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UV SPECTROPHOTOMETRIC ANALYSIS OF THE ANTIOXIDANT ACTIVITY AND ANTIBACTERIAL ASSAY OF THE LEAF, STEM AND ROOT BARKS OF *CHRYSOPHYLLUM ALBIDUM* (SAPOTACEAE)

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ABSTRACT

Methanol extracts of the leaf, stem and root barks of *Chrysophyllum albidum* were analyzed for their total polyphenol contents, flavonoids concentration and *in vitro* antioxidant activity using ultraviolet (UV) spectrophotometric analysis of the gallic acid equivalent (GAE), quercetin equivalent (QE), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) reduction capability respectively, while the antibacterial activities were determined using standard procedures. The phytochemical screening revealed the presence of alkaloids, terpenoids, anthraquinone, flavonoids, saponins, carbohydrates and phenolic acids in the extract of the various parts of *C. albidum.* The phenolic contents of the leaves, stem and root barks were 11.0, 14.1, 13.0 (% mg/g GAE) respectively while the total flavonoids content of the respective parts were; 96.8, 176.6, and 127.8 (mg/g QE) respectively. Consequently, the IC_{50} Of the leaf, root and stem barks were found to be 0.0675, 0.0385, and 0.0245 mg/ml respectively. The antibacterial assay showed that the root and stem samples had mild antibacterial activity while the leaf sample showed no inhibition. There was a positive correlation between the polyphenols contents and the *in vitro* antioxidant activity. These results suggest that different parts of *C. albidum* possess varying levels of polyphenols, antioxidant and antibacterial capacities. The study should therefore form the basis for the choice of the plant part to be utilized for specific pharmacological application.

KEYWORDS: Chrysophyllum albidum; Antibacterial; Antioxidant, UV Spectrophotometry.

1.0 INTRODUCTION

For centuries, plants have been an integral part of human food as well as means of management of various diseases and ailments.[1] Plants owe their nutritional and therapeutic effectiveness to its metabolic products such as alkaloids, polyphenol and glycosides.[2] Antioxidants are substances that donate electrons to react with the unpaired electrons of free radicals thus counteracting their oxidative effect.[3] Antioxidants such as vitamin A, ascorbic acid (vitamin C), vitamin E, polyphenol and certain minerals (such as selenium) enhance immune system function by quenching free radicals.

Antibacterials are compounds that suppress the growth or destroy bacteria. It is important to note therefore, that antibiotics is used synonymously with antibacterial agents and remains the largest, widely known and most studied class of anti-microbial agents.[4] Diseases caused by microbe are so encompassing, and some of these organisms include *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aereus*, *Klebsiella pneumoniea*, *Candida albicans*.

Chrysophyllum albidum is a plant that is widely used as application to sprains, bruises and wounds in herbal medicine among the indigenous people across southern Nigeria. The seed and root extracts of *C. albidium* effectively arrested bleeding from fresh wounds, inhibited microbial growth of known wound contaminants and accelerated wound healing process.[5] The people of south western Nigeria have been using *C. albidum* leaves for the management of infections and other ailments since prehistoric times.[6] The roots and leaves of the plant have been widely used for medicinal purposes.[7] In addition, its seeds are a source of oil, which is used for diverse purposes.[8] *C. albidum* is used in folklore in the treatment of yellow fever, malaria, diarrhea, vaginal and dermatological infections.[9,10] *Chrysophyllum albidum* is established to have haematinic potentials.[11]

2. MATERIALS AND METHOD

2.1 *Plant Material*

The various parts of *Chrysophyllum albidum*, which included the leaves, stem bark and root bark were harvested from University of Port-Harcourt Botanical Garden, Choba, Nigeria in mid-October, 2016. The plant was authenticated and sample deposited at the herbarium section, Plant Science and Biotechnology Department, University of Port Harcourt and a voucher number (UPH/C/022) assigned.

2.2 *Reagents and Chemicals Used*

All the reagents used were of analytical grade: Methanol (Sigma Aldrich, Germany), n-hexane (Sigma Aldrich, Germany), Dragendorfs' reagent, Fehling's solution A and B, Sodium hydroxide, Hydrochloric acid, Molish reagent, Sulphuric acid, Ferric chloride, Gallic acid (Sigma Aldrich, Germany), Quercetin, Folin-Ciocalteu's reagent (Sigma Aldrich, Germany), Aluminium chloride, sodium carbonate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Ascorbic acid (Kermel), single strength Muller Hinton agar , Gentamicin 0.1 mg/ml.

2.3 *Extraction of the Plant Material*

Fresh leaves, seeds, stem and root barks of the plant were harvested and air-dried for 14 days after which they were powdered using mechanical grinder. Two hundred gram (200 g) of the powdered dried leaves, seeds, stem, and root barks were each defatted by soxhlet extraction using 2.5 litres of n-hexane over a period of 48 h. The defatted marc was dried and weighed. The dried marcs were separately extracted with 2.5 ml of methanol over a period of 48 h and the methanol extracts from the different plant parts were collected, dried and stored separately in a refrigerator.

The percentage yield was calculated using the formula

$$\%\,Yield = \frac{weight\ of\ extract}{weight\ of\ crude\ powder} \times 100$$

2.4 *Determination of Antioxidant Capacity Using Dpph Radical Scavenging Capacity*

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of the leaf, stem, and root extracts were measured by colorimetric method.[12] In brief, an aliquot of 2 ml of sample solution at different concentrations (0.002- 0.2 mg/ml) was mixed with 2 ml of DPPH solution (0.075% in methanol). The reaction mixture was incubated for 30 min in the dark at room temperature. The absorbance of the resulting solution was measured at 517 nm with a spectrophotometer. Controls were prepared in a similar way as for the test group except for the replacement of the test sample with the corresponding extraction solvent. The radical scavenging capacity of the tested samples was measured using the equation below. All determinations were performed in triplicate. Ascorbic acid was used as the standard. IC_{5O} was used to determine the level of antioxidant activity. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation.[13]

$$\% \text{ Inhibition} = \frac{A - B}{A} \times 100$$

Where A is the absorbance of pure DPPH in oxidized form while B is the absorbance of sample taken after 30 minutes of reaction with DPPH.

2.5 *Determination of Total Phenolic Content*

Total phenolic content was quantified and expressed as Gallic acid equivalents (GAE) according to a method proposed by Singleton *et al.*[14] The amount of the total polyphenols in leaves, stem and root extracts was determined with the Folin-Ciocalteu's reagent. 1 ml of standard solution of concentrations 0.0625, 0.125, 0.25, 0.5 and 1.0 mg/ml of gallic acid were prepared in methanol. Concentrations of 0.1 g/ml of plant extracts were also prepared in methanol and 0.5 ml of each sample was introduced into test tubes and mixed with 2.5 ml of a 5-fold dilute Folin-Ciocalteu's reagent and 4 ml of 10% sodium carbonate. The tubes were covered with aluminum foil and allowed to stand for 45 min at room temperature and the absorbance was read at wavelength 560 nm using a spectrophotometer.

Gallic acid was used as a standard and the total phenolics were expressed as gallic acid equivalents (GAE mg/g) using this formula:

TPC = GAE × V/M

Where;

TPC= Total content of phenolic compounds (% w/w)

GAE = Gallic acid equivalent (obtained from the standard curve using the regression equation)

V=volume of extract in ml;

M= weight of plant extract in g.

2.6 *Determination of total Flavonoid Content using Aluminium Chloride Colorimeteric Method*

In this method[15], quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in methanol and then diluted to 6.25, 12.5, 25, 50, 80, and 100 µg/ml. A calibration curve was made by measuring the absorbance of the dilutions at 440 nm (λ_{max} of quercetin) with a Jenway 6405-UV/Vis spectrophotometer (Jenway 6405, England).

In brief, a volume of 2.0 ml of 2% $AlCl_3$ in ethanol solution was added to 2.0 ml of different plant extracts. After 1h incubation at room temperature, the absorbance was measured at 440

nm. Appearance of yellow colour indicated the presence of flavonoids. The plant extracts were evaluated at a final concentration of 0.1 g/ml. Total flavonoids contents were calculated as quercetin equivalent (QE mg/g) using this formula:

TFC = QE × V/M

Where;

QE= Quercetin equivalence obtained from the standard curve

V=volume of extract in ml;

M= weight of plant extract in g.

2.7 *Antibacterial Activity Test*

A 250 ml volume of single strength Muller Hinton agar was prepared. 20 ml of each of the preparation was transferred into a well labelled agar pour bottle and after this the bottles were autoclaved at a temperature of 120 ^{0}C and pressure of 15 psi for 15 min. After autoclaving, the agar was allowed to cool a little, then 0.1 ml of a standardized culture containing *Pseudomonas aeruginosa* [gram-negative organism] was transferred into separate well labelled agar pour bottles, the organism and the agar were properly mixed together, and the mixtures aseptically transferred into well labelled petri-dish and allowed to solidify. The same procedure was carried out using 0.1ml of a standard culture containing *Staphylococcus aureus* [gram-positive organism]. Upon solidification, four holes with diameter of 6 mm each were punched aseptically on each of the plates using a sterile cork borer, and few drops of the control (Gentamicin 0.1 mg/ml) which is enough to fill the hole was transferred into one of the holes in each plate. Then to the other holes, the root, stem and leaf samples were transferred into them as labelled in each plate. After these, the plates were incubated at a temperature of 37 ^{0}C for 24 h.

3.0 RESULTS

The result of the phytochemical screening (Table 1) shows that *Chrysophyllum albidum* contains flavonoid, cardiac glycoside, alkaloid, anthraquinone, carbohydrate, saponins, phenolic acid, terpenoids and reducing sugars. However, only the leaf and the root bark contain tannins while the leaf and stem bark contain cardiac glycosides. Alkaloid was absent in the leaf sample.

Table. 1. Comparison of phytochemical constituents present in various parts of *C. albidum*.

Test	Leaf	Stem	Root
Alkaloid	-	+	+
Tannin	+	-	+
Saponnin	+	+	+
Flavonoid	+	+	+
Carbohydrate	+	+	+
Anthraquinone	+	+	+
Phenolic acids	+	+	+
Terpenoids	+	+	+
Reducing sugar	+	+	+
Cardiac glycosides	+	+	-

Key: + = Present - = Absent

Table 2 shows the total flavonoids (mg/g quercetin equivalent), total polyphenol (% mg/g gallic acid equivalent), and antioxidant activities (% DPPH reduction) of the leaf, seed, stem and root barks of *Chrysophyllum albidum*. Table 3 is the result of the comparison of the IC_{50} of ascorbic acid and that the various plant parts tested.

Table. 2: Comparative analysis of the total flavonoid content, total phenolic content and IC_{50} of the leaf, root and stem barks of *Chrysophyllum albidum*.

Plant part	Total Flavonoid Content (mg/g)	Total Phenolic Content (mg/g)	IC_{50} (mg/ml)
Stem bark	176.6	14.1	0.0245
Root Bark	127.8	13	0.0385
Leaf	96.8	11	0.0675

Table. 3: Comparative IC_{50} of ascorbic acid and the various parts of *C. albidum*.

S/No	Plant Part	Ic_{50}
1	Ascorbic acid	0.0039
2	Leaves	0.0535
3	Stem	0.084
4	Root	0.062

The total flavonoids content of the *C. albidum* was found to be: stem bark (176.6 mg/g QE) > root bark (127.8 mg/g QE) > leaf (96.8 mg/g QE). The total polyphenols contents on the other hand were: stem bark (14.1%mg/g GAE) > root bark (13% mg/g GAE) > leaf (11% mg/g GAE). The IC_{50} results were: leaf (0.0675 mg/ml) > root bark (0.0385 mg/ml) > stem bark (0.0245 mg/ml).

For the antibiotic assay (Table 4), the stem sample had inhibition zone diameters of 12 mm for both organisms (*staphylococcus aureus* and *pseudomonas aeruginosa*), the root sample had inhibition zone diameters of 10 mm and 13 mm respectively for *staphylococcus aureus* and *pseudomonas aeruginosa*. However, the leaf sample showed no inhibition zone diameter for the two organisms. The standard (gentamicin (0.1 mg/ml)) had inhibition zone diameter of 14 mm each for the two organisms.

Table. 4: Inhibition zone diameter of the different parts of *C. albidum*.

Organism	Different plant parts (10 mg/ml)			Gentamicin (0.1 mg/ml)
	Stem	Root	Leaf	
Staphylococcus aureus	12 mm	10 mm	_	14 mm
Pseudomonas aeruginosa	12 mm	13 mm	_	14mm

4.0 DISCUSSION

The percentage DPPH reduction basically shows the dose dependent antioxidant activities of the various parts of *C. albidum* while the antioxidant activity represented as mg/ml IC_{50} defined the potency of each part. It is worthy of note that the IC_{50} value decreases with increasing potency/antioxidant activity. The antioxidant properties of the various part from the most active are: stem (0.0245 mg/ml) > root (0.0385 mg/ml) > leaf (0.0675 mg/ml), however, the IC_{50} of the standard antioxidant was found to be 0.009 mg/ml. This result shows that the methanol extract of the stem of C. *albidum* is more potent than that of other parts (root and leaf samples).

Generally, the phytochemical analysis showed that the leaf, stem and root barks of *C. albidum* were rich in flavonoids and polyphenols. The presence of polyphenols in the various parts of *C. albidum* is of nutritional and medicinal significance. Polyphenols have been recognized as antioxidant agents and free radical scavengers with therapeutic and physiological functions.[16] It has been reported that compounds such as flavonoids, which contain phenolic hydroxyl groups, are responsible for the radical scavenging and chelating effects of many plants used as food and medicine.[17] Such free radical scavenging potentials could prevent oxidative stress–related diseases such as cancer, diabetes, hypertension, and other age-related disorders.[18,19]

The antibacterial activity of the samples may be due to the presence of tannins, flavonoids, saponins, alkaloids and anthraquinones. These phytochemical compounds are biologically

active and may be responsible for the antimicrobial activities of *C. albidum.*[20] Phytochemicals exert antimicrobial activity through different mechanisms; tannins for example, act by iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes[21] in microbial cells. Herbs that have tannins as their component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery[22] thus exhibiting antimicrobial activity. Li *et al*[23] reviewed the biological activities of tannins and observed that tannins have remarkable activity in cancer prevention and anticancer, thus suggesting that *C. albidum* could be a possible source of important bioactive molecules for the treatment and prevention of cancer. In addition to its antimicrobial and anticancer activities, tannins function as stable and potent antioxidants.[24] Alkaloids are reported to have analgesic, anti-inflammatory and adaptogenic activities which help to alleviate pains, develop resistance against diseases and endurance against stress.[25] Tannins and alkaloids were absent in the leaf sample during phytochemical screening but present in the root and stem bark samples. It is probable that these bioactive compounds might be responsible for the antibacterial activity exhibited by *C. albidum*.

5.0 CONCLUSION

The variations in the content and distribution of bioactive components among different parts of the same plant underscores the need for a detailed independent study of various parts of a plant before any generalization could be made on the plant's pharmacological or therapeutic potentials. In this study, the total phenolic and flavonoid contents, as well as the *in vitro* antioxidant capacity of *C. albidum* were found to be highest in the stem bark sample, followed by the root and leaf samples. Antibacterial assay revealed similar trend. Thus, the stem bark of the plant should be preferentially utilized as a valuable resource for natural antioxidant and antibiotic remedies.

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