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Phytochemical analysis and Antioxidant Evaluation of Lemon Grass (*Cymbopogon citratus* DC.) Stapf Leaves

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ABSTRACT: *Cymbopogon citratus* commonly called lemon grass is claimed to possess diverse medicinal value among different cultures. The present study determined the phytochemicals and evaluated the antioxidant potential of *Cymbopogon citratus* leaves. The phytochemical and proximate analysis of the powdered leaves were carried out using standard methods. The antioxidant activity of the crude methanol extract and its fractions (n-hexane, ethyl acetate and chloroform) was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays. The total phenolic and flavonoid contents were assessed using the Folin-Ciocalteu and aluminium chloride colorimetric methods, respectively. The phytochemical analysis revealed the presence of carbohydrates, reducing sugars, saponins, tannins, flavonoids and other phenolics compounds. The moisture, ash, fat, crude fibre, crude protein, water soluble ash and acid insoluble ash contents were 13.00%, 7.63%, 2.44%, 29.40%, 4.45%, 6.13% and 4.00%, respectively. Among the extract and fractions tested, the ethyl acetate fraction exhibited the highest antioxidant activity. The ethyl acetate fraction also had the highest phenolic and flavonoid contents. There was a strong relationship between the polyphenolic content and antioxidant activity of the extract and fractions with a coefficient of determination (r^2) of 0.889 and 0.920 for total phenols and total flavonoids, respectively. The present study showed that the leaves of *Cymbopogon citratus* especially the ethyl acetate fraction possess good antioxidant activity and could serve as potentially source of natural antioxidants.

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The benefits of phytomedicines cannot be overemphasized and at present, medicinal plants occupy a key position in plant research and medicine (Roger *et al.,* 2015). The medicinal value of plant lies in the bioactive phytochemical constituents of the plant (Sheikh *et al.,* 2013). The most important of these bioactive phytochemicals are alkaloids, tannins, flavonoids, phenolics (Edeoga *et al.,* 2005), phlobatannins, saponins and cardiac glycosides (Adegbegi *et al.,* 2012). Recently, there has been an upsurge of interest in the therapeutic potentials of medicinal plants and some fruits as antioxidants in reducing free radical induced tissue injury. Free radicals are chemical compounds which contain an unpaired electron spinning on the peripheral layer around the nucleus. The family of free radicals generated from oxygen are called reactive oxygen species (ROS) and those generated from nitrogen are called reactive nitrogen species (RNS) (Imieje and Falodun, 2013). Free radicals such as the reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated through endogenous processes such as metabolism, respiration and phagocytosis. They are also generated by exogenous systems such as pesticides, some pollutants, organic solvents as well as radiation (Erharuyi and Falodun, 2012). They are chemically aggressive molecules which react with different types of macromolecules in the body to cause damage to vital cell constituents such as DNA, proteins and lipids. ROS include free radicals such as superoxide anion radicals (O_2^-) and hydroxyl radicals ($OH^\bullet$), as well as non-free radicals such as hydrogen peroxide (H_2O_2) and singlet oxygen (Imieje and Falodun, 2013). Despite the harmful effects of free radicals, ROS and RNS play an important role in the physiological functions of our body when present in moderate or low concentrations such as in the signal transduction pathway, smooth muscle relaxation, defense against infectious agents and cell growth. However, accumulation of free radicals in our body causes a phenomenon called oxidative stress which is defined as imbalance between oxidants and antioxidants in favour of oxidants, potentially leading to cellular damage (Phang *et al.,* 2011). The generation of these free radicals is normally balanced by an equivalent production of antioxidants, through our natural antioxidant defense mechanism which are enzymatic antioxidants (superoxide dismutase,

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glutathione peroxidase, quinone reductase and catalases) and the non-enzymatic antioxidant (ascorbic acid, α-tocopherol, melatonin, β-carotene) obtained from the diet (Erharuyi and Falodun, 2012). *Cymbopogon citratus,* commonly known as lemon grass, is a tropical perennial herb belonging to the family Poaceae (true grasses) (Uraku *et al.,* 2015). It is an aromatic perennial tall grass with rhizomes and densely tufted fibrous root and it is used in folk medicine in the treatment of nervous and gastrointestinal disturbances, fever and hypertension (Adegbegi *et al.,* 2012). The present study seek to evaluate the antioxidant potential of this plant.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials: Fresh *Cymbopogon citratus* leaves were collected in June 2016 from Ekosodin Community, Benin City, Nigeria. The leaves were identified and authenticated by Dr. Akinnibosun H. Adewale of the Department of Plant Biology and Biotechnology, University of Benin where a voucher specimen was prepared and herbarium specimen number UBH_c 0287 was deposited. The leaves were air dried and powdered. The crude powdered sample was stored in an air-tight container until ready for use.

Phytochemical and Proximate analyses: The phytochemical screening and proximate analysis of the powdered leaves were done according to standard procedures (African Pharmacopoeia 1986; AOAC, 1984; Sofowora, 1982; Evans, 2002).

Extraction and fractionation: The powdered plant material (750 g) was extracted with methanol (4.25 L) by maceration at room temperature for 7 days. The extract was concentrated to dryness using a rotary evaporator at reduced pressure. The crude methanol extract (17.71 g) was dissolved in aqueous methanol (4:1, 100 mL). The solution was extracted successively with n-hexane (600 mL), chloroform (600 mL) and ethyl acetate (500 mL). The various fractions were concentrated to dryness and stored in the refrigerator until use.

DPPH Radical Scavenging Assay: The radical scavenging activity of the crude methanol extract and fractions was evaluated using method previously described with slight modification (Jain *et al.,* 2008). A solution of 0.2 mM DPPH in methanol was prepared, and 1.0 mL of this solution was mixed with 3.0 mL of extract in methanol containing 0.001 – 0.2 µg/mL of the extract. The reaction mixture was mixed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as reference standard. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(A_0 - A_1)}{A_0} \text{ x } 100$$

Where: A_0 = Absorbance of DPPH radical in methanol, A_1 = Absorbance of DPPH radical + sample extract/standard.

The 50% inhibitory concentration (IC_{50}) was determined by making a logarithmic plot of the concentration-response relationship and the IC_{50} calculated from the equation of the plot.

Ferric Reducing Antioxidant Power (FRAP) Assay: The FRAP assay was done according to method previously described by Benzie and Strain (1996) with some modifications. The fresh working FRAP solution was prepared by mixing 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM Tripyridyltriazine (TPTZ) solution in 40 mM HCl, and 2.5 mL of 20 mM ferric chloride hexahydrate ($FeCl_3.6H_2O$) solution and then warmed at 37°C before using. The extract (1.5 mL of 1 mg/mL) was mixed with 2.8 mL of the FRAP solution, and incubated at room temperature for 30 min in the dark. Readings of the coloured product (ferrous tripyridyltriazine complex) was then taken at 593 nm. The calibration curve was constructed using ferrous sulphate heptahydrate ($FeSO_4.7H_2O$), at concentrations of 0.01, 0.02, 0.04, 0.08, 0.1, 0.12 and 0.15 mM, and the absorbance values were measured as for sample solutions. Results were expressed as millimolar of ferrous sulphate equivalent per gram of extract (mM FSE/g extract).

Total Phenol: Total phenol content in the extracts was determined by the method previously described by Kim *et al.* (2003). Exactly 0.5 mL of extract solution (1 mg/mL) was added to 4.5 mL of distilled water, and 0.5 mL of Folin–Ciocalteu's reagent (previously diluted with water 1:10, v/v) was added to the solution. After mixing, the tubes were maintained at room temperature for 5 min followed by the addition of 5 mL of 7% sodium carbonate and 2 mL of distilled water.

After shaking, the resultant mixtures were incubated at room temperature for 90 min. The absorbance was measured at 750 nm. The total phenolic content was expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract). The standard curve was prepared using gallic acid in six different concentrations (12.5, 25, 50, 75, 100 and 150 mg/L).

Total Flavonoid: Total flavonoid content was estimated using the method previously described by Ebrahimzadeh *et al.* (2008). Briefly, 0.5 mL of extract sample (1 mg/mL) was mixed with 1.5 mL of methanol and then, 0.1 mL of 10 % aluminium chloride was added, followed by 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 min. The absorbance was measured spectrophotometrically at 415 nm. The results were expressed as milligrams quercetin equivalent per gram of extract (mg QE/g extract). The standard curve was prepared using quercetin in six different concentrations (12.5, 25, 50, 75, 100 and 150 mg/L).

Statistical Analysis: Results were expressed as means ± standard deviation (SD) of three replicates. Where applicable, the data were subjected to one-way analysis of variance (ANOVA) and difference between means were determined by Duncan multiple range test using statistical analysis system (SPSS statistics 17.0). Correlation between polyphenolic content and antioxidant activity was determined by regression analysis at 95% confidence level. P-value < 0.05 was regarded as significant.

RESULTS AND DISCUSSION

Proximate and phytochemical analyses: Proximate analysis is a pharmacopoeia standard for authentication of crude powdered plant material. It refers to the determination of the major constituents of feed and other crude plant materials. It is used to assess if the feed is within its normal compositional parameters or has been adulterated. The present study determined the proximate parameters including; moisture content, ash, alcohol and water extractive values, crude fat, crude fibre, crude protein and nitrogen free extract of the powdered leaf of *Cymbopogon citratus*.

Table 1: Proximate analysis of the leaves of *Cymbopogon citratus*

Parameters	Mean ± SD
Total ash	7.63 ± 0.23
Moisture content	13.00 ± 2.92
Alcohol soluble extractive value	14.50 ± 0.50
Water soluble extractive value	9.67 ± 1.16
Acid insoluble ash	4.00 ± 1.08
Water soluble ash	6.13 ± 0.48
Crude fat	2.44 ± 0.58
Crude fibre	29.40 ± 1.03
Crude protein	4.45 ± 0.13

The results showed that *C. citratus* leaf has moisture (13.00 ± 2.92%), ash (7.63 ± 0.23%), crude protein (4.45 ± 0.13%), crude fat (2.44 ± 0.58%), and crude fiber (29.40 ± 1.03%) (Table 1). The phytochemical constituents of the leaf of *Cymbopogon citratus* are presented in Table 2. The result revealed the presence of tannins, saponins, reducing sugars, flavonoids and other phenolics compounds.

Table 2: Phytochemical Composition of the Leaves of *Cymbopogon citratus*

Phytochemicals	Inference
Alkaloids	–
Carbohydrates	+
Reducing sugars	+
Saponins	+
Tannins	+
Flavonoids	+
Phenolics compounds	+
Phytosterol	–
Proteins	+
Essential oil	+

+: indicates presence of component; –: indicates absence of component

Evaluation of Antioxidant activity: The present study evaluated the antioxidant activity of the crude methanol extract and fractions (n-hexane, chloroform and ethyl acetate) of *Cymbopogon citratus* leaf using the free radical scavenging and ferric reducing antioxidant power assays.

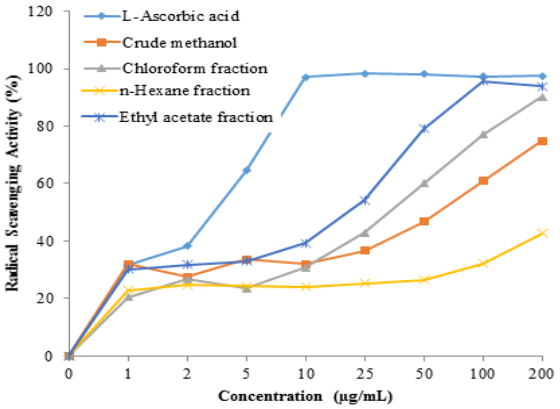

Fig 1: DPPH radical scavenging activity of the extract and fractions of *Cymbopogon citratus* Leaf

Figure 1 shows the scavenging activity of the extract and fractions of the leaf of *Cymbopogon citratus.* The 50% inhibitory concentrations (IC_{50}) for the extract and fractions as well as ascorbic acid are shown in Table 3 while the ferric reducing antioxidant power are presented in Table 4 [results were expressed as millimolar of ferrous sulphate equivalent per gram of extract (mM FSE/g extract) from ferrous sulphate calibration curve (Figure 2)]. Natural antioxidants present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. The stable DPPH radical has been widely used to assess the ability of compounds to act as free radical scavengers or hydrogen donors and thus to evaluate the antioxidant activity.

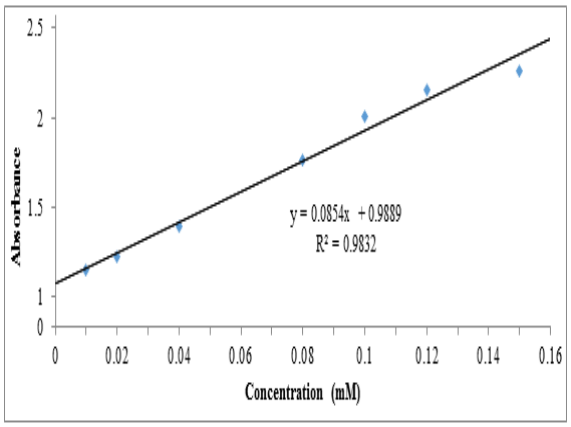

Fig 2: Ferrous sulphate calibration curve

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical is a stable nitrogen radical with a deep purple colour. It can readily undergo reduction by an antioxidant and the degree of discolouration indicates the scavenging potentials of the antioxidant (Yamaguchi *et al*., 1998; Jayaprakasha *et al*., 2004). Due to the ease and convenience of this reaction, the DPPH radical scavenging assay is widely used as a measure of antioxidant activity (Eyob *et al*., 2008). In the present investigation, the extract and fractions exhibited appreciably high and concentration-dependent radical scavenging activity except the n-hexane fraction which showed low radical scavenging activity (Figure 1). The ethyl acetate fraction exhibited the highest activity (IC_{50} = 4.53 ± 0.71 µg/mL) compared to the crude extract and other fractions. This was followed by the chloroform fraction (IC_{50} 5.3 ± 1.01 µg/mL), the methanol extract (IC_{50} 6.65 ± 0.11 µg/mL) and the n-hexane fraction (IC_{50} 12.6 ± 1.40 µg/mL) (Table 3).

Table 3: IC_{50} values of extract and fractions of *Cymbopogon citratus* and ascorbic acid

Samples	IC_{50}Value (µg/mL)
L-ascorbic acid	3.44 ± 0.03
Methanol extract	6.65 ± 0.11
N-hexane fraction	12.60 ± 1.40
Chloroform fraction	5.30 ± 1.01
Ethyl acetate fraction	4.53 ± 0.71

FRAP is a simple direct test for measuring antioxidant capacity. This method was initially developed to assay plasma antioxidant capacity, but now also used with plant extracts (Gourine *et al*., 2010). FRAP assay measures the reducing ability of antioxidants against oxidative effects of reactive oxygen species. The antioxidative activity is estimated by measuring the increase in absorbance caused by the formation of ferrous ions from FRAP reagent (containing TPTZ (2,4,6-Tri (2-pyridyl)-s-triazine) and $FeCl_3.6H_2O$). The ferric (Fe (III)-TPTZ) complex undergoes reduction to a coloured ferrous (Fe) II-TPTZ) complex at low pH). Increasing absorbance indicates an increase in the reductive ability. The absorbance is measured spectrophotometrically at 593 nm (Benzie and Strain, 1996). In the present study, n-hexane, chloroform, ethyl acetate fractions and crude methanol extract of *Cymbopogon citratus* exhibited promising antioxidant power with FRAP values of 157.55, 195.32, 212.02, and 243.91 mM Ferrous sulphate equivalent per gram of extract for n-hexane fraction, chloroform fraction, crude methanol extract and ethyl acetate fraction, respectively (Table 4). The result obtained from this assay suggest that extract of *Cymbopogon citratus* may play a protective role against oxidative damage by sequestering Fe^{2+} ions.

Table 4: FRAP values of the Extract/Fractions of *Cymbopogon citatus* leaf

Extract/Fraction	FRAP Values
Methanol extract	212.02
N-hexane fraction	157.55
Chloroform fraction	195.32
Ethyl acetate fraction	243.91

Polyphenolic content: Plant phenolic compounds are natural antioxidants and have the potential to donate hydrogen atoms to the radical and make them stable (Goupy *et al*., 2003). Their antioxidant ability has been attributed to the presence of hydroxyl substituents and their aromatic structure, which enables them to scavenge free radicals (Kefalas *et al*., 2003). Figure 3 shows the phenolic content of the crude methanol extract of *C. citratus* and its fractions.

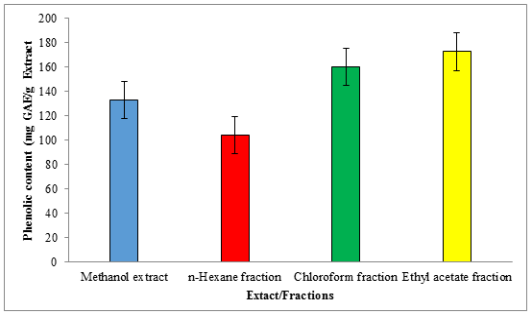

Fig 3: Total phenolic content of the extract and fractions of *Cymbopogon citratus* leaf

The ethyl acetate fraction had the highest phenolic content (172.5 mgGAE/g extract) among the extract and fractions. This was followed by the chloroform fraction (160.0 mg GAE/g extract), methanol extract (132.5 mg GAE/g extract) and n-hexane fraction (104.0 mg GAE/g extract). The total flavonoids contents were also evaluated, and the results revealed high content of flavonoids. The ethyl acetate fraction also had the highest flavonoid content (192.6 mgQE/g Extract), followed by the chloroform fraction (153.0 mgQE/g Extract), crude methanol extract (143.0 mgQE/g Extract) and n-hexane fraction (80.2 mgQE/g Extract) (Figure 4).

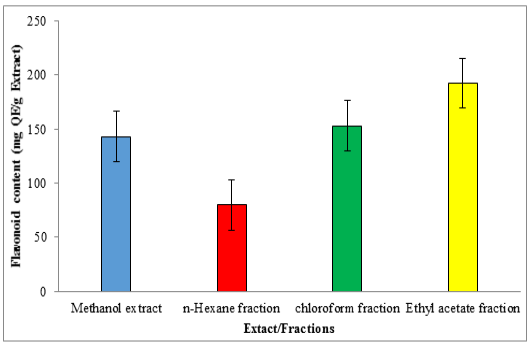

Fig 4: Total flavonoid content of the extract and fractions of *Cymbopogon citratus* leaf

Flavonoids are one of the most important natural products that impart antioxidant potential and other medicinal properties to plants due to their phenolic hydroxyl groups. They can delay or inhibit oxidation process of lipid by inhibiting the initiation of oxidative chain reaction. They also chelate ions and often decreasing the metal ion pro-oxidant activity (Mira *et al*., 2002). The higher value of the phenolic and flavonoid contents in the ethyl acetate fraction compared to other fractions may be due to the polar nature of these components. Phenolic compounds are generally polar and solvents appears to play a significant role in their extraction so that polar solvents tend to contain more of these components compared to the less polar or non-polar solvents. Several studies have reported a positive correlation of the antioxidant activity of medicinal plants and their phenolic content (Parr and Bolwell, 2000; Amarowicz *et al*., 2004; Sathishsekar and Subramanian, 2005; Elmastas *et al*., 2007; Maizura *et al*., 2011). In the present study, a positive correlation was found between the polyphenolic content and antioxidant activity of the leaves of *Cympobogon citratus* with Pearson correlation coefficient (r) of 0.942 and 0.959 for total phenol and total flavonoid content, respectively (Figure 5). This observation shows that the antioxidant activity of *Cymbopogon citratus* may be attributed to their phenolic and flavonoid contents.

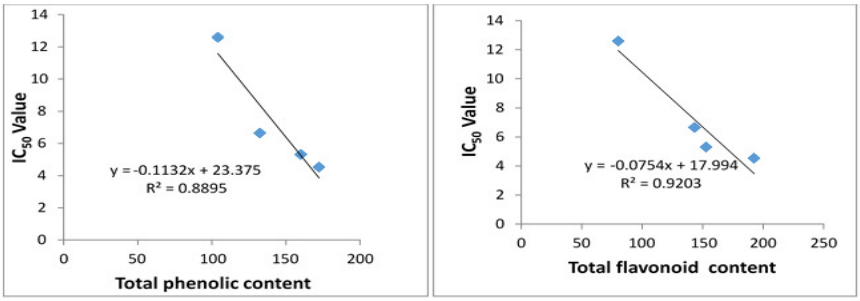

Fig 5: Correlation between total phenolic and total flavonoid contents and DPPH scavenging activity (IC_{50}) in extract and fractions of *Cymbopogon citratus* Leaf

Conclusion: The results of this study have shown that the leaves of *Cymbopogon citratus* have antioxidant activity with the ethyl acetate fraction being the most active. The study has also shown that the leaves of *Cymbopogon citratus* contains high content of polyphenolic compounds and that its antioxidant activity may be attributed to the presence of these constituents.

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