CHEMICAL COMPOSITION AND BACTERICIDAL ACTIVITY OF *Cymbopogon Citratus*

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ABSRACT
Cymbopogon citratus was analyzed for its chemical constituents and bactericidal activity. Proximate analysis revealed that the sample contained moisture content: 4.20%, crude protein 23.6% ash content: 1.3%, crude fat 26.00%, crude fibre: 39.25% and carbohydrate 5.65%. The phytochemical analysis of the aqueous and ethanolic extracts of cymbopogon citratus indicated that it had alkaloid, saponin, tannins, phenols, cardiac glycosides and terpenoid. Test for bactericidal activity using agar diffusion method showed that bactericidal activity was active on salmonella, typhi and pseudomonas but inactive on Eschorichea Coli and staphilocolus aureus.

Keywords: Cymbopogon citrates, bactericidal activity, Proximate analysis, pseudomonas Eschorichea Coli, staphilocolus aureus

1 INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Lemon grass (*Cymbopogan Citratus*) is aromatic perennial tall grass with rhizomes and densely tufted fibrous root. From afar, the plant looks like a huge, large tuft of grass with slender and stiff stems. As its name suggests, lemon grass has a strong alluring, citrusy or lemon fragrance with grassy note and a hint of ginger-like spices. The plant is a native herb from India and is cultivated in other tropical and subtropical countries (Figuerinha *et al*, 2008, Mehraban *et al*, 2005).

The extracted oil of *Cymbopogon Citratus* is already in use by the pharmaceutical industries as a source of new phytochemical molecule for the development of new drugs. Previous studies on the leaf extracted oil of lemon grass revealed, antitityrosinase and antioxidant activities in human cell (Saeio *et al*, 2011), anti-inflammatory in rats (Gebou *et al*, 2013), anti-carcinogenic effect and cholesterol reduction in human (Costa *et al*, 2011, Bidinotto *et al*, 2012)
Lemon grass is of west Indian origin and yields an essential oil with high content of citral (>70%) (Paranagama, 2003, Handique and Singh, 1990).

Previous studies on the leaf extracted oil of lemon grass revealed, antitityrosinase and antioxidant activities in human cell (Saeio *et al*, 2011), anti-inflammatory in rats (Gebou *et al*, 2013), anti-carcinogenic effect and cholesterol reduction in human (Costa*et al*, 2011, Bidinotto*et al*, 2012)

Furthermore, many studies have reported the antimicrobial activity of lemon grass oil originating from different parts of the globe against adverse range of micro-organism comprising Gram positive and Gram negative bacteria, yeast and fungi, namely *Bacillus cereus, bacillus subtilis, Escherichia Coli, kolebsiella pneumonia*(Naik *et al*, 2010).

2 MATERIALS AND METHODS

2.2 MATERIAL

The leaves of *cymbopogon citratus* were collected from Oko, Orumba north, local government area, Anambra state, Nigeria.

The samples were sorted, washed thorough by with distilled water to remove dirt and debris cut into smaller places before these were shade dried for 3weeks at room temperature (28±3^{o}c). The dried leaves were pulverized into fine powder with electric blender. The powdered materials were stored in air tight polythene bag protected from direct sunlight until required for use. The plant samples were crushed and blended into smaller pieces to enhance the penetration of the extracting solvent into the plant cells, thus facilitating the release of the active principles. The cold maceration method as described by Ewansiha *et al* (2012) was used.

Preparation of the extracts

300g of powered leaves of *cymbopogon citratus* were soaked in 500ml of 95% ethanol and water (aqueous) for four days respectively. The extracts were filtered and the filtrates were concentrated by rotary evaporation to form ethanolic and water (aqueous) extracts respectively.

PROXIMATE ANALYSIS OF LEMON GRASS

Moisture content

The moisture content was determined using the AOAC, (2002) method no. 945.38. 2g of the sample was weighed into a clean dry and pre weighed crucible. The sample and crucible were dried in a moisture extraction oven for 4 hours at 110^{o}C. The sample was allowed to cool in a desiccator and reweighed. Then it was kept in the oven until a constant weight is obtained.

The crude fat determination

Crude fat determination was carried out using the (AOAC, 2002) method no. 920.39A. 5g of the air dried ground sample was weighed into a filter paper (Whatman No 1) and carefully wrapped and was put into the sample holder of the soxhlet apparatus. A clean dried 2250ml soxhlet extraction flask was weighed and half filled with 150ml of n-hexane, then the whole extraction apparatus was assembled together and the flask placed of the heating mantle and heated at 60^{o}C for 3 hours. Then the flask was disconnected.

Crude fiber determination

The (AOAC, 2002) method no. 942.05 was used. 2g of the deflated sample was weighed into a 250ml beaker containing 200ml of 0.125M H_2SO_4. The mixture was heated in a steam bath for 2 hours at 70^{o}C and allowed to cool. The cooled mixture was filtered using a muslin cloth over Buckner funnel. The residue left was washed 3 times with hot water to remove the acid then it was transferred into a beaker 200ml of KOH solution. The mixture was again heated in a steam bath for 2 hours and allowed to cool. The cooled solution was filtered and the residue washed 3 times with hot water. The final residue obtained was transferred into a clean, dried and pre weighed crucible and dried in oven at 120^{o}C to a constant weight. Then sit was transferred to muffle furnace with a tong and ashed at 550^{o}c until it becomes ash-white.

Crude protein determination

The (AOAC, 2002) method no. 955.04C called the Kjedahl method was used. This method involves 3 steps, digestion, distillation and titration. 0.5g of the ground sample was weighed into was clean dried kjedahl flask for digestion, 0.5g $CuSO_4$ crystal, 0.5g of Na_2S0_4 crystal and 35ml of concentrated H_2SO_4 were added to the flask with anti-bumping granules. Then the Kjedahl flask was transferred into fume cupboard for digestion. The digestion process was continued with constant rotation of flask until the sample colour changes from black to a light blue or colourless colour.

Distillation process of the sample was performed using 0.1M NaOH solution, the reaction that took place gives out Ammonia which is absorbed in 0.1N H_2SO_4 acid.

% Carbohydrate determination

The carbohydrate content is the difference between the summation of (% moisture, fat, fiber and protein) and 100.

PHYTOCHEMICAL ANALYSIS OF THE EXTRACTS

Phytochemical analysis was performed to screen the extracts for the pressure of the following active principles: Tannins, flavonoids, volatile oils, phenol, glycosides, saponnins, alkaloids and terpenoids. All procedures were described by Ewansiha, (2012).

Test for Tannins

Zero point five milliliters (0.5mls) of each extract was added to 10.0ml of distilled water and mixed with few drops of ferric chloride ($FeCl_3$) solution. An immediate visible green precipitate is indicative of positive test (Ewansiha *et al*, 2012).

Test for flavonoids

One point zero milliliters (1.0mls) of each extract was dissolved in sodium hydroxide (NaOH) solution. The appearance of yellow solution which disappeared on addition of HCl, indicates the presence of flavonoid (Ewansiha *et al*, 2012).

Test for volatile oils

Volatile oils are characterized by their odor, oil-like appearance and ability to volatilize at room temperature. The plant materials were distilled with water by stream distillation and the distillates were collected in a graduated tube. The aqueous portion which separate automatically was returned to the distillation flask. The formation of emulsion which floats on top of the aqueous phase owing to its low density is indicative of the presence of volatile oil (Ewansiha *et al*, 2012).

Test for Carbohydrates

Three grams of powered samples each of cymbopogon citratus leaf were boiled separately in 50ml of distilled water on a water bath for 3 minutes. The mixtures were filtered while hot and the resulting filtrate was allowed to cool. A few drops of mohischis reagent was added to concentrated sulphemic acid was added and allowed to from a lower layer. A purple ring at the interface of the liquid will indicated the presence of carbohydrates. The mixtures were shaken, allowed to stand for two minutes and then dilute with 5ml of water. A purple precipitate also indicated the presence of carbohydrates. (Ewansiha *et al*, 2012).

Test for Glycosides

The test for glycosides was carried out with "borntrager's test method". 2ml of hydro lysate was added to 3ml of chloroform layer was separated then 3ml of 10% ammonia solution was

added into the mixture. A light pink color which indicate the presence of glycoside was observed.

Test for Saponnins

5ml of the sample extract was diluted with distilled water upto 20ml. the suspension formed is shaken in a graduated cylinder. A 2cm layer of foam is formed which indicates the presence of saponnins.

Test for alkaloids

The test for alkaloid was performed using the "Wagner's test method". 3 drops of the Wagner's reagent was pipetted into 3ml of the sample extract. A reddish brown coloration appeared which indicates a positive test for alkaloids.

Test for terpenoid

5ml of the extract was added to 2ml of chloroform and 2ml of H_2SO_4 was carefully added to it to form a layer, a reddish brown coloration of the interface shows the presence of terpenoid.

Preparation of extracts

300g of powered leaves of cymbopogon citratus were soaked in 500ml of 95% ethanol and water (aqueous) for four days respectively. The extracts were filtered and the filtrates were concentrated by rotary evaporation to form ethanolic and water (aqueous) extracts respectively.

DETERMINATION OF ANTIBACTERIAL ACTIVITY

Test organisms

The microorganisms used were gram positive bacterial staphylococcus aureus and gram negative bacterial Escherichia coli, pseudomonas aeruginosa and salmonella typhi. They were obtained from Glanson Laboratory at Awka and from NAFDAC at Agulu all in Anambra State, Nigeria.

Procedure

Antibacterial assay

The antibacterial activity was performed by disc diffusion method. The bacterial strains were grown in nutrients broth. Nutrients agar was use for the antibacterial test. The 24 hours broth culture of the test organisms were serially diluted to 10^{-3} dilution. Sterile swab sticks was used to inoculate the diluted test organisms on surface of the already prepared nutrients agar plate. Sterile paper disc of about 0.5cm in diameter was soaked with the extract and allowed to dry. This was then placed on the surface of the inoculated plate. The plates were then incubated at about 37^{o}C for 24 hours. After incubation, the diameter zones of inhibition were measured using meter rule.

3 RESULTS

The result of the proximate analysis and phytochemical analysis of *cymbopogon citratus* leaf are shown in the tables below

Table 1: Nutrient composition of *cymbopogon citratus*

NUTRIENT	COMPOSITION (%)
Moisture	4.20
Fat	26
Ash	1.3
Fiber	39.25

Protein	23.6
Carbohydrate	5.65

Table 2: Phytochemical analysis of *cymbopogon citratus*

PHYTOCHEMICAL		RESULT
Glycosides	+	
Alkaloid	+	
Saponnins	+	
Terpenoid	+	
Flavonoid		+
Tannin		+
Alkaloids		+
Volatile oil		+
Phenol		+

The result of bactericidal activities of ethanol and aqueous extract of cymbopogon citratus leaf is shown in the table below

Table 3: The result of the bactericidal activity of ethanolic and aqueous extracts of *cymbopogon citratus.*

TEST ORGANISM	DIAMETER ZONE OF INHIBITION (MM)	
	WATER EXTRACT	ETHANOLEXTRACT
Escharicgia Coli	_	_
Staphylococcus Aurues	_	_
Salmonella Typhii	_	19
Pseudomonas	_	17

Key – no inhibition.

4 DISCUSSION

The result of proximate analysis (Table 1) showed the low moisture content (4.20%)of *cymbopogon citratus* is desirable as it will prevent microbial attacks and allow for high storage capacity. The carbohydrate content is low (5.65%) compared to the carbohydrate content of the work of Asaolu *et al* whose report was recorded as 55.00%. the crude fiber content (39.25) of *cymbopogon citratus* on the other hand is higher than the report for other leaves (Tiwari *et al*, 2010). This makes *cymbopogon citratus* to be a good source of crude fiber than other conventional leaves. The ash content on *cymbopogon citratus* to be 1.3% which is low compared to work of Asaolu *et al*, 2013 (20.30%). The protein content is (23.6%) which is very highand compares favourably with those obtained by Asaolu *et al*, (2013) for *cymbopogon citratus*.

The result of phytochemical analysis (Table 2) showed that flavonoid, tannins, saponins, terpenoid, cardiac glycoside and volatile oil were present in ethanol and aqueous extracts of *cymbopogon citratus.*

The presence of volatile oil, also called essential oil and according to International Union of pure and applied chemistry (1995), the presence of a volatile oil gives plant their specific aromas which was confirmed by the aroma produced by this plant and are extracted by solvent extraction.

The presence of volatile oil also confirmed the report of Seenirasan *et al.,* (2006) of the application of *cymbopogon citratus* in perfumery, cosmetic and soap industry.

According to Kolodziej and Kiderlen (2005), tannin and phenolic compound have been found to inhibit bacterial and fungal growth and also capable of protecting certain plants against infection. According to the report of plant database, (2008), that phytochemical compounds has bactericidal properties were confirmed in this study.

The presence of tannins in the *cymbopogon citratus* extracts agrees with the report of IUPAC, (1995), that tannins are important in herbal medicine and they are applied in arresting bleeding and wound healing.

The presence of alkaloid in the *cymbopogon citratus* may be responsible for the anti-malaria property of the plant as was reported by Mirghani *et al.,* (2012).

Saponins appear to inhibit or kill cancer cells. They may also be able to do it without killing normal cell. Ambrosy and Andrew, (2014). It is likely that the presence of saponins maybe responsible for the anti-cancer activity of *cymbopogon citratus* as was reported by Finkel, (2003).

Terpenoid increase the potential of cannabis based medicinal extracts to treat pain, inflammation, fungal etc. Ambrosy and Andrew (2014). The presence of terpenoid may be responsible for the anti-inflammatory activity of *cymbopogon citratus* as reported by Gebuo *et al.,* (2013).

The beneficial medicinal uses of cardiac glycoside are as treatment for congestive heart and cardiac arrhythmias, however their relative toxicity prevent them from being widely used. Ambrosy and Andrew, (2014).

Ethanolextract of *cymbopogon citratus* showed bactericidal activity (Table 3), in this research work on salmonella and pseudomonas. The result is different from the result obtained by Kolodziej and Kiderlen, (2005) who reported that methanol extract which has similar property with ethanol did not show inhibition on salmonella. The inhibitory activity of *cymbopogon citratus* extract on test organism indicates that the plant possess active ingredients which may be ethanol soluble. Actually, the inhibition of the plant extract on test organisms was low compared to the inhibition shown by chloramphenicol used as control.

5 CONCLUSION

The result of the study on *cymbopogon citratus* have led to the following conclusions: The extract of *cymbopogon citratus* leaf (Ethanol and Water) possessed intermediate bactericidal activity against *salmonella and pseudomonas. Escherichia Coli* and *staphilococus aureus* were markedly resistant to *cymbopogon citratus* aqueous and ethanolic extracts. However, *cymbopogon citratus* has low bactericidal activity on test organisms as compared to the control (chloramphenicol).

The phytochemical analysis conducted in this research revealed that there are active ingredients in *cymbopogon citratus* such as flavonoids, tannins, volatile oils, saponnins, terpenoids, and cardiac glycosides while the proximate analysis represented content of crude protein (23.6%), ash (1.3%), crude fat (26.00%), crude fiber (39.25%), carbohydrate (5.65%) and moisture (4.20%) respectively.

5.1 RECOMMENDATIONS

Further research should be carried out on the plant (lemon grass) from the leaf to the root to know or determine the active compounds that might be present in them.

The use of lemon grass leaf by the scientist, doctors, pharmacologist etc. for the production or synthesis of modern drugs should be encouraged since not everyone has affinity for herbal drugs.

Finally the knowledge of this economic and medicinal plant should be made known to everybody both poor and the rich, learned and illiterates among the populace.

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