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Determination of Antibiotic Potency of N-Hexane, Ethanolic and Methanolic Leaf Extracts of *Cymbopogon Citratus* on Some Selected Hospital Bacteria

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ABSTRACT

Medicinal plants have served as valuable starting materials for drug development in both developing and developed countries. This study investigated the antimicrobial activity and potency of *Cymbopogon citratus* using ethanol, methanol and N-Hexane as extraction solvents. The standard organisms for the research study were *Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa,* were obtained from stock cultures in the Departmental Microbiology Laboratory of Nnamdi Azikiwe University, Awka. The phytochemical screening (qualitative) revealed the presence of Saponins, Tannin, alkaloids, phenolics, glycosides and absence of Steroids, while the results of GC- FID analysis (quantitative) revealed high concentration of spartein (22.9010 µg/ml) and Cyanogenic glycoside (22.4972ppm) for N-Hexane extract, flavanzol (21.1844ppm), ribalinidine (42.0783µg/ml), cyanogenic glycoside (22.8784ppm), phytate (25.6937µg/ml), epihedrine (18.0922 µg/ml), naringin (18.1835 µg/ml) for ethanolic extract, naringin (17.9012µg/ml), flavanzol (21.1923ppm), ribalinidine (25.1726µg/ml), cyanogenic glycoside (16.9569ppm), flavonones (16.3116ppm), Steroids (17.2251ppm), phytate (19.2509µg/ml), epihedrine (13.1275 µg/ml) for methanolic extract. Agar Well diffusion technique was used for the antimicrobial activity studies which revealed 9.0mm (*Staphylococcus aureus*) at 250 mg/ml and 16.4mm (*Escherichia coli*) at 250 mg/ml concentration as the highest zone of inhibition for ethanolic extract with *E. coli* and *Cryptococcus neoformans* exhibiting the highest and the lowest zone of inhibition respectively at 200 mg/ml of ethanolic extract. The results showed that ethanolic extract exhibited the highest antimicrobial activity with MIC value of 50 mg/ml on all the test organisms. *Staphylococcus aureus* and *Klebsiella pneumoniae* were the most susceptible organisms showing sensitivity with MBC value of 250 mg/ml of the ethanolic extract and *Cryptococcus neoformans* for methanolic extract. Hence, the high level of antibacterial efficacy exhibited by *Cymbopogon citratus* reaffirms its prospective use in the management of infections and also as a potential source of antimicrobial agents. It can be concluded that despite the inhibitory effects of concentrations of ethanolic extracts of this plants on the growth of pathogenic bacteria especially Gram-positive, to introduce it as an alternative to chemical antimicrobial drugs, further investigation is required.

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KEYWORDS: *N-Hexane, Ethanolic, Methenolic, Potency, Cymbopogon Citrus.*

INTRODUCTION

Herbal medicine is regarded as an important part of the health care industry by more than two-thirds of the population in developing countries, according to the WHO (Okemy *et al*., 2015).

Cymbopogon citratus is a tropical and sub-tropical aromatic high sedge that is grown in Southern Asia and Africa. *Cymbopogon citratus* is high and enduring sedge which throws a short rhizome into dense fascicles. The inflorescence is about a meter-long spike. Flowers are borne on decompounds spathulate; 30 to more than 60 cm long panicles (Haque *et al*., 2018).

Plants consist of various kinds of chemical constituents known as phytoconstituents (Mercy *et al*., 2017). The presence of classes of phytochemicals such as; flavonoid, alkaloid, tannin showed cytotoxic effect (Chaudhary, *et al.,* 2017). These plant-derived compounds flavonoid, alkaloid, tannin, terpenoids conduct certain biological functions that enhance therapeutic activities such as anti-carcinogenic, anti-inflammatory, and antioxidant properties (Batiha *et al.,* 2020). The process was qualitative which is termed phytochemical screening. Also quantitative phytochemical screening was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector which identified active substances such as naringin, Spartein, Rutin, ribalinidine, cardiac glycoside, phytate, kaempferol and others.

Statement of problems

It has been observed that the conventional antibiotics have not been used to reduce resistance possessed by microorganism, hence, the need for novel substances and active compounds with less resistance. Most of the plants do not poses bioactive substance that prevents disease in our society, meanwhile there was little or no scientific knowledge and research on specific organism *Cymbopogon citratus* inhibit, specific infections it can cure, at what concentration the organism will be effectively inhibited and the phytochemical component responsible for its inhibition, hence the need for this research.

Aim and Objective

Aim

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

Specific Objectives of this Study are to:

1. determine the bioactive substance of *Cymbopogon citratus*,
2. determine the antimicrobial activity of N-hexane, Ethanolic and Methanolic extracts of *Cymbopogon citratus* on *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes* and *Klebsiella pneumonia.*
3. determine the Minimum Inhibitory concentration values of the N-hexane, Ethanolic and Methanolic extracts of *Cymbopogon citratus* and
4. determine the Minimum Bactericidal Concentration values of the N-hexane, Ethanolic and Methanolic extracts of *Cymbopogon citratus*.

Materials and Methods

Materials

The materials, chemicals, equipment, reagents (Ethanol, Methanol & N-Hexane) used for this study were of highest analytical grade available and were obtained from recognized suppliers.

StudyArea/site:

The study was carried out at Dochy Labouratories Quatar, Awka (Phytochemical screening), Zidan Medical Diagnostic Center, Ifite Awka (Antimicrobial Activities).

Study Duration:

The research study started on 12th October 2023 and ended on 24th April, 2024.

Sample Collection

Fresh leaves sample of *Cymbopogon citratus* was collected in a clean polythene bag at Urunaba Nsukwu Abatete in Idemili North Local Government Area Anambra State, and transported to Nnamdi Azikiwe University, Awka**.** It was identified and authenticated at the Botany Department of Nnamdi Azikiwe University, Awka by Dr. Gabriel Ogbusobe.

Sample preparation for Analysis

The *Cymbopogon citratus* was washed and cleaned under running tap water and then dried at room temperature of 25 °C for 14 days. Upon drying, the leaves were pounded using mortar and pestle into smaller particles and then grounded into powder using an electric blender. The powder was sieved using a sieve of pore size of 80 microns. The powder (100 g) was then stored in an airtight container and kept under room temperature until required.

Sterilization of Material used for the study:

All the glassware used were washed thoroughly, rinsed with distilled water, air dried and sterilized in an oven (hot air) at 160 ^{o}C for 2 hours. Each glassware was wrapped with aluminium foil before sterilization. Distilled water and all prepared media were sterilized in the autoclave at 121 ^{o}C for 15 minutes. Cork- borers and glass-rods were sterilized by dipping into 70 % alcohol prior to flaming in a Bunsen burner. The working bench was swabbed with 70 % alcohol before and after each experiment.

Sterility Proofing of Extracts

One loopful of the extracts was inoculated in Mueller Hinton Agar by streaking, which was incubated at 37

°C for 48 hrs for bacteria and 96 hrs for fungi growth. Absence of visible growth indicated sterility of the extracts.

N-Hexane, Ethanol and Methanol Extraction

Extraction was done according to Balakrishman *et al, (*2014) with slight modifications, as followed; 10 g, 20 g and 40 g of *Cymbopogon citratus* leaves powder was added to100 ml each of Methanol, Ethanol and N-Hexane, to get concentrations of 100, 200 and 400 mg/ml respectively, and shaked for 3 hrs at 40 ºC, which was then filtered with Whatman filter paper, followed by collection of the extract which was poured in conical flask in electric-oven at 40 ºC until dried, obtained and stored in refrigerator until use.

Phytochemical Screening

Phytochemical screening was carried out in the leaves extracts using the established protocols as described by (Thusa and Mulmi, 2017) and (Nwauche *et al*., 2023). Phytochemical Examination was carried out separately using the three solvents (Metanol, Ethanol and N- Hexanes)

Qualitative Phytochemical Analysis

Phytochemical examination was carried out for all the extracts as per the standard methods (Thusa and Mulmi, 2017).

Test for Reducing Sugar

Mixture of 15 mls of Fehlings solution A and B (equal parts) was added to 2 g of the powdered plant materials in the test tube. These were heated in a boiling water-bath for five minutes. A brick red precipitate indicates a positive result. The heating was necessary since the reduction of cupric oxide to cuprous oxide in certain cases take place near the boiling point. The brick red precipitated is cuprous oxide which is a reduction product of cupric oxide of Fehling's solution.

Test for Terpenoids

Addition of 5 ml of each extract in 2 ml of chloroform (Numex, India), and concentrated H_2So_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the inner face was formed to show positive results for the presence of terpenoids.

Test for Alkaloids

Test tubes containing 1.0 ml of extracts, 5.0 ml of 2% HCl was heated in a water bath (Memmert) for 10 minutes**,** then filtered using Whatmann No 1 filter paper**.** The filtrate was used for the following tests below.

Wagner's reagent test

Principle: Alkaloids under acidic condition and at room temperature reacted with iodine and potassium iodide to give reddish brown precipitate.

Reagent: Wagner's reagent: 2 g of iodide and 3 g of potassium iodide were weighed, mixed and dissolved in 30 ml distilled water and made up to 100 ml with distilled water.

Procedure: Pipette of 1.0 ml of filtrate was made in a test tube with addition of 1.0 ml of Wagner's reagent. These was mixed properly and a reddish brown precipitate was observed which indicates the presence of alkaloids.

Meyer's reagent test

Principle: Alkaloids under acidic condition and at room temperature reacted with mercuric chloride and potassium iodide to give a cream colour or precipitate.

Reagents: Dissolve Meyer's reagent: 1.4 of mercuric chloride was added in 60 ml distilled water and 4.5 g of potassium iodide in 20 ml distilled water. The two solutions were mixed and diluted to a 100 ml with distilled water.

Procedure: Pipette of 1.0 ml of filtrate and 1.0 ml of Meyer's reagent was made in a test tube**,** these was properly mixed and observed for colour change. Cream colour precipitate was observed which indicates presence of alkaloids.

Test for Cardiac Glycosides

One (1 ml) of the extract was added in 10 ml of 50 % H_2So_4 and was heated in boiling H_2O for 5 minutes. 10ml of fehlings solution (5 ml of each solution A and B) was added and boiled. A brick red precipate indicating presence of glycosides was observed.

Test for Saponins

Two grams of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. Then 10 ml of the filterate was mixed with 5 ml of distilled water and shaked vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaked vigorously, then observed for the formation of emulsion.

Test for Flavonoid

Ferric chloride test for phenolic nucleus:

Principle: Phenolic nucleus reacts with ferric chloride at room temperature to give greenish brown or black colour or precipitate.

Reagent: 10 % $FeCL_2$ (10.0 g $FeCL_2$was weighed and dissolved in 100 ml of distilled water).

Procedure: Pipette of 1.0ml extract and 1.0 ml 10 % ferric chloride was added in the same test tube, which was then properly mixed together**. A** greenish brown or black colour/precipitate was observed which indicates presence of phenolic nucleus.

Lead Acetate Test

Principle: Flavonoids at room temperature reacts with lead acetate to give a yellow color or precipitate.

Reagent: 10 % lead acetate (10.0 g of lead acetate was weighed and dissolved in 100 ml distilled water).

Procedure: Pipette 1.0 ml extract into a test tube. Pipette 1.0 ml of 10 % lead acetate solution in the same test tube**.** These were mixed properly and precipitate was observed which indicates presence of flavonoids.

Sodium Hydroxide Test

Principle: Flavonoids at room temperature and under alkaline pH forms observable precipitate.

Reagent: Dilute NaOH: (40 g of NaOH was weighed and dissolved in 1 litre of distilled water).

Procedure: Pipette of 1.0 ml extract and 1.0 ml of dilute NaOH solution was madein the same test tube**.** These were mixed and observed for color change. There was formation of precipitate which indicates presence of flavonoids.

Test for Tannins

Acid Test

Principle: Phlobotannins under acidic condition reacts with dilute HCL to give a red colour or precipitate.

Reagent: 1 % HCL, 1.0 ml concentrated HCL was pipetted and made up to 100 ml distilled water.

Procedure: Three milliliters of extract was added to 2.0 of 1 % HCL**.** There was presence of red colour or precipitate which indicates the presence of phlobotannins

Lead Acetate Test

Principle: Phlobotannins reacts at room temperature with lead acetate to give dark-blue to black precipitate.

Reagent: 5 % lead acetate: 5.0 g lead acetate were weighed and dissolved in 100 ml distilled water

Procedure: Two milliliters of extract was added in a test tube and 3 drops of 5 % lead acetate solution was also added to the extract**.** There was a dark blue to black precipitate which indicates presence of phlobo-tannins.

Test for Resins

The powdered materials (0.2 g) was extracted with 15 ml of 90% ethanol. The alcoholic extract was then poured into 20 ml distilled water in a beaker. A precipitate occurring indicates the presence of resins**.**

Test for Carbohydrates

Molisch's test for Glucose

1 gram of the powdered samples was boiled with 10 ml of water in the test tube and filtered. Few drops of Molisch's reagent was added to the filtrates in the test tubes then 5 ml of concentrated H_2So_4 was then gently poured down the side of these tubes to form a lower layer. Formation of a ring with a purple colour at the outer surface was considered positive.

Test for Steroids

The crude plant extracts (1 mg) was taken in a test tube and dissolved with chloroform (0.5 ml), then added equal volume (1 mg) of concentrated sulphuric acid to the test tube by sides. There was no colour change at the upper layer in the test tube and sulphuric acid layer (lower layer).

Test for Phenolics

Each extract was separately stirred in 10 mls of distilled water and filtered. Few drops of 5 %$Fecl_3$ reagent was added to the filterate. Blue-green or blue-black Colouration or precipitation was taken as indication of the presence of phenolics.

Test for volatile oils

Volatile oils are characterized by their odour, oil-like appearance and ability to volatilize at room temperature. The plant materials were distilled with water by steam distillation and the distillates were collected in a graduated tube. The aqueous portion which separates automatically was returned to the distillation flask. The formation of emulsion which floats on top of the aqueous phase owing to its less density is indicative of the presence of volatile oils.

Quantitative Phytochemical Analysis (GasChromatography)

Quantification by GC-FID

The analysis of phytochemical was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15 meter MXT-1 column (15 m x 250 um x 0.15 um) was used. The injector temperature was 280 ^oC with splitless injection of 2 ul of sample and a linear velocity of 30 mms^{-1}, Helium 5.0 pa.s was the carrier gas with a flow rate of 40 $mlmin^{-1}$. The oven operated initially at 200 ^oC, it was heated to 330 ^oC at a rate of 3 $^oC\ min^{-1}$ and was kept at this temperature for 5 min. The detector operated at a temperature of 320 ^oC.

Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentration of the different phytochemicals expres in ug/g A. D. Buss., M.S. (2010), Kelly, (2014).

Culture Media

Mueller Hinton Agar (MA) used includes; Nutrient Agar, Sabouraud Dextrose Agar.

Test organisms

The standard organisms for the research study were *Escherichia coli, Staphylococcus aureus,*

Pseudomonasa eruginosa, Aspergillus niger, Candida albican, Cryptococcus neoformans, Streptococcus pyogenes and *Klebsiella pneumonia* obtained from stock cultures in the Departmental Microbiology Labouratory of Nnamdi Azikiwe University, Awka Anambra State, Nigeria. They were sub-cultured and identified based on their colonial morphology, microscopic appearance and specific biochemical reaction. The test organisms were sub- cultured in 10 ml broth each and incubated at 37 °C for 18 to 24 hours. After 24 hours, the organisms were sub-cultured into fresh Muller Hinton Broth and incubated for 3 hours which was used for all analyses.

Biochemical Tests Reaction

Biochemical Testing was carried out for confirmation of the bacteria isolates. The procedure was carried out as described by Amirreza, 2022; Bulbul *et al.,* 2023.

Catalase Test

A loopful of bacteria growth was transfered to a surface of clean, dry glass slide using a wire loop. A drop of 3 % H_2O_2 was added on the slide and mix. The presence of Catalase was indicated by bubbles gas (oxygen) while absence of bubbles indicates absence for Catalase.

Indole Test

A loopful of each isolate was inoculated onto 5 ml of sterile peptone water enriched with 1% tryptophan and incubated at 35 °C for 48 hours. To the tubes, 0.5 ml kovac's indole reagent was added. The tubes were shaken gently after an interval for 15 minutes. Development of cherry red colour in the top layer of the tube was a positive test. Absence of red colouration was indole negative.

Methyl Red Test (MRtest)

A loopful of each isolates were inoculated into appropriately labeled tubes containing MR broth. Uninoculated tube was kept as control. The tubes were incubated at 37 °C for 24 hours. After proper incubation 3 drop of Methyl red indicator was added to the tubes including control. MR-positive test was indicated by red colour formation while no colour denotes negative.

Voges-Proskauer Test (VP-Test)

A loopful of each isolates was inoculated into glucose phosphate peptone water medium and was incubated at 37 °C for 2 days. Ethanolic solution of 5 % a-naphthol (1.2 ml) and 0.1 ml of 4 % potassium hydroxide solution was then sequentially added to 2 ml of culture, sharked vigorously and placed in a sloping position (for maximum exposure of the culture of air). It was examined after 30 to 60 minutes. The red colour starting from the liquid air interface within an hour indicates a positive test for voges-proskauer.

Citrate Test

Using sterile technique Simmons citrate agar slant was inoculated with the test organism by means of a streak inoculation. An uninoculated tube was kept as control. The tubes were incubated at 37 °C for 24 hours and was observed for growth and colouration of the medium. Colour of the medium if turned blue shows a positive result. If colour of the medium remains green, it shows negative result.

Urease Test

A loopful of each isolate was inoculated into urea agar and was incubated at 37 °C for 24 hours. A positive test was indicated by liberation of pink colour as a result of ammonia production while initial yellow colour indicates negative.

Antimicrobial Sensitivity Testing of the Extract

Muller Hinton agar media was streaked uniformly with a loopful each of *Escherichia coli, Staphylococcus aureus, Pseudomonasa eruginosa, Streptococcus pyogenes* and *Klebsiella pneumonia* and was labeled appropriately.

A sterile stainless steel cork borer of 5 mm in diameter was used to make wells, 4 wells were made on each plate with one of the wells set aside for control. The holes were filled with 1 ml each of leave extracts. Each well was appropriately labeled. Controls were also carried out by leaving the well empty. The extracts were introduced into the holes with the aid of rubber pipette. The inoculated Petri dishes were left for an hour at room temperature for extracts to diffuse before placing in the incubator at 37 °C for 24 hours for bacteria and then 2-6 days for fungi growth of test organisms after which zones of inhibition were observed. The diameter of the zones of inhibition were measured with a ruler and recorded in "mm". The antimicrobial studies were done in triplicates and the mean of the diameters of the zones of inhibition in mm were taken and the size of the cork borer which is '5 mm was subtracted from the values (Kigigha *et al.,* 2018; Izah and Aseibai, 2018).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined against bacteria after the antimicrobial test was performed. The MIC of the methanolic, ethanolic and N- hexane extract of the lemon grass plant was determined at various concentration 50, 100 and 200 mg/ml. Then 9 ml of sterile peptone water was dispensed in each test tube, then 1 ml of each of the extract at different concentrations were introduced and mixed in a test tube. Then 0.1 ml of inoculums was added to each test tube. The tubes were incubated aerobically at 37 °C for 24 hours (for bacteria). Two control tubes were maintained for each test batch.

These included control (that is, the tube containing the extract, growth medium but with no inoculum) and organism control (that is, the tube containing the growth media and inoculums). The lowest concentration of the extract that produced no visible bacterial growth (i.e no turbidity) when compared with the control tube was regarded as minimum inhibitory concentration (MIC). The concentration of tubes with absence of turbidity was used for the determination of Minimum Bactericidal Concentration MBC (Izah and Aseibai, 2018).

Determination of Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) of the extracts was determined using (Kigigha *et al.,* 2018) method with little modifications. Samples were taken from the plates with no visible growth in the MIC assay and sub-cultured onto a freshly prepared Nutrient agar medium (for bacteria) and then incubated at 37 °C for 48 hours. The MBC was taken at the lowest concentration of the extract in which there was no bacterial.

Results

Table 1, 2 and 3: shows the phytochemical (qualitative) analysis of *Cymbopogon citratus* which revealed the presence of alkaloids, flavonoids, saponins, cardiac glycosides, terpenoids in all the extraction solvents used. Reducing sugar and steroids were totally absent in all the extracts. Tanin was absent in methanolic and N-Hexane extracts while in ethanolic extract resin was absent.

Table 1: **Qualitative Phytochemical analysis of N-Hexane extracts**

Test	Inference
Alkaloids	++
Flavonoids	++
Phenols	+
Reducing sugar	-
Tannin	-
Saponin	++
Cardiac glycoside	++
Terpenoids	++
Steroids	-
Resin	++

++ =moderately present
- =absence
+ =weakly present

Table 2: **Qualitative Phytochemical analysis of Ethanolic extracts**

Test	Inference
Alkaloids	++
Flavonoids	++
Phenol	+
Reducing sugar	-
Tannin	++
Saponin	++
Cardiac glycoside	+
Terpenoids	++
Steroids	-
Resin	-

++ = moderately present
+ =weakly present
- = absence

Table 3: **Qualitative Phytochemical analysis of Methanolic extracts**

Test	Inference
Alkaloids	++
Flavonoids	+
Phenol	+
Reducing sugar	-
Tannin	-
Saponin	+
Cardiac glycoside	+
Terpenoids	++
Steroids	-
Resin	++

++ = moderately present
+ =weakly present
- = absence

Table 4: shows the colonial and microscopic characteristic of bacteria isolated on nutrient agar, which shows the presence of two gram positive organisms (*E. coli* and *Pseudomonas*) both been motile and flagellated, also the presence of three gram negative organisms (*Staphylococcus, Streptococcus, and Klebsiella*) which were non-motile and non-flagellated.

Table 4: Colonial and Microscopic Characteristics of Bacteria Isolate in Nutrient Agar

Sample Isolates	MACROSCOPY		MICROSCOPY		Isolate	Organisms
	Colony Morphology	Texture	Motility	Flagellium		
1	Creamy to white shiny color	Smooth round convex	+	+	Bacteria	*E. coli*
2	white to creamy with slightly yellowish hue	Slightly wrinkle	-	-	Bacteria	*Staphylococcus spp*
3	Translucent white to grayish color	Smooth slightly rough	-	-	Bacteria	*Streptococcuspp*
4	Creamy white to pale yellow	Mucoid smooth glistening appearance.	-	-	Bacteria	*Klebsiella spp*
5	Greenish blue with Irregular edges	Flat, fruity odor	+	+	Bacteria	*Pseudomonaspp*

+ = Positive - = Negative

Table 5: shows the bacteria characteristics which help in the confirmation of the actual bacteria isolate.

Staphylococcus aureus, klebsiella pneumoniae and *Pseudomonas aeuroginosa* were catalase positive; *E.coli, Streptococcus pyogenes* and *klebsiella pneumonia* were positive for sugar fermentation; all organisms were positive for citrate, coagulase and urease test while *E.coli* was the only organism positive for indole test.

Table 5: Biochemical Test for the Bacteria Isolates

Test			Results		
Catalase	-	+	-	+	+
Sugar fermentation	+	-	+	+	-
Citrate	+	+	+	+	+
Indole	+	-	-	-	-
Coagulase	+	+	+	+	+
Urease	+	+	+	+	+
Oxidase	-	-	-	-	+
Organisms	*E. coli*	*Staphylococcus spp*	*Streptococcus spp*	*Klebsella spp*	*Pseudomonas spp*

+ = Positive - = Negative

Table 7: shows the sensitive test done using well diffusion method for all the organisms (bacteria) isolated at different concentration which ranges between 100 mg/ml - 250 mg/ml against the extract (methanol, ethanol and N-Hexane). Total clearance was observed across the extracts at varying concentration with different organisms which revealed 9.0 mm (*Staphylococcus aureus*) at 250 mg/ml and 16.4 mm (*Escherichia coli)* at 250mg/ml concentration as the highest zone of inhibition for ethanolic extract, with *E.coli* and *Cryptococcus neoformans* exhibiting the highest and the lowest zone of inhibition respectively at 100 mg/ml of the ethanolic extract. At concentration of 100 mg/ml, *Streptococcus pyogenes* of 2.4mm exhibited the highest zone of inhibition while *Cryptococcus neoformans*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* exhibited the lowest zone of inhibition of 1.0mm for methanolic extract.

Table 7: Sensitivity-Zone of clearance/inhibition in mm

Conc.(mg/ml)	Methanolic(mm) 100 200 250	Ethanolic(mm) 100 200 250	N-Hexane(mm) 100 200 250	Control
Test organism				
Cryptococcus neoformas	1.0 4.0 6.0	2.5 5.5 7.5	NIL NIL NIL	NIL
Streptococcus pyogenes	2.4 4.4 6.4	3.5 5.5 7.5	NIL NIL NIL	NIL
Klebsiella pneumoniae	1.0 2.0 6.0	2.8 3.0 7.0	NIL NIL NIL	NIL
Staphlococcus aureus	1.8 0.8 1.8	4.0 5.0 9.0	NIL NIL NIL	NIL
E. coli	2.0 3.0 4.0	5.9 9.9 16.4	NIL NIL NIL	NIL
Pseudomonas aeruginosa	1.0 2.5 3.0	3.0 4.5 5.5	NIL NIL NIL	NIL

TC= Total clearance NIL = No zone of inhibition

At table 8: the results showed that ethanolic extract exhibited the highest antimicrobial activity with a constant MIC value of 50, 100 and 200mg/ml on all the test organism followed by methanolic extract which was able to inhibit only *Streptococcus pyogenes, Cryptococcus neoformans* at constant MIC value of 50, 100 and 200mg/ml, then N-hexane extract exerted poor inhibition of the organisms as most of the organisms were not inhibited.

NT= not turbid (no growth indicating "inhibition")

T = Turbid (growth indicating "no inhibition")

Table 8: Minimum Inhibitory Concentration (MIC) of Extract on Bacteria Isolates

Organism	Extract	50 mg/ml	100 mg/ml	200 mg/ml
Staphylococcus aureus	Ethanol	NT	NT	NT
	Methanol	NT	NT	NT
	N-Hexane	T	T	NT
Streptococcus aeruginosa	Ethanol	NT	NT	NT
	Methanol	NT	NT	NT
	N-Hexane	T	T	NT
Pseudomonas aeruginosa	Ethanol	NT	NT	NT
	Methanol	T	T	NT
	N-Hexane	T	T	T
Klebsiella pneumonia	Ethanol	NT	NT	NT
	Methanol	T	T	NT
	N-Hexane	T	T	T
E.coli	Ethanol	NT	NT	NT
	Methanol	T	NT	NT
	N-Hexane	T	T	T

NT=Not Turbid (no growth indicating "inhibition")
T=Turbid (growth indicating "no inhibition")

Table 9: revealed *Klebsiella pneumonia* and *Staphylococcus aureus* as the most susceptible organism showing sensitivity with MBC value of 250 mg/ml of the ethanolic extract and also *Streptococcus aeruginosa* was susceptible at MBC value at lowest concentration of 250 mg/ml of N- hexane.

TABLE 9: Minimum Bactericidal Concentration (MBC)

Conc.(mg/ml)	METHANOL			ETHANOL			N-HEXANE		
	250	500	1000	250	500	1000	250	500	1000
Streptococcus aeruginosa	G	G	G	G	NG	NG	NG	NG	NG
E.coli	G	NG	NG	G	NG	NG	G	G	G
Pseudomonas aeruginosa	G	G	NG	G	NG	NG	G	G	G
Klebsiella pneumonia	G	G	NG	NG	NG	NG	G	G	G
Staphylococcus aureus	G	G	NG	NG	NG	NG	G	G	G

NG =No Growth **G =Growth**

Discussion

Phytochemical screening carried out indicated the presence of biologically active constituents including Saponin, tannin, glycosides, alkaloids, phenol, flavonoids, resin and reducing sugar. These metabolites have been reported to possess antibacterial and antifungal properties (Jafari *et al*., 2012; Ewansiha *et al.,* 2012). In this study, the ethanolic extract had the highest Concentration of the assayed phytochemicals, followed by the methanolic extract, then N-hexane extract. This study also revealed that the ethanolic extract of *Cymbopogon citratus* possesses high antimicrobial activity as it showed the broadest spectra against all organisms tested. 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 accordance with this phenomenon include the works of Hindumathy (2011), Ewansiha *et al*., (2012) Kruthi *et al.,* (2014). Also, ethanol as an organic solvent has a wider propensity of dissolving phytoconsituents present in plants, compared to Methanol and N-hexane due to its chemical Composition (2 carbon atoms, 6 hydrogen atoms, 0ne oxygen and functional group), structure and Complexity (Karkala and Ganjewala , 2009; Ekpeyong *et al.,* 2015).

Extraction and phytochemical screening of bioactive agents from medicinal plants permits, the

demonstration of their physiologic activities. The phytochemical analysis showed that flavonoids, alkaloids, saponin, resin, terpenoids and cardiac glycosides were present in N-hexane extract, ethanolic extract and Methanolic extract while volatile oils was only present in N-Hexane extract. Phytochemical screening of *Cymbopogon citratus* also revealed the presence of volatile oil, also called essential oils. According to (International union of pure and Applied chemistry - IUPAC (1995)), the presence of volatile oil gives plants their specific aromas which is Confirmed by the aromas produced by this plant and are extracted by solvent extraction. The presence of volatile oils also confirms the report of Seenivasan *et al.,* 2006 of the application of *Cymbopogon citratus* in perfumery, Cosmetics and Soap industry.

Various volatile oils in plants have been reported to have medicinal values ranging from skin treatment to remedy for Cancer (Komiya *et al.,* 2006). The extraction of volatile oils in lemon grass confirms the activity showed against the test organisms by this plant and also in part confirms the report of Babay, *et al.,* 2004 of the oil extracted from same plant by distillation to exhibit great antibacterial activity and also confirms the potency of this particular plant against skin cancer prevention as reported by Nakamura *et al.,* 2003. Lemon grass oils was found to be among the most active against human dermatophyte strain inhibiting 80% of strains as reported by Lima *et al.,* 1993 and Akiyama *et al,* 2001, this is confirmed by the antifungal activity of lemon grass against strains of fungi species used as test organism and also confirms reports by traditional users of lemon grass against ring worm infections. According to Kolodzieji *et al.,*(2005), tannin and phenolic compounds have been found to inhibit bacterial and fungal growth and also capable of protecting certain plants against infection.

According to the report by (Dutabare, 2008) phytochemical Components have antifungal properties which were confirmed in this study. The presence of tannin in the plant extract agrees with the report of (International union of pure and Applied Chemistry (IUPAC, **1995**) that tannin 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 protein and render them resistant to attack by proteolytic enzymes, internally; they form a pellicle of coagulated protein over the linning of the alimentary tract. This is no doubt confirm partly the report that *Cymbopogon citratus* has been used against gastrointestinal disturbances (Nakamura *et al.,* 2003) but might require high dosage due to the level of antimicrobial activity it showed in this research result. Tannin have also been reported to have anti-diarrheal, homeostatic and anti-hemorrhagic activity (Akiyama *et al*., 2001). The presence of Terpenoid and Phenol also explain some of the pharmacological action of the leaves extract. Phenolic compounds are important components in vegetable foods, infusions and teas for their beneficial effect in human health (Ozcan *et al*., 2009).

Generally, Cardiac glycosides serve as defense mechanism against cardiovascular diseases as reported by (Schneider and Wolfling, 2004). This may therefore explain its therapeutic effect against cardiovascular and digestive problems. It has been reported that Tannins usually forms insoluble complexes with protein, thereby interfering with their bioavailability (Enujiugha and Agbede, 2000). However, differences in antimicrobial activities of medicinal plant are obviously related to difference in their content of active compound (Boakye-Yiadom and Konning, 1975).

Cymbopogon citratus did not contain steroid, these result agreed with Soares *et al.,* 2018, that plants and their natural constituents were subject to variation due to difference in their locations and climatic condition of where they thrive Ojo *et al*., 2010. Differences between proportions of components may be due to differences in plant which varies according to the geographical origin, genetic differences, age/stage of maturity, season of harvest, part of the plant used and method of extraction (Ekpenyong *et al*., 2014).

Alkaloids play some important metabolic role in living organisms, causing some plysiological changes and are involved in protective function in animals, thus are used in making medicines. They have been shown to have important pharmacological functions such as anticancer, Psychedelics and antimalarial (Cordell, 2008) analgesic, antispasmodic and bactericidal (Okuru De, 2004), antioxidant and stimulating activities. Saponins are reported to exhibit broad range of pharmacological actions, such as ability to heal wounds and inflamed mucous membrane (Okwu De, 2004). It also has anti-hyper cholesterol and haemolytic (Souza *et al*., 2007); (Redly *et al*., 2007). The extract is rich in flavonoids which are the most common polyphenols found in human diet and which have been implicated in many human diseases including lipid lowering, hepatoprotective, anti-inflammatory, antioxidant, antimalarial and antimicrobial activities by acting as antioxidant (Okwu De, 2004).

Anthocyanins are flavonoid found in virtually all vegetables, fruits and other plant parts, and are

reported to show antioxidant properties (Cashine *et al*., 2005). They also exhibit anti-inflammatory activity (Middleton *et al*., 2000). Anthocyanins have been reported to play a beneficial role in visual acuity, stress protestants, treatment of cancer, heart disease, age-related neurodegenerative disorders and in angiogenesis (Roy *et al*., 2009). Phenols are commonly found in plants and have diverse physiological functions, including "anti-inflammatory, antioxidant and antimalarial activities (Han *et al.,* 2007);(Ovenden *et al*., 2011). Naringenin which is the most abundant flavonoid in the extract is found mainly in citrus fruits and tomatoes and have been shown to be beneficial in the management of cancer, cardiovascular diseases and osteoporosis (Okwu De, 2004). Recently, it has shown to cause a significant reduction in the liver injury in rats (Cushine *et al.*, 2005).

It was also observed that the Gram-negative bacteria used in this study (*Pseudomonas aeruginosa*, *klebsiella pneumonia* and *E. coli)* were less susceptible with lesser zones of inhibition to the extracts compared to Gram-positive organisms (*Staphylococcus aureus*, *Streptococcus pyogenes*). This is as a result of the variation in cell wall structure and Complexity of these bacteria (Khan *et al.,* 2011; Ewansiha *et al.,* 2012). This observation also agrees with the reports of (Jafari *et al.,* 2012) and (Kruthi *et al*., 2014).

This results are consistent with findings of other researcher (Hindumathy, 2011; Ojo *et al*, 2010, Mothana *et al.,* 2010, Duraipandiyan *et al.,* 2016, Caccioni *et al.,* 2000; Kraft *et al.,* 2004) that most plant extracts have inhibition effect on gram positive bacteria and little effect on gram negative bacteria. All the concentrations tested had a little inhibition effect on *Peudomonas aeruginosa* in which its probable cause was the presence of cell wall polysaccharides which prevented the active compounds, essential oils and extracts, from reaching the cytoplasmic membrane of gram negative bacteria (Duraipandiyan *et al*., 2006). In general, herbal product leads to granular cytoplasm and cytoplasmic membrane rupture (Caccioni *et al.,* 2000) and inactivation or inhibition of intracellular and extracellular enzymes activity and being disintegrated into cell wall (Kraft *et al*., 2004). Bacteria cell lyses could be one of the reasons for the observed antimicrobial property. Moreover, there is consensus that surface binding and damage to membrane function are the most important mechanisms for the inhibition of bacteria by *Cymbopogon citratus* (Yamanaka *et al*., 2005; Clement *et al*., 1994). This study is in agreement with that of Mohamodally *et al.*(2005) who reported greater activity of his extract against gram positive *Staphylococcus aureus* but is in contrast with that of Morteza-semnani *et al*.,(2005) who reported best activity of their organic extract against gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. Although, the zone of inhibition was seen in ethanol extract but it was less when compared with methanol extract. This can be attributed to the fact that in ethanol extract the component of the plant are more extracted than in methanol extraction because methanol is considered to have large dipole molecules and a high dielectric constant.

This infers that the inhibitory compound of the plant extracts is more efficacious and more soluble in alcohol than N-hexane. However important factor of solvent effectiveness is the active components of the plants. Higher plant extract exhibiting antimicrobial activity has increased in the recent year and several reports on the subject have been published (Fransworth, 1994; Benzi and Ceci, 1997: etha Bagglii 2000). The alcoholic (methanol and ethanol) extract showed greater activity than the N-hexane extract which implied that the antimicrobial compound resided in concordance with that of some other workers (Voravuthikunchai *et al.,* 2004; Pareklu *et al.,* 2005). This means based on the compound miscibility, some will have antimicrobial action and others will not. It is important to note also that the effectiveness of extracts is directly related to the concentration of the extract.

Table 5 shows a total of five bacteria species that were identified using their biochemical characteristics. The bacteria were *Escherichia coli, Pseudomonas aeroginosa, klebsiella pneumonia, Streptococcus pyogenes, and Staphylococcus aureus. Escherichia coli, Klebsiella pneumonia and Pseudomonas aeroginosa* were confirmed to be gram negative while *Staphylococcus aureus* and *Streptococcus pyogenes* were confirmed to be gram positive because of the nature of the cell walls and their component which gives different characteristic reaction to the dye used for their staining. *Staphylococcus aureus, klebsiella pneumonia,* and *Pseudomonas aeroginosa* were catalase positive meaning it produced the enzyme catalase while *Escherichia coli* was catalase negative. Generally, the positive result of the organisms to citrate, urease signifies that the organism produces enzyme such as citrate and urease respectively. The coagulase positive showed clumping of cells in the bacterial suspension mixed with the plasma while urease positive indicate ammonia production. The positive result observed for motility of *Pseudomonas*

aeruginosa and *Escherichia coli* implies that they are motile due to the presence of flagella, cilia etc. They are spore negative because they are not spore formers. *Staphylococcus aureus* is a facultative anaerobes gram positive coccus, which appears as grape like clusters when viewed through a microscope that grow by aerobic respiration or by fermentation that yields principal lactic acid (Kluytanans*et al*., 1997). Ryan and Ray (2004) reported similar result where the *Staphylococcus aureus* isolated were coagulase positive although he admitted that other *Staphylococcus spp* could be coagulase negative.This inferred that the cultures used were pure and true culture of the isolate.

The result in table 8 and 9 in this study suggested that *Cymbopogon citratus* demonstrated the ability to inhibit growth of some bacteria and significantly lower MIC values. The result demonstrated a wide range of activities of the ethanol extract against the isolates. Ethanolic extract exhibited the highest antimicrobial activity with MIC value of 50, 100 and 200mg/ml on all the test organisms followed by methanolic extract which was able to inhibit only *Streptococcus pyogenes,* at constant MIC value of 50, 100 and 200mg/ml, N-hexane extract exerted poor inhibition of the organisms as most of the organisms were not inhibited. Table 9 revealed *Klebsiella pneumonia* and *Staphylococcus aureus* as the most susceptible organism showing sensitivity with MBC value of 250 mg/ml of the ethanolic extract and at the lowest concentration of 250 mg/ml for methanolic extract, also *Streptococcus aeruginosa* and susceptible at MBC value at lowest concentration of 250 mg/ml of N- hexane.

The highest MIC of the extracts against *Escherichia coli is* an indication that the plant extract is less effective on gram negative bacteria because the bigger the inhibition zone the lower the MIC. This result also emphasizes the high antimicrobial potency of extracts of *Cymbopogon citratus* because a low MIC indicates a high efficacy of the extract (Choi *et al*., 2011). These results is in accordance with most research studies that have been done where the MIC and MBC of lemon grass are considerably lower ranging from 0.16-20µg/ml for most bacteria and 0.25-10µg/ml for most fungi (Duke *et al.,* 2003; Schwiertz *et al.,* 2006). Result obtained in this study also agreed with other research, which shows *Cymbopogon citratus* start exhibiting antibacterial activity at 30.6mg/ml (Packiyasothy and Kyle 2002). Hence, extracts of *Cymbopogon citratus* can effectively lyse bacterial cells at low concentration, thus confirming the aforementioned high antimicrobial efficacy of the plant.

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.

Conclusion

Ethanolic extract of lemon grass demonstrated the greatest antimicrobial activity among the three extracts, closely followed by the methanolic extract and then, N-Hexane extract. The bactericidal effects of these extracts indicated that the plant has a promising potential as effective antimicrobial agent. The antimicrobial activity of the three extracts can be enhanced if the components are purified. Lemon grass (*Cymbopogon citratus*) also possesses qualities such as its fine lemony scent which makes it not only useful as a drug but for domestic (spice for cooking), agriculture (pesticides, insecticides) and industrial purposes (detergents, cosmetics, perfumes)

REFERENCES

[1] Ablaa, H. A, Al-Mehana, W. N, Mahdi, I. K. and Hasan, T. H. (2021). Gene therapy by studying the effect of single nucleotide polymorphism at the BcL11A gene on the severity of beta-thalassemia disease in the province of AL-Najaf. *International Journal of Pharmaceutical Research*, *13*(1).

[2] Abusaiba, T. H. H. (2020). Prevalence of hepatitis virus B and C in patients in Al Najaf Governorate, *Iraq International Journal of Pharmaceutical Research*, **12**(3):1297-1303.

[3] Aćimović, M, Kiprovski, B, Gvozdenac, S. (2020). Application of *Cymbopogon citratus* in agro-food industry. *Journal of Agronomy Technology and Engineering Management,* **3** (3) 423-436.

[4] AlAboody, M. S, Mickymaray, S. (2020). Anti-fungal efficacy and mechanisms of flavonoids

[5] Amirreza, T. (2022). Guidelines for Quick Application of Biochemical Tests to identify unknown Bacteria. Antibiotics, **9** (2).

[6] Aourach, M, Barbero, G. F, González de Peredo, A. V, Diakite, A, El Boukari, M, Essalmani, H (2021). Composition and antifungal effects of aqueous extracts of *Cymbopogon citratus, Laurus nobilis* and *Santolina chamaecyparissus* on the growth of *Fusarium oxysporum.* Plant Protection, **54** (19–20) 2141-2159.

[7] Avoseh, O, Oyedeji, O, Rungqu, P, Nkeh-Chungag, B, Oyedeji, A. (2015).*Cymbopogon species,* ethnopharmacology, phytochemistry and the pharmacological importance. Molecules, **20** (5).

[8] Azimirad, M, Dezfulian, A, Alebouyeh, M, Bahreiny Esfehani, R, Shahrokh, S, Zali, M. R. (2017). Infection with enterotoxigenic *Staphylococcus aureus* as a concern in patients with gastroenteritis. *Journal of Global Antimicrobal Resistance,* **9**:11-14.

[9] Barut, E. N, Engin, A, Saygın, I, Kaya-Yasar, Y, Arici, S, Sezen, S. F. (2021). Alpha-lipoic acid, a promising adjuvant for nonsteroidal anti-inflammatory drugs therapy with improved efficacy and gastroprotection. Drug Development Research, *82* (6).

[10] Bastidas-Bacca, M. A. (2023). Ethnobotanical insights, qualitative analysis of medicinal plants in Colón Putumayo for traditional knowledge preservation. Plants,**12** (19).

[11] Batiha, G. F. and Beshbishy, A. M. (2020). Gas Chromatography, mass spectrometry analysis, phytochemical screening and anti-Protozoal effects of the methaolic *Viola tricolor* and acetonic *Laureus nobilis* extracts. BMC Complemetary Medicine Therapies, **20** (87).

[12] Bieski, I. G. C. (2015). Ethnobotanical study of medicinal plants by population of Valley of Juruena Region, Legal Amazon, Mato Grosso, Brazil. *Journal of Ethnopharmacology,* 173383-423,

[13] Biharee, A, Sharma, A, Kumar, A, Jaitak, V. (2020). Antimicrobial flavonoids as a potential substitute for overcoming antimicrobial resistance. Fitoterapia, 146.

[14] Boeira, O, (2020). Phytochemical characterization and antimicrobial activity of *Cymbopogon citratus* extract for application as natural antioxidant in fresh sausage. Food Chemistry, 319.

[15] Boudechicha, A. (2023). Microfluidizing technique application for Algerian *Cymbopogon citratus* (DC.) Stapf effects enhanced volatile content, antimicrobial, and anti-mycotoxigenic properties. Molecules, **28** (14).

[16] Boukhalfa, D, Yaha, N. E. Y, Nabti, B, Boukhalfa, D. (2023). Phytochemical analysis and biological activity of the essential oil of lemongrass from the Algerian Sahara. BioPharmaceutical Science, **22** (3).

[17] Bulbul, R, Trisha, D. and Sayan, B. (2023). Overview on old & New Biochemical Tests to for Bacterial identification.

[18] Butnariu, M, Sharifi-Rad, J. (2022). Status Natural Compounds of Medicinal Plants, Herbs, and Spices, Polyphenols, Tannins, Volatile Oils. Isolation, Characterization, and Therapeutic Applications of Natural Bioactive Compounds, IGI Global, 22-66.

[19] Butnariu, M. (2023). Plant Genome Engineering for Improved Flavonoids Production Plants as Bioreactors for Industrial Molecules, John Wiley & Sons, Limited.

[20] Caner, A, Onal, M. G, Silici, S. (2021). The effect of bee bread (Perga) with chemotherapy on MDA-MB-231 cells. Molecular Biology Reports, **48** (3)2299-2306.

[21] Chaudhary, A, Sharma, S, Mittal, A, Gupta, S. and Dua, A. (2020). Phytochemical & antioxidant profiling of *Ocimum Sanctum. Journal of food sciece and technology*, **57**(10).

[22] Choi, J, Damte, D, Lee, S, Kim, J. and Park, S. (2011). Antimicrobial

[23] Clement, S. L, Kaiser, W. J, and Eichenseer, H.(1994). Acremonium endophytes in germplasms of major grasses and their utilization for insect resistance in Biotechnology of Endophytic Fungi of Grasses, 185-199.