

Comparative Studies on the Chemical Composition and Antimicrobial Activities of the Ethanolic Extracts of Lemon Grass Leaves and Stems

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Abstract: The aim of this study is to examine the phytochemical screening, chemical composition and antimicrobial activities of ethanol extracts of both the leaves and stems of *Cymbopogon citratus*. For proximate analysis, results for the leaves on the Moisture content, ash content, fat content, crude fibre, crude protein and carbohydrate were: 13.50, 11.17, 10.00, 19.54, 17.50 and 28.29% while that of the stem were: 7.98, 14.29, 13.50, 12.5, 17.50 and 34.23%, respectively. Also, the extracts was screened against two gram-negative bacteria, *Klebsiella pneumoniae* and *Proteus vulgaris*, two gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus* and fungi strain *Penicillium* and *mucor* at three different concentrations [1:1, 1:2 and 1:3] using Disc diffusion method. For both the leaves and stem extracts, the phytochemical analysis revealed the present of tannins, flavonoid, phlobotannins and cardiac glycosides but absence of alkaloid and saponin.

Keywords: Antimicrobial activity, *Cymbopogon citratus*, phytochemical analysis, proximate analysis

INTRODUCTION

Cymbopogon citratus (lemon grass) is an aromatic perennial tall grass with rhizomes and densely tufted fibrous root. It has short underground stem with ringed segments, coarse green slightly leathery leaves in dense clusters. The plant is a native herb from India and is cultivated in other tropical and subtropical countries (Figueirinha *et al.*, 2008; Mehraban *et al.*, 2005). It is used as traditional folk medicine in the treatment of nervous gastrointestinal disturbances fever, hypertension (Borrelli and Izzo, 2000; Melo *et al.*, 2001 and Abubakar *et al.*, 2008). Recent attention has been paid to extracts of biologically active components isolated from plant species. The medicinal value of plants lies in some chemical substances that produce a definite physiological action in human body. The most important of these bioactive constituents are alkaloids, tannins, flavonoids, phlobotannins, saponins and cardiac glycoside (Edeoga *et al.*, 2005; Abubakar *et al.*, 2008). Microorganism is a term used instead of microbes, they are small organism that belonging to various groups: bacteria, fungi, protozoa, one can add viruses, characterized by their unicellular structure (Ekberg *et al.*, 2003). Antimicrobial agents are natural substance produce by certain groups of microorganism and chemotherapeutic agents, which are chemically

synthesized. A hybrid substance is a semi synthetic antibiotic, where, in a molecular version produced by the microbes is subsequently modified by the chemist to achieve desired properties (Langford and Roland, 2004). Furthermore, some antimicrobial compounds originally discovered as products of microorganism, can be synthesized entirely by chemical means. In medical and pharmaceutical worlds, these entire antimicrobial agents used in the treatment of diseases are referred to as antibiotic, interpreting the word literally. Most of the natural antibiotics that are being used in agriculture and medicine are produced by these unrelated groups of microbes including eukaryotic moulds and 2 types of spore-forming bacteria (Babayi *et al.*, 2004; Shariff and Shariff, 2001). *Cymbopogon citratus* (lemon grass) plant generally, are used traditionally for the treatment of malaria and typhoid fever. Concoction prepared from the combination of the leaves and grass of these plants or the boiling of the individual plant leaves have been used in the treatment of ailments like typhoid fever, stomach ache etc., (Udeh *et al.*, 2001). *Cymbopogon citratus* has application in the soap, cosmetics and perfumery industries.

The aim of this study is to examine the phytochemical screening, chemical composition and antimicrobial activities of ethanol extracts from both the leaves and stems of *Cymbopogon citratus* (Lemon grass).

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MATERIALS AND METHODS

Materials:

Collection of plant materials: Whole plant material (*Cymbopogon citratus*) containing both the leaves and the stems were planted, cultivated and harvested.

Pre-treatment of the sample (*Cymbopogon citratus*):

The samples were collected and washed with clean water for 2-3 times, it was sliced and then spread on a dried clean nylon under the room temperature. The sample were milled and sieved. The powdery sample were partitioned in three parts and labeled before the analysis.

This research was carried out in the department of Science Laboratory Technology, Faculty of Food Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria between 2011-2012.

Methods:

Antimicrobial procedure: One hundred grams of the powdered sample were extracted with 500 mL of organic solvent (ethanol). The powdered sample was soaked in the solvent for 72 h after which it was left to be air dried. One gram each was weighed from it, into three different test tubes labeled 'A, B and C'. The 3 test tubes were arranged in the test-tube rack. One milliliter of distilled water to the first test tube labeled A, 2 mL of distilled water to test tube B and 3 mL of distilled water to test tube C. Two grams of nutrient agar was weighed into a clean conical flask, 4 mL of distilled water was added to it also and aluminium fuel was used to cover it, 2 g of Potato Dextrose Agar (PDA) was weighed into another conical flask, 40 mL of distilled water was added to it and also covered with aluminum fuel. The 2 conical flasks and 8 Petri-dishes were both autoclaved until it made a sound which indicates that it has been sterilized. Ethanol was used to sterilize the table in which the Petri-dishes were placed; it was numbered 1, 2 and 3 on each Petri-dish with paper. Pour plate method was used to apply the microorganism as used by Ramesh *et al.* (2001); cork boring method was used for extract application. The principle used was the diffusion of extracts into the already inoculated agar medium.

A drop (0.1 mL) of test organism was aseptically injected into separate sterile Petri-dishes from broth culture. Sterilized nutrient agar of about 20 mL was poured on top of the test organism after it has cooled to about 45°C. It was rotated gently for when distributing microorganism.

However, the same was done for plate containing fungi; potato dextrose agar was used for the cultivation. The plates were allowed to solidify. Well were bored on the agar on the agar plates using sterile cork borer of 7 mm in diameter. Different concentration of the extract

inside the 3 test tubes placed in the test-tube rack was introduced into the wells in the plates, sterile water and antibiotics were introduced into another well of the sample plates which serves as negative control. The plate containing bacteria were incubated for 24 h at 37°C while those of fungi were incubated for 3 days at 27°C and observed for zone of inhibition.

Phytochemical screening: Phytochemical screening of the extract were carried out in order to identify the constituents, using standard phytochemical methods as it was described by Trease and Evans (1989) and Sofowora (1993). The phytochemical screening involves detection of alkaloids, flavonoids, saponins, tannins, phlobotannins, anthraquinones and cardiac glycosides.

Lieberman's test: (0.5 g) of the sample was dissolved in 2 mL of acetic anhydride and cooled in ice. Sulphuric acid was carefully added until the color changed to brownish yellow color.

Salkowski test: (0.5 g) of the sample was dissolved in 2 mL of glacial acetic acid. One milliliter of ferric chloride was added to it also. Reddish brown color was observed which indicate the presence of cardenoides.

For proximate analysis: Proximate analysis of the sample was performed according to AOAC (1984). Proximate analysis are performed to know the % Moisture content, % Ash content, % Crude Fibre, % Fat content, % Crude Protein and % Carbohydrate. Proximate analysis constitutes the classes of food present in the sample.

RESULTS AND DISCUSSION

The proximate, phytochemical and antimicrobial analysis were conducted on the ethanolic extract of *Cymbopogon citratus* stems and leaves. The result obtained are presented in the tables. The fairly highly moisture content of the leaves than the stems indicates

Table 1: Chemical composition of *Cymbopogon citratus* leaves and stems

Proximate composition	Percentage (%) dry weight	
	Leaves	Stems
Moisture content	13.50	7.98
Ash content	11.17	14.29
Fat content	10.00	17.50
Crude Fibre	19.54	13.50
Crude Protein	17.50	34.23
Carbohydrate	28.29	12.50

Table 2: Antimicrobial activity screening of *Cymbopogon citratus* leaves using disc diffusion method

Test organism	Plant extract	Min. inhibition concentration (mg/mL)			
		1 mg/mL	2 mg/mL	3 mg/mL	
<i>Klebsiella pneumonia</i>	Leaves	1.00	0.00	0.10	0.20
	Stem	1.10	0.00	0.00	0.30
<i>Proteus vulgaris</i>	Leaves	1.10	0.00	0.00	0.10
	Stem	1.20	0.01	0.20	0.30
<i>Bacillus subtilis</i>	Leaves	1.00	0.00	0.10	0.10
	Stem	1.10	0.00	0.00	0.00
<i>Staphylococcus aureus</i>	Leaves	1.10	0.20	0.10	0.10
	Stem	1.00	0.00	0.20	0.30
<i>Penicillium</i>	Leaves	0.70	0.00	0.00	0.00
	Stem	1.10	0.00	0.00	0.00
<i>Mucor</i>	Leaves	0.00	0.00	0.00	0.00
	Stem	1.10	0.00	0.00	0.00

that *Cymbopogon citratus* leaves may be susceptible to microbial growth. Fat content and crude Fibre is said to be higher in stem than the leaves, while moisture content is higher in leaves than the stem as was determined. The results showed the average ash content of *Cymbopogon citratus* stem to be 14.29% which is reasonably high. This is an indication that *Cymbopogon citratus* has a reasonable amount of inorganic nutrients and allows for high storage capacity. The carbohydrate content of the leaves extract is high (28.29%). This shows that *Cymbopogon citratus* is a very good source of energy. The protein content of leaves extract is low (17.5%) as compared with the Stem extract. The crude fibre content (19.54%) of *Cymbopogon citratus* leaves on the hand is higher. This makes *Cymbopogon citratus* to be a good source of crude fibre than the stem extracts and any other conventional leaves. The result of the proximate analysis of *Cymbopogon citratus* stem is as shown in Table 1.

The results on antimicrobial showed that the extracts were screened against two gram-negative bacteria, *Klebsiella pneumoniae* and *Proteus vulgaris*, two gram positive bacterial: *Bacillus subtilis* and *Staphylococcus aureus* and 2 fungi strains *Penicillium* and *Mucor* at 3 different concentration (1:1, 1:2 and 1:3) using disc diffusion method which showed that lemon grass possessed bactericidal. The ethanolic extract of *Cymbopogon citratus* stem inhibited the entire tested organism more than the leaves. However the percentage inhibition varied with the type of leaves extracts, extract concentration as well as the type of bacteria. The results showed that inhibition of microbial growth was greater at high concentration of the ethanolic extracts and less inhibition was observed as the concentration was lowered in the stem than in the leaves.

The results of antibacterial activity of the extracts against *E. ewil*, *S. typhi*, *S. avreus*, *B. Cereus*, *A. niger* and *Penicillium*, showed the ethanolic extract to inhabit only the growth of 8 coiled *S. aureus* were not inhibited (Table 2). The mechanism of action of the

Table 3: Qualitative analysis of the phytochemical component of ethanolic extract of *Cymbopogon citratus* leaves and stems

Chemical constituents	Leaves	Stem
Alkaloid	-	-
Tannins	+	+
Saponins	-	-
Phlobotannins	+	+
Flavonoid	+	+
Cardiac glycoside Salkowski	+	+
Keller killiani	+	+

ethanolic extract seems to be dose-dependent, as similar results has been reported by Oboh (2001). From the present study. it could be seen that ethanolic extract of *Cymbopogon citratus* exhibits antibacterial activity against *S. typhi* while the growth of this microbe (*S. typhi*) was affected by the extract. *Cymbopogon citratus* is a good source of carbohydrate, crude fibre and nutritive elements.

The present study of ethanolic extract of *Cymbopogon* leaves tested positive for Tannins, flavonoid, phlobotannins, and cardiac glycoside (Table 3). Generally, cardiac glycosides serve as defence mechanism against cardiovascular disease as reported by Schneider and Wolfing (2004). This may therefore explain its therapeutic effect against cardiovascular and digestive problems. The cardiac glycoside compounds are important component in vegetable foods and teas for their beneficial effects in human health (Ozcan *et al.*, 2009).

CONCLUSION

In conclusion, according to the present study, ethanolic extract of *Cymbopogon citratus* (both leaves and stems) have antimicrobial activity with the stem having higher content. *Cymbopogon citratus* leaves is one of the medicinal plants since they contains cardiac glycosides, tannins, phlobotannins and flavonoids. Antimicrobial properties of the stem is higher than that of the leaves and both part of the plants are desirable tools in the control of undesirable microorganisms especially in the treatment of infectious disease and in food spoilage. *Cymbopogon citratus* are recommended

to be used for the treatment of nervous gastrointestinal disturbance, fever, hypertension since the plant contain cardiac glycoside and the cardiac glycosides serves as defence mechanisms against cardiovascular disease and digestive problems. *Cymbopogon citratus* (Lemon grass) whole plant materials are recommended to be taken because it has many beneficial effects in human health (Ozcan *et al.*, 2009).

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