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Effect of Extraction Solvents on Yield, Mineral Composition, Phytochemical Constituents, Antioxidant and Antimicrobial Properties of *Chrysophyllum albidum* (African Star Apple) Leaf

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Abstract

Chrysophyllum albidum is a tropical plant that belongs to the family *Sapotaceae* with about 800 species that make up almost half of the order *Ericales* The yield, mineral, phytochemical constituents, antioxidant and antimicrobial properties of different solvent extracts of *Chrysophyllum albidum* leaves were investigated. The leaves of *C. albidum* were successively extracted in acetone, ethyl acetate, petroleum ether, ethanol and distilled water. All obtained crude extracts were evaluated for yield, minerals, phytochemical constituents, free radical scavenging (2, 2-diphenyl-1- picryl hydrazyl - DPPH and Ferric Reducing Antioxidant Power Assay-FRAP) activities and antimicrobial properties. The ethanol extract has the best yield (10.41%) while petroleum ether has the least (2.22%). Phytochemical screening of the crude extracts showed the highest presence of tannin (4.25±0.01), total phenolic content (77.48±0.02) and flavonoid (18.39±0.06) in acetone extract, saponin (1.43±0.30) and alkaloid (3.76±0.10) were highest in aqueous extract while all were absent in petroleum ether extract. Aqueous extract showed a higher presence of magnesium (6.103±0.00), potassium 27.578±0.00), sodium (2.969±0.00), zinc (0.036±0.00) and manganese (0.082±0.00) except calcium (1.001±0.00) that was higher in ethyl acetate extract and iron (0.161±0.00) in acetone extract. The antioxidant activity of Acetone (IC = 83.10±0.52 µg mL) and ethanol (IC = 86.71±0.06 µg mL) extract showed 2, 2-diphenyl-1- picryl hydrazyl (DPPH) scavenging activity which was comparable with that of standard ascorbic acid (IC = 95.14±0.51 µg mL). Ferric Reducing Antioxidant Power Assay (FRAP) scavenging activity showed a maximum effect in acetone extract while the least was observed in pet ether extract. The in vitro antimicrobial activity was done by agar disc diffusion method against *Staphylococcus aureus*, *Aeromonas hydrophila*, *E. coli*, *Pseudomonas aeruginosa, Salmonella spp*, and *Bacillus spp*. Maximum -antibacterial activity (zone of inhibition) was shown by acetone and ethanol extracts against all the tested organisms whereas ethyl acetate extract showed no activity. This study has revealed that the leaf extracts of *C. albidum* possess potent phytonutrients, antioxidants and free radical scavenging activity which may be due to the presence of flavonoids and total phenolic content (TPC) as well as antimicrobial effects against some of the tested bacteria.

1.0 Introduction

The African star apple, *Chrysophyllum albidum* (G. Don) is a tropical plant that belongs to the family *Sapotaceae* with about 800 species that make up almost half of the order *Ericales* (Ehiagbonare *et al.*, 2008). It is

primarily a forest tree and its natural occurrences have been reported in diverse zones in Nigeria, Uganda, Niger Republic, Cameroon and Cote d'Ivoire (Bada, 1997). It is distributed throughout the Southern part of Nigeria where it is called 'Agbalumo' (Yoruba); 'Udara' (Igbo), while in the Northern part of Nigeria, it is called 'Khada'(Hausa) (Madubuike and Ogbonnaya 2003; Idowu *et al*., 2006). Ecologically, the tree has efficient nutrient cycling and the high rate of mineralization of the leaves improves the quality of the topsoil (Adesina, 2005). The skin or peel is orange to golden yellow when ripe and the pulp within the peel may be orange, pinkish, or light yellow, within the pulp are three to five seeds that are not usually eaten. The seed coats are hard, bony, shiny, and dark brown, and when broken reveal white-coloured cotyledons. The fruit has been found to have the highest content of ascorbic acid per 100g of edible fruit or about 100 times that of oranges and 10 times of that of guava or cashew (Pearson, 1976). The bark is used for the treatment of malaria and yellow fever, while the leaf is used as an emollient and for the treatment of skin eruption, stomach ache and diarrhea (Idowu *et al.*, 2006). Seed and root extracts of *C. albidium* are used to arrest bleeding from fresh wounds, inhibit microbial growth of known wound contaminants and also enhance the wound healing process as they have astringent characteristics (Okoli and Okere, 2010). Tannins, flavonoids, terpenoids, proteins, carbohydrates, and resins are the phytochemicals that have been reported in *Chrysophyllum albidum* (Akaneme, 2008). It has antioxidant properties by scavenging free radicals, decreasing lipid peroxidation and increasing the endogenous blood antioxidant enzymes level (Adebayo *et al*, 2010). The leaves of *Chrysophyllum albidum* were shown to reveal the presence of Alkaloids, Cardiac glycoside, Anthraquinones, Flavonoids, Terpenoids, and Steriods, which are useful substances that have medicinal and physiological activities (Fasogbon *et al*., 2017).

Bioactive molecules serve as starting materials for laboratory synthesis of drugs as well as a model for the production of biologically active compounds (Dhanani *et al.*, 2017). Phytochemical processing of raw plant materials is essentially required to optimize the concentration of known constituents and also to maintain their activities (Aziz *et al*., 2003). The role of free radicals in disease initiation cannot be overemphasized. Most free radicals such as, hydroxyl radical (OH^-), superoxide radical (O^{2-}), lipid peroxide radicals and hydrogen peroxide (H_2O_2) are being implicated in some disease conditions (Adebayo *et al.*, 2011). These reactive oxygen species (ROS) are generated as a result of normal biochemical metabolism in the body which is due to the high level of exposure to xenobiotics (Pourmorad *et al.*, 2006; Kumpulainen *et al.*, 1999; Cook and Samman, 1996). Pathological conditions result when the generation of ROS induced by stimuli in the organism exceeds the antioxidant capacity of the organism (Yang *et al.*, 2008). The harmful effect of these reactive species in normal metabolic processes which lead to disease conditions is a consequence of their interaction with some biological compounds within and outside the cells (Adebayo *et al*., 2011). Oxidative stress occurs as a consequence of excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and it is ameliorated by endogenous antioxidant enzyme activity and exogenous dietary antioxidants (Sugino, 2007). Antioxidants present in the diet can delay lipid peroxidation by inhibiting the initiation or propagation phase of oxidizing chain reactions by scavenging free radicals (Sherwin, 1990). Recently, many natural and synthetic free radical scavengers and antioxidants have been employed in protecting biomolecules against free radical-mediated damage (Adebayo *et al*., 2011). The solvent extracts of the leaves of *C. albidum* have been reported to demonstrate antioxidant activity by a variety of in-vitro methodologies (Adebayo *et al*., 2011). This study has therefore demonstrated that the leaf extracts of *C. albidum* possess potent phytonutrients, antioxidants and free radical

scavenging activity which may be due to the presence of flavonoids and total phenolic content (TPC) as well as antimicrobial effects against some of the tested bacteria.

2.0 Materials and Methods:

Sample Collection and Identification

The leaves of *Chrysophyllum albidum* (African Star Apple) plant were obtained from Arowomole Area, Osuru, Ogbomoso South Local Government, Ogbomoso, Oyo State, Nigeria. A voucher specimen was identified at the Department of Botany, University of Ibadan with a Voucher Number: UIH – 22831.

2.1.0 Chrysophyllum albidum leaves preparation

The leaves of *Chrysophyllum albidum* were air-dried for 14 days with the leaves turned periodically to expose all the leaves to air. They were weighed frequently until a constant weight was obtained and thus considered dry. The dried leaves were ground in a blender into powder and processed for extraction with solvents.

2.1.1 Sample extraction with Solvents:

Crude solvent extraction of *Chrysophyllum albidum* leaves was carried out by soaking the dried and powdered samples in analytical grade ethyl acetate, acetone, ethanol, petroleum ether and distilled water respectively and filtered after 72 hours under room temperature. Each 400 g of the leaves was extracted with 2.5 litres of the individual solvent in glass jars. The samples were then filtrated by passing through Whatman No. 1 filter paper. The filtrates were evaporated to dryness using a rotary evaporator. The crudes were weighed with a sensitive scale to determine their yields.

2.2.0 Phytochemical Screening:

Qualitative Determination of Phytochemicals in *Chrysophyllum albidum*

Qualitative tests were performed to quantify the presence of Alkaloids, Flavonoids, and Saponins in the leaf extracts according to the procedures of Williams, 2005, Adegoke *et al*, 2010 and Manjulika, *et al*, 2014.

i. **Test for alkaloids**: 3ml extract of *C. albidum* will be dissolved in 3ml of 1% hydrochloric acid (HCl) in a steam bath. Mayer's reagent will then be added to the mixture. Turbidity of the resulting precipitate will be taken as positive evidence of the presence of alkaloids in the extract (Hager's Test).
ii. **Test for flavonoids**: About 0.2g of the leaf extract of *C. albidum* will be dissolved in a dilute sodium hydroxide (NaOH) solution, and an equal amount of hydrochloric acid will be added. A yellow solution that turned colourless will indicate the presence of flavonoids in the extract.
iii. **Test for saponins:** About 0.2g of *C. albidum* leaf extract will be mixed with distilled water and heated to a boil. Frothing (appearance of a creamy mix of small bubbles) will show the presence of Saponins in Methanol while red in Distilled water (Foam Test).
iv. **Test for steroids:** Acetic anhydride (2ml) will be added to 0.5g of the leaf extract of *C. albidum* in a test tube. It will then be followed by the addition of 2ml of sulfuric acid. A colour change from violet to blue or green will indicate the presence of steroids in the extract (Salkowaski Test).
v. **Test for terpenoids:** The *C. albidum* leaf extract (0.2g) will be mixed with 2ml of chloroform, and 3ml of concentrated H_2SO_4 will be carefully added to form a layer. A reddish brown interface will be formed which will indicate the presence of terpenoids in the extract.
vi. **Test for Tannins:** A small quantity of the extract will be mixed with distilled water and heated in a water bath. The mixture will be filtered and ferric chloride will be added to the filtrate. A blue solution will indicate the absence of tannins in distilled water and dark green colour will indicate its presence in methanol (Braymer's Test).
vi. **Liebermann's test for cardiac glycosides:** 3g of the extract will be dissolved in 3mls of chloroform

respectively. Afterward, there will be an addition of 3mls of acetic acid and the solution will be well-cooled inside the ice. After the careful addition of tetraoxosulphate (VI) acid, there will be a colour change from violet to blue, then to green which will indicate the presence of a glycone portion of glycoside.

vii. **Borntrager's test for anthraquinone derivatives:** Chloroform extract of the material was obtained by boiling it in a water bath. 1ml of diluted (10%) ammonia will be added to 3g of the extract respectively and the mixture will be shaken. Any colour change will be recorded. Anthracene derivatives will be indicated by a pink colour in the ammoniacal (lower) layer; these will be done in triplicates for proper statistical analysis (Arvind *et al.*, 2010).

2.3.0 Quantitative Phytochemical Analysis

i. **Determination of saponin:** Saponin was determined by Obadoni and Ochuko method. 20 g of each sample were put into a conical flask and 100 cm^3 of 20 % aqueous ethanol was added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55^0 C. The mixture was filtered and the residue was re-extracted with another 200 mL of 20 % aqueous ethanol. The combined extracts were reduced to 40 mL over a water bath at about 90^oC. The concentrate was transferred into a 250 mL Separating funnel and 20 mL of di-ethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded, the purification process was repeated. 60ml of n- butanol was added, the combined n – butanol extract was washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath after evaporation the samples were dried in the oven to constant weight. the Saponin content was calculated as a percentage.

ii. **Determination of total alkaloids:** 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated in a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added in drops 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.

iii. **Determination of total phenolic content:** The concentration of phenolics in plant extract samples was determined using the spectrophotometric method (Folin-Ciocalteu assay). The reaction mixture of 1 mL of extract and 9 mL of distilled water was taken in a volumetric flask (25 mL). 1 mL of Folin-Ciocalteu phenol reagent was added to the mixture and shaken well. After 5 minutes, 10 mL of 7 % Sodium carbonate (Na_2CO_3) solution was treated to the mixture. The volume was made up to 25 mL. A set of standard solutions of Gallic acid (20, 40, 40, 60, 80 and 100 µg/mL) were prepared and incubated for 90 min at room temperature. The absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Ultraviolet (UV) /Visible spectrophotometer. Total phenol content was expressed as mg of GAE/gm of extract.

iv. **Determination of tannin content:** The tannins were determined using Folin - Ciocalteu assay method. 0.1 ml of the sample solution was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35 % Na_2CO_3 solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions

were measured against the blank at 725 nm with a Spectrumlab 752s UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract.

v. **Determination of total flavonoid content:** Total flavonoid content was measured by the aluminum chloride colorimetric assay. The reaction mixture consists of 1 ml of extract and 4 ml of distilled water was taken in a 10 ml volumetric flask. In the flask, 0.30 ml of 5 % sodium nitrite was treated and after 5 minutes, 0.3 ml of 10 % aluminum chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with the UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract.

vi. **Determination of mineral composition of *Chrysophyllum albidum* leaves**
The mineral composition of the sample was determined using the methods as recommended by Association of Official Analytical Chemists (AOAC, 2011 and Sahrawat *et. al.,* 2002). 0.5 g of dried powered *C. albidum* leaves extract was put into a Kjeldahl digestion flask in which 24cm^3 of the mixtures of the concentrated nitric acid (HNO_3), conc. H_2SO_4 and 60% $HClO_4$ (9:2:1 v/v) was added. The flask was placed in a stationary position overnight to prevent excess foaming. Afterward, the flask was digested in a clear solution, made cool and emptied into a volumetric flask of 50cm^3 after it was subjected to heat. The solution was thereafter diluted to the volume with distilled water. Similarly, another solution was prepared without the addition of the sample. The solution was used for the mineral analysis. The mineral contents (calcium, magnesium, iron, zinc, copper and manganese) were analyzed using Atomic Absorption Spectrophotometer (AAS). Sodium and Potassium were analyzed using Atomic Emission Spectrometry and total phosphorus was determined by colorimetry using the Vanadomolybdate (blue) method (AOAC, 2011).

2.4.0 DPPH radical scavenging activity of the crude extracts

The radical scavenging activity of the crude extracts of *C. albidum* was determined according to the method of Gyamfi *et al,* 1999 with slight modification by using DPPH (2, 2-diphenyl-1-picrylhydrazyl). Appropriate solution of the extracts was mixed with 1ml, 0.4 Methanolic solution containing DPPH and left for 30 minutes and the absorbance was taken at 517nM in the spectrophotometer. The DPPH radical scavenging activity was calculated as:

DPPH radical scavenging activity (%)

$$= \frac{(A_{Control} - A_{Sample})}{(A_{Control})} \times 100$$

where $A_{Control}$ is the absorbance of the control reaction (containing all reagents except the test compound), and A_{Sample} is the absorbance of the test compound (extract). Ascorbic acid was used as positive control. The tests were carried out in triplicate.

2.5.0 Ferric reducing power assay

The reducing power of the compound was evaluated according to the procedures of Oyaizu, 1986 with slight modification. This was done by mixing 2.5ml aliquot of the sample with 2.5ml 200mM of Sodium Phosphate buffer (pH 6.6) and 2.5ml of 1% Potassium Ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated at 50^{0}C for 20 minutes and then 2.5ml 10% TCA was added. The mixture was centrifuged at 650rpm for 10 minutes. 5ml of the supernatant was mixed with an equal volume of water and 1% Ferric Chloride ($FeCl_3$). The absorbance was

measured at 700nm with a spectrometer. An increase in absorbance of the reaction mixture indicates increased reducing power. The experiment was conducted in triplicates and values were expressed as equivalents of ascorbic acid in µg / mg of extract.

2.6.0 Antimicrobial activity screening of the leaf extracts

The antimicrobial activities of the extract of *C. albidum* leaves was determined. The pathogenic organisms used *(Staphylococcus aureus, Aeromonas hydrophila, E. coli, Pseudomonas aeruginosa, Salmonella spp*. and *Bacillus spp.)* were obtained from the Department of Microbiology of the University of Ibadan. They were sub-cultured on nutrient agar and incubated at 37^0C for 18-24 hours to obtain pure culture and kept on Agar slants (Fagbemi *et. al.,* 2009) for further use. Different solvent (acetone, ethyl acetate, petroleum ether, ethanol and distilled water) extracts of *Chrysophyllum albidum* leaf were assessed for their antimicrobial activities against the listed pathogens. 10mg/ml concentration of the extract was prepared by dissolving them in Dimethyl Sulphur Oxide (DMSO). Mueller Hinton Agar (MHA) was prepared according to the manufacturer's instructions and sterilized in an autoclave at 121^0C for 15 minutes. After the temperature has cooled to 45^0C, they were poured aseptically into petri dishes and the plates were allowed to solidify before use.

Inoculum standard preparation

Inoculum standard was prepared from 18 to 24-hour old culture of the selected fish pathogens by comparing the turbidity with the McFarland standard. Each of the inoculum standards of the pathogen was spread on MHA plates prepared. After this, a 7mm diameter cork borer was used to make holes in the plates and each of the extracts was placed in the holes and labelled appropriately. The hole for both positive control (Streptomycin) and negative control (Dimethyl Sulphur Oxide) was also made. The plates were incubated aerobically at 37^oC for 18-24 hours thereafter, the zones of inhibition of the growths were observed and measurements of the zones were taken using a transparent ruler (Richard *et al*., 2013) and recorded properly.

3.0 Statistical Analysis

All experiments were carried out in triplicates and data expressed as mean ± standard error of the mean (SEM) using Excel and Graphpad Prism 8.

4.0 Results and Discussion

4.1.0 Percentage yield of the extract of *C. albidum* leaf

In order to identify antibacterial active compounds of a medicinal plant, its enormous therapeutic potential due to the presence of several antibacterial substances should be taken into consideration including the extraction and bioassay techniques used (Sinivassan *et al*., 2001). Besides, the type of solvent used for the extraction plays a vital role in the solubility of the active principles of plant materials that not only affected the amount of representative compounds which will influence the antibacterial activity of the extract (Nair *et al*., 2005). Table 1 shows the amount of extract yield that was obtained from a 400g dried sample of the leaves of *C. albidum* macerated with analytical grade acetone, ethyl acetate, petroleum ether, ethanol and distilled water. It showed ethanolic extract had the best yield (10.41%) followed by aqueous (9.26) and acetone (5.18%) while the lowest value was obtained in the pet ether extract (2.22%).

Table 1: Percentage yield of crude extract from *Chrysophyllum albidum* by several solvent extractions Solvent

	Weight of powdered leaf (g)	Weight of crude extract (g)	Yield (%)
Aqueous	400	37.03	9.26
Ethanol	400	41.62	10.41
Acetone	400	20.70	5.18
Ethyl Acetate	400	13.43	3.36
Pet Ether	400	8.89	2.22

4.2.0 Mineral composition of C. ***albidum*** **leaf extracts**

As shown in Table 2, calcium, magnesium, potassium, sodium, iron, zinc and manganese were all present in the different extracts. However, the values obtained for aqueous extract revealed that magnesium (6.10±0.00 mg/g), potassium (27.58±0.00 mg/g), sodium (2.97±0.00 mg/g), zinc (0.04±0.00 mg/g) and manganese (0.08±0.00 mg/g) were higher compared to all the other solvents except for calcium that had higher value (1.00±0.00 mg/g) in ethyl acetate extract and iron that had higher value (0.16±0.00 mg/g) in acetone as compared to other extracts investigated.

Table 2: Mineral composition of *Chrysophyllum albidum* leaf extractsConc. (µg/mL)

	Acetone	Ethyl acetate	Ethanol	Aqueous	Pet ether
Ca (mg/g)	0.76±0.01^{c}	1.00±0.00^{a}	0.79±0.01^{b}	0.31±0.00^{d}	0.21±0.00^{e}
Mg (mg/g)	1.53±0.01^{c}	1.01±0.00^{d}	1.82±0.08^{b}	6.10±0.00^{a}	0.31±0.00^{e}
K (mg/g)	0.79±0.00^{d}	2.22±0.00^{b}	0.93±0.00^{c}	27.58±0.00^{a}	0.16±0.00^{e}
Na (mg/g)	0.16±0.00^{d}	0.33±0.00^{b}	0.21±0.00^{c}	2.97±0.00^{a}	0.09±0.00^{e}
Fe (mg/g)	0.16±0.00^{a}	0.11±0.000^{c}	0.13±0.00^{b}	0.08±0.00^{d}	0.00±0.00^{e}
Zn (mg/g)	0.01±0.00^{c}	0.01±0.00^{c}	0.01±0.00^{c}	0.04±0.00^{a}	0.03±0.00^{b}
Mn (mg/g)	0.01±0.00^{b}	0.01±0.00^{b}	0.01±0.00^{b}	0.08±0.00^{a}	0.00±0.00^{c}

*Values are presented as mean ± standard error of the mean; the different superscripts in the same column are significantly different ($p<0.05$)

NOTE: Ca= Calcium; Mg= Magnesium; K= Potassium; Na= Sodium; Fe= Iron; Zn= Zinc; Mn= Manganese

4.3.0 Qualitative Phytochemical Screening of *C. albidum* Leaf Extracts

The results of the preliminary qualitative phytochemical screening of *C. albidum* leaf extracts show the presence of tannin, saponin, coumarin, alkaloids, steroids, tannins, cardiac glycoside and phenol (Table 3). The aqueous, ethanol, acetone and ethyl acetate extracts

revealed the presence of alkaloid and coumarin except the pet ether extract. Tannin, saponin and flavonoid were found to be present in aqueous, ethanol and acetone extracts while steroid was found in ethanol, acetone and ethyl acetate extracts. Charcones were present in aqueous and ethanol extracts but only petroleum ether extract showed the presence of cardiac glycoside

Table 3: Qualitative phytochemical screening *C. albidum* leaf extracts

Phytochemicals	Aqueous	Ethanol	Acetone	Ethyl acetate	Pet ether
Saponin (Foam Test)	+ve	+ve	+ve	-ve	-ve
Tannin (Braymer's Test)	+ve	+ve	+ve	-ve	-ve
Flavonoid	+ve	+ve	+ve	-ve	-ve
Steroid (Salkowaski Test)	-ve	+ve	+ve	+ve	+ve
Phlobatannin (Precipitate test)	-ve	-ve	-ve	-ve	-ve
Terpenoid	-ve	-ve	-ve	-ve	-ve
Coumarin	+ve	+ve	+ve	+ve	-ve
Emodin	-ve	-ve	-ve	-ve	-ve
Anthraquinone (Borntrager's Test)	-ve	-ve	-ve	-ve	-ve
Anthrocyanins	-ve	-ve	-ve	-ve	-ve
Alkaloid (Hager's Test)	+ve	+ve	+ve	+ve	-ve
Cardiac Glycosides (Legal's Test)	-ve	-ve	-ve	-ve	+ve
Charcones	+ve	+ve	-ve	-ve	-ve
Phenols	+ve	+ve	+ve	-ve	-ve

4.4.0 Quantitative Phytochemical Screening of *C. albidum* Leaves Extracts

The quantitative analysis of the chemical constituents of *C. albidum* extracts revealed (Table 4) that the presence of saponin and alkaloid were higher in aqueous extract (1.43±0.03 %); (3.76±0.01%) compared to acetone (0.56±0.20%); (3.48±0.40%) and ethanol (0.53± 0.20%); (3.38±0.13%). Ethyl acetate showed the absence of saponin but the least value for alkaloid (3.32±0.09%). Meanwhile, the acetone extract showed larger presence of tannin (4.25±0.01 mg/g), total phenolic content (77.48±0.23 mg/g) and flavonoid (18.40±0.06mg/g) higher than that of ethanol (3.78±0.00mg/g); (67.11±0.35mg/g);

(12.40±0.06mg/g) and aqueous (0.64±0.01mg/g); (14.60±0.17mg/g); (10.88±0.01mg/g) extracts.

Table 4: Quantitative phytochemical screening of Chrysophyllum albidum leaf extracts Phytochemical

	Aqueous	Ethanol	Acetone	Ethyl acetate	Pet ether	Method
% Saponin	1.43±0.02^{a}	0.53±0.01^{c}	0.56±0.01^{b}	NIL	NIL	Obadoni and Ochuko, 2001
Tannin mg GAE/g	0.64±0.01^{c}	3.78±0.00^{b}	4.25±0.01^{a}	NIL	NIL	Folin-Ciocalteu method *in* Mythili *et al*., 2014
% Alkaloid	3.76±0.01^{a}	3.38±0.08^{c}	3.48±0.02^{b}	3.32±0.05^{c}	NIL	Harborne J. B., 1973
TPC mg GAE/g	14.60±0.10^{c}	67.11±0.20^{b}	77.48±0.13^{a}	NIL	NIL	Singleton,1999
Flavonoid mg/g	10.88±0.01^{c}	12.39±0.04^{b}	18.39±0.04^{a}	NIL	NIL	Aluminium Chloride Colorimetric Assay

*Values are presented as mean ± standard error of the mean; the different superscripts in the same column are significantly different ($p<0.05$)

4.5.0 Result of Antioxidant Activities of *C. albidum* Leaves Extracts (DPPH and FRAP Scavenging Activities)

The DPPH radical scavenging activity of the solvent extracts of *C. albidum* leaf at varying concentrations was measured along with standard ascorbic acid. The entire solvent extracts investigated showed appreciable free radical scavenging activities. The IC50 values (Table 5) for the leaf extracts were found to be highest in ethanol (86.71±0.06 µg/mL) and acetone (83.10±0.52 µg/mL) extracts respectively**.** However, the table showed acetone extract as having considerable scavenging activities at all concentrations. This is further represented in Fig. 1.

Table 5: DPPH radical scavenging activities of the *Chrysophyllum albidum* leaf extracts and the standard ascorbic acid

Conc. (µg/mL)	Acetone	Ethyl acetate	Ethanol	Aqueous	Pet ether	Ascorbic acid
100.00	91.69±0.02^{b}	75.07±0.07^{d}	88.33±0.03^{c}	45.45±0.12^{e}	28.37±0.04^{f}	98.77±0.00^{a}
50.00	83.10±0.30^{c}	38.23±0.10^{d}	86.71±0.04^{b}	36.78±0.13^{d}	23.49±0.03^{e}	95.14±0.30^{a}
25.00	77.01±0.25^{b}	15.79±0.45^{f}	55.40±0.40^{c}	17.97±0.04^{e}	19.29±0.00^{d}	91.32±0.19^{a}
12.50	46.54±0.38^{b}	8.61±0.28^{f}	29.64±0.47^{c}	13.63±0.03^{e}	16.77±0.05^{d}	86.28±0.11^{a}
6.25	28.53±0.76^{b}	1.940±0.27^{e}	17.18±0.25^{c}	1.240±0.01^{f}	7.08±0.01^{d}	66.73±1.33^{a}

*Values are presented as mean ± standard error of the mean; the different superscripts in the same column are significantly different ($p<0.05$)

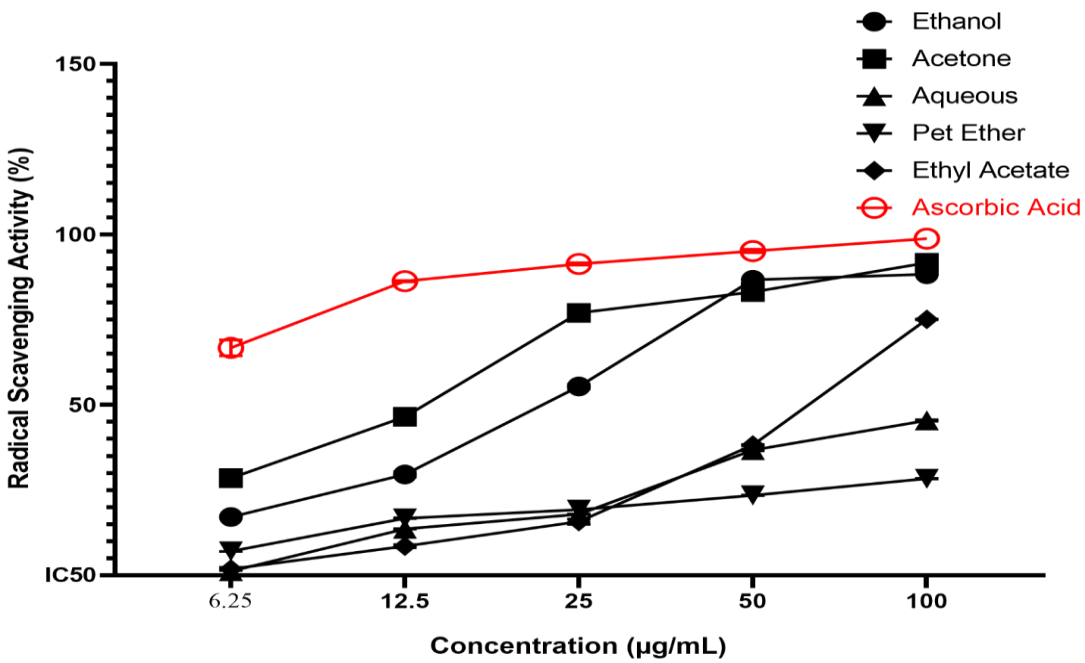

Figure 1: Graphical representation of the DPPH radical scavenging activities of the solvent extracts of *Chrysophyllum albidum* leaf

In addition, as depicted in Table 6, the ferric reducing antioxidant properties (FRAP) of the leaf extracts showed that acetone extract (269.14± 1.00 Conc. mg AAE/g) has higher activities as compared to other extracts in the fold while pet ether extract (59.98± 2.46 Conc. mg AAE/g) presented the least activity. This is further explained in a bar chart as represented in Fig. 2.

Table 6: FRAP analysis of ethanol, acetone, ethyl acetate, aqueous and pet ether extracts of *Chrysophyllum albidum* leaf

Solvent extract	1	2	3	Conc. mg AAE/g
Pet Ether	61.40	61.40	57.14	59.98 ± 1.42^{e}
Acetone	270.14	268.14	269.14	269.14 ± 0.58^{a}
Ethanol	186.14	184.14	181.14	183.81 ± 1.46^{b}
Aqueous	151.14	158.14	159.14	156.14 ± 2.52^{c}
Ethyl Acetate	136.14	134.14	136.14	135.47 ± 0.67^{d}

*Values are presented as mean ± standard error of the mean; the different superscripts in the same column are significantly different ($p<0.05$)

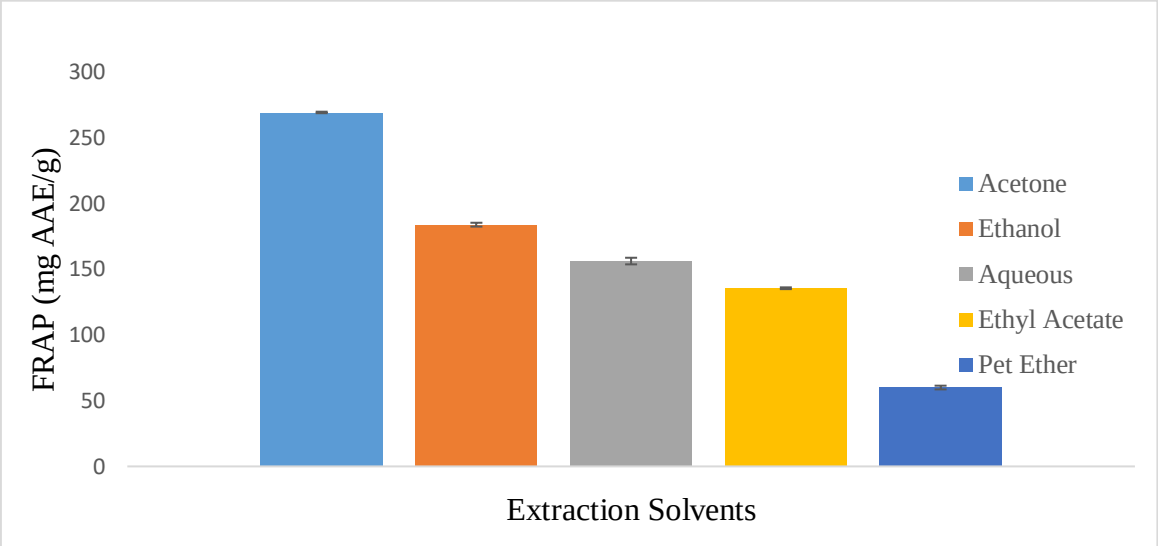

Figure 2: FRAP radical scavenging activities of the solvent extracts of *Chrysophyllum albidum* leaf

4.6.0 Antibacterial Activity of *C. albidum* Leaves Extracts

The antimicrobial activity of C. *albidum* leaves extracts were evaluated for minimum inhibitory concentration against different strains of bacteria and the results were compared with the activity of the standards. C. *albidum* leaves recorded varying inhibition zones against tested bacteria ranging from 6.0 ± 0.00 mm for *Samonella spp*. to 13.5 ± 0.50 for *P. aeruginosa* (Table 7). The results showed that acetone and ethanol extracts extract exhibited antibacterial activity against both gram-positive bacteria and gram-negative bacteria tested (*Staphylococcus aureus, Aeromonas hydrophila, E. coli, Pseudomonas aeruginosa, Salmonella sp,* and *Bacillus sp*) except aqueous and pet ether extracts that showed inhibition against only *P. aeruginosa* and ethyl acetate extract that did not inhibit any of the organisms investigated. Fig 3 showed in the bar chart the various activities recorded by the organisms against each of the solvent extracts.

Table 7: Result of Antimicrobial Activities of *Chrysophyllum albidum* Leaves Extracts against Selected Fish Pathogens (Zone of inhibitions of the extracts on selected fish pathogens)

Microorganisms	Ethanol (mm)	Acetone (mm)	Ethyl Acetate (mm)	Aqueous (mm)	Pet Ether (mm)	Positive Control (mm)	Negative Control (mm)
Staphylococcus aureus	8.0 ±0.00	6.5 ±0.50	-	-	-	23.0 ±1.00	-
Aeromonas hydrophila	8.5 ±0.50	10.0 ±0.00	-	-	-	12.0 ±0.00	-
Escherichia coli	9.5 ±0.50	8.5 ±0.50	-	-	-	-	-
P. aeruginosa	11.5±0.50	13.5 ±0.50	-	11.0 ±1.00	8.0±0.00	21.5 ±0,50	-
Salmonella spp.	6.0 ±0.00	7.0 ±0.00	-	-	-	14.0 ±0.00	-
Bacillus spp.	9.5 ±0.50	8.0 ±0.00	-	-	-	26.0 ±0.00	-

KEY: Positive Control: Streptomycin; Negative control: Di-Methyl Sulphur Oxide (DMSO)

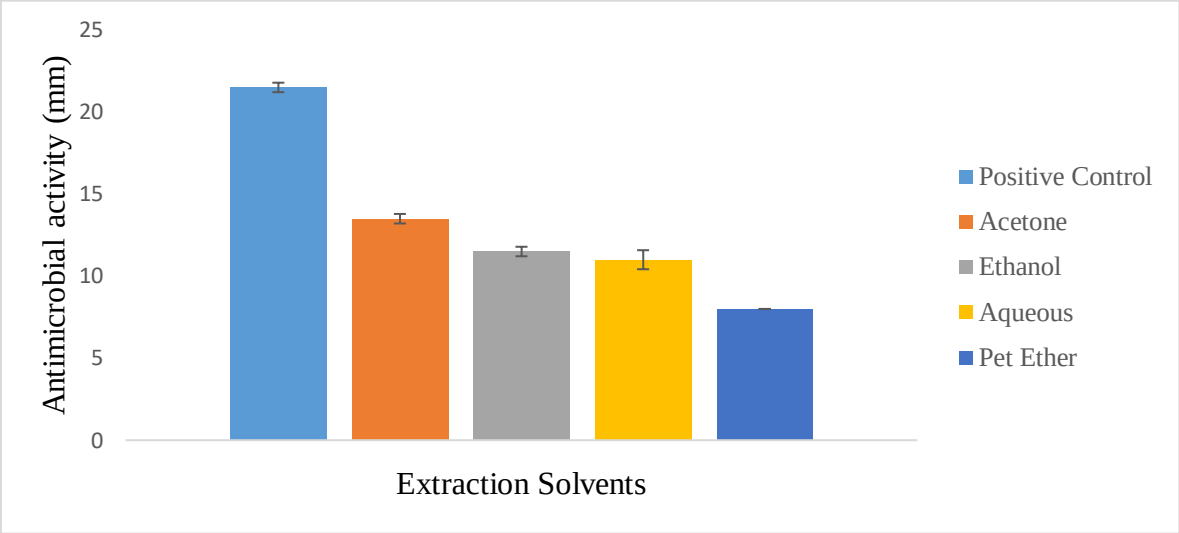

Figure 3: Antimicrobial activities of C*hrysophyllum albidum* leaf extracts against selected fish pathogens (zone of inhibitions of the extracts on selected fish pathogens)

5.0 Discussion

The yield as represented in Table 1 showed the dominance of aqueous extract followed by ethanol and acetone respectively while petroleum ether extract yield was the least. The investigated mineral content of leaf extracts of *C. albidum* revealed the presence of calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), zinc (Zn) and manganese (Mn) in various proportions as represented in Table 2. The amount of minerals in food is of interest because of their essential nature and effects on living system (Oktem *et al.*, 2005). According to Vallee and Auld (1990) iron (Fe) aids in the transport of oxygen in red blood cells and muscles. Zinc is required for the optimum functioning of many enzymes involved in catalytic functions, maintenance of structural stability, and regulatory functions (Colak *et al*., 2005). Essential metals can produce toxic effects when the metal intake is excessively high. The iron content of the extracts was low, and much of it has to be consumed before a substantial amount can be obtained. Ukana *et al*. (2012) reported that *C. albidum* peel and pulp had iron contents of 37.330 and 40.110 mg/kg respectively which were higher than the results obtained in this study. Iron is an important constituent of succinate dehydrogenase as well as a part of the heme of hemoglobin (Hb), myoglobin and cytochromes. Magnesium is an active component of several enzyme systems in which thymine pyrophosphate is a cofactor (Murray *et al*., 2000). *C. albidum* aqueous leaf extract had higher amount of magnesium as compared to the other extracts. High intake of Manganese that is above 10 mg/kg which is the recommended dose by WHO/FAO (1984) is regarded as a neurotoxic substance and the contents of the extract were within the limit. Potassium and Sodium had higher values in the aqueous extract than in all the other extracts. Ukana *et al*. (2012) reported lower sodium contents in the peel and pulp compared to the results obtained in this work. Sodium is the principal extracellular cation and is used for acid-base balance and osmoregulation in intermodular fluid (Crook, 2006).

In the phytochemical screening and quantitative estimation of the crude amount of the chemical constituents of *C. albidum* leaves studied (Tables 2 and 3), the aqueous, ethanol and acetone extracts showed the presence of saponins, tannins, alkaloids, flavonoids and phenols. Tannins are known to hasten the healing of wounds and inflamed mucous membranes (Manjulika *et al*, 2014). Flavonoids are regarded as a potent water-soluble antioxidants and free radical

scavengers, which prevent oxidative cell damage and also have strong anticancer activity (Stauth, 1993). The leaves have been shown to contain alkaloids, cardiac glycoside, anthraquinone, flavonoids, terpenoids, and steroids, which are useful substances that have medicinal and physiological activities (Sofowora 1993). Phenolic and flavonoids have been shown to have antibacterial, antiviral, anti-inflammatory, anti-allergic, antithrombotic and vasodilatory activities (Miller, 1996 and Mazur *et al*., 2005).

The plant could also be employed as a source of natural antioxidant boosters in the treatment of some oxidative stress disorders in which free radicals have been implicated (Orijajogun *et al.*, 2013). *Chrysophyllum albidum* has antioxidant properties by scavenging free radicals, decreasing lipid peroxidation and increasing the endogenous blood antioxidant enzymes level (Adebayo et al., 2010).. The presence of various active ingredients or metabolites (Ibegbulem *et al*., 2003) as revealed by the phytochemical screening (Table 3) supports the resourcefulness of the plant extracts (Sofowora, 1993). The actions of secondary plant metabolites varied when ingested by animals (Trease and Evans, 1983). For example, saponins have a positive role in cholesterol metabolism (Price *et al*., 1987). In this study, the acetone, ethyl acetate, ethanol, petroleum ether and water extracts of *C. albidum* showed zone of inhibition against the isolated fish pathogens with varying diameters. The results of the present study on the antimicrobial potentials of *Chrysophyllum albidum* leaves extracts (Table 7) against *Staphylococcus aureus, Aeromonas hydrophila, Escherichia coli, Pseudomonas aeruginosa, Salmonella spp. and Bacillus spp.* have shown that acetone and ethanol extracts inhibited the growth of all the test isolates. There is an indication that the extracts possess substances that can inhibit the growth of some micro-organisms. The phenolic compounds in *Chrysophyllum albidum* may be responsible for the therapeutic, antiseptic, antifungal or bacterial properties of the plant which agreed with the findings of Adewusi, (1997) who reported that latex or exudates from *Chrysophyllum albidum* have antimicrobial properties against S*taphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Streptococcus pyogenes and Candida albican.* However, the ability of the extracts to inhibit the growth of these organisms point to the fact that these organisms do not possess a mechanism inactivating the active ingredients in the extracts or other mechanisms which include exclusion of the substance from the cell and modification of the target site of the substance (Adegoke *et al*., 2010). The zone of inhibition exhibited by acetone and ethanol leaf extracts of *C. albidum* on all the organisms investigated in this work is of great importance because of the enormous threat these organisms pose. As noted by Okwu and Iroabuchi, 2001 and Okwu and Morah, 2017, the presence of phenolic compounds in the plant parts indicates that *C. albidium* contains antimicrobial agents. Phenolic compounds are considered to be bacteriostatic and fungistatic. Therefore, they can be used as an alternative to synthetic antibiotics as they are much cheaper and also in this case of drug resistance by these organisms as they frequently develop resistance to orthodox antibiotics (Singleton, 1999). The orthodox antibiotics are usually accompanied by contraindications while most medicinal plants are relatively free of such contraindications (Adegoke *et al.*, 2010). The potential of this plant could help in putting to rest the fear of therapeutic failure of synthetic antibiotics to some serious infectious diseases in aquaculture and could herald opportunities for animal health industries in the use of the plant material as a drug against disease pathogens.

6.0 Conclusion

This work has revealed that crude extracts of the leaves of *Chrysophyllum albidum* possessed phytoconstituents that has a rich mineral profile, radical scavenging abilities and antimicrobial properties. It can therefore be deduced that ethanol and acetone extracts exhibited better activities which may be

referred to as broad-spectrum based on their radical scavenging and antimicrobial potentials by which they were able to inhibit the growth of both gram-negative and gram-positive bacteria. The implication of these results established that *Chrysophyllum albidum* leaf extracts could be used as an easy and accessible source of natural antioxidant and antibacterial agents as a potential source of useful drugs.

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