

Phytochemical and Antioxidants Screening of *Chrysophyllum albidum*, *Mezoneuron benthamianum*, *Phyllanthus muellerianus* And *Acalypha fimbriata*

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Abstract: Qualitative and quantitative phytochemical screening of *Chrysophyllum albidum*, *Mezoneuron benthamianum*, *Phyllanthus muellerianus* and *Acalypha fimbriata*. All the four plants contained alkaloids, tannin and flavonoids. Steroids and anthraquinone were present in *C. albidum*, *M. benthamianum*, *A. fimbriata*. Saponin was present in *C. albidum*, *M. benthamianum* and *P. muellerianus*. Terpenoids were found in *C. albidum* and *A. fimbriata*. Cardiac glycosides was present in *C. albidum*, *P. muellerianus* and *A. fimbriata*. The antioxidant activities of the plants were evaluated using DPPH free radical scavenging activity, Nitric oxide scavenging activity and Reducing power activity. *Chrysophyllum albidum* had the lowest calculated IC₅₀ for DPPH and NO assessment, 0.913 mg/ml and 117.818 µg/ml respectively. *C. albidum* also showed the highest value of total antioxidant capacity, 70.36. *Acalypha fimbriata* had the lowest IC₅₀ for Reducing power potential, 11.007 µg/ml but a total antioxidant capacity of 48.9. The Iron II chelation ability of the plants were dose dependent with *Acalypha fimbriata* showing the closest potential to the standard EDTA and *Chrysophyllum albidum* showing the least potential. The research has shown that the phytoconstituents and the antioxidant properties of these medicinal plants would be responsible for the therapeutic claims of the plants.

Keywords: Phytochemical, Antioxidant, Chelation

Introduction

Medicinal plants are of great importance in drug development and humans have used them for different diseases from the beginning of human history (Rahman et al, 2017). The medicinal power of plants lies in phytochemical constituents that cause definite pharmacological actions on the human body (Akinmoladun et al, 2007). It is believed that crude extract from medicinal plants are more biologically active than isolated compounds due to their synergistic effects (Jana and Shekhawat, 2010). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Doss, 2009). Majority of phytochemicals have been known to bear valuable therapeutic activities such as insecticides (Kambu et al, 1982), antibacterial, antifungal (Lemos et al, 1990), anticonstipative (Ferdous et al, 1992), spasmolytic (Sontos et al 1998), antiplasmodial (Benotivical et al, 2001) and antioxidant activities (Kaur and Mondal, 2014).

A number of studies have focused on the biological activities of phenolic compounds which are potential antioxidants and free radical scavengers (Rice-Evans et al, 1995; Cespedes et al, 2008; Reddy et al, 2008; Chanda and Dave, 2009). A free radical is defined as any atom or molecule possessing unpaired electrons. It can be formed in living organisms by both endogenously (respiration, peroxisomes stimulation of polymorphonuclear leucocytes and macrophages) and exogenously (ionizing radiation, tobacco smoke, pollutants, pesticides and organic solvents) (Irshad and Chaudhuri, 2002). These free radicals are produced by our body to stabilize the body's natural function. The excess amount of free radical could cause oxidative cell and tissue damage (Sen et al, 2010). It can also cause oxidative damage to proteins, lipids and DNA and chronic diseases such as cancer, diabetes, aging and other degenerative diseases in humans (Aiyegoro and Okoh, 2010). Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and wellbeing (Van et al, 2000).

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Iron overload is the excess iron in the body. The body has limited capacity to excrete excess iron. Though iron deficiency can cause anemia, its excess in the body increases the body susceptibility to infection and promotes free radical tissue damage. Iron overload impairs host immune defence mechanism, promotes replication and growth of pathogens. Thus removal of excess iron during infection in iron overload host may be beneficial by restoring the body immunity and denying the pathogen of excess iron for its replication and growth (Cronje and Bornman, 2005).

Chrysophyllum albidum, African star apple, belongs to the plant family Sapotaceae. The plant is a lowland rain forest tree species that grows up to 25 to 37 m in height at maturity with a girth varying from 1.5 to 2 m. The seeds of the plant could remove metal ions from aqueous solution (Oboh et al, 2009). The plant extracts possess hepatoprotective activity (Adebayo et al, 2011). The ethanol root bark extract showed anti-fertility activity (Onyeka et al, 2012). The roots, barks and leaves of *C. albidum* is/are widely used as an application to sprains, bruises and wounds in southern Nigeria (Olorunnisola et al, 2008; Mac Donald et al, 2014). The bark is used for the treatment of yellow fever and malaria. The root and stem barks are used in urinary related infections (Florence and Adiaha, 2015). The leaf is used as an emollient and for the treatment of skin eruption, stomachache and diarrhea (Adisa, 2000) and for cancer remedy in Cuba (MacDonald et al, 2014).

Aqueous extract of *Mezoneuron benthamianum* had significant vasorelaxing, antioxidant and aphrodisiac properties (Zamble et al, 2008). An infusion of the dried roots of the plant is drunk or used as a bath against general malaise. Gallic acid and gallic acid derivatives have been isolated from the leaves of *Mezoneuron benthamianum* (Binutu and Cordell, 2000). A decoction of roots, bark and leaves is used to cure urethral discharge (Schmeizer et al, 2008). Traditionally, *Mezoneuron benthamianum* is used in management of erectile dysfunction, dysentery, urethral discharges, skin diseases and wounds (Akosua et al, 2011). Gallic acid and its methyl ester (methylgallate) inhibit the growth of both Gram-negative and Gram-positive bacteria, but this other gallate derivatives only suppress Gram-positive bacteria (Osho, 2014).

Phyllanthus muellerianus is widely used to treat intestinal troubles. An infusion of the young shoots is taken to treat severe dysentery (Schmelzer et al, 2008). Phthalates compounds have been isolated from *Phyllanthus muellerianus* (Euphorbiaceae) (Saleem et al, 2009). Decoction of *Acalypha*

fimbriata is used as laxatives (Kola et al, 2008). The leaves of *acalypha fimbriata* are used in asthma, rheumatism, syphilis, ulcers and also as antihelmintic, antimicrobial and antifungal in Nigeria (Odugbemi, 2008). The aim of this research is to study the phytochemical constituents and the antioxidant potential of these four medicinal plants of which their anti-mycobacterium tuberculosis had been earlier reported.

MATERIALS AND METHODS

Collection of Plant Samples:

Batches of *Chrysophyllum albidum* fruits, *Phyllanthus muellerianus* and *Acalypha fimbriata* were purchased in Mushin market in Lagos State, while *Mezoneuron benthamianum* plant was obtained from Iberekodo market in Ogun State, Nigeria.

Phytochemical Screening:

Qualitative and quantitative phytochemical screening of the plants was carried out according to Sofoluwe (1993), Ejikeme et al (2014), Boham and Kocipai (1994), Obadoni and Ochuko (2002), Amadi et al (2004), Mahdu et al (2016), Muhammad and Abubakar (2016), Harborne (1993) and AOAC (1990).

Test for alkaloids:

A 5 mg sample of the extract dissolved in 3 ml of acidified ethanol was warmed slightly and then filtered. Few drops of Mayer's reagent and 1 ml of Dragendroff's reagent were added to 1 ml of the filtrate and turbidity was observed.

Test for Saponins:

5 ml of the extract solution was shaken vigorously for a stable persistent froth. The frothing was mixed with olive oil and was shaken vigorously. The formation of emulsion indicated the presence of saponins in the samples.

Test for Tannins:

0.25 g of various solvent extract was dissolved in 10 ml distilled water and filtered. 1% aqueous Iron chloride (FeCl_3) solution was added to the filtrate. The appearance of intense green, purple, blue or black colour indicated the presence of tannins in the test sample.

Test for Phlobatannins:

Two millilitres (2 mL) of the aqueous solution of the extract were added into 1% aqueous hydrochloric acid and was then boiled with the help of Hot plate stirrer. Formation of red colored precipitate confirmed a positive result.

Test for Anthraquinones:

One gram (1 g) of the powdered seed was placed in a dry test tube and 20 mL of chloroform was added. This was heated in steam bath for 5 min. The extract was filtered while hot and allowed to cool. To the filtrate was added with an equal volume of 10% ammonia solution. This was shaken and the upper aqueous layer was observed for bright pink colouration as indicative of the presence of Anthraquinones. .

Test for Terpenoids (Salkowski test):

5 ml of solvent extract was mixed in 2 ml of chloroform and 3 ml of concentrated H₂SO₄ was carefully added. A layer of the reddish brown colouration was formed at the interface thus indicating a positive result for the presence of terpenoids.

Test for Flavonoids:

0.5 g of various extract was shaken with petroleum ether to remove the fatty materials (lipid layer). The defatted residue was dissolved in 20 ml of 80% ethanol and filtered. The filtrate was used for the following tests: (a) 3 ml of the filtrate was mixed with 4 ml of 1% aluminium chloride in methanol. Formation of yellow colour indicated the presence of flavonols, flavones and chalcones. (b) 3 ml of the filtrate was mixed with 4 ml of 1% potassium hydroxide. A dark yellow colour indicated the presence of Flavonoids. (c) 5 ml of the dilute ammonia solution was added to the portion of the aqueous filtrate of each plant extract followed by the addition of concentrated H₂SO₄. The appearance of the yellow colouration indicated the presence of flavonoids.

Test for steroids:

A 5 ml sample of the extract was added to 2 ml acetic anhydride and 2 ml H₂SO₄. The colour change from violet to blue or green in some samples indicated the presence of steroids.

Test for Cardiac glycosides (Keller-Killani test):

1ml of the extracts were dissolved in 1ml of glacial acetic acid and cooled, after cooling, 2-3 drops of ferric chloride was added. To this solution 2ml of conc. sulphuric acid was added carefully along the walls of the test tube. Reddish brown ring was formed at the interface which indicated the presence of deoxysugar of cardenoloides.

Estimation of alkaloids:

200 cm³ of 10% acetic acid in ethanol was added to each plant powder sample (2.50 g) in a 250 cm³ beaker and allowed to stand for 4 hours. This

was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed and the percentage of alkaloid is expressed mathematically as

$$\% \text{ Alkaloid} = \frac{\text{Weight of alkaloid} \times 100}{\text{Weight of sample}}$$

Estimation of flavonoid:

Flavonoid determination was by the method reported by Ejikeme et al. and Boham and Kocipai. 50 cm³ of 80% aqueous methanol was added to 2.50 g of sample in a 250 cm³ beaker, covered, and allowed to stand for 24 hours at room temperature. After discarding the supernatant, the residue was re extracted (three times) with the same volume of ethanol and the solution was filtered through whatman filter paper No 42 (125 mm). Each plant sample filtrate was later transferred into a crucible and evaporated to dryness over a water bath. The content in the crucible was cooled in a desiccator and weighed until constant weight was obtained. The percentage of flavonoid was calculated as

$$\% \text{ Flavonoid} = \frac{\text{Weight of flavonoid} \times 100}{\text{Weight of sample}}$$

Estimation of Saponins:

Saponin quantitative determination was carried out using the method reported by Ejikeme et al. and Obadoni and Ochuko. 100 ml of 20% aqueous ethanol was added to 5 grams of plant powder sample in a 250 cm³ conical flask. The mixture was heated over a hot water bath for 4 hours with continuous stirring at a temperature of 55°C. The mixture was filtered and the residue re-extracted with another 100 ml of 20% ethanol. The combined extract was evaporated to 40 cm³ over water bath at 90°C. 20 cm³ of diethyl ether was added to the concentrate in a 250 cm³ separator funnel and vigorously agitated from which the aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added and was washed twice with 10 ml of 5% aqueous sodium chloride. After discarding the sodium chloride layer, the remaining solution was heated in a water bath. After evaporation the sample were dried in the oven into a constant weight. The saponin content was calculated as a percentage:

$$\% \text{ Saponins} = \frac{\text{Weight of saponins} \times 100}{\text{Weight of sample}}$$

Estimation of Tannins:

Analytical method for quantitative determination of tannin was according to Amadi et al. and Ejikeme et al. By dissolving 50 g of sodium tungstate (Na_2WO_4) in 37 cm^3 of distilled water, Folin-Denis reagent was made. To the reagent prepared above, 10 g of phosphomolybdic acid ($\text{H}_3\text{PMO}_{12}\text{O}_{40}$) and 25 cm^3 of orthophosphoric acid (H_3PO_4) were added. Two-hour reflux of the mixture was carried out, cooled, and diluted to 500 cm^3 with distilled water. One gram of each plant powder in a conical flask was added to 100 cm^3 of distilled water. This was boiled gently for 1 hour on an electric hot plate and filtered using number 42 (125 mm) Whatman filter paper in a 100 cm^3 volumetric flask. Addition of 5.0 cm^3 Folin-Denis reagent and 10 cm^3 of saturated Na_2CO_3 solution into 50 cm^3 of distilled water and 10 cm^3 of diluted extract (aliquot volume) was carried out after being pipetted into a 100 cm^3 conical flask for colour development. The solution was allowed to stand for 30 minutes in a water bath at a temperature of 25°C after thorough agitation. With the aid of a Spectrum Lab 23A spectrophotometer optical density was measured at 700 nm and compared on a standard tannic acid curve. Dissolution of 0.20 g of tannic acid in distilled water and dilution to 200 cm^3 mark ($1 \text{ mg}/\text{cm}^3$) were used to obtain tannic standard curve. Varying concentrations ($0.2\text{--}1.0 \text{ mg}/\text{cm}^3$) of the standard tannic acid solution were pipetted into five different test tubes to which Folin-Denis reagent (5 cm^3) and saturated Na_2CO_3 (10 cm^3) solution were added and made up to the 100 cm^3 mark with distilled water. The solution was left to stand for 30 minutes in a water bath at 25°C . Optical density was ascertained at 700 nm with the aid of a Spectrum Lab 23A spectrophotometer. Optical density (absorbance) versus tannic acid concentration was plotted.

The following formula was used in the calculation:

$$\frac{\text{Tannic acid (mg/100g)}}{\frac{C \times \text{extract volume} \times 100}{\text{Aliquot volume} \times \text{weight of sample}}} =$$

where C is concentration of tannic acid read off the graph

Estimation of Steroids:

1 ml of text extract of steroid solution was transferred into 10 ml volumetric flask. Sulphuric acid (4N, 2 ml) and iron (III) chloride (0.5% w/v, 2 ml) were added followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated on a water bath maintained at $70 \pm 2^\circ \text{C}$ for 30

minutes with occasional shaking and was diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank (Mahdu et al, 2016)

Estimation of Phenols:

The determination of total phenolics based on Folin-Ciocalteu reagent assay. An aliquot (1ml) of extracts and standard solution of Gallic acid (100 mg/ml) was added to 25 ml volumetric flask, containing 9 ml distilled water. The distilled water itself was used as blank. One ml of Folin-Ciocalteu reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added to the mixture. The solution was diluted to volume (25 ml) with distilled water and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 750 nm with an UV-Vis Spectrophotometer. The total phenolic content of root extracts expressed as mg Gallic acid equivalents (GAE)/100 G fresh weights

Estimation of terpenoids:

About 2 g of the plant powder was weighed and soaked in 50 ml of 95 % ethanol for 24 h. The extract was filtered and the filtrate was extracted with petroleum ether (60 to 80°C) and concentrated to dryness. The dried ether extract was treated as total terpenoids (Ferguson, 1956).

Estimation of Cardiac Glycosides (using Muhammad and Abubakar, 2016)

8 ml of plant extract was transferred to 100 ml volumetric flask and 60 ml of H_2O and 12.5 % of lead acetate were added, mixed and filtered. 50 ml of the filtrate was transferred into another 100ml flask and 8 ml of 47 % Na_2HPO_4 were added to precipitate excess Pb^{2+} ion. This was mixed and completed to volume with water. The mixture was filtered twice through same filter paper to remove excess. 10 ml of purified filtrate was transferred into clean Erlenmeyer flask and treated with 10 ml Balfet reagent. A blank titration was carried out using 10 ml distilled water and 10 ml Balfet reagent. This was allowed to stand for one hour for complete colour development. The colour intensity was measured colorimetrically at 495 nm.

Calculation

$$\% \text{ of total glycosides} = \frac{A \times 100 \text{ g} \%}{77}$$

Where A = Absorbance

DPPH radical scavenging assay:

DPPH (2, 2-diphenyl picryl hydrazyl) is a commercially available stable free radical, which is purple in colour. The antioxidant molecules present in the herbal extracts, when incubated, react with

DPPH and convert it into di-phenyl hydrazine, which is yellow in colour. The degree of discoloration of purple to yellow was measured at 520 nm, which is a measure of scavenging potential of plant extracts. 10 µl of plant extract was added to 100 µl of DPPH solution (0.2mM DPPH in methanol) in a microtitre plate. The reaction mixture was incubated at 25 °C for 5 minutes, after that the absorbance was measured at 520 nm. The DPPH with corresponding solvents (without plant material) serves as the control. The methanol with respective plant extracts serves as blank. The DPPH radical scavenging activity of the plant extract was calculated as the percentage inhibition.

NITRIC OXIDE Scavenging Activity:

NO. generated from sodium nitroprusside (SNP) was measured according to the method of Marcocci et al. (1994). Briefly, the reaction mixture (5.0 ml) containing SNP (5 mM) in phosphatebuffered saline (pH 7.3), with or without the plant extract at different concentrations, was incubated at 25°C for 180 min in front of a visible polychromatic light source (25W tungsten lamp). The NO. radical thus generated interacted with oxygen to produce the nitrite ion (NO₂⁻) which was assayed at 30 min intervals by mixing 1.0 ml of incubation mixture with an equal amount of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediaminedihydrochloride). The absorbance of the chromophore (purple azo dye) formed during the diazotisation of nitrite ions with sulphanilamide and subsequent coupling with naphthylethylenediaminedihydrochloride was measured at 546 nm. The nitrite generated in the presence or absence of the plant extract was estimated using a standard curve based on sodium nitrite solutions of known concentrations. Each experiment was carried out at least three times and the data presented as an average of three independent determinations.

Reducing power assay:

The sample extracts of the plants were put in 1 ml of phosphate buffer in a test tube and 5 ml of 0.2 M phosphate buffer, pH 6.6, was added. To this, 5 ml of 1 % potassium ferricyanide solution was added. The mixture was then incubated at 50° C for 20 min. 5 ml of 10 % TCA was added after the incubation and the content was centrifuged at 1000 rpm for 10 min. The upper layer of the supernatant (5 ml) was mixed with 5 ml of distilled water. 1 ml of ferric chloride was then added and vortexed. Then, the absorbance of the reaction mixture was read spectrophotometrically at 700 nm against water blank (Oyaizu, 1986).

Metal Chelating Activity:

The chelating ability of the plants extracts was examined using Dinis et al, 1994. 50 µl of 2 mM FeCl₂ was added to 1 ml of different concentration of the extract (2, 3, 4, 5 mg/ml). The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 minutes. The absorbance of the solution was thereafter measured at 562 nm. The percentage inhibition of ferrozine-Fe²⁺ complex formation was calculated as [(A₀ - A_s)/A_s] x 100, where A₀ was the absorbance of the control and A_s was the absorbance of the extract/standard. Na₂EDTA was used as positive control.

RESULTS AND DISCUSSION:

Evidence from laboratory studies show that phytochemicals have therapeutic effect against some severe disorders. In this study, qualitative and quantitative phytochemical screening of *Chrysophyllum albidum*, *Mezoneuron benthamianum*, *Phyllanthus muellerianus* and *Acalypha fimbriata* were investigated. Table 1 shows the result of the phytochemical screening.

Table 1: Qualitative phytochemical screening.

	<i>Chrysophyllum albidum</i>	<i>Mezoneuron benthamianum</i>	<i>Phyllanthus muellerianus</i>	<i>Acalypha fimbriata</i>
Alkaloids	+	+	+	+
Saponin	+	+	+	-
Tannin	+	+	+	+
Phlobatannin	-	-	-	-
Anthraquinone	+	+	-	+
Flavonoid	+	+	+	+
Steroid	+	+	-	+
Terpenoid	+	-	-	+
Cardiac glycoside	+	-	+	+

Alkaloids, saponin, tannin, anthraquinone, flavonoid, steroid, terpenoid and cardiac glycosides were present in the seed cotyledon of *Chrysophyllum albidum*. The quantitative determination of the phytochemical of the seed cotyledon gave alkaloid 81 %, saponin 48.9 %, terpenoid 14.8 %, cardiac glycoside 12.2 %, flavonoid 12 %, steroid 8.9 % and tannin 0.2 % as shown in Figure 1. Eleagnine, an alkaloid isolated from the plant, had been reported to be antimicrobial (Idowu et al, 2003) and to be antinociceptive, anti-inflammatory and antioxidant (Idowu et al, 2006). The seed of *C. albidum* was shown to be hypoglycemic (lowering blood sugar) and hypolipidemic (lowering cholesterol) by Olorunnisola et al. The hypoglycemic and the hypolipidemic effect could be due to the presence of saponin in the plant since saponin had been reported to be useful in treating hyperglycaemia (Malinow et al, 1977). The property could also be due to the presence of glycosidic flavonoid. A glycosidic flavonoid, Myricetin rhamnoside, identified in the leaves of the plant had been reported to exhibit an excellent radical scavenging activity by Adebayo et al.

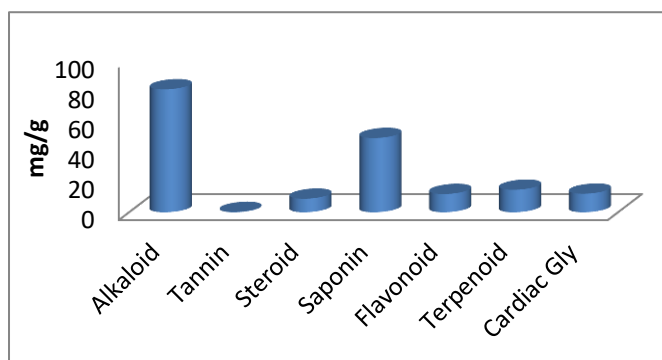


Figure 1: Quantitative phytochemical analysis of the seed cotyledon of *Chrysophyllum albidum*.

The qualitative phytochemical screening of the leaves of *Mezoneuron benthamianum* showed the presence of alkaloid, saponin, tannin, anthraquinone, flavonoids and steroids. The result of the quantitative determination as shown in Figure 2 shows tannin to be most abundant. The phytochemicals in this plant would be responsible for the reported antiplasmodial activity (Jansen et al, 2017), antibacterial and antioxidant ((Akosua et al, 2007), analgesic, antipyretic and anti-inflammatory (Mbagwu et al, 2007), antidiarrhoeal (Mbagwu and Adeyemi, 2008), anticandidal and antioxidant potential (Fayemi et al, 2012).

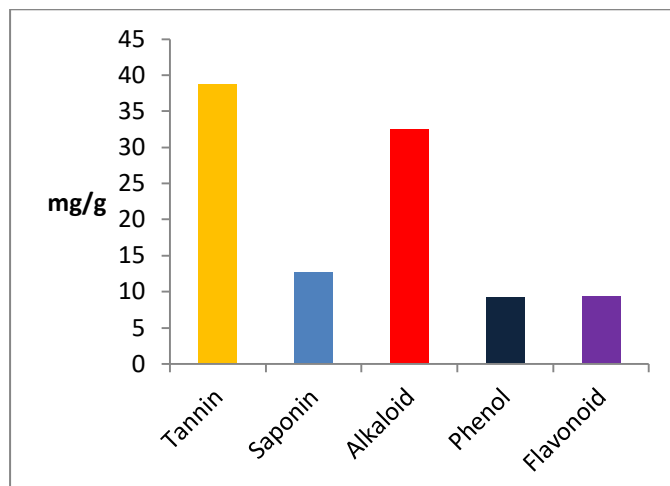


Figure 2: Quantitative phytochemical analysis of the leaves of *Mezoneuron benthamianum*.

The leaves of *Phyllanthus muellerianus* contained alkaloids, saponin, tannin, flavonoid and cardiac glycoside. The quantitative analysis gave 19.8 mg/g saponin, 18.7 mg/g cardiac glycosides, 7.8 mg/g alkaloid, 6.5 mg/g flavonoids and 1.9 mg/g tannin. The biological activity of geraniin and ellagitannin isolated from the plant had been reported (Boakye et al, 2016; Agyere et al, 2010).

Ofokansi et al reported the antibacterial activity of the leaf extract of *P. muellerianus* while Doughari and Sunday showed that the plant extracts contained tannins, flavonoids, saponins alkaloids and anthraquinones.

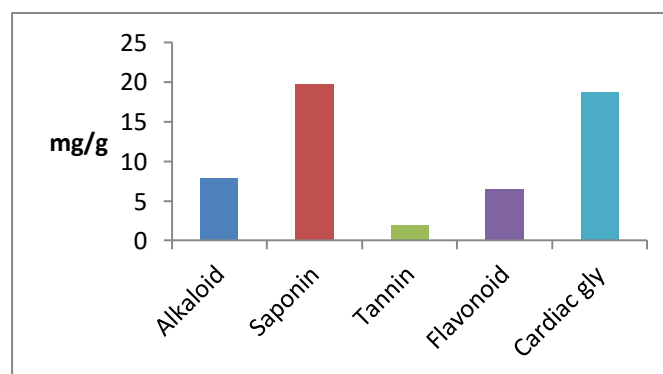


Figure 3: Quantitative phytochemical analysis of the leaves of *Phyllanthus muellerianus*.

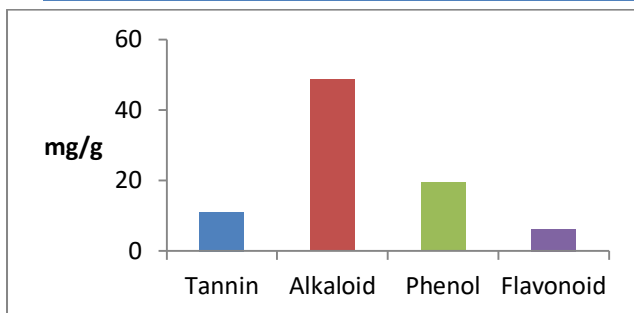


Figure 4: Quantitative phytochemical screening of the leaves of *Acalypha fimbriata*

The leaves of *Acalypha fimbriata* contained alkaloids, tannins, anthraquinones, flavonoids

steroids, terpenoids and cardiac glycosides while the quantitative analysis as shown in figure 4 shows alkaloids to be the most abundant. Akinbuluma et al had already reported the presence of alkaloids, tannins, flavonoids in the methanolic extract of the plants. The antiemetic and the antimicrobial activities of the plant had been reported (Quds et al, 2012; Kasim et al, 2011).

The DPPH free radical scavenging, Nitric Oxide scavenging, Reducing power potential and Iron II chelation ability of the four plants were assessed. The results of the antioxidant activities were shown graphically in Figures 5-9. The IC_{50} values calculated were shown in Table 2.

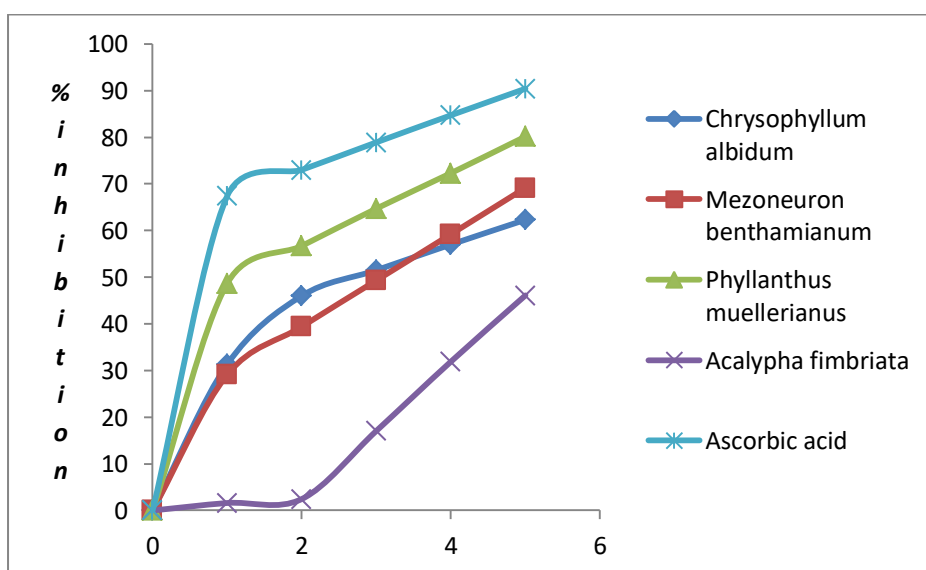


Figure 5: DPPH free radical scavenging activity

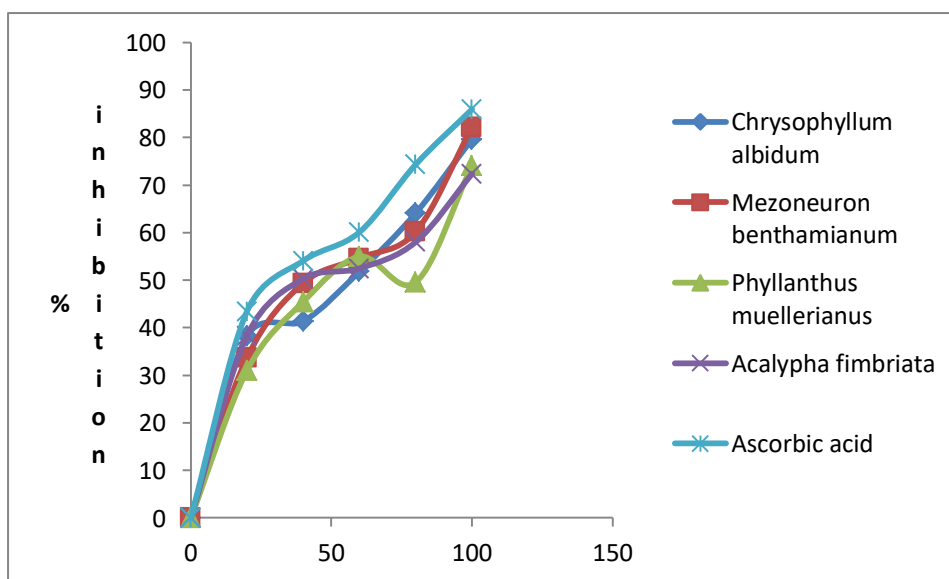


Figure 6: Nitric Oxide scavenging activity

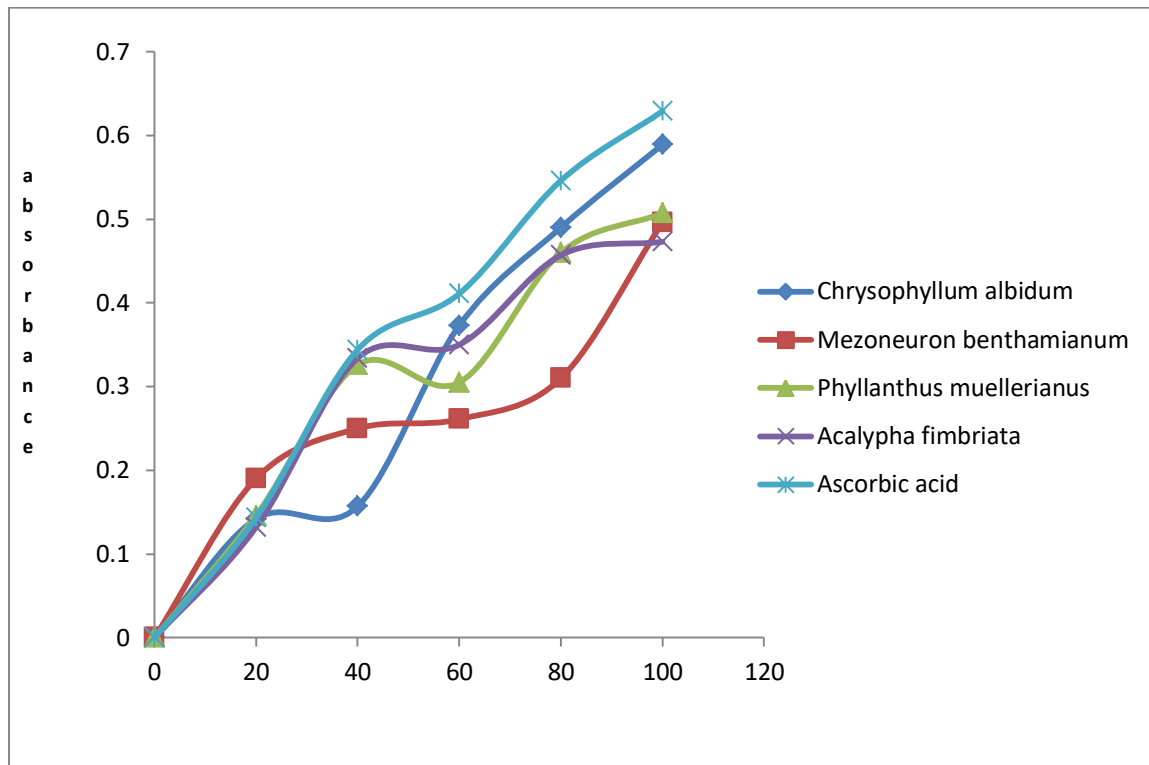


Figure 7: Reducing power antioxidant activity

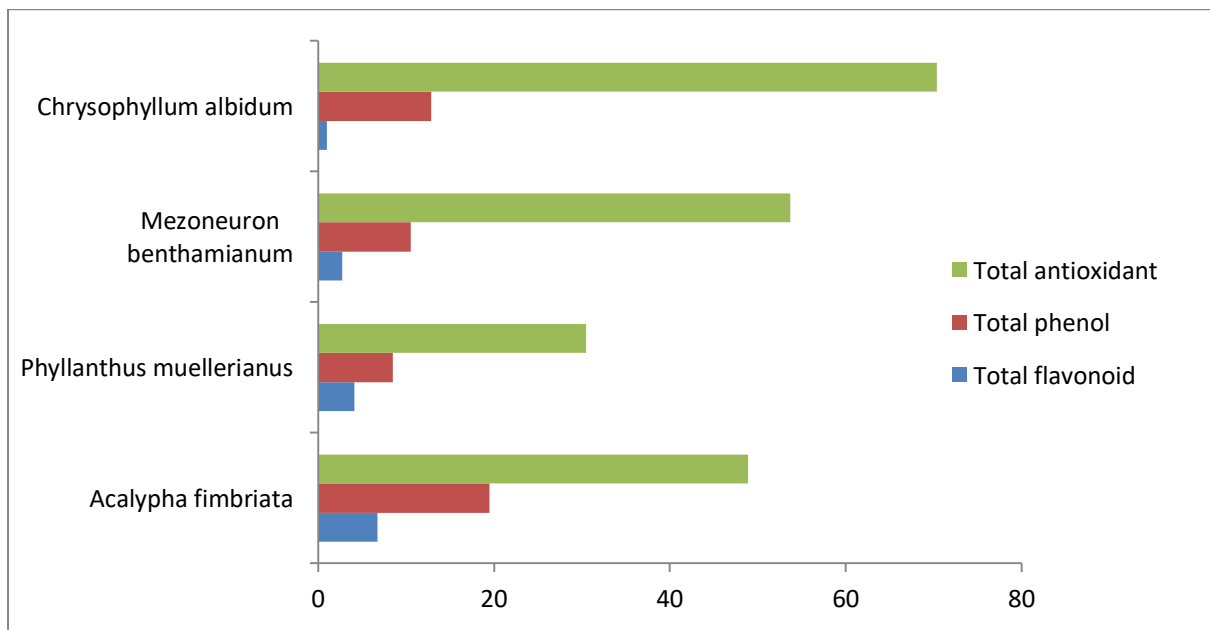


Figure 8: Total antioxidant capacity

Table 2: IC₅₀ Values

Samples	DPPH scavenging(mg/ml)	NO Scavenging(µg/ml)	Reducing power(µg/ml)
<i>Chrysophyllum albidum</i>	0.913	117.818	61.582
<i>Mezoneuron benthamianum</i>	12.574	433.127	186.801
<i>Phyllanthus muellerianus</i>	10.949	461.217	299.768
<i>Acalypha fimbriata</i>	3.907	426.284	11.007
Ascorbic acid	9.400	324.259	234.381

Both *Chrysophyllum albidum* and *Acalypha fimbriata* have IC₅₀ values lower than that of the standard ascorbic acid in DPPH scavenging activity as shown in Table 3. Also observed from the table 3, the IC₅₀ values of Reducing Power of *Acalypha fimbriata*, *Chrysophyllum albidum* and *Mezoneuron benthamianum* are lower than that of the ascorbic acid. Figure 12 shows *Chrysophyllum albidum* has the highest total antioxidant capacity (70.36) but its phenol and flavonoid values were not the highest. This suggests that there are other phytoconstituents or/and other metabolic processes responsible for the

antioxidant activities of *Chrysophyllum albidum*. *Chrysophyllum albidum* was reported to exhibit antioxidant properties by scavenging free radicals, decreasing lipid peroxidation and increasing the endogenous blood antioxidant levels. Myricetin rhamnoside, a glycosidic flavonoid isolated from the *C. albidum* exhibited antioxidant property (Adebayo et al, 2011). Eleagnine, an alkaloid, isolated and identified from *Chrysophyllum albidum* had been reported to possess antioxidant property (Idowu et al, 2006).

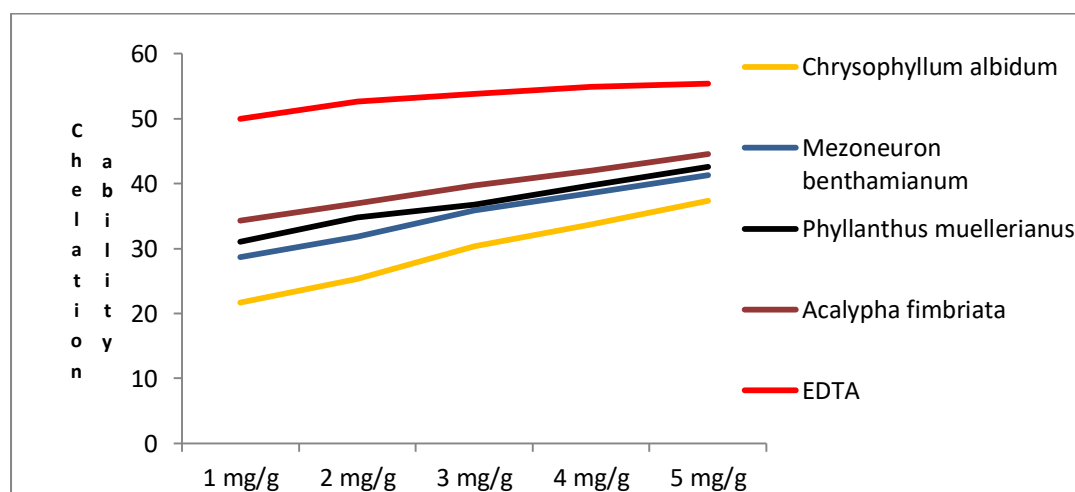


Figure 9: Iron II chelation ability

Metal chelation is beneficial when iron contributes to increased infection and also increases the progression of infection to clinical diseases. The result shown in Figure 9 showed the Fe²⁺ chelation ability of the plants extracts to be concentration dependent. EDTA was used as standard. From figure 12, the values of the total flavonoids of the plants were 6.72 for *A. fimbriata*, 4.085 for *P. muellerianus*, 2.777 for *M. benthamianum* and 1.003 for *Chrysophyllum albidum*. The Fe²⁺ chelation ability of the extracts showed the chelating ability of *A.fimbriata* > *P. muellerianus* > *M. benthamianum* > *C. albidum*. Thus there is a direct relationship between the flavonoid content and chelation potential of the plants. *Acalypha fimbriata* has the lowest IC₅₀ value for

reducing power (11.007 µg/ml) and the highest concentration dependent Fe²⁺ chelation ability. Excess Iron is stored as Fe³⁺ in Ferritin and iron overload sustains for a long period if the stored iron is not getting reduced and released for metal chelating drugs (Sarkar et al, 2012). Thus *A. fimbriata* with significant ability to reduce Fe³⁺ and good Fe²⁺ chelation potential seems a promising medicinal plant with a flavonoid that could be of health benefit to iron overload infected people. In general, the selected medicinal plants, *C. albidum*, *M. benthamianum*, *P. muellerianus* and *A. fimbriata* would possess several health benefits by virtue of their antioxidant properties.

Conclusion

This research work showed that plants contained phytochemicals that could be responsible for their medicinal values. The four medicinal plants showed strong antioxidant activity with *Chrysophyllum albidum* having the highest total antioxidant capacity. *Acalypha fimbriata* had the lowest IC₅₀ value for reduction potential and the highest dose dependent Iron II chelation ability. These properties of *Acalypha fimbriata* could be due to the presence of flavonoids in the plants. Isolation and identification of the bioactive compounds in the plants would help more in their ethnomedicinal uses.

Conflict of Interest

The authors declare no conflict of interest.

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