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Comparative evaluation of chemical Profile of *Chrysophyllum albidum* seed cotyledon and leaf

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Abstract
The seed cotyledon and leaf of *Chrysophyllum albidum* were analyzed for their phytochemical and proximate compositions. The results obtained from the anaylsis of the seed cotyledon and leaves of *C. albidum* were compared. It was observed that, the *C. albidum* contain substantial amount of saponins, tannins, flavonoids, alkaloids and phenols. The alkaloids and tannins present in the seed cotyledon were higher compared with the level observed in the leaves. There was generally low percentage proximate fractions of the leaves in terms of fats, ash and fiber and lower percentage fractions of ash and fibers were observed in the seed cotyledons. Protein and carbohydrate and ash compositions of the leaves were observed to be higher compared with the compositions present in the seed cotyledon. This study has shown that *C. albidum* seed cotyledon and leave are good source of phytochemicals that are biologically important, thus they can be potential sources of useful drugs in the management of some ailments.

Keywords: Phytochemicals; proximate; leaf; seed cotyledon

Introduction
Plants and their derivatives play key role in world health and have long been known to possess biological activity. Thirty percent of all modern drugs were derived from plants (Riaz *et al*., 2010; Omoboyowa *et al*., 2013) [6, 5]. According to the World Health Organization about 80% of the world population relies essentially on plants for primary health care (Omoboyowa *et al*., 2013) [5]. There is growing interest in exploiting plants for medicinal purposes especially in Africa (Adeniyi *et al*., 2012; Amusa *et al*., 2003) [15, 1]. They are distributed in Nigeria, Uganda, Niger, Cameroon and Cote d'Ivore (Adebayo *et al*., 2010) [8]. It is often called the white star apple and distributed throughout the southern part of Nigeria (Idowu *et al*., 2006) [11]. Across Nigeria, it is known by several local names and is generally regarded as a plant with diverse ethno-medicinal uses (Amusa *et al*., 2003) [15]. In South-Western Nigeria, the fruit is called "Agbalumo" and know as "Udara" in South-Eastern Nigeria. The bark is used for treatment of malaria and yellow fever, while the leaf is bused as an emollient and for the treatment of skin eruption, stomach ache and diarrhea (Idowu *et al*., 2006) [11]. The cotyledons from the seeds of *Chrysophyllum albidum* are used as ointments in the treatment of vaginal and dermatological infections in Western Nigeria.
The fruit pulp is rich in Vitamin C iron and excellent source of raw material for industries (Akubugwo and Ugbogu, 2007) [9]. Tannins, flavonoids, proteins, carbohydrates and resins are the phytochemicals that have been reported in *Chrysophyllum albidum* (Akaneme 2003) [16]. Eleagrine, tetrahydro-2-methyl Harman and Skatole have been isolated from the plant and Eleagrine was the main compound responsible for it's antimicrobial activity (Idowu *et al*., 2006) [11]. The seed cotyledon has been reported to possess antihyperglycemic and hypolopidemic effects (Olorunnisola *et al*., 2003) [13]. Thus, the study investigated the chemical compositions of the seed cotyledon and leaf samples of *Chrysophyllum albidum*

Materials and Methods
Plant material
The fresh leaves and seed cotyledon of *Chrysophyllum Albidum* (Africa star Apple) was collected from Unwana environment, Afikpo North Local Government Area of Ebonyi State. It was identified by taxonomist at the Department of Botany, University of Nigeria, Nsukka.
The seed cotyledons were removed from the seeds with the aid of a sterilized knife, the cotyledon was cleaned, air dried and carefully ground into a coarse form by the use of a mechanical blender. The leaves was plucked with a clean cutlass, air dried at room temperature, ground with a mechanical blender into a coarse form.

Preparation of fat free sample
Two grams (2 g) of the samples were defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 hours.

Phytochemical Analysis
Phytochemical analysis
Chemical tests were carried out on the samples for the quantitative determination of phytochemical constituents.

Alkaloid determination
The alkaloid content was determined gravimetrically. Five grams of the sample were weighed into a 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added and covered to stand for 4 h. This was filtered and the extract was concentrated using a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration using Whatman filter paper No. 4 (125 mm) and weighed (Obadoni and Ochuko, 2001) [3].

Saponin determination
Saponin content was determined using the method described by Obadoni and Ochuko, (2001) [3]. Twenty grams (20 g) of each ground samples were dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55 ºC. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 ºC. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The mixture of n-butanol and extracts was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath at about 90 ºC. The samples were dried in an oven at 100 ºC until a constant weight was obtained. The saponin content was calculated in percentage (Obadoni and Ochuko, 2001) [3].

Tannin determination
Tannin content was determined going by the method described by Van-Burden and Robinson (1981) [7]. Five hundred miligrams of the sample was weighed into 100 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipette out into a tube and mixed with 3 ml of 0.1M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120 nm wavelength, within 10 min. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured (Van-Burden and Robinson, 1981) [7].

Flavonoid determination
Flavonoid content was determined using the method described by Boham and Kocipai (1994) [17]. Ten grams of the ground samples were extracted repeatedly with 300 ml of methanol: water (80:20) at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed (Boham and Kocipai, 1994) [17].

Determination of total phenols
Total phenols were determined using the method described by Obadoni and Ochuko (2001) [3]. For the extraction of phenolic component, the fat free sample was boiled with 50 ml of ether for 15 min. 5 ml of the extract was pipette into a 50 ml volumetric flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. The absorbance of the solution was read using a spectrophotometer at 505 nm wavelengths (Obadoni and Ochuko, 2001) [3].

Proximate Composition Analysis
This was carried out according to the method of AOAC (1990) [2]

Moisture Content Determination
Two grams of each of the sample was weighed into dried weighed crucible. The samples was put into a moisture extraction oven at 105 ^{0}C and heated for 3 hours. The dried samples was put into desiccators, allowed to cool and reweighed. The process was reported until constant weight was obtained the difference in weight was calculated as a percentage of the original sample

$$\text{Percentage moisture} = \frac{W_2 - W_1}{W_2 - W_3} \times \frac{100}{1}$$

Where
W_1 = Initial weight of empty dish
W_2 = Weight of dish + Un-dried sample
W_3 = Weight of dish + dried sample

Ash Content Determination
Two grams of each of the samples was weighed into crucible, heated in a moisture extraction oven for 3hour at 100 ^{0}C before being transferred into a muffle furnace at 550 ^{0}C until it turned white and free of carbon. The sample was then removed from the furnace, cooled in a desicator to a room temperature and reweighed immediately. The weight of the residual ash was then calculated as

Ash Content

$$\frac{\text{Percentage Ash} = \text{Weight of Ash}}{\text{Weight of original of sample}} \times \frac{100}{1}$$

Crude Protein Determination
The micro kjeldahl method described by A.O.A.C (1990) was used. Two grams of each of the sample was mixed with 10ml of concentrated H_2SO_4 in a heating tube. One table of selenium catalysts was added to the tube and mixture heated inside a fume cupboard. The digest was transferred into distilled water. Ten millimeter portion of the digest mixed with equal volume of 45% NaOH. Solution and poured into a kjeldahl distillation apparatus. The mixture was distilled and the distilled collected into 4% boric acid solution containing 3 drops of methyl red indicator. A total of 50ml distillate was collected and titrated as well. The sample was duplicated and the average value taken.

The nitrogen content was calculated and multiplied with 6.25 to obtain the crude protein content. This is given as percentage Nitrogen

$$= \frac{(100 \times N \times 14 \times VF)\ T}{100 \times Va}$$

Where
N = Normality of the titrate (0.1N)
VF = Total volume of the digest = 100ml
T = Titre value
Va = Aliquot volume distilled

Crude Fiber Determination

Two grams (2g) sample and 1g asbestos were put into 200ml of 1.25% of H_2SO_4 and boiled for 30 minutes. The solution and content then poured into Buchner funnel equipped with muslin cloth and secured with elastic band. This was allowed to filter and residue was then put into 200ml boiled NaOH and boiling continued for 30 minutes, then transferred to the Buchner funnel and filtered. It was then washed twice with alcohol. The material obtained washed thrice with petroleum ether. The residue obtained was put in a clean dry crucible and dried in the moisture extraction oven to a constant weight. The dried crucible was removed, cooled and weighed. Then, difference of weight (i.e. loss in ignition) is recorded as crucible fiber and expressed in percentage crude fiber,

$$= \frac{W_1 - W_2}{W_3} \times \frac{100}{1}$$

Where
W_1 = Weight of sample before incineration
W_2 = Weight of sample after incineration
W_3 = Weight of original sample

Fat Content Determination

Two grams of the sample was loosely wrapped with a filter paper and put into the thimble which was filled to a clean round bottom flask, which has been cleaned, dried and weighed. The flask contained 120ml of petroleum ether. The sample was heated with a heating mantle and allowed to reflux for 5hours. The heating was then stopped and the thimbles with the spent samples kept and later weighed. The difference in weight was received as mass of fat and is expressed in percentage of the sample. The percentage oil content is percentage fat

$$= \frac{W_2 - W_1}{W_3} \times \frac{100}{1}$$

Where
W_1 = Weight of the empty extraction flask
W_2 = Weight of the flask and oil extracted
W_3 = Weight of the sample

Carbohydrate Content Determination

The nitrogen free method described by A.O.A.C (1990) was used. The carbohydrate is calculated as weight by difference between 100 and the summation of other proximate parameters as Nitrogen Free Extract (NFE) percentage carbohydrate
(NFE) = $100 - (M + P + F_1 + A + F_2)$

Where
M = moisture
P = protein
F_1 = fat
A = ash
F_2 = crude fiber.

Statistical analysis

Three analytical determinations were carried out on each independent replication for every parameter. Three independent replicates (n = 3) were obtained from each treatment and the results presented in tables and are reported as means ± standard error of mean (SEM). Data were analysed by t-test (P<0.05).

Results

Table 1 summarizes the quantitative determination of phytochemical constituents of *C. albidum* seed cotyledon and *C. albidum* leaf. The result reveals that both plants contain substantial amount of saponnins, tannins, flavonoids and phenol. The concentration of alkaloids present in the *C. albidum* seed cotyledon was higher compared with the level observed in *C. albidum* leaf.

Table: 1: Phytochemical composition of methanol extract of *C. albidum* (udara) seed cotyledon extract and leaf extract.

Phytochemical Composition	*C. albudium* seed Cotyledon (mg/100g)	*C. albidum* Leaf (mg/100g)
Saponnins	0.20 ± 0.002	0.028 ± 0.003
Tannins	127.77 ± 0.002	135.55 ± 0.003
Akaloids	226.96 ± 0.002	513.48 ± 0.002
Flavonoids	106.46 ± 0.04	112.60 ± 0.002
Phenol	385.81 ± 0.00	222.56 ± 0.003

Date represented in Mean ± SEM

The result of proximate analysis of the seed of *C. albidum* and leaf were presented in table 2. There was generally low percentage proximate fractions of *C. albidum* seed cotyledon in terms ash fibre and fats and low percentage fractions of ash, and fiber were observed in the leaf. Protein and fats composition of *C. albidum* leaf were observed to be significantly higher compared with the compositions present in *C. albidum* seed cotyledon while the percentage moisture and fats composition in seed cotyledon of *C. albidum* were revealed to be significantly higher compared with the concentration of these proximate compounds in *C. albidum* were revealed to be significantly higher with the concentration of these proximate compounds in *C. albidum* leaf (table 2).

Table 2: Proximate composition of methanol extract of *C. albidum* (udara) seed cotyledon extract and leaf extract.

Proximate Composition	*C. albudium* seed Cotyledon (%)	*C. albidum* Leaf (%)
Proteins	13.14 ± 0.002	7.45 ± 0.002
Ash	2.62 ± 0.003	2.18 ± 0.002
Fat	0.82 ± 0.002	3.42 ± 0.002
Fibre	2.96 ± 0.01	3.42 ± 0.002
Moisture	9.39 ± 0.002	47.68 ± 0.39
Carbohydrate	71.40 ± 0.19	36.82 ± 0.01

Date represented in Mean ± SEM

Discussion

The bioactive components observed to be present in both the seed cotyledon and leaf of *C. albidum* are known to exhibit medicinal as well as physiological activity (Adeniyi *et al*; 2012) [1]. The presence of alkaloid may be toxic but are chemical element in the cotyledon of seed, used as a remedy for fever, while the leaf is used as emollients and for the treatment of skin eruptions, diarrhea and stomachache reactions as stated by Adisa (2000). This confirms the efficacy of the seed against vaginal and dermatological infections (Idowu *et al.,* 2006) [11]. This further explains the therapeutic and medicinal properties of *C. albidum* and support the use of this plant as an external application for skin eruptions diseases.

The value of flavonoids was higher in the seed cotyledon (112.575mg/100g) when compared with the leaf (106.459mg/100) flavonoids are potent water soluble super antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and inhibit tumor growth (Stauth, 1993) [18] Flavonoids has been shown to inhibit the development of fluids that result in diarrhea by targeting the intestinal cystic fibrosis trans-membrane conductance regulator (Schuier *et al* ; 2005) [14]. Flavonoids in intestinal tracts lower the risk of heart disease (Okwu, 2004) [4]. The biological functions of flavonoids include protection against allergies, inflammatory, free radical scavenging, platelets aggregation, microbes, ulcers, hepatoxins, viruses and coronary heart disease (Burits and Bucar, 2002) [10]. Hence, people that are prone to such infection can feed on *C. albidum* fruit as a source of natural antioxidants.

The presence of tannins in seed cotyledon and lave also been reported by other researchers, and this plant parts has anti-inflammatory effect which help control all indications of gastritis, esophagttis, enteritis and irritating bowel disorders (Dharmananda, 2001) [19].

The saponin content was high in the leave (0.1995mg/100g-1) compared with the seed cotyledon (0.025mg/100g). The high saponin content of *C. albidum* leaves justifies the use of the extracts to control human cardiovascular disease and reduce blood cholesterol as documented by Aletor (1993) [20]. The phenolic content was higher in the leave (385.80mg /*100*) but the seed cotyledon (222.554$\pm$0.0025). The presence of phenolic compounds in the plant parts indicates that *C. albidum* contain antimicrobial agents.

Evaluation of the potentials of *C. albidum* in wound care showed that the cotyledon extract exhibited haemostatic, antimicrobial and wound healing activities (Faleyimu and Oluwalana, 2008) [21]. The cotyledon extract mixed with shear butter oil arrest bleeding from fresh wounds by reducing bleeding and blood clotting time. The haemostatic effect may be due to increase in the coagulation process with the consequence reduction in clotting time as well as vasoconstriction which are necessary in limiting blood loss from damage vessels. The phytochemical may contribute to the wound healing activity by supposing inflammatory reaction involved by tissues (Lotito *et al.,* 2006) [22].

Conclusion

This study has shown that *C. albidum* seed cotyledon and leaves are good source of phytochemicals that are biologically important, thus they can be potential source of useful herbs in the management of some ailments.

Also, this plant (*C. albidum*) contains appreciable level of proximate compounds, vitamins and minerals that are readily available; they could be consumed to supplement the source or non-available sources of nutrient. The extracts of the seed cotyledon and leaves have good potentials as anti-inflammatory, anti-diarrheal and anti-hemorrhoids compound and further provide a rationale for the use of the seed extracts of this plant (*C. albidum*) in traditional medicine practice in Nigeria.

The food value of *C. albidum* fruit is not very high; however, the fruit is very high in antioxidants. The total phenols and flavonoids concentrations are much stronger in antioxidants capacities.

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