. This agrees with the work of Kolodziej and Kormas [20] and Komiya et al. [21] who reported that phytochemicals such as tannins have antibacterial and antileishmanial activity due to their immune modulatory effects on the microbial antigenic receptors. Other phytochemicals were analyzed by phenolic extraction, which includes diverse group of chemical compounds, such as flavonoids, lignins, tannins, phenolic acids, coumarins, phenols, phenylpropanoids, quinines, stilbenoids and xanthenes [22].

All concentrations of the acetone extract did not show activity on *Escherichia coli* i.e. the organism was not susceptible at 25, 50 and 100 mg/ml concentrations of the acetone extract. Also, *Staphylococcus aureus* was not susceptible at 25, 50 and 100 mg/ml concentrations of the chloroform extract, but *Salmonella typhi* and *Escherichia coli* are sensitive to various

concentrations of acetone and chloroform leaf extracts used.

The minimum inhibitory concentrations of ethanol extract were found to be 50 mg/ml, 100 mg/ml and 100 mg/ml for *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* respectively. The MICs of acetone extract were found to be 50 mg/ml, 25 mg/ml and 25 mg/ml for *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* respectively. While, the MIC of the chloroform extract recorded 100 mg/ml, 100 mg/ml and 50 mg/ml for *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* respectively. Therefore, the extracts of acetone are more effective in killing *Salmonella typhi* and *Staphylococcus aureus* at lower dosage.

Based on the results obtained, lemongrass has demonstrated varying degree of antibacterial activity against *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. Therefore, this signifies that some bacteria that have not been tested with lemongrass extract in this research may also be susceptible to the antibacterial effect of lemongrass.

4. CONCLUSION

The preliminary phytochemical screening of the ethanol leaf extracts revealed the presence of flavonoids, tannins, alkaloids, glycosides, phenols, steroids and phytosteroids and carbohydrates, some of which could be used as medical regimens. Lemongrass has demonstrated antimicrobial properties which could be harnessed for the development of alternative means of therapeutic control of bacterial pathogens.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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