

Yield Potentials of *Chrysophyllum albidum* G. Don Seed Oil for Therapeutic and Industrial Uses

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Abstract

This study determined the extractable yield, phytochemical constituents and physicochemical properties of *Chrysophyllum albidum* seed oil. Seeds of *Chrysophyllum albidum* were collected from two locations, air-dried, milled and cold macerated. The seed oils were extracted and purified using activated charcoal before qualitative screening to identify their phytochemical contents. The physicochemical properties were quantitatively determined. Cold maceration resulted in low seed oil yield. The phytochemical constituents included alkaloids, anthraquinones, terpenoids and cardiac glycosides, while tannins and flavonoids were absent. Physicochemical properties included: acid value (6.31 – 8.10 mg KOH/g), saponification value (254.70 – 356.60 mg KOH/g), iodine value (65.30 – 68.30 mg/g), ester value (247.50 – 348.50), pH (2.93 – 4.04), relative density (0.787 – 0.874 g/cm³) and refractive index at 28°C (1.45 – 1.46). There were slight variations in the yield and physicochemical constituents of the seed oils from the two locations, which exhibited non-drying characteristics.

Keywords: Acid value, Activated charcoal, Ester value, Seed oil, Phytochemicals

Introduction

Tropical forests are enriched with numerous fruit trees that are good sources of vitamins, antioxidants, and food supplements, but these fruits are underutilised in many parts of Africa (1, 2). One of such fruit species is *Chrysophyllum albidum* G. Don, whose fleshy parts are consumed and the seeds discarded (3). However, it has been documented that seed oil plant such as *Chrysophyllum albidum* have great potentials in the development of products in the food, cosmetics and pharmaceutical industries (1, 4, 5). *Chrysophyllum albidum* (family Sapotaceae) is a tropical, perennial crop, attributed to the rain forests and the coastal regions of tropical Africa (6, 7, 8). It is one of the indigenous tree species used in agroforestry farms because it provides non-timber forest products (NTFPs) for

household consumption as well as for local, regional, and international trade (9). It is commonly known as the ‘white star apple’ and grows to heights of 25 – 37 m with a mature girth ranging from 1.5 - 2.0 m (5, 10). In Nigeria, it is naturally distributed in the southwestern and southeastern parts and locally known as Agbalumo (Yoruba), Udara (Igbo) and Agwaluma (Hausa-Fulani) (11, 12). The importance of *C. albidum* for local community livelihood improvement and food security have been reported in previous studies (13, 15, 16, 17). The seed oil of *C. albidum* has been identified as a potential raw material that could be valuable in developing countries of Africa (5). Hence, with the therapeutic qualities of *C. albidum* already established, more research is required to ascertain inherent properties of the seed oil.

Therefore, this study was designed to determine the extractable yield, phytochemical constituents and physicochemical properties of seed oil of *C. albidum* fruits obtained from two locations in Oyo state, Nigeria.

Materials and Methods

Experimental Site and Sample Collection

The study was carried out at the Biomedical Research Centre, Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan and the Pharmaceutical Chemistry Laboratory, University of Ibadan, Nigeria.

Fruits of *C. albidum* were collected from a mother tree within the Forestry Research Institute of Nigeria (FRIN) (latitude 7°26'11"N and longitude 3°54'69"E) and from Akesan market in Oyo town (latitude 7°51'3"N and longitude 3°55'53"E). The fruits were depulped to obtain the seeds. Thereafter, the seeds were cracked open to extract the seed kernels. The kernels were air-dried to a constant weight and then oven-dried at 50°C for 24 hours. This ensured a standard moisture level that protected the kernels from mycotoxins. The dried kernels were then milled to a fine powder in order to achieve maximum extraction rate.

Seed Oil Extraction

The milled seed kernels of *C. albidum* were cold macerated with analytical grade N-Hexane (99%). Milled sample (130 g) was placed in a glass container, and five-fold excess of analytical grade N-Hexane was added and the container was stirred vigorously. The mixture was allowed to stand for 72 hours with constant agitation

every 2 hours. The filtrates were concentrated to separate the N-Hexane from the extracted oil *in vacuo* at 35°C. Further concentration was done using a vacuum oven at 30°C and 600 mmHg to ensure absolute removal of the solvent of extraction from the oils. The percentage yields of the oils were then determined (18).

Purification of Seed Oils

Decolourization is one of the major processes when refining fats and oils. The process is designed to remove a wide range of impurities and pigments from crude fats and oils for both commercial and health reasons (19). The seed oils were purified using activated charcoal following a standard analytical procedure (20).

The oil samples (10% v/v) were prepared using hexane to make 100 mL. Activated charcoal powder (30g) was packed into the column with the aid of a vacuum pump. The prepared oil solution was then run through the activated charcoal, the effluent collected, and then concentrated *in vacuo* at 35°C. This was further concentrated using a vacuum at 40°C and 600 mmHg. The percentage yield was then calculated.

Qualitative Phytochemical Screening

The oils were qualitatively screened to ascertain the phytochemicals present in them, following the methods below (21 and 22).

Anthraquinones

Seed oil (0.5 mL) was boiled with 10 mL of concentrated H₂SO₄ and filtered while hot. The filtrate was shaken with 5mL of chloroform. The chloroform layer was pipetted into another clean test tube and 1mL of dilute ammonia (10%) solution was added. The resulting solution was observed

for colour changes at the Ammoniacal phase (pink colouration indicated the presence of anthraquinones).

Terpenoids (Salkowski test)

Seed oil sample (0.5 mL) was added to 2 mL of chloroform and 3 mL of concentrated H₂SO₄ was carefully added to form a layer. A reddish-brown colouration at the interface indicated the presence of terpenoids.

Flavonoids

Three methods were used to test for the presence of flavonoids. First, diluted ammonia (5 mL) was added to 2 mL of an aqueous filtrate of the seed oil, then concentrated H₂SO₄ (1 mL) was added. A yellow colouration that disappears on standing indicated the presence of flavonoids. Second, 0.5 mL of 1% Aluminium solution was added to 2 mL of the filtrate. A yellow colouration indicated the presence of flavonoids. Third, 1 g of the sample was heated with 10 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute Ammonia solution. A yellow colouration indicated the presence of flavonoids.

Saponins

Seed oil (5 mL) was added to 5 mL of distilled water in a test tube and heated on a water bath. The solution was shaken vigorously and observed for stable persistent frothing. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Tannins

Seed oil (0.5 mL) was boiled in 10 mL of distilled water in a test tube and then filtered. A few drops (0.5 mL) of 0.1% ferric chloride was added and the mixture was observed for brownish green or a blue-black colouration.

Alkaloids

Seed oil (0.5 mL) was diluted to 10 mL with acid alcohol, boiled, and filtered. Then, 5 mL of the filtrate was added to 2 mL of diluted ammonia and 5 mL of chloroform. The solution was gently shaken to extract the alkaloidal base. The chloroform layer was extracted with 10 mL of acetic acid. The solution was divided into two portions. Mayer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of creamy (with Mayer's reagent) or reddish-brown precipitate (with Dragendorff's reagent) was regarded as a positive indication of the presence of alkaloids.

Cardiac Glycosides (Keller-Killiani test)

The seed oil (0.5 mL) was diluted to 5 mL in water and glacial acetic acid (2 mL) containing one drop of ferric chloride solution was added. This was under laid with 1 mL of concentrated H₂SO₄. A brown ring at the interface indicated the presence of a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Determination of Physicochemical Properties

Selected physicochemical properties of the seed oils were determined using standard procedures of AOAC (18).

Determination of Acid Value

Twenty five milliliters (25 mL) of alcohol (95%), ether (25 mL) and phenolphthalein (1 mL) solutions were mixed and neutralized by adding 0.5 mL of dilute alkali drop until a pale pink colouration was obtained. The seed oil samples (10 g) were accurately weighed into different conical flask (250 mL) and the prepared solvent was added. After the seed oil had completely dissolved, it was then titrated against the prepared 0.1 M aqueous KOH, shaking constantly until the pink colour that persists for 15 seconds was obtained. The number of mL required was noted. The determination was duplicated and the acid value was calculated using the Eqn. i:

$$\text{Acid Value} = 56.1V \times \frac{N}{W} \dots \dots \text{Eqn. (i)}$$

Where; V = Volume in mL of standard potassium hydroxide or sodium hydroxide used, N = Normality of the KOH or NaOH solution, W = Weight in gram of the sample, 56.1= mg of KOH contained in 1 mL of a 0.1 M solution

Determination of Saponification Value

The seed oil samples (2.0g) were weighed into a 250 mL quick fit flask and exactly 25.0 mL of approximately 1 M alcoholic KOH was added using a burette. A reflux condenser was attached and the mixture was refluxed for 1 hour on a water-bath, swirling the contents, frequently. The flask was then removed from the water-bath, and

5 mL of phenolphthalein solution was poured down the condenser (in order to wash the latter without diluting the contents of the flask) and the flask was allowed to cool for 5 minutes under the tap water. The mixture was then titrated against 0.5 M HCl. A blank was prepared under similar conditions. The number of mL of 0.5 M HCl required was noted. The test was replicated and the SV was calculated using Eqn ii:

$$SV = (A - B) \times N \times \frac{56.1}{W} \dots \dots \text{Eqn (ii)}$$

Where; A = 0.5 M HCl for blank (mL), B = 0.5 M HCl for sample (mL), W = weight of sample (dry basis) (g), N = normality HCl solution, 56.1 = equivalent weight of Potassium Hydroxide

Determination of Iodine Value

The iodine value of the seed oil was determined using the method described by (23). The oil samples (0.17 g) were weighed into a dry 500 mL iodine flask and 10 mL of carbon tetrachloride was added. After oil dissolution, 20.0 mL of 0.2 M iodine monochloride solution was added using a burette, and a stopper moistened with KI solution was inserted, the mixture was allowed to stand in the dark for 30 minutes at a temperature of 15 – 25°C. The stopper was partly removed and 15 mL of 10% w/v KI solution was poured over the stopper into the flask. Distilled water (100 mL) was also added in the same way. The stopper was re-inserted and the flask was shaken vigorously. The content of the flask was then titrated with 0.1 M Sodium thiosulphate solution, using starch mucilage as the indicator (added towards the end-point). The same procedure was

used to titrate a blank solution without the oil sample. The procedure was duplicated and iodine values were calculated using Eqn. iii:

$$\text{Iodine value} = (B - S) \times N \times 12.69 / W \dots$$

Eqn. (iii)

Where; B = the volume of Sodium thiosulphate required for the blank (mL), S = the volume of Sodium thiosulphate required for the sample (mL), N = Normality of Sodium thiosulphate solution (Eq/L), 12.69 = conversion factor from mEq sodium thiosulphate to grams of iodine, W = weight of the sample (g)

Ester Value

This is the number of mg of KOH required to neutralize the fatty acid obtained solely by hydrolysis of the glycerides contained in 1 g of the substance. It is therefore, the difference between saponification value (SV) and the acid value (AV) obtained for each of the seed oils;

$$\text{Ester value} = SV - AV \dots \dots \dots \text{Eqn (iv)}$$

Determination of pH Value

The pH is the value characteristics of an aqueous solution which represents conventionally its acidity or alkalinity. The pH meter was used to determine the pH values of the different seed oil samples by immersing the pH electrode into the oils. The potential differences between the electrodes immersed in standard as well as the oils were taken as the pH values.

Determination of Relative Density

To determining the relative density of the seed oils, a 10 mL relative density bottle was used. The weight of the dried, clean empty relative density bottle (10 mL) was taken (W_0), the bottle was then filled with distilled water to the graduated mark and the weight recorded (W_1). The bottle was dried using acetone and then filled with the oil to the graduated mark and the weight taken (W_2). The relative density of the seed oils were calculated using Eqn. v:

$$\text{Relative density} = \frac{(W_2 - W_0)}{(W_1 - W_0)} \dots \text{Eqn. (v)}$$

Where, W_0 = weight of empty relative density bottle (g), W_1 = weight of water + relative density bottle (g), W_2 = weight of test sample + relative density bottle (g).

Determination of Refractive Index

An Abbey refractometer was used to determine the refractive index of the seed oils. Three (3) drops of the seed oil were dropped on the prism of the refractometer, which was then closed, adjusted and viewed through the eye-piece and the refractive index was recorded.

Results and Discussion

Seeds obtained from FRIN had a higher oil yield ($1.50 \pm 0.02\%$) than those from Oyo ($1.10 \pm 0.01\%$). This could be due to the fact that fruits from Oyo were harvested some days before purchase from the market and they were a mix of fruits from different trees. Whereas, fruits from FRIN were freshly harvested from one parent tree and processed immediately. This corroborates previous work on olive oil that mentioned that time of harvesting before processing

affected oil yield (24). The yield was low when compared with previous studies on the same species (6, 7, 18, 24, 25, 26). This implies that large quantities of *C. albidum* seeds would be required to obtain an appreciable amount of oil, if the cold maceration method is used. In addition, the particle size may have affected the yield, because it has been previously confirmed that larger particle sizes (500 µm) of the powdered seed kernel were more suitable for solid-liquid extraction of *C. albidum* seeds oil (24). However, the milled samples used in this study were finely blended (200 µm). The crude seed oil had a dark brown colour and a pleasant odour, while the purified oil revealed a higher amount of impurities in the seeds from Oyo (Table 1). The dark brown colour and pleasant sweet

odour agrees with the findings of Umaru *et al.* (6). However, the purified oils had a colourless appearance and were not denatured.

The qualitative screening revealed that terpenoids, anthraquinones, alkaloids, saponins and cardiac glycosides were present in the seed oils, while tannins and flavonoids were absent (Table 2). The aqueous seed extract of *C. albidum* has been shown to have antibacterial and antifungal actions probably due to the presence of phytochemicals (27, 28). The secondary metabolites in the seed oils were similar to those found in the leaves and fruits of *C. albidum* (29, 30, 31). The presence of saponins in the seed oil from FRIN suggest the presence of anthelmintic properties in the oil (32).

Table 1: Yield and colour of seed oils of *Chrysophyllum albidum* obtained from two sources

Sources	Weight of Milled Seed kernel (g)	Volume obtained (mL)	Percentage Yield (w/w)	Colour
Crude Seed Oil Extracts				
FRIN	1130	16.95	1.50±0.02	Dark brown
Oyo	1130	12.43	1.10±0.01	Dark brown
Purified Seed Oil Extracts				
FRIN	10	9	90.00±0.05	Colourless
Oyo	10	8.85	88.50±0.03	Colourless

± Standard Deviation (SD)

The acid value which determines the freshness and edibility of the oil was highest (9.45 mg KOH/g) in crude seed oil from Oyo, and least in the purified seed oil from FRIN (Table 3). The AV was above the permissible level for edible oils (<0.6 mgKOH/g) (33). Moreover, the AV was higher than those previously reported for the same species (25, 26). Although, it was lower than that of *Landolphia owariensis* (15.33 mg KOH/g) and palm kernel oil

(14.04 mg KOH/g) (26). The high AV could limit its potential use as an edible oil. The highest saponification value (356.60 mg KOH/g) was obtained in the crude seed oil from FRIN, while the least was from purified seed oil from Oyo (254.70 mg KOH/g). The results revealed that *C. albidum* seed oil from the two sources had high saponification values but low molecular weight of fatty acids in the oil fractions (34).

Table 2: Phytochemical composition of *Chrysophyllum albidum* seed oil

Constituents	FRIN	Oyo
Anthraquinones	+	+
Alkaloids	+	+
Cardiac glycosides	+	+
Flavonoids	-	-
Saponins	+	-
Tannins	-	-
Terpenoids	++	++

Interpretation: ++ = Abundant, + = Present, - = Absent

The high SV of the crude *C. albidum* oil (FRIN and Oyo) connotes a high amount of triacylglycerol congruous with the high ester value, which confirmed their soap-making potentials (35). However, the saponification values of the seed oils were higher than the permissible SV stipulated by the Codex Alimentarius (250-260 mg KOH/g of oil), except for the purified *C. albidum* oil from Oyo (36).

Iodine value which indicates the degree of unsaturation in fats and oils was highest (68.30 mg/g) in purified seed oil from Oyo and least (65.30 mg/g) in crude seed oil from FRIN (Table 3). The standard limit of IV specified by (37) ranges from 6.3 - 10.6 mg/g. The *C. albidum* seed oils had very high IV (65.30 – 68.30 mg/g) when compared with the Codex standard. This indicates a greater degree of unsaturation and low rancidity in the seed oils. Hence, the *C. albidum* seed oil may be classified as non-drying oils (IV <100 mg/g), suitable for industrial uses (soap making, lubricating oils, and candles) (18, 38).

Highest EV (348.50) was recorded in the seed oil from FRIN, while the least

(247.50) was obtained for the purified seed oil from Oyo. The high ester values in the crude seed oils indicated the low molecular weight of the fatty acid content (39, 40).

The pH which is an important characteristics for determining the quality and shelf life of oils ranged from slightly acidic to acidic. Therefore, *Chrysophyllum albidum* seed oil may be stored for extended periods. Low acidity or high alkalinity makes oils to be susceptible to microorganisms and subsequent degradation (41). The crude seed oil from FRIN had a higher relative density (0.874 g/cm³) compared to that from Oyo (0.787 g/cm³). The relative densities of the seed oils were less than that of water and similar to the bulk and tapped density of *C. albidum* fruit exudates reported by Eichie *et al.* (41). The refractive index which reveals the risk of oxidation, spoiling and rancidity did not differ among the seed oils. All the seed oils were within the allowed refractive index range of 1.4620–1.4640 (42, 43).

Conclusion

Chrysophyllum albidum seed had a low extractable oil yield. Apart from the absence of saponins in seeds from Oyo, all other variables had similar characteristics. Hence, the presence of phytochemicals and the physicochemical properties of the seed oil highlight its potentials in pharmaceutical, cosmetic and manufacturing industries. This portends opportunities for future product development and sustainable use of this multipurpose tree species.

Table 4: Physicochemical parameters of crude and purified oils of *Chrysophyllum albidum* seeds from two locations

Parameters		<i>C. albidum</i> crude oil (FRIN)	<i>C. albidum</i> crude oil (Oyo)	<i>C. albidum</i> purified oil (FRIN)	<i>C. albidum</i> purified oil (Oyo)
Acid value (mgKOH/g)		8.10	9.45	6.31*	7.20
Saponification value (mgKOH/g of oil)		356.6*	339.70*	288.70	254.70
Iodine value (mg/g)		65.30	65.50	66.80	68.30*
Ester value		348.5*	330.2*	282.39	247.50
pH value		3.91*	2.93*	4.76	4.04
Relative density (g/cm ³)		0.874	0.787*	0.882	0.826
Refractive index @ 28°C		1.4596	1.4597	1.4590*	1.4591

*There is significant difference between the treatments @ P<0.05 using ANOVA

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