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Antibacterial, GC-MS and FTIR Chemical Profiling of Methanol Leaf Extract of *Cymbopogon citratus* Linn

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Abstract: *This study was carried out in order to determine the phytochemical constituents using analytical methods (Phytochemicals, GC-MS and FTIR) and antimicrobial potential of Cymbopogon citratus methanol leaf extract. The plant leaves were extracted using the cold maceration process from dried crushed leaves. Qualitative analyses exhibited the presence of alkaloids, flavonoids, Saponin tannins, glycosides, steroids and terpenoids. A total of 17 compounds were identified by GC-MS which ranges from high molecular weight to low molecular weight compounds. The FTIR characterization carried out on the extract confirmed the presence of functional groups such as: C-H, C=O, N-H and C=C indicating peak of alkane, carbonyl group, amine and monosubstituted benzene. An antimicrobial study of the extracts was carried out against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosaand Salmonella typhi using agar—well diffusion method. The extract was found to be effective against all the test organisms with the highest zone of inhibition at 100 mg/ml, Escherichia coli, (zone of inhibition = 24), Staphylococcus aureus (zone of inhibition = 20)Pseudomonas aeruginosa (zone of inhibition = 21)and Salmonella typhi (zone of inhibition = 15)*

Keywords: *Antibacterial activity; Bacterial pathogens; Cymbopogon citratus. GC-MS and FTIR*

1. INTRODUCTION

Medicinal plants have long been an integral part of traditional medicine systems, particularly in developing nations with little access to basic health care, and they continue to play an important role in several heath care systems today. Herbal drugs that are more dependable, secure, and affordable are gaining popularity in both urban and rural areas. [1]. Plants have become a rich source of medicinal products for the treatment of a variety of ailments, owing to the presence of a plethora of different metabolites that have a variety of therapeutic effects. There is no doubt that plants used in folk medicine have been of great importance for the production of drugs [2].The discovery of medicinal plant drugs makes new and substantial advances against many pharmacological targets, including cancer, HIV, Alzheimer's and malaria [3]. More recently, the isolation of active chemicals has expanded to include the use of plants as medicines, beginning with the isolation of early narcotics like cocaine. [4]. There is a resurgence of interest in herbal medicine, as well as a growing desire for more medications derived from plants.

The growing popularity of plant-based medicines stems from the widespread perception that green medicine is both safer (with less side effects) and more effective than costly synthetic pharmaceuticals, many of which have significant side effects. [5]. Despite the availability of modern medicine in some cultures, herbal medicines (medicinal plants) have remained popular for historical and cultural reasons, in addition to their safety, effectiveness, and reduce costs [6].

They can also serve as a source of potentially lucrative new medicinal chemicals, as all portions of a plant, from roots to seed heads and flowers, can provide lead compounds[7].

As a result, developing a treatment using plant extracts or plant-derived compounds has become a natural blueprint for new drug development.. [8]. There are around 270,000 higher plants that exist on this planet. Nigeria's southeastern region, in particular, boasts some of the world's most diverse plant

species. However, for phytochemical and biological investigations, only a small portion has been explored. It looks like we have just scratched the surface of this world's wonderful resource. The search for drugs that are both safe and non-toxic for the treatment of numerous human illnesses is thus a major research priority. Screening for bioactive chemicals in medicinal plants is required as a basis for subsequent biomedical research. With advances in phytochemical techniques, certain active components of many medicinal plants have been identified and used as valuable medications in current medical systems.

The most frequent bioactive chemicals are alkaloids, flavonoids, tannins, and phenolic compounds [9]. They are important raw materials for the manufacture of pharmaceutical products and other neutriceucals [10]. *Cymbopogon citratus* is an important fragrant plant that contains numerous phytonutrients and is widely utilized for its pleasant flavor and therapeutic potential.

Cymbopogon citratus is of great interest in food technology and traditional medicine because of its commercially important essential oils[11-12]. *Cymbopogon citratus* are generally referred to as citronella grass or lemongrass, is aperennial aromatic tall grass with rhizomes and densely tufted fibrous root. It has small underground stems with ringed parts, slightly leathery coarse, green leaves in thick clusters [13].

The plant is used to treat digestive diseases, mental disorders, fevers, menstrual disorders, rheumatism, and other joint ailments in various parts of the world.

Lemon grass aerial components have long been utilized in folk medicine as an infusion or stew.

The plant's main phytoconstituents include essential oils, flavonoids, and phenolic chemicals.

The plant also contains alkaloids, saponins, tannins, anthraquinones, and steroids[14][13]. *C. Citratus* leaves have historically been used as tea to protect against influenza, fever, pneumonia, added to non-alcoholic drinks and baked foods, and used in confections and cuisines as a flavoring agent and preservative.

The leaf contains essential bioactive compounds that establish the plant's anti-inflammatory, antiseptic, anti-dyspeptic, anti-fever, anti-spasmodic, analgesic, anti-pyretic, tranquilizing, anti-hermetic and diuretic properties [15-17].

Its essential oils are used as a fragrance in the production of perfumes, soaps, detergents, and creams in cosmetic industries, as well as additives in candles and other insect repellents [17]. 18]. It has been utilized as a snake and reptile repellent in certain Asian and African countries.

Researchers have discovered that lemongrass revitalizes the body and promotes overall health.

It improves digestion by regulating xenobiotic-metabolizing enzymes in the liver and intestines, as well as preventing carcinogenesis caused by chemicals. [15].

2. MATERIALS AND METHODS

Collection and identification of *Cymbopogon citratusis*

The leaves of Cymbopogon citratus were harvested from a botanical garden in the National Root Crop Research Institute, Umudike Abia State, Nigeria on the 18th May, 2021.

The plant was taxonomically identified and authenticated by Dr. O. Emmanuel at the Department of Plant Science and Biotechnology, Abia State University, Uturu, Nigeria. A voucher specimen [ABSU/ CC/1289] has been deposited in the Botanical Department of Biological Sciences School.

2.1. Preparation of *Cymbopogon citratusis* Leaf Extracts

Fresh plant leaves were washed with running tap water for 5 minutes to remove the dust and debris and rinsed with sterile distilled water.

The sample was air dried on the laboratory bench for fifteen days at room temperature to prevent decomposition of thermo labile compounds. The dried specimen was milled into coarse powder using an electric blender. 300 g of the pulverized leaf material was mixed with 500 ml of solvent (95 % methanol) and introduced into a rotary shaker operating at 100 rpm for twenty-four hours. Whatman

No.1 filter paper was used to filter out the resulting solution. The extract was concentrated under reduced pressure using Digital Heidolph Rotary evaporator (4000 series) and the supernatant plant extract was decanted after complete removal of the solvent.

2.2. Instrumentation for GC-MS Analysis

The HP 7890 GC instrument integrated with the Agilent 5975C MSD mass spectrometer (Agilent, Santa Clara, CA, USA) was used for the GC-MS study of the methanol leaf extract of *Cymbopogon citratusis*. The capillary column was Agilent HP-5MS (30 m x 0.25 mm i.d. x 0.25 NM film thickness), the carrier gas was Helium (purity > 99.999%), and the flow rate was 1 mL / minute.The temperature of the injector was 2500 ^{0}C, and the injection mode was split less. The temperature of the G.C oven was kept for 5 minutes at 500 ^{0}C, which was raised to 2100 ^{o}C at a rate of 30 ^{0}C / minutes, sustained for 3 minutes at 2100 ^{0}C, and finally raised to 2300 ^{0}C at 1500 ^{0}C / minutes. The conditions of the mass spectrometer were as follows: ionization energy, 70 eV; temperature of the ion source, 2300 ^{0}C; quadruple temperature, 150 ^{0}C; quadruple mass spectrometer scan range 30 to 500 atomic mass units (amu); solvent delay time 2.8 minutes.

2.3. Infrared Spectroscopy Fourier-Transform (FTIR)

FTIR has been shown to be a valuable instrument for characterizing and distinguishing compounds or functional groups (chemical bonds) present in an unknown plant mixture [19] Furthermore, FTIR spectra are normally so special that they are like a molecular "fingerprint." The spectrum of an unknown compound can be described by reference to a library of recognized compounds for the most common plant compounds. FTIR analysis was conducted using Agilient spectrophotometer system, which was used to detect the characteristic peaks and their functional groups using Attenuated Total Reflectance (ATR) accessory. The FTIR scan was performed in the wave number region of 4000-550 cm^{-1} (mid- infrared range**.**

2.4. Identification of Compounds

The National Institute of Standard and Technology (NIST) database with more than 62,000 patterns was used to interpret the GC-MS profile. In addition, this analysis obtained information on components of the test materials, such as retention time, name, molecular weight and structure. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library, determining the name, molecular weight and component structure of the test materials. [20] [12]

2.5. Phytochemical Screening

Phytochemical analysis was carried out for identification of tannins, terpenoids, flavonoid, alkaloid, phenol, glycosides, and saponins. Standard procedures were followed to identify the constituents as described by [21-22].

2.6. Antibacterial Assay of the Plant Leaf Extract

Standard strains of one gram-positive (*S. aureus*) and three gram-negative *(E. coli, S. typhi, and P. aeruginosa*) were used for antibacterial effect determination. The antibacterial activity of the plant leaf extract of *Cymbopogon citratus* for different concentration (25 mg/ml, 50 mg/ml, 75 mg/ml and 100mg/ml) were determined using the agar well diffusion method as described by [23]. A standardized inoculum was used for the inoculation of plates Muller Hinton agar was prepared according to the specifications and allowed to cool to about 40-50°C. The freshly prepared and cooled media was poured into Petri dishes and allowed to solidify at room temperature. A sterile swab stick was used to spread about 0.2 ml ofthe standardized test inoculum evenly on the surface of the solidified media. Five equidistant wells of 5 mm in diameter were then made on the seeded agar plate using a sterile cork borer and the plant extracts with concentrations ranging from 25-100 mg/ml were introduced into the bored holes. The plates were then incubated at 37°C for 24 hrs. 0.1 mg mL ciprofloxacin solution (positive control) Zones of inhibition around the isolates were measured with graduated scale after the period of incubation.

Table1. *Antibacterial Effects of Cymbopogon citratusPlant leaf Extract on Selected Strains of Bacteria*

Test sample	**Concentration Mg ML**	**Zone of inhibition/ Bacteria Strain (mm)**		
*C. ciratus*SA	100	EC	PA	SAL
24	75	20	21	15
18		17	19	13
50	13	11	10	11
25	07	09	05	10
Ciprofloxacin	0.127	24	20	19

SA= *Staphylococcus aureus, EC = Escherichia coli, PA=* Pseudomonas aeruginosa*SAL= Salmonella typhi*

Table2. *Qualitative analysis of phytochemical constituent present in C. citratus extract*

S.N	**Phytochemical test**	***C. citratus* Methanol Extract**
1	Flavanoid	+
2	Alkaloid	+
3	Tanin	+
4	Saponin	+
5	Steroid	+
6	Terpenoid	+
7	Cardiac Glycoside	+

(+): Positive result (Presence of the phytochemical)

(-): Negative result(Absence of the phytochemical)

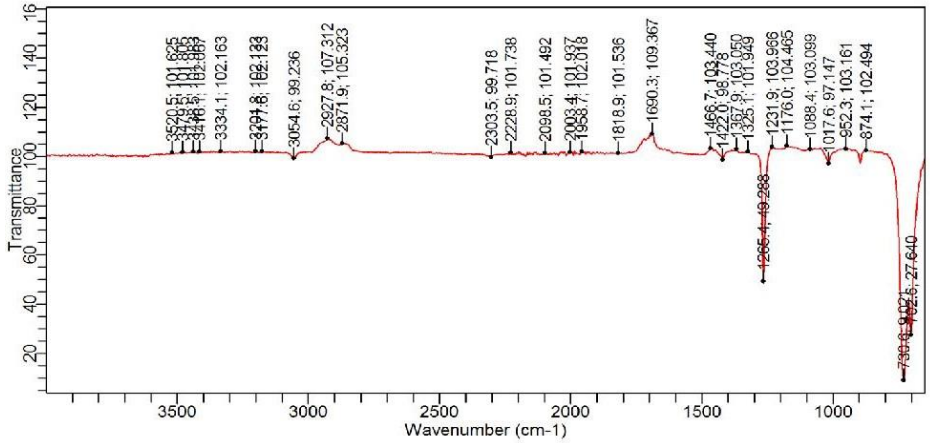
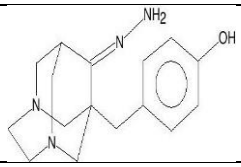
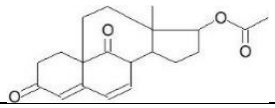
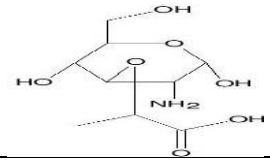
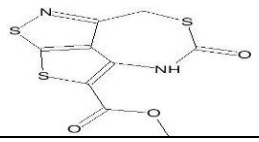
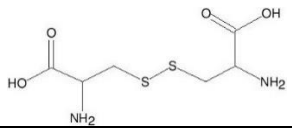
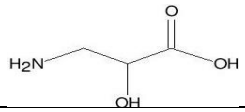
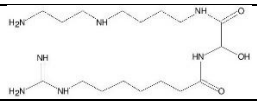
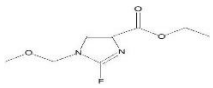
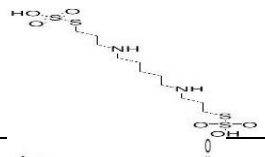
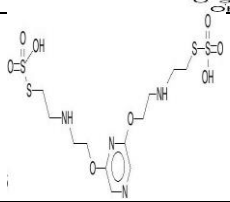


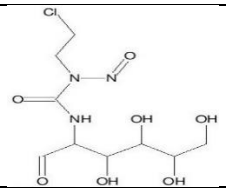
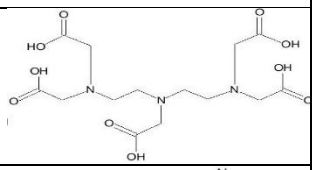
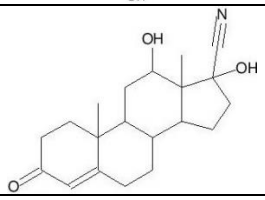
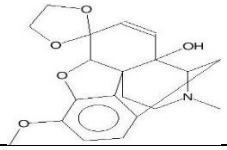
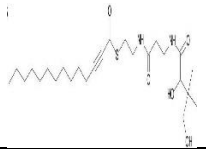
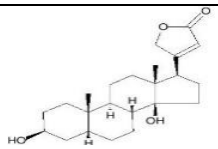
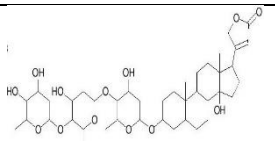
Figure1. *FTIR Spectrum Cymbopogon citratus*

Table3. *FTIR Analysis of Cymbopogon citratus*

Absorption frequency	Functional group	Stretching/bending vibration
3054.6	Amine	N-H
2927.8	Alkanes	C-H
2871.9	Alkanes	C-H
1690.3	Carbonyl	C=O
1466.7	Aliphatic	C-H
1422.0	Aliphatic	C-H
1266.4	Amine	N-H
1017.6	Aliphatic Fluoro compounds	C-F
703.6	Monosubstituted benzene	C=C

Table4. *Phytochemicals identified in Cymbopogon citratusextract by GC-MS*

SN	Phytochemical compound	Formula	Molecular weight	R.T	Chemical structure
1	1-(4-Hydroxyphenylmethyl)-3,6-diazahomoadamantan-9-one hydrazone	$C_{16}H_{22}N_4O$	286	0.576	
2	10,11-Cyclo-9,11-secoestra-4,6-diene-3,9-dione,	$C_{20}H_{24}O_4$	328	1.876	
3	Muramic acid	$C_9H_{17}NO_7$	251	1.913	
4	2,8-Diaza-3,5,10-trithiatricyclo[5.4.1.0(4.12)]dodeca-1,4(12),6-trien-6-carboxylic acid,	$C_9H_6N_2O_3S_3$	286	2.342	
5	Cystine	$C_6H_{12}N_2O_4S_2$	240	2.754	
6	Isoserine	$C_3H_7NO_3$	105	3.906	
7	Deoxyspergualin	$C_{17}H_{37}N_7O_3$	387	4.678	
8	Imidazole-4-carboxylic acid, 2-fluoro-1-methoxymethyl	$C_8H_{11}FN_2O_3$	202	5.243	
9	N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid]	$C_{11}H_{26}N_2O_6S_4$	410	6.765	
10	2,6-Bis[2-[2-S-thiosulfuroethylamino]ethoxy]pyrazine	$C_{12}H_{22}N_4O8S_4$	478	8.345	
11	Chlorozotocin	$C_9H_{16}ClN_3O_7$	313	10.345	

					
11	Pentetic Acid	$C_{14}H_{23}N_3O_{10}$	393	12.787	
12	Preg-4-en-3-one	$C_{20}H_{27}NO_3$	329	14.659	
13	Morphinan-6-one,	$C_{20}H_{23}NO_5$	357	16.617	
					
15	2-Myristynoyl pantetheine	$C_{25}H_{44}N_2O_5S$	484	22.528	
16	Digitoxigenin	$C_{23}H_{34}O_4$	374	23.786	
17	Digitoxin	$C_{41}H_{64}O_{13}$	764	25.408	

3. Results and Discussion

3.1. Analysis of Phytochemical Constituents

The phytochemical screening of Cymbopogon citratus methanol leaf extract as presented in (table 2) revealed the presence of flavonoid, phenol, alkaloid, tannin, terpenoid, and glycoside chemicals, which is consistent with previous studies [24]. The significant abundance of metabolites in plant extracts could explain their use in traditional medicine for the treatment of infectious disorders [25]. has reported the anti-inflammatoryimmune system stimulating and antimicrobial properties of saponin particularly against bacteria. Tannins which are polyphenolic compounds and have sufficient hydroxyls and other suitable groups have been shown to be active against many gram-negative rods [26]. Tannins also have antibacterial and antileshmanial activity due to their immune modulatory effects on the microbial antigenic receptors [27-28]. Alkaloids which were also present contain basic nitrogen atoms and have reported use as local anaesthetic and stimulant [29]. Flavonoids which are present in *C. citratus* plant leaf extract shows a wide range of therapeutic potentials, one of which is their ability to scavenge for hydroxyl radicals, and superoxide anion radicals, and thus improving

health [30]. Flavonoids also exhibit anti-inflammatory, anti-allergic effects, analgesic and antioxidant properties [31]. The synergistic effects of these metabolites are known to have inhibitory activity against diseases caused by pathogens. Therefore, it can be used pharmacologically to develop new compounds for health benefit.

3.2. Antibacterial Activity

The result from the antibacterial activity has shown that the methanol leaf extract of *Cymbopogon citratus*has antibacterial activity against the four tested pathogens as presented on table1. The inhibitory action was more on the gram-positive organism (*S. aureus* compared to the gram-negative organisms used in the analysis. *Staphylococcus aureus* had the highest diameter zone of inhibition with 24 mm at 100mg/ml. At concentrations of 25-100 mg/ml, the extracts possess antibacterial activity (from being partially active to very active). The maximum effect on the pathogens was observed at 100 mg/ml and the minimum was at 25 mg/ml the extract was found to be active against the test organisms, even at low concentrations.

3.3. GC-MS Analysis of the Extracts

In the GC-MS *Cymbopogon citratus methanol leaf extract* 17 compounds were identified. The identification of the phytochemicals were determined by their retention time (RT), molecular formula, molecular weight (MW) and chemical structure are presented in Table 4. Some of the compound present in the *Cymbopogon citratus (Lemon grass)* were Deoxyspergualin,Cystine, N,N'-Pentamethylenebis [s-3-aminopropyl thiosulfuric acid], 2,8-Diaza-3,5,10-trithiatricyclo [5.4.1.0(4.12)] dodeca-1,4(12),6-trien-6-carboxylic acid and various other compounds were identified. These phytochemicals are responsible for various pharmacological actions like antimicrobial ,anti-oxidant anti-inflammation, ,antiseptic, , antidepressant activities of the herb. The presence of various phytocompounds on the methanol leaf extract can be attributed to its widely use in traditional medicines [32].

3.4. FTIR Analysis of the Extracts

The Fourier Transform Infrared Spectroscopy was used to identify the functional groups as presented in Figure 3 and Table 1. Figure 3 shows the FTIR spectrum of *Cymbopogon citratus* methanol leaf extract. The peak at 3054.6 and 1266.4 cm^{-1} revealed the presence of amines (N-H stretch and bending). The peak at 2927.8 and 2871.9 cm^{-1} refers to the presence of alkanes (C–H stretch). The peak at 1690.3 cm^{-1} corresponds carbonyl functional group (C=O stretch). A peak at 703.6 cm^{-1} shows the presence of alkene and aromatic compound (C=C bend). The results of FT-IR spectroscopy confirm the presence of various chemical constituents such as amines, alkanes, aromatic compounds, and monosubstituted benzene.

4. CONCLUSION

Our investigation has revealed that the leaf extract of lemon grass was found to have potent antibacterial activity against the microorganisms in our investigation. The identified metabolites alkaloids, flavonoids, saponin, and tannins are believed to be responsible for this effect, validating the plant's use in traditional medicine. As a result, these plant metabolites can be included in the formulation of broad-spectrum antimicrobial agent. Thus, Isolation, identification, and purification of these phytoconstituents, as well as determination of their distinct antimicrobial potencies and toxicological evaluation, are all recommended in order to develop novel chemotherapeutic drugs with broad-spectrum affinity.

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