



Phytochemical and Antimicrobial Activities of the Leaves and Roots Extracts of Lemon Grass (*Cymbopogon Citrates*) from Basiri, Ado-Ekiti, Ekiti State, Nigeria

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Abstract

The research study aimed to extract *Cymbopogon citratus* leaf and root using various solvents with view to determine the phytochemical analysis and antimicrobial effect of the plant extracts on selected microorganisms. All the extract was subjected to phytochemical and antimicrobial activities for the presence and absence of various primary and secondary metabolites. Phytochemicals such as alkaloids, tannins, phenol, reducing sugar and saponin were present in both leaf and root extracts. And this revealed that the presence of the phytochemical which could be used for medical regimens. Antimicrobial screening showed that the plant is biologically active against *Staphylococcus aureus* and *Escherichia coli*.

Keywords: *Cymbopogon Citratus*, Phytochemistry, Antimicrobial screening, and biological pathogens.

Introduction

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans as medicinal ingredients and nutrients [1]. They protect plants from disease and damage, and also contribute to the plant's colour, aroma and flavour. In general, the plant chemicals that protect plants from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called phytochemicals [2]. Recently, it has been clearly shown that they also have roles in the protection of human health, when their dietary intake is significant [3]. *Cymbopogon citratus* is an aromatic plant belonging to the Gramineae family [4]. It is a tall, clumped perennial grass growing to a height of 1 m. The leaf-blade is linear, tapered at both ends and can grow

to a length of 50 cm and width of 1.5 cm [5]. Lemon grass (*Cymbopogon citratus*) are commonly cultivated as culinary and medicinal herbs because of their scent, resembling that of lemons (*Citrus limon*). Lemon grass common names include barbed wire grass, silky heads, citronella grass, and fever grass, amongst many others. Lemon Grass (*Cymbopogon citratus*) and lemon (*Citrus limon*) can be used in treating HIV complications, especially secondary bacterial infections.[6], 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 [7].The general objective of the study is to investigate the qualitative phytochemical analysis and antimicrobial activities of leaves and roots extract of lemon grass and to determine the functional group present in the leaves and roots extract of *Cymbopogon citratus* with FT-IR spectroscopy.

Materials and Methods

Sample Collection and Identification

The leaves and roots of *Cymbopogon citratus* (lemongrass) was collected from Basiri street in Ado-Ekiti in Ekiti State, Nigeria in month of January, 2020 and identified at the department of science technology (microbiology unit) the Federal Polytechnic Ado Ekiti. The plant leaves and roots were washed in running water to remove adhesive contaminants. The plant leaves and roots were dried in laboratory bench at room temperature for three weeks, and grind to obtain the dried powdered plant, which was kept in an airtight container before processing.

Procedure for the Extraction

50g of each dried sample of the leaves and roots of the Lemon grass (*Cymbopogon citratus*) was added separately to 500ml of ethanol. The samples were allowed to stand overnight at room temperature with intermittent shaking. Each sample was filtered using Whatman no 1 filter paper. 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.

Phytochemical Analysis Determination (Qualitative Analysis)

Phytochemical screening was carried out on the leaf and the root extracts of the Lemongrass (*Cymbopogon citratus*) using standard procedures according to the guide by Harborne J. B [8] and Sofowora [6]. The following test were carried out: test for Alkaloids, flavonoids, tannins, saponins, terpenoids, reducing sugar, Cardiac Glycosides, steroids and phenols.

Antimicrobial Activities of the Cymbopogon Citratus Collection of Micro Organism

Micro-organisms was collected in micro biology laboratory (microbiology research unit) in Federal polytechnic Ado Ekiti, Ekiti State and the bacterial species were *Staphylococcus aureus*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa* and *Escherichia coli*.

Preparation of Sample Solution

50g powder of lemongrass was blended with 150ml of ethanol, Orbital shaker was used for the extraction purpose in which the sample subjected to continuous shaking for 24hours. The sample was then filtered out using Whatman No. 1 filter paper, and then the filtrates were evaporated using a rotary evaporator under reduced pressure at 4°C. The extract was pooled and dried and stored at 4°C in a refrigerator until screened for antibacterial

Preparation of Neutrient Agar

36g of Muller Hinton nutrient agar was measured on an analytical balance and it was dissolved in 100ml of distilled water under aseptic condition in order to avoid it been contaminated with external microbes, the agar solution was then sterilized in an autoclave at the temperature of 110°C and 5kpacal pressure for 1 hour after which the viscous sterilized agar was poured into plate and was allowed to solidified in the dish

Sterilization of Petri Dishes

Clean petri-dish was sterilized in the oven under 120°C of about 30mintes.

Screening Activities

Agar well diffusion method was employed to assess the antibacterial activity of *Cymbopogon citratus* ethanol extracts against the human pathogenic bacteria. The overnight bacterial culture was taken to prepare the inoculums and adjust to 0.5 McFarland standards in 0.9% autoclaved normal saline. The Muller Hinton agar medium was prepared and autoclaved at the 121°C for 15 min. The media was poured into each petri dish and set aside to solidify under the laminar hood. After solidifying of the media, a sterile cotton swab was used to spread the inoculums throughout the medium uniformly. Wells were made using a 6 mm diameter cork borer. Then, 100 µl of each extract adjusted to the same concentration (50 mg/ml) was totaled to a respective well. The agar plate was allowed to rest for 1 hour under the laminar hood and incubated later at 37°C for one daytime. The sensitivity of the test microorganisms were found by assessing the diameter of the zone of inhibition in which significant susceptibility was taken as ≥ 7 mm in diameter.

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy spectrum of *C. citratus* leaf and root was obtained using FTIR spectrophotometer (Perkin Elmer). FTIR used for chemical identification as each molecule and chemical structure creates a unique spectra. The IR spectra were accounted in

% transmittance. The wave number region for analysis was 4000–400 cm^{-1} (mid-infrared range.) with resolution of 0.15 cm^{-1} .

Results and Discussion

Phytochemical Screening Results

Phytochemical analysis of leaves and roots extract of *Cymbopogon citratus* is shown in the table 1 below:

Table 1. Summary of Phytochemical Analysis of Leaf and Root Extract of *Cymbopogon Citratus* Result

Parameters	Leaves A	Root B
Alkaloids	++	++
Flavonoids	-	++
Phenol	++	++
Saponins	++	-
Tannins	+	++
Steroids	-	-
Cardiac Glycosides	-	++
Reducing sugar	++	++
Terpenoids	++	+

Keys: ++ = Highly present, + = Present, - = Absent

From the table 1, it was observed that extraction and Phytochemical screening of bioactive agents from medicinal plants permits the demonstration of their physiological activities. The Phytochemical analyses showed that alkaloids, phenols, tannins, reducing sugar and terpenoids were present in both ethanolic leaves and roots extract. Flavonoids and cardiac glycosides were present in the ethanolic root extracts and absent in the leaves extract, saponins is present in the leaves and absent in the roots while steroids are both absent in both leaves and roots extracts. The medicinal value of plants lies in some chemical substances that have definite physiological functions in the human body. Different phytochemicals have been found to possess a wide range of medicinal properties, which may help in protection against various diseases. For example, alkaloids protect against chronic diseases; saponins protect against hypercholesterolemia and steroids and triterpenoids show the analgesic properties [9]. According to Kolodziej and Kiderlen (2005), tannins and phenolic compounds have been found to inhibit bacterial and fungal growth and also capable of protecting certain plants against infection, Phytochemical component has antifungal properties which were confirmed in this study. The presence of tannins in the plant extract agrees with the report of IUPAC (1995) that tannins are important in herbal medicine and they are applied in arresting bleeding and wound healing. Tannins and tannic acid own their stringent action to the fact that they precipitate protect and render them resistant to attack by proteolytic enzymes, internally; they form a pellicle of coagulated protein over the lining of the alimentary tract.

Antimicrobial Activities

The results for anti-microbial activities result of leave extract of *Cymbopogon citratus* was shown table 2 below

Table 2

Test organisms	Concentration (g/mL)			
	Diameter of zones of inhibition (mm)			
	0.2	0.4	0.6	0.8 Control
<i>Pseudomonas aeruginosa</i>	10	12	15	18 -
<i>Escherichia coli</i>	-	-	11	14 -
<i>Staphylococcus aureus</i>	20	22	28	30 -
<i>Klebsiella sp</i>	14	28	22	24 -

Keys : - = No zone of inhibition, ++ = Zone of inhibition

The results for anti-microbial activities result of roots extract of *Cymbopogon citratus* was shown table 3 below

Table 3

Test organisms	Concentration (g/mL)			
	Diameter of zones of inhibition (mm)			
	0.2	0.4	0.6	0.8 Control
<i>Pseudomonas aeruginosa</i>	8	10	12	15 -
<i>Escherichia coli</i>	10	14	18	20 -
<i>Staphylococcus aureus</i>	14	21	24	28 -
<i>Klebsiella sp</i>	-	12	15	17 -

Keys , - = No zone of inhibition , ++ = Zone of inhibition

From the table 2 and 3 above shows results of the experiments were carried out on the antimicrobial activity of dried leaves and root extract of lemongrass with ethanol against with different microorganisms as shown in table 2 and 3. This study also revealed that the root ethanolic extract of lemon grass (*Cymbopogon citratus*) possesses high antibacterial activity as it showed in table 2 the broadest spectra against all bacteria tested except *Klebsiella spp* at 0.2g/mL concentration with no active effect. This could be attributed to the presence of a high concentration of phytochemicals which in turn is facilitated by the solubility capabilities of ethanol used as an extraction solvent. Similar reports in concordance with this phenomenon include the works of Hindumathy (2011)[10], Kruthi *et al.* (2014)[11]. Table 2 results of the antibacterial activity of the extracts of *Cymbopogon citratus* against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella spp* and *Staphylococcus aureus* showed that ethanol leaf extract was active against all the test organisms at the concentration of 0.6 and 0.8g/mL. The ethanol leaf extract recorded better antimicrobial activity when compared with root extracts. This may be as result of varying polarity and the ability of ethanol to extract more of the plant active components from the leaves more than the root used. The highest mean zone of inhibition (30 mm) of *Staphylococcus aureus* by the ethanolic extract was recorded at 0.8g/mL while the lowest (10 mm) was at 0.2g/mL in *Pseudomonas aeruginosa*.

It was also observed in both tables that, Gram-negative bacteria used in this study *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were less susceptible (with lesser zones of inhibition) to the extracts compared to the Gram-positive organisms *Staphylococcus aureus*, being the most susceptible. This is as a result of the variation in cell wall structures and complexity of these bacteria [12]. This observation also agrees with the reports of Kruthi *et al.* (2014).

Based on the results obtained, lemongrass has demonstrated varying degree of antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* 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. Lemongrass has demonstrated antimicrobial properties which could be harnessed for the development of alternative means of therapeutic control of bacterial pathogens.

The results of this study therefore imply that *Cymbopogon citratus* has great antibacterial activities and contains biologically active compounds with pharmacologic properties. Hence, it is a reliable source of therapy for infections caused by the test organisms. Further modifications of the plant extracts will increase the scope of its use for prophylaxis and therapy.

Fourier Transform Infrared Spectroscopy (FTIR) Result

The Fourier transform infrared spectroscopy (FTIR) result of the leaves and roots extract were shown table 4 and 5 with the spectrograph in fig., 1 & 2 below.

Table 4. IR absorption spectra of lemon grass leaves

S/N	Peaks	Inference
1	3324	N-H
2	2974	C-H
3	2929	C-H
4	2886	OH
5	2132	OH
6	1379	C-N
7	1274	C-O
8	1088	C-O
9	1043	C-H

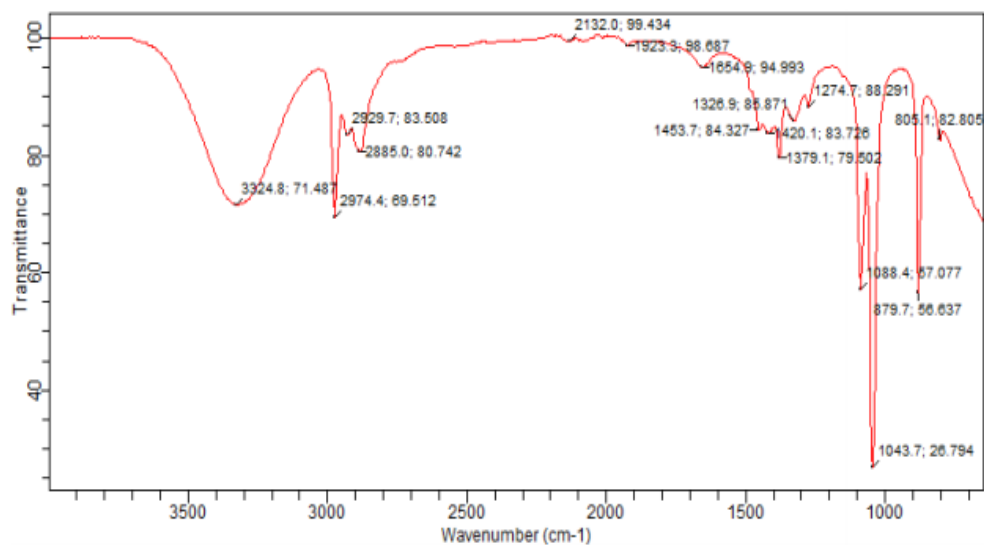


Figure 1. FT-IR spectrograph of the Lemon grass leaf extract

Table 5. IR absorption spectra of lemon grass leaves

S/N	Peaks	Inference
1	3324	N-H
2	2944	C-H
3	2974	C-H
4	2832	O-H
5	1923	C=O
6	1654	C-N
7	1412	C-O
8	1449	C-O
9	1382	C-O

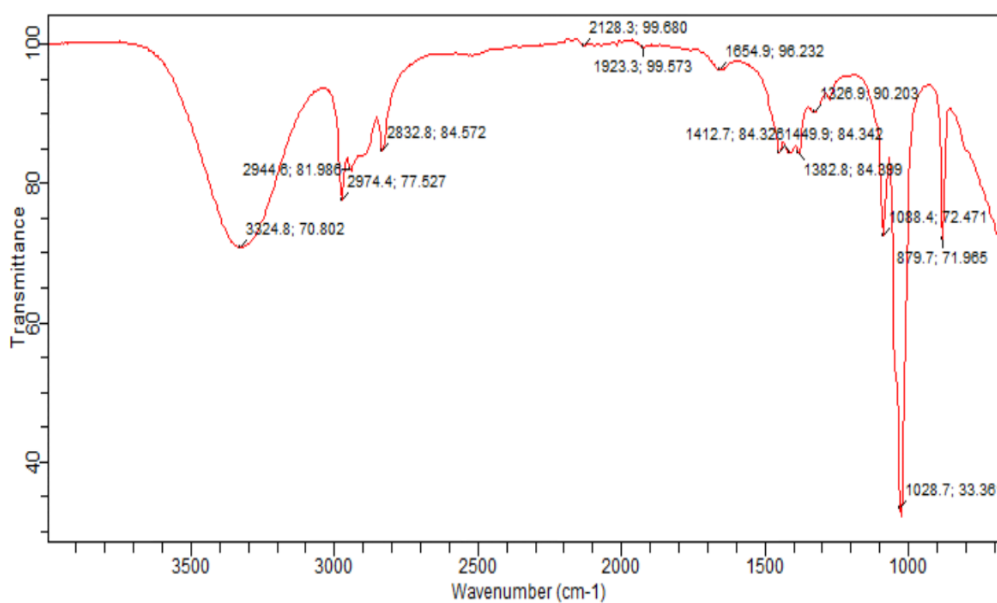


Figure 2. FT-IR spectrograph of the Lemon grass root extract

From table 4 and the spectra above in figure 1, the IR spectra of lemon grass leaf having strong characteristic peaks at 3324cm^{-1} show the presence of N-H and peaks at 2974, and 2929cm^{-1} , show the C-H stretching, 2886 and 2132cm^{-1} shows the presence of O-H group and 1379,1274,1088 and 1043cm^{-1} shows the presence of C-O group.

From table 5 and spectra in figure 2, shows the IR of lemongrass root having strong peaks at 3324cm^{-1} show the presence of N-H, the peak at 2944 and 2974cm^{-1} show the presence of C-H stretching, and peak at 2832cm^{-1} shows the presence of O-H group and peak at 1923cm^{-1} shows the presence of C=O and also the peak at 1412, 1449 and 1382cm^{-1}

Conclusion

Phytochemical test carried out on the leaves and root extracts of the plant *Cymbopogon citratus* showed that it contains alkaloid, tannin and saponins. The antimicrobial studies showed that the root and the leaves extract are active against *Staphylococcus aureus* and *Escherichia coli* at concentration not below 0.2 g/ml. This results has confirmed the use of this plant for the treatment of disease caused by *Staphylococcus aureus* and *E. coli* such disease include fever. The FTIR analysis showed that the leaves and root extracts contains amine, alkene, carboxylic and ester groups.

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