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Research Paper

Chemical profile and antimicrobial activity of *Cymbopogon citratus* leaves

Omotade I. Oloyede*

Department of Biochemistry, University of Ado-Ekiti, Nigeria.

*Corresponding author

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ABSTRACT

Chemical composition and antibacterial activity of aqueous extract of *Cymbopogon citratus* leaves were studied. Moisture, Crude protein, Crude fibre and Carbohydrate contents were 12.36%, 15.68%, 27.72% and 29.58% respectively. Phosphorus was found to be the most abundant (15.58mg/100g) followed by Potassium (8.60mg/100g). Zinc an important microelement was present in considerable amount (0.93mg/100g). Lead and Chromium which are toxic at low concentrations were not detected. Phytate and Oxalate contents were 0.48±0.02 and 0.48±0.05mg/g respectively. Terpenoid, Cardiac glycosides and Phenol were also present. Antimicrobial activities of aqueous and ethanol extract of *Cymbopogon citratus* leaves were compared against four bacteria by disc diffusion method. Both extracts were found to exhibit selective inhibition against the isolates. Ethanol extract exhibited high inhibitory activity against all the tested bacteria in order of sensitivity as *Staphylococcus aureus*>*Salmonella typhi*>*Bacillus aureus*>*Escherichia coli*, while aqueous extract was more active against *Salmonella typhi*, at the tested concentrations. In view of the nutrient and antinutrient contents determined, *Cymbopogon citratus* leaves could be considered safe and good as a therapeutic agent in traditional medicine practice and as dietary supplements.

Keywords: *Cymbopogon citrates*; Antinutrient; Antimicrobial activities.

INTRODUCTION

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 and phenol (Edeoga, et al., 2005; Abubakar, et al., 2008).

Cymbopogon citratus (Lemon grass) is an aromatic perennial tall grass with rhizomes and densely tufted fibrous root. It has short underground stems with ringed segments, coarse, green slightly leathery leaves in dense clusters. (Carlin, et al., 1986) The plant is a native herb from lndia and is cultivated in other tropical and subtropical countries.(Figueirinha, et al., 2008) It is used as traditional folk medicine in the

treatment of nervous, gastrointestinal disturbances fevers and hypertension.(Borrelli,2000; Melo, et al., 2001).

Lemon grass is also a folk remedy for coughs, consumption, elephantiasis flu, gingivitis, headache leprosy, malaria, ophthalmia, pneumonia and vascular disorders. It is principally taken as tea to remedy digestive problems, diarrhoea and stomach ache (Carlin, et al., 1986). As a medicinal plant, lemon grass has been considered a carminative and insect repellant. Studies on extracts from *Cymbopogon citratus* leaves have demonstrated anti-inflammatory, vasorelaxing, diuretic and valuable remedy in treating ringworm as a local application(Melo, et al., 2001; Runnie, 2004) In developing countries where medicine are quite expensive, investigation on antimicrobial activities from ethnomedicinal plants may still be needed.(Cowan,1999). The present study is designed to determine the chemical constituents of the leaves of *Cymbopogon citratus* and its antimicrobial activity against some bacteria.

MATERIALS AND METHODS

***Collection of Plant materials*:** Fresh samples of *Cymbopogon citratus* leaves were collected from Ado-Ekiti metropolis. The identity was confirmed in Plant Science Department, University of Ado-Ekiti, Nigeria by the Technologist Mr Femi Omotayo.

Extraction procedure: The green leaves of *Cymbopogon citratus* were airdried at room temperature and grounded into powdery form with an electric blender. .Aqueous extract was prepared by soaking the dry powdered leaves in distilled water (5%w/v) the resulting suspension was left overnight at room temperature. Thereafter, the suspension was filtered and the filtrate kept in the refrigerator at 4°C prior to analysis. Ethanol extract was prepared by soxhlet extraction. The leaves were extracted with 90% ethanol for 72 hours to get the ethanol extract. The extract was concentrated under reduced pressure. Residue used for the analysis.

Microorganisms: The following bacteria were used in this study; *Esherichia coli, Salmonella typhi, Staphylococcus aureus and Bacillus cereus.* These organisms were collected from stock culture of Microbiology Department, University of Ado-Ekiti, Nigeria.

***Bioassay procedure**:* The extracts of the leaves of *Cymbopogon citratus* were tested for antimicrobial activity against the tested organism using the agar diffusion method of Deeni and Hussain, 1991. Cultures of these bacteria were grown in nutrient agar at 37°C and maintained on slopes of nutrient agar. Each of the organisms was transferred into a separate test- tube containing nutrient broth to reactivate them by culturing overnight at 37°C. The plates were allowed to air-dry for 5- 10min. Sterile aper discs (6 mm diameter) prepared from Whatman Number 1 filter paper were impregnated with the crude extracts. Each disc contained 10mg/ml, 5mg/ml, 2.5mg/ml and 1.25mg/ml of the extracts respectively. Four discs and one of control were arranged and firmly pressed on to the agar surface of each seeded plate. Sterile 80% (v/v) ethylene glycol in 0.1M phosphate buffer pH 7.4 was used as the solvent in preparing the stock solution and for impregnating control discs, which had no extracts added. Plates were then incubated aerobically at 37 °C for 24h. The zones of inhibition were measured at the end of incubation period. Antimicrobial activities of the plant extracts were expressed by - (negative), + (zone of inhibition $\leq$ 8mm in diameter) and ++ (zone of inhibition $\geq$ 8 mm in diameter).The result recorded for each bioassay was the average of quadruplicated test.

***Determination of the Minimum Inhibitory Concentration (MIC*):** Different dilutions of both extracts were introduced to the prepared nutrient agar plates containing

specific bacteria on which different wells have been made. The plates were incubated at 37°C for 24h. Zones of inhibition were measured at the end of incubation period. The MIC was taken to be the lowest dilution inhibiting growth of the organism.

Phytochemical tests: Phytochemical tests for alkaloids, anthraquinones, phlobatannins, saponins and tannins were carried out as described by Trease and Evans (1987) and each of the tests was qualitatively expressed as negative (-ve) or positive (+ve).

Proximate Analysis: Proximate analyses were carried out according to the procedure of Association of Official Analytical Chemist(AOAC) This constitute the class of food present in samples such as carbohydrate, protein, fat, free sugar, starch, fiber, ash content and moisture content.

Determination of Mineral Composition: The extracts were dry ashed at 550°C.The ash was boiled with 10ml of 20% hydrochloric acid in a beaker and then filtered into a 100ml standard flask .It was made up to the mark with deionised water. The minerals were determined from the resulting solution using Atomic Adsorption Spectroscopy (Pye Unican Sp9,Cambridge,UK).

***Determination of Antinutrients*:** Tannin, Oxalate and Phytate were used in this study. Tannin was determined according to the method of Makkar et al., 1993 which is based on the ability of tannin-like compounds to reduce phosphorus tungstomolybdic acid in alkaline solution to produce a highly coloured blue solution. The absorbance was measured at 725nm.The method of (Asiber, 1987) was used to determine Phenol, Oxalate and Phytate respectively.

RESULTS AND DISCUSSION

Proximate and mineral analyses were conducted on the leaves of *Cymbopogon citratus*. The results obtained are presented in Table 1. Potassium is essential in the maintenance of cellular water balance, pH regulation in the body and it is also associated with protein and carbohydrate metabolism (Berry, 1998). The high potassium content could be utilized for the management of hypertension and other cardiovascular conditions. The low level of sodium is desirable because high dietary sodium has been associated with essential hypertension (Latham, 1997). Zinc boost the immune system and act as antioxidant (Ferguson, et al., 1993). Presence of zinc in the leaves contributes to the antioxidant activity demonstrated by the plant.

Table- 1: Chemical Composition of *Cymbopogon citratus* Leaves

Proximate Compositions	**% Dry Weight**	**Mineral Composition**	**Values (mg/100g**
Moisture Content	12.36±0.36	Na	1.06
Ash Content	13.43±0.02	K	8.60
Crude Protein	15.68 ± 0.83	Ca	0.45
Crude Fibre	27.72 ± 0.02	Mg	0.89
Fat Content	1.25 ± 0.01	Zn	0.93
Carbohydrate	38.44 ± 0.77	Fe	0.42
		P	15.58
		Cu	0.30
		Pb	ND
		Cr	ND

- Values are mean ± SD of 2 determinations
- ND= Not Detected

The fairly high moisture content indicates that *Cymbopogon citratus* may be susceptible to microbial growth but the moderate levels of carbohydrate and protein shows that it can form part of human diet and also considered as a good source of plant protein. Lead and Cadmium were not present in detectable amount and this is a great advantage to consumers since these minerals have been reported to be toxic at low concentrations (Oberleas, 1983).

In the present study aqueous extract of *Cymbopogon citratus* leaves tested positive for terpenoid phenol and cardiac glycoside (Table 2). Many phytochemicals are present in the plants as glycosides (with a sugar moiety attached) 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. 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 effects in human health (Ozcan, et al., 2009).

Besides the nutritional importance of plants, they also contain certain antinutritional factors (Ewaidah, 1993) such as phytate, tannins and oxalate (Table 2). Phytate is an antinutrient in plant foods which has ability to chelate divalent elements like calcium, iron and zinc (Oberleas, 1983; Leshenne, et al., 2005) thus decreasing their bioavailability for normal metabolic function. The present study on Cymbopogon citratus leaves revealed low concentrations of Phytate and oxalate (0.48mg/g) Tannin was not detected. Leaves of *Cymbopogon citratus* could be considered safe with regards to tannin poisoning also it has been reported that tannin usually forms insoluble complexes with protein, thereby interfering with their bioavailability (Enujiugha and Agbede, 2000). The low levels of phytate and oxalate is of nutritional significance because it may allow bioavailability of many essential minerals and therapeutic effects of the leaves is guaranteed. Phytate is beneficial in human as it reduced the incidence of heart disease and act as anticarcinogens.(Ferguision, et al., 1993) However, the pharmacological actions of the leaves cannot be ascertained by the result of Proximate, Mineral, antinutrient and phytochemical analysis only.

Table-2: Phytochemical Screening and Antinutrient Composition(mg/g) of *Cymbopogon citratus* Leaves.

Test	Result
Tannin	-ve
Alkaloids	-ve
Terpenoid	+ve
Phenol	+ve
Flavonoid	-ve
Anthraquinones	-ve
Phlobatannins	-ve
Steroid	-ve
Saponins	-ve
Cardiac glycoside	+ve
Tannin	ND
Phenol	1.0005±0.005
Phytate	0.48±0.02
Oxalate	0.48±0.05

- Values are mean±SD of two determinations
- ND=Not detected

Antibacterial activities of aqueous and ethanol extract of *Cymbopogon citratus* leaves were compared in this study (Table 3).The zones of inhibition of growth of the microorganisms are a function of relative antibacterial activity of the extracts. The extracts showed selective levels of activities against the organism. *Staphylococcus aureus* was sensitive at 1.25, 2.5, 5 and 10mg/ml of ethanol extract (Table 3) with 1.9, 1.7, 1.2 and 1.0 mm zones of inhibition while it was only sensitive at 10mg/ml of aqueous extract. *Salmonella typhi* was sensitive at 2.5, 5 and 10mg/ml of both extract. However, highest sensitivity was demonstrated with ethanol extract with zones of inhibition of 3.0mm.This can be deduced to the ability of ethanol to extract more of essential oils and secondary plant metabolites which are believed to exert antibacterial activity on the test organism. Aqueous extract showed minimal antibacterial activity. The variation in activities of the extracts may be due to insolubility of active compounds in water or the presence of inhibitors to the antimicrobial components.

Table-3: Effect of *Cymbopogon citratus* Leaves extract against Microbial Isolates Diameter zones of inhibition(mm).

Plant extract	Test Organism	Minimum Inhibitory Concentration (mg/ml)	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml
Aqueous extract	*Escherichia coli*	5.0	1.6	1.1	-	-
	Salmonella typhi	2.5	2.6	2.0	0.9	-
	Staphylococcus aureus	10.0	1.7	-	-	-
	Bacillus cereus	10.0	1.5	-	-	-
Ethanol extract	*Escherichia coli*	2.5	1.4	1.1	0.8	
	Salmonella typhi	2.5	3.0	2.7	1.6	
	Staphylococcus aureus	1.25	1.9	1.7	1.2	1.0
	Bacillus cereus	2.5	1.7	1.5	1.2	-

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