



PHYTOCHEMICAL SCREENING, ANTIMICROBIAL ACTIVITY AND TLC PROFILING OF LEMON GRASS (*Cymbopogon citratus*)

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ABSTRACT

Medicinal plants are the source of treatment for many diseases and ailments throughout the developing world. Studies on them could lead to the finding of novel drugs for effective treatment of various diseases. We have chosen one of the most commonly used plants of *Cymbopogon citratus* leaves for the present study which was screened for phytochemical and antibacterial properties. The antibacterial activity test was evaluated qualitatively through the use of diffusion method on a solid medium and direct contact method using the following microbial isolate: *Staphylococcus aureus*, and *E. coli*. The study revealed that *Cymbopogon citratus* extracts contained rich availability of tannins, flavonoids, saponins, glycoside, alkaloids and steroids as the secondary metabolites. Furthermore, the extracts of plant leaves were found to have possessed the antibacterial properties for wide a range of gram-positive and gram-negative bacterial strains and demonstrated the best inhibition against *Escherichia coli* with an inhibition zone of 21 mm at a concentration 300 mg/ml.

Keywords: antimicrobial, *Cymbopogon citratus*, phytochemical screening, TLC

INTRODUCTION

Plants have been identified as excellent producers of therapeutic agents, due to the presence of nutritional (minerals and vitamins) and non-nutritional (fibres, active phytochemicals etc.) components. Different researches have been studied on the chemical composition, therapeutic use and enzyme inhibition of *Cymbopogon sp.* from different times. (Ranitha *et al.*, 2014),

Medicinal plants are the basis for the treatment of various diseases, Nearly 80% of people living in developing countries still depend on plant-based traditional medicine for their primary health care and almost three-fourths of the herbal drugs used worldwide are derived from medicinal plants. (Rao *et al.*, 2004), However, the quality control of herbal medicine remains a challenge because there is a high variability in the active constituents involved (Agun *et al.*, 2003). Medicinal plants are the source of treatment for many diseases and ailments throughout the developing world, they contain various bioactive principles which have the potential to cause beneficial and/or detrimental effects. (Rao *et al.*, 2004).

Cymbopogon citratus (*C. citratus*) is commonly known as lemongrass, barbed wire grass, citronella grass, fever grass and tangled but due to its broad distribution (Oladeji *et al.*, 2019) *C. citratus* flourishes in sunny, warm, humid conditions of the tropics and grown in a wide variety of soil ranging from rich loam to poor laterite, but calcareous and water-logged soils are unsuitable for its cultivation (Farooqi and Sreeramu, 2001). *C. citratus* is rich in bioactive compounds and the isolated and identified phytochemicals from its leaves mainly include flavonoids, alkaloids, saponin, tannins and phenolic compounds, which consist of quercetin, luteolin, apigenin, isoorientin 2'-O-rhamnoside and kaempferol that are known to have many benefits, especially in the fields of pharmacy, food, health and agriculture (Hasim *et al.*, 2015; Erminawati *et al.*, 2019). The other compounds identified in *C. citratus* are mainly alcohols, aldehydes, ketones, esters and terpenes (Hasim *et al.*, 2015)

MATERIALS AND METHODS

Collection and Preparation of sample

Fresh leaves of the plant *Cymbopogon citratus* (lemongrass) were collected from Safana, Katsina State, January, 2022, and

identified and authenticated at the Herbarium of the Department of Biological Sciences, Federal University Dutsin-ma. The plant leaves were washed in running water to remove adhesive contaminants and allowed to dry at room temperature. It is then cut, air-dried and ground with mortar and pestle. 50 grams of dried powder of *Cymbopogon citratus* leaves was packed in a separate round bottom flask for sample extraction using hexane, ethyl acetate and ethanol as solvent. The extraction is conducted with 200 ml of the solvent mixture for 72 hours. At the end of the extraction the extract is collected and stored for further analysis.

Phytochemicals analysis

Test for alkaloids

Wagner's test: About 10 ml of plant extract was taken and a few drops of Wagner's reagent were added and the formation of a reddish-brown precipitate indicates the presence of alkaloids (Harborne 1987).

Test for Flavanoids

Shinoda Test: 10 ml of extract was added to a pinch of magnesium turnings and 1-2 drops of concentrated hydrochloric acid were added. The Formation of pink color indicates the presence of Flavanoids. Lead acetate test: 10 ml of extract was taken and few drops of 10% lead acetate solution were added. The Appearance of a yellow colour precipitate indicates the presence of flavonoids (Harborne 1987).

Tannins

Lead acetate test: 10 ml of extract was taken and 0.5 ml of 1% lead acetate solution was added and the formation of a precipitate indicates the presence of tannins and phenolic compounds.

Ferric chloride test: Five mg of extract was taken and 0.5 ml of 5% ferric chloride was added. The development of dark bluish-black color indicates the presence of tannins (Harborne, 1998)

Test for Phenols

Five ml of extract was dissolved in 0.5 ml of 20% sulphuric acid solution. Followed by addition of a few drops of aqueous

sodium hydroxide solution, it turns blue which indicates the presence of phenols (Harborne 1987).

Test for steroids

Salkowski's test: Five ml of extract was dissolved in 2 ml of chloroform and an equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and the lower layer turns yellow with green fluorescence, indicating the presence of the steroids (Siddiqui, 1997).

Test for Saponins

Foam test: 0.5 mg of extract was diluted with 20 ml distilled water and shaken well in a graduated cylinder for 15 min. The formation of foam to a length of 1cm indicated the presence of saponins and steroids (Harborne 1987).

Test for Glycosides

Glycoside test: 0.1 mg of extract was dissolved in 1 ml of water and then an aqueous NaOH solution was added. The Formation of yellow color indicates the presence of glycosides (Siddiqui, 1997).

Antibacterial test

Preparation of Antibacterial Analysis

The antibacterial screening of leaves extracts was determined using some pathogenic microbes such as (*staphylococcus aureus*, *Escherichia coli*). The microbes were obtained from the Department of Microbiology Federal university Dutsinma. The Diffusion method was the method used for screening the extract. Muller Hinton agar was the medium used as the growth medium for the microbes.

Preparation of Test Sample for Antibacterial Analysis

1g of the extracts and 2ml of (DMSO) was measured and used for serial dilution and sent to the autoclave before use for sterilization. This gave a stock solution that contained a concentration of 500 mg/ml. The solution was then

transferred into a test tube and mixed well by shaking. Three dilutions were additionally made from each of the extracts as 500mg/ml, 300 mg/ml, 200 mg/ml and 100 mg/ml respectively. (Asuquo, 2011).

Preparation of Mueller Hinton Agar

The medium was prepared according to the manufacturer's instructions, sterilized at 121 °C for 15 minutes, poured into sterile Petri dishes and allowed to cool and solidify.

Determination of Zones of inhibition growth

The sterilized medium was seeded with 0.1 ml of the standard inoculum of the test microbe, the inoculum was spread evenly over the surface of the medium by the use of a sterilized swab. By the use of the standard cork borer of 6 mm in diameter, a well was cut at the center of each inoculated medium. 0.1 ml of the solution of the extract of the concentration of 50 mg/ml was then seeded into the well on the inoculated medium. Incubation was made at 37°C for 24 hrs, after which the plates of the medium were observed for the zone of inhibition of growth. The zone was measured with a transparent ruler and the results were recorded in millimeters. (Yanah, 2020).

Thin-layer Chromatography (TLC)

Thin-layer chromatography was carried out using TLC pre coated plates by one-way ascending technique. The TLC was carried out to ascertain the number of components in each crude extract. A little quantity of each of the extracts was dissolved in dichloromethane to form a solution. The extract was then spotted manually on a TLC plate using a capillary tube and allowed to dry. After drying the plate was developed in an air-tight chromatographic tank using a perceived solvent system. (Deepak, 2017). The various solvent systems were used to develop the plates, including: Hexane: Ethyl acetate (5:5), developed chromatograms were air dried and visualized under normal day-light and then under ultraviolet light (254 nm & 366 nm).

RESULTS

Table 1: Yield in grams of each plant extract of *Cymbopogon citrates*

S/N	EXTRACT	W1 (gram)	W2 (gram)	W3 (gram)
1	N-Hexane	171.81	177.50	5.69
2	Ethyl acetate	284.95	291.13	6.18
3	Ethanol	175.82	180.82	5.0

Table 1 shows the various amounts of extracts obtained after the extraction of the plant material using using n-hexane, ethyl acetate and ethanol as solvents. The W1 is the empty bottle W2 contain bottle with extract and W3 is the actual yield of the extract

Phytochemical screening test result

Table 2: Phytochemical analysis of ethanol and ethyl acetate extract from leaves of *Cymbopogon citrates*

S/N	Parameter	ethanol	ethyl acetate
1.	Flavonoids	+	+
2.	Alkaloids	+	+
3.	Saponins	-	-
4.	Phenols	+	+
5.	Tannins	+	+
6.	Steroids	+	+
7.	Glycosides	+	-

Key + =present --absent

Table 2 revealed the presence of phytochemicals screening of *Cymbopogon citrates*. The extracts show the presence of all the phytochemicals except for phenol and glycosides which were absent in ethyl acetate extracts.

Antimicrobial Test Result**Table 3: Antimicrobial activity of ethanol extract of *Cymbopogon citratus* against *Staphylococcus aureus*, *Escherichia coli***

Bacteria	Concentrations		
	300 mg/ml	200 mg/ml	125 mg/ml
<i>Staphylococcus aureus</i>	15		10
<i>Escherichia coli</i>	19		ND

Table 4: Antimicrobial activity of N-hexane extract of *Cymbopogon citratus* against *Staphylococcus aureus*, *Escherichia coli*

Bacteria	Concentrations		
	300 mg/ml	200 mg/ml	125 mg/ml
<i>Staphylococcus aureus</i>	14		ND
<i>Escherichia coli</i>	21		15

Key ND =Not detected Control (Ciprofloxacin) Bacteria

Table 5: Antimicrobial activity of ethyl acetate extract of *Cymbopogon citratus* against *Staphylococcus aureus*, *Escherichia coli*

Bacteria	Concentrations		
	300 mg/ml	200 mg/ml	125 mg/ml
<i>Staphylococcus aureus</i>	15		10
<i>Escherichia coli</i>	16		11

Tables: 3, 4 and 5, show the sensitivity of two pathogenic gram-positive and gram-negative bacteria to the leaf extracts of *Cymbopogon citratus* were tested and compared to that of the antibacterial antibiotic Ampicillin. The results shown Table 4 are the average zones of inhibition for each extract. All the plant extracts tested showed antibacterial activity;

however, the plant extracts differ in their activities against the microorganisms tested. Highest antibacterial activity was observed with hexane extract of *Cymbopogon citratus* against *E. coli* (21 mm) and *S. aureus* (14 mm), while minimum activity was observed against *S. aureus* (10 mm)

TLC Result**Table 6: Represents the TLC profile of ethanol extract**

Spot	Distance traveled by spot(mm)	Distance traveled by solvent(mm)	Retention factor (mm)	Inference
A	0.35	0.66	0.53	Alkaloids
B	0.39	0.66	0.59	Alkaloids
C	0.48	0.66	0.72	Flavonoids
D	0.52	0.66	0.78	Flavonoids

Figure 1 and 2, shows the TLC plate of ethanol extract.

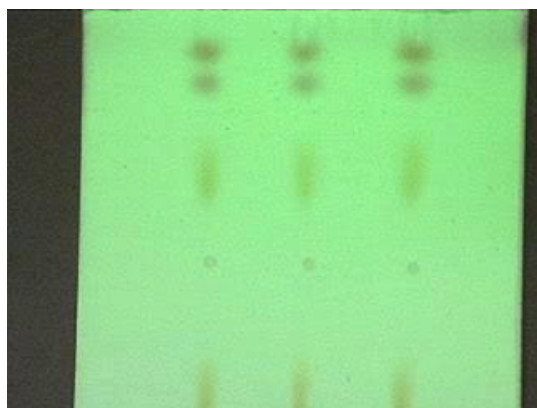


Fig. 1:



Fig. 2:

Table 6 represent the thin layer chromatographic analysis of crude ethanol shows the presence of 4 spots using ethyl acetate and n-hexane solvent system (ethyl acetate 5 ml : 5 ml n-Hexane) revealed the presence of some spots as shown above, while the figure shows the TLC plate for the extract.

The TLC profiling of the extract revealed the presence of several phytochemicals. Various phytochemicals gives different R_f values in a different solvent system. This variation in R_f values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in the

selection of the appropriate solvent system for separation of pure compounds by column chromatography.

DISCUSSION

The results of the phytochemical analysis of lemon grass extracts revealed the presence of flavonoids, alkaloids, tannins, saponins, steroids, and glycosides in the ethanol extract and the absence of saponins and glycosides in the ethyl acetate extract. As a result, these findings are consistent with the study by (Owusu *et al.*, 2021). The lack of saponins contradicts the findings of Rotimi, 2019, who claimed that lemon grass contains them. The results of the current study are supported by investigations conducted by numerous scientists addressing the phytochemical screening of lemon grass (*C. citratus*) leaves (Ekpenyong *et al.*, 2014). The presence of glycosides in lemon grass did not conform to (Zhou *et al.*, 2016). This could be attributed to the extraction method since most polar molecules are extracted by polar solvents. According to (Njar *et al.*, 2006) dry lemon grass contains higher phenol and flavonoids than fresh lemon grass (Geetha and Geetha, 2014). However, the findings of this study showed that lemongrass contains the phytochemicals mentioned above.

According to Assous *et al.*, (2013) and Gazwi (2020), environmental factors like climate, altitude, and rainfall are to blame for the variances in phytochemicals mentioned above. The study's findings so imply that the detected phytochemical compounds may be the bioactive components, and these plants are demonstrating their value as a source of bioactive substances with significant medical value. (Shendurse *et al.*, 2021). According to (Owusu-Ansah *et al.*, 2023) who observed that tannins are vital in herbal medicine and are used to control bleeding injuries, the presence of tannins and flavonoids in plant extracts is consistent with this. Tannins and tannin acid are a pellicle of coagulated protein over the lining of the digestive tract, and they have stringent functions precipitating and inwardly resisting protein attacks.

The antibacterial activity of an extract made from *Cymbopogon citratus* leaves was investigated in this work. The results of the observation of the antibacterial activity of aqueous leaf extract against various Gram-positive and Gram-negative bacteria are displayed in the [Table 3,4 5]. The zone of inhibition reveals that all the extracts showed higher activity against all the tested microbes. The hexane extract was found to have the highest activity, *E. coli*, with 21 mm, 18 mm and 15 mm at concentrations of 300 mg/ml, 200 mg/ml and 100 mg/ml respectively and *Staphylococcus aureus* with 15 mm, 13 mm and 10 mm at concentrations of 300 mg/ml, 200 mg/ml and 100 mg/ml with ethyl acetate extract to be the highest. This study and (Madan, *et al.*, 2015) were comparable. While this study found that *Cymbopogon citratus* showed more activity in bacteria and that its results were consistent with those of (Di Pasqua, *et al.*, 2007), another study conducted by Hammer, *et al.* (2005) claimed that the herb is more fungicidal in nature. Based on the findings of numerous scientists who found that lemongrass has antibacterial action against a wide variety of microorganisms, including gram-positive and gram-negative germs, yeast, and fungi, the plant can be used to treat many bacterial infections. (Shendurse, *et al.*, 2021).

CONCLUSION

The study of Phytochemical of *Cymbopogon citratus* conclude that *Cymbopogon citratus* is one of the most important plant having therapeutic uses. The phytochemical study reveals that plants contain many phytochemicals such as alkaloids, flavonoids, saponin, glycoside, tannins, phenolic

compounds and steroids. The antimicrobial test result also shows that the plant used in the research exhibit antimicrobial property on the bacteria and act as a source of medicinal purpose.

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