American Journal of Microbiology and Biotechnology
2017; 4(5): 61-66
http://www.aascit.org/journal/ajmb
ISSN: 2375-3005

AASCIT American Association for Science and Technology

Keywords
Phytochemical,
Antimicrobial,
Cymbopogon citratus,
Bacteria Strains

Received: June 15, 2017
Accepted: August 8, 2017
Published: September 8, 2017

Preliminary Phytochemical Screening and Antimicrobial Activity of *Cymbopogon citratus* (DC.)Stapf. (Poaceae) Leaf Ethanol Extract Against Selected Microbes

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Citation
Ukwubile Cletus Anes, Troy Salvia Malgwi, Musa Yusuf Dibal, Otalu Otalu Jr., Aliyu Nuhu. Preliminary Phytochemical Screening and Antimicrobial Activity of *Cymbopogon citratus* (DC.)Stapf. (Poaceae) Leaf Ethanol Extract Against Selected Microbes. *American Journal of Microbiology and Biotechnology.* Vol. 4, No. 5, 2017, pp. 61-66.

Abstract
This study was carried out in order to determine the phytochemical constituents and antimicrobial potential of *Cymbopogon citratus* ethanol extract. The result revealed that the leaf extract contained alkaloids, carbohydrates, tannins and flavonoids, while sterols, saponins and cardiac glycosides were not detected. Antibacterial activity by agar well diffusion method and using a standard antibiotic (ceftriaxone USP) and distilled water as controls against selected Gram positive and Gram negative strains; *Klebsiella pneumonia ATCC10031*, *Bacillus subtilis MCTC8230, Salmonella typhi ATCC9184* and *Staphylococcus aureus* and a fungus *Candida albican ATCC19231*, showed that *Staphylococcus aureus* had the highest zone of inhibition of 26.5±0.1mm of the extract followed by *Bacillus subtilis* ACTC8230 with 25.25±0.1mm, *Candida albican ATCC1984* with 23.5±0.2mm, *Klebsiella pneumonia ATCC10031* with 22.0±0.1mm while *Salmonella typhi ATCC9184* has 20.5±0.1mm as the least. These results were compared to the diameter zone of inhibition of the standard drug at $p \leq 0.05$ (one-way ANOVA). The study showed that the plant extract possessed antibacterial activity against the microbes with more potency on Gram positive bacteria strains than Gram negative strains. This study therefore, justifies the ethnomedicinal use of the plant in the treatment of typhoid fever and sexually transmitted infections caused by some of these microbes.

1. Introduction

Even though pharmaceutical industries have produced a number of antibiotics in the last decades, resistance to these drugs by microorganisms has increased. In general, microbes have the genetic ability to transmit and acquire resistance to drugs, which are

utilized as therapeutic agents [1]. For quite sometimes, plants have been a valuable source of natural products for maintaining human health. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments [2]. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant [3].

Plants are potential source of medicinal compounds. They are traditionally used in oral health and to treat many infectious diseases including diarrhoea, fever, cold and stomach disorder [4]. These plants are ingested as decoctions, teas or juice as well as poultice and applied directly on infected wounds or burns [5].

According to the world health organization (WHO) report, early investigation revealed that four billion people, representing 80% of the world's population, presently used herbal medicine for some aspects of primary health care. Herbal medicine is a major component in all indigenous people's tradition; a common element in Ayurvedic, homeopathic, traditional oriental and native American - Indian medicines. It is also estimated that of the 199 plant-derived pharmaceutical medicines, about 74% are used in modern medicines in ways that correlate directly with traditional uses of plants by native cultures [6].

Cymbopogon citratus is usually used in folk medicine for the treatment of neurological and gastro-intestinal disorder and as an antispasmodic, analgesic, antibacterial, antifungal, anti-inflammatory, anti-pyretic, diuretic and sedative [7]. It is called in various Nigerian languages as: Igbo (*Acharaehi*), Yoruba (*kooko Oba*), Hausa (*tsauri*). Lemon grass (as it commonly called in English) extract has the ability to control bacteria growth and fungal pollutant in food [8]. The leaf extract is applied for its medicinal value to cure ache, oily skin, scabies, burns, boils, eczema and wounds. Extract of both the leaves and stalks of *Cymbopogon citratus* are also used in herbal medicine to wash open wounds, and also to treat nervous condition and inflammation [9].

In Nigeria, *Cymbopogon citratus* is used for arresting stomach problems, headache, blood circulation problems, and it is also used in combination with other plants for effective treatment of malaria and typhoid. The plant is locally used as insecticide and as poultice on snake bites and bee stings among others [10-11]. Though there are limited scientific data on the medicinal claims of lemon grass, it has been used in traditional medicine for treatment of several ailments in different parts of the world.

This present study therefore, investigated the phyto-constituents and antimicrobial potency of ethanol leaf extract on selected pathogenic microbes.

2. Materials and Methods

2.1. Material: Microorganisms

The microorganisms used in this research were obtained from the Department of Microbiology, National Research Institute for Chemical Technology (NARICT) Zaria, Nigeria. The micro organisms were: *Klebsiella pneumonia ATCC10031, Bacillus subtilisNCTC8230, Salmonella typhi ATCC9184, Candida albican ATCC19231,* and *Staphylococcus aureus* (Clinical isolate).

2.2. Methods

2.2.1. Collection and Identification of Plant Materials

Fresh leaves of *Cymbopogon citratus* were collected from a bush located in Bali Local Government Area, Taraba State, Nigeria and was identified by Mr. Ukwubile Cletus. A. of the Department of Science and Laboratory Technology, Federal Polytechnic, Bali, Nigeria, where a voucher number of *POA001* was deposited for the plant.

2.2.2. Preparation of the Plant Material

The fresh leaves were washed thoroughly with tap water followed by distilled water in order to remove any dirt or filthy particles present, and were air- dried under shade at room temperature for two weeks. The dried leaves were grind in an electronic blender to fine powder. 600 g of the powdered leaves were weighed using electronic balance and stored at 4°C in an air tight container in a cupboard for future use.

2.2.3. Extraction of Plant Material

600 g of the powdered leaf was soaked in a 1000 mL capacity separating funnel containing 800 mL 100% v/v absolute ethanol (Sigma Aldrich) for 48 by cold maceration technique. It was concentrated in vacuo using rotary evaporator after filtering the filtrate using VLC apparatus. The extract yielded 11.66% and stored in desiccator for further use.

2.2.4. Culture Media Preparation

The media were prepared by weighing 40g of the nutrient agar from the stock container and was dissolved in 120 mL distilled water. The media were sterilized in an autoclave at 121°C for 15 min. The prepared media was then poured into culturing plates and left for 10 min to solidify.

2.2.5. Preliminary Phytochemical Screening of Ethanol Leaf Extract

The preliminary phytochemical screening of ethanol leaf extract of *Cymbopogon citratus* was carried out by the methods previously described [12].

Test for glycosides (general test)

To a portion of the extract, 5 mL of dilute sulphuric acid was added and boiled on water bath for 10-15 min. It was then cooled and neutralized with 20% KOH and then divided into two portions:(i) To the first portion, 5 mL of the mixture Fehling's solution A and B was added and boiled. A brick red precipitate shows the release of reducing sugar as a result of hydrolysis of glycoside. (ii) To the second portion, 3 mL of Ferric chloride solution was added. A green to blue color

was produced due the release of phenolic aglycones due the hydrolysis.

Test for unsaturated sterols

Salkowski test

To a small portion of the extract, 2 drops of concentrated sulphuric acid was added to the test tube. Note immediate color changes at the interface of the extract over one hour period (cherry red color) indicate the presence of unsaturated sterols.

Test for cardiac glycoside

Keller-Kiliani test

A portion of the extract was dissolved in 1mL of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1mL of concentrated sulphuric acid was added downside of the test tube to form a lower ring at the bottom, and observed carefully at the interphase for purple-brown ring. This indicates the presence of deoxy-sugars and a pale-green color in the upper acetic layer indicates the presence of cardiac glycosides.

Keddes test

To a portion of the extract, 1mL of 2% solution of 3,5-dinitrobenzoic acid in 95% alcohol was added. The solution was made alkaline with 5% sodium hydroxide. Appearance of purple-blue color, indicates the presence of cardenolides.

Test for saponins

Frothing test

About 10 mL of distilled water was added to a portion of the extract and was shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30 min. A honeycomb froth that persists for 10 min after heating indicates the presence of saponins.

Test for tannins

Ferric chloride test

To a portion of the extract, 3 drops of ferric chloride was added. A greenish-black precipitate indicates the presence of condensed tannins while hydrolysable tannins gave a brownish-blue precipitate.

Bromine water test

Few drops of bromine water were added to the extract in a test tube. A buff colored precipitate indicates condensed tannins while hydrolysable tannins give none at all.

Lead sub-acetate test

To a small portion of the extract, 3 drops of the lead sub-acetate solution were added. A coloured precipitate indicates the presence of tannins.

Test for flavonoids

Sodium hydroxide test

Few drops of 10% sodium hydroxide were added to the extract. Yellow coloration indicates the presence of flavonoids.

Ferric chloride test

Few drops of ferric chloride solution were added to a portion of the extract, a green precipitate indicate the presence of phenolic nucleus.

Test for alkaloids

Mayer reagent

To a portion of the extract, few drops of Mayer's reagent were added. A cream precipitate indicates presence of alkaloids

Dragendorff's reagent

To apportion of the extract, few drops of Dragendorff's reagent were added. A reddish brown precipitate indicate presence of alkaloids.

Picric acid test

Few drops of 1% picric acid solution were added to a portion of the extract in a test tube. Yellow coloration indicates the presence of alkaloids.

2.2.6. Antimicrobial Evaluation of *C. citratus* Leaf Extract

Agar well-diffusion method was used to determine the antimicrobial activity. Nutrient agar plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of *Bacillus subtilis MCTC8230*, *Salmonella typhi ATCC9184, Candida albican ATCC19231, Klebsiella pneumonia ATCC10031* and *Staphylococcus aureus* bacteria. Wells (10mm diameter and about 2 cm a part) were made in each plates using sterile cork borer 5 mm diameter. Stock solution 0.1 mL of the plant extract was added using a sterile syringe into the wells and left for 24 h. Control experiments comprising a standard antibiotic Ceftriaxone USP; Reyoung Pharmaceuticals, China and distilled water inoculums, were set up in order to compare their sensitivity. The plates were incubated at 37°C±2°C for 24 h. The diameter zones of inhibition (mm) were measured using transparent ruler. The readings were taken in three different fixed directions in all 3 replicates, and the average values were tabulated [12]. A positive control (ceftriaxone 5 mL) and negative control (distilled 10 mL) were also seeded in the same way as above to compare the level of significance between the extract and the standard drug.

Determination of minimum inhibitory concentration (MIC) of the extract

The MIC was carried out on the microorganisms that showed sensitivity to the extract. This was done using broth dilution methods. The aim was to know the least concentration of the extract that will inhibit or prevent the growth of the bacteria. Nutrient broth was prepared according to the manufacturer's instructions. Ions of the broth was disposed into 5 test tubes each for the bacteria and sterilized at 121°C for 15 minutes. MC-Farland turbidity standard scale number 0.5 was prepared to give a turbid suspensions of the microorganisms. Normal saline was also prepared. The microbes were inoculated into the normal saline until the turbidity marched that of the MC-Farland scale by visual comparison. At this point, the microbes have a concentration of 1.5×10^8cfa/mL. Dilution of the extracts in the nutrient broth was done for each set of the organisms to obtain concentrations of 20 mg/mL, 10 mg/mL, 5mg/mL, 2.5 mg/mL and 1.25 mg/mL. Using a sterile pipette, 0.2 mL of the microbe suspension was transferred into each test tubes, and then incubated at 37°C for 24 hours. The tubes were observed for turbidity or growth. Test tube with the lowest concentration of the extract showing a clear solution (no turbidity) was regarded as the MIC [13-15].

Determination of minimum bactericidal concentration (MBC) of the extract

The MBC was carried out in order to determine whether the microbes were killed or only their growths were inhibited. Blood agar was prepared according to the manufacturer's instructions and sterilized at 121°C for 15 minutes. It was poured into sterile Petri dishes and left to solidify. The plates were labeled accordingly to correspond to the MIC test tubes. The contents of the MIC in the proceeding test tubes in the serial dilution were sub cultured into the plates by dipping a sterile wire loop and streaking the surface of the agar, in the plates. The plates were then incubated at 37°C for 24 hours after which they were checked for growth. The plate with the lowest concentration of extract without growth was taken as the MBC [15-16].

3. Statistical Analysis

All statistical analysis was based on one-way analysis of variance (one-way ANOVA) using SPSS version 22. Data were expressed as mean±SD for triplicate readings. Significance different was compared at $p \leq 0.05$.

4. Results and Discussion

The result of the phytochemical screening (Table 1) showed that leaf ethanol extract of *Cymbopogon citratus* contains alkaloids, tannins, carbohydrates and flavonoids while sterols cardiac glycoside and saponins were absent, and his result was also obtained by [17-18]. These metabolites were responsible for the observed antimicrobial activity of the leaf extract. For instance, many classes of alkaloids had been shown to possessed antimicrobial properties against *Methicillin Resistance Staph. aureus* by denaturing their transposons making the bacterium susceptible to antibiotics [18]. It has also been shown that saponins are active antifungal agents while tannins have been reported to prevent the development of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable for them [19-21]. Classes of alkaloids are also among the major powerful poisons known with some been proved to be useful in correcting renal disorders and exhibit antibacterial activities no matter how small they occur in the plant [21]. This report was not different from that obtained in the present study.

Antibacterial activities of *Cymbopogon citratus* ethanol extract by agar well diffusion method (Tables 2 and 3) revealed that the extract displayed various superior diameter zones of inhibition against the microbes than the standard broad spectrum antibiotic ceftriaxone. In Table 2, *Staphylococcus aureus* had the highest diameter zone of inhibition with 26.5±0.1mm, and it was followed by *Bacillus subtilis NCTC8230* with diameter zone of inhibition of 25.5±0.1 mm, *Candida albican ATCC9184* with diameter zone of inhibition of 23.5±0.2 mm, while *Salmonella typhi ATCC9184* with diameter zone of inhibition of 20.5± 0.2 mm and *Klebsiella pneumonia ATCC10031* with diameter zone of inhibition of 22.0±0.2 mm had the least.

Antibacterial agents affect the synthesis of peptidoglycan around a bacterial cell and the cell will die by osmotic shock. These agents with low activity against an organism have a high MIC while a highly active antibacterial agents gives a low MIC, and from this study, *Bacillus subtilisNCTC8230* showed highest activities than the other bacteria microbes tested. However, the fact that these organisms were sensitive to the crude leaf ethanol extracts, suggested that the leaf extract can be used to treat diseases caused by these organisms. For instance, staphylococcus infection caused by *S. aureus* as well as other sexually transmitted infections caused by these microbes. These results obtained from this study was comparable to that of the standard antibiotic (ceftriaxone USP) at $p \leq 0.05$ (one-way ANOVA).

The antimicrobial activity of the leaf ethanol extract of *C. citratus* can be attributed to the presence of flavonoids, alkaloids, sterols and tannins. These secondary metabolites were responsible for the antibacterial activity of the crude extract against the Gram positive and Gram negative bacterial strains as well as the fungus used in this study.

Table 1. *Phytochemical screening of Cymbopogon citratus leaf ethanol extract.*

Constituent	Test	Observation	Inference
Carbohydrate:	Molisch	Reddish coloured at the interface	+ +
	Fehling	Brick red precipitate	+
Sterols:	Salkowski	No colour change	-
C/glycoside:	Kedde's	No colour change	-
	Keller-Kiliani	No colour change	-
Tannins:	Ferric chloride	Green colour	+ +
	Lead sub-acetate	Coloured precipitate	+ +
Saponins:	Frothing	No foam 10 min later	-
Flavonoids:	NaOH	Yellow colour	+
	Ferric chloride	Green precipitate	+
Alkaloids:	Mayer's	Cream precipitate	+
	Dragendorff's	Reddish precipitate	+ +
	Picric acid	No colour change	-

+ (moderately detected), + + (strongly detected), **-** (not detected).

Table 2. *Diameter zone of inhibition of the extract against the microbes.*

Microbes	Zone of inhibition (mm)			Bacterial group
	Extract	Positive Control	Negative Control	
Bacillus subtilis NCTC8230	25.25±0.1	24.0±0.1	0.00	+
Candida albican ATCC19231	23.5±0.2	15.0±0.2	0.00	NA
Staphylococcus aureus	26.5±0.1	*14.5±0.2	0.00	+
Salmonella typhi ATCC9184	20.5 ±0.21	9.0 ±0.1	0.00	-
Klebsiella pneumonia ATCC10031	22.0 ±0.2 1	9.0±0.1	0.00	-

+(Gram positive strain), - (Gram negative strain), NA (not applicable),*(highest zone of inhibition), Results are mean ± SD, The positive control is ceftriaxone 10 mL USP. The negative control is water, * Significant different at $p \leq 0.05$ (one-way ANOVA), n= 3.

Table 3. *Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extract against the microbes.*

Organism	MIC (µg/mL)					MBC (µg/mL)				
	20	10	5	2.5	1.25	20	10	5	2.5	1.25
Bacillus subtilis NCTC8230	-	-	-	O*	++	-	-	O[a]	+	+ +
Candida albican ATCC19231	-	-	o*	+	++	-	o[a]	+	++	+++
Staphylococcus aureus	-	-	-	-	o*	-	-	-	o[a]	+++
Salmonella typhi ATCC9184	-	o*	+	++	+++	o[a]	+	++	+++	+++
Klebsiella pneumonia ATCC10031	-	o*	+	++	+++	o[a]	+	++	+++	+++

- (clear), O* (MIC), 0a (MBC), + (light growth), ++ (moderate growth), +++ (dense growth).

5. Conclusion

Our study revealed that the leaf extract of lemon grass demonstrated a strong antimicrobial activity against the microbes. This activity was possibly due to the identified metabolites alkaloids, flavonoids, sterols, tannins, which further confirm the use of the plant in health care delivery in traditional medicine. Thus, these metabolites from the plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial infections such as gonorrhea, pneumonia, eye infections, and a fungus infection candidiasis. However, isolation, identification and purification of these phytoconstituents and determination of their respective antimicrobial potencies as well as toxicological evaluation is further recommended, with a view to formulating novel chemo therapeutic agents with broad-spectrum affinity.

Competing Interests

We have no competing interests.

Acknowledgements

We are thankful to Mr. Mika Nurah of the Department of Microbiology, National Research Institute for Chemical Technology (NARICT), Zaria, Nigeria for supplying us with the microbes on demand.

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