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Exploring the Antimicrobial Activities of *Chrysophyllum albidum* Seed Oil in Pharmaceutical and the Seeds in Food Industry

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Abstract: The anti- microbial activity tests of the seed oil, NaOH and ash soaps of the seed oil, of C. *albidum* were carried out to determine their effectiveness against gram positive, gram negative bacteria and some fungi strains. The proximate analysis of C. *albidum* seeds was performed to determine its nutritional benefits to mankind. Agar diffusion tests using Disc diffusion for the pure oils and soaps while the cup plate test method for dilutions was used in the determination of in-vitro activities. Standard methods were used for proximate determinations. The results showed the activity of the oil against *Klebsiella pneumonia* at a concentration as low as 10 mg/mL. The ash-soap of the leaves shows the highest antimicrobial activities because its MIC is the closest to the standard, Streptomycin. It possesses activity against *Escherichia coli* and *Klebsiella pneumonia* at a concentration as low as 5 mg/mL. The % proximate analysis composition shows Moisture content, Ash content, Crude Fibre, Fat/Oil, Protein, Carbohydrates as follows (14.98, 1.90, 2.09, 2.38, 12.14 and 67.65) respectively. The study concluded by unleashing the great potential of *Chrysophyllum albidum* seeds as a useful raw material in food, cosmetics and pharmaceutical industries rather than being regarded as waste in the environment.

Keywords: Antimicrobial activity, Proximate analysis**,** *Chrysophyllum albidum* seeds, Soaps, Seed oil

1. INTRODUCTION

The burgeoning applications of seed oils have become one of the most vital agro – industrial activities in today's global economy. Oils from seeds and animals are important materials in the formulation of drugs, soap, food and cosmetics [1] and also important as raw material in biodiesel production. The common seed oils which have been developed for maximum production of oil are groundnuts, soya beans, coconut, cotton seeds, oil palm and sunflower [2]. There are yet unexploited and under-utilized seeds which could serve as important source of oil for food and industrial purpose. One of such is the African star apple (*Chrysophyllum albidum*) seeds.

Chrysophyllum albidum has been classified as a wild uncultivated fruit tree which occurs naturally in the high forests or bushes and seldom planted as a fruit tree. Boys, girls, women and men, generally pick the fruits from the wild forest for consumption and sales. *C. albidum* fruit is a great source of economic empowerment to rural dwellers. The roots and leaves are used for medicinal purposes [3], while the seeds are used for local games, ointments, treatment of vaginal and dermatological infections in western Nigeria. The seed extracts are used to arrest bleeding from fresh wounds, and to inhibit microbial growth of known wound contaminants and to enhance wound healing process. The seeds contain phenols, have anti-oxidant activities, sugar lowering and cholesterol reducing effects. The fruits are not only consumed fresh but also used to produce stewed fruit, manmade syrup and several types of soft drinks [4].

Chrysophyllum albidum seeds are brownish black in colour, obliquely ellipsoid to obovoid, up to 2.8 cm long and 1.2 cm wide; its coat is hard, bony, shiny and dark brown and when broken reveals white or coloured cotyledons [5]. The seed of *Chrysophyllum albidum* has been reported to be one of the nonconventional and underexploited oilseeds [6]. There had been few reports about the seed extracts and the seed oil from *C. albidum*. The reports available were majorly on antimicrobial activities of seed extract of *C. albidum* , there is little or no information regarding the microbial activities of the seed oil. Although, physical characterization had been carried out on the seed oil, but not much is seen regarding the antimicrobial activities of the seed oil. Duyilemi and co-worker 2012, reported that the aqueous seed extract of *C. albidum* only showed inhibition against *Candida albican* [7]. However, the study carried out by George et al., 2018 reported the inhibition of *S. aureus, S. Aureus* (MRSA) and *S. Aureus* ATCC 25923*, E. coli* (ESBL), *E. coli, Shigella flexneri, Klebsiella pneumonia* and *Candida albicans* to the seed extract of *Chrysophyllum albidum* [8].

The leaves are used as emollients for the treatment of skin eruptions, diarrhoea and stomach aches which are as a result of infections and/or inflammatory reactions [9]. It also has great medicinal benefits which include lowering blood cholesterol, reducing the rate of sugar uptake as well as detoxifying actions. The sweet fleshy fruit is an excellent source of nutrients such as vitamin C, iron and it is used as a thickener for flour, also used as raw material for some manufacturing industries [10]. The plant could be employed as a source of natural antioxidant boosters in the treatment of some oxidative stress disorders in which free radicals have been implicated [11].

Proximate and nutrient analysis of edible fruits and vegetables plays a crucial role in assessing their nutritional significance [12]. The nutritional quality of any feed or ingredient can be judged by its biological, chemical and physical content [13]. The chemical content has proven to be a very important tool in food chemistry because it tends to examine the nutritional quality based on the proximate composition of the food and feed which includes the protein, carbohydrates, lipid, moisture, ash and fibre content respectively [14]. According to [12], some essential nutrients reported to be present in the seeds of *Chrysophyllum albidum* are K, P, S, Ca, Mg and Zn.

This study seeks to investigate the antimicrobial properties of *Chrysophyllum albidum* seed oil against Gram positive, Gram negative bacteria and fungi strains. The study also seeks to understand its nutritional composition with a view to establish its potential as food or food supplement, exploring the application of the seed oil in cosmetic and pharmaceutical industries.

2. MATERIAL AND METHODS

2.1. Collection and Preparation of Sample

Figure 1. Fruits, Leaves and Seeds of *Chrysophyllum albidum*

2.2. Extraction of Oil

Dried *Chrysophyllum albidum* seeds were purchased from Gbongan Market, Gbongan town in Osun State. The cotyledons were separated from the endocarp and powdered. 1000 g of the powdered sample was soaked in 4 L methanol for exhaustive extraction. The methanol extract was concentrated *in vacuo* on a rotatory evaporator. The methanolic crude extract obtained was suspended in methanol/water (1:1) and subjected to liquid-liquid partitioning using *n*-hexane (4x2 L) to separate the oil from the aqueous layer in a 5 L separating funnel. The *n*-hexane layer obtained was concentrated in a rotatory evaporator to obtain the oil.

2.3. Preparation of Leaf Ash

The leaves of C. *albidum* were washed with double distilled water and dried in an oven to constant weight. The dried leaves were ashed in a porcelain crucible placed in a Gallenkamp muffle furnace for 6 hours by stepwise increase of the temperature up to 500°C. The ashed samples were homogenized in porcelain mortar and pestle and sieved. 100 g of the sample was weighed into polyethylene buckets of 2 L capacities and 1 L of water was added [15, 16]. The buckets were covered to prevent contamination and extractions were done for 24 hours. These extracts were alkaline to litmus paper and pH was taken and recorded.

2.3.1. Saponification Reaction using the Ash-Extracts

Two hundred milliliters of the ashed peel extract was concentrated to 50% by heating in a beaker [17]. The concentrated extract was heated to 70°C and 15 g of oil was gradually charged into the pot with continuous stirring maintained at 70°C, distilled water was added and formation of semi solid brown soap was seen. The process was completed by salting–out, scooping of soap to separate it from lye and the washing of soap severally with water.

2.3.2. Antimicrobial Activity Assays

Test Organisms

Microorganisms used include reference and clinical isolates comprising of Gram-positive and Gram-negative bacteria and fungi strains. These include *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213), *Clostridium sporogens*(NCIB 532), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (Clinical strain), *Salmonella enterica*var*typhii* (ATCC).

The strains were from stocks of culture collections maintained in the Pharmaceutical Microbiology Laboratory of the Department of Pharmaceutics, Faculty of Pharmacy, Obafemi Awolowo University where the experiments were performed.

Agar Diffusion Tests: Disc Diffusion and Cup Plate Methods

The disc diffusion test was used for the pure oils and the soaps while the cup plate test was used for their dilutions. The oil samples and their soap preparations were dissolved in MeOH/H_2O to give varying concentration of (5.0, 10.0, 20.0, 40.0 and 80.0) mg/mL. Surface plating of the organisms were done for the 20 mL oven dried Mueller Hinton Agar used for the overnight grown bacteria. For the dilutions, holes of diameter 9 mm were made in the agar plates using a sterile metal cup-borer. Two drops of each dilution and control were put in each hole under aseptic condition, kept at room temperature for 1 hour to allow the agents to diffuse into the agar medium and incubated accordingly. For the pure oil and soap, each of these were used to soak sterile 6 mm Whatman paper discs and subsequently placed on the agar plates, allowed for diffusion and incubated. Streptomycin (1 mg/mL) was used as positive controls for bacteria. MeOH/H_2O. The plates were incubated at 37°C for 24 h for the bacterial strains.

2.4. Chemical Composition Analysis

The cotyledons separated from the endocarp were powdered and appropriate quantities of the sample were weighed for the chemical composition analysis. The chemical composition was carried out using Official Methods of Analysis (OMA). Association of Official Analytical Chemists (AOAC, 1990) method was used for moisture content and AOAC, (1984) as used by [18] was used for carbohydrates, ash and crude fibre contents. Kjeldahl method described by [19] was used to determine the total nitrogen and multiply by a factor of 6.25 to determine the protein content. Determination of crude fat was done by solvent extraction gravimetric method described by [20].

3. RESULTS AND DISCUSSIONS

3.1. Microbial Analysis of Oil and Soaps

Table 1. The Physical Properties of the Oil and Ash Extract

Colour of the oil	Dark brown
Refractive Index of the oil	1.51
pH of the ash-extract	10.46

Figure 2. The *Chrysophyllum albidum Seed Oil*

Table 2. In-vitro Antimicrobial Activity of the Oil and the Soaps against Selected Reference and Clinical Strains of Organisms

Organsims/Agents (mg/mL)	Zones of Inhibition in mm*					
	Pseudomonas aeruginosa (ATCC 27853)	*Staphylococcus aureus* (ATCC 29213)	*Clostridium sporogens*(NCIB 532)	*Escherichia coli* (ATCC 25922)	*Klebsiella pneumonia* (Clinical strain)	*Salmonella entericavartyphii* (ATCC)
Oil						
5	-	-	-	-	-	-
10	-	-	-	-	10	-
20	-	10	-	10	10	-
40	-	10	-	12	11	11
80	-	15	13	16	15	15
NaOH Soap						
5	-	-	-	-	-	-
10	-	-	-	13	10	-
20	-	9	-	13	13	-
40	-	10	-	15	13	9
80	-	11	-	18	13	10
Ash Soap						
5	-	-	-	10	10	-
10	-	10	-	13	10	10
20	-	13	-	13	11	12
40	-	13	11	15	11	13
80	-	15	13	21	15	16
Streptomycin						
1	20	20	16	20	19	19

- = No Inhibition*cup size = 6mm

Table 3. Minimum Inhibitory Concentration (MIC) in mg/mL of the agents

Organisms /Agents (mg/mL)	Minimum Inhibitory Concentration (mg/mL)					
	Pseudomonas aeruginosa (ATCC 27853)	*Staphylococcus aureus* (ATCC 29213)	*Clostridium sporogens* (NCIB 532)	*Escherichia coli* (ATCC 25922)	*Klebsiella pneumonia* (Clinical strain)	*Salmonella entericavartyphii* (ATCC)
Oil	-	16.98	50.12	15.85	10.0	22.39
NaOH Soap	-	19.95	-	7.08	8.3	34.67
Ash Soap	-	8.51	25.12	1.76	0.67	8.91
Streptomycin	0.5	0.125	0.125	0.125	0.125	0.25

The results of antimicrobial evaluation show that the seed oil possesses useful antimicrobial activities as antibacterial inhibitory activities were obtained at concentrations of 20, 40 and 80 mg/mL. These organisms have been implicated in skin and mucous membrane infections with reports of morbidity and mortality [21].

Duyilemi et.al, 2012, reported that the aqueous seed extract of *C. albidum* only showed inhibition against *Candida albican* [7]. While George et al., 2018 [8{] reported the inhibition of *S. aureus, S. Aureus* (MRSA) and *S. Aureus* ATCC 25923, *E. coli* (ESBL), *E. coli, Shigella flexneri, Klebsiella pneumonia* and *Candida albicans* to the seed extract of *Chrysophyllum albidum* [8]. In this study the seed oil of *C. albidum showed inhibition against Staphylococcus aureus* (ATCC 29213), *Clostridium sporogens* (NCIB 532), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (Clinical strain) and *Salmonella enterica*var*typhii* (ATCC). These showed the seed oil and the seed extracts have similar content and activities to some extent. The seed oil of *C. albidum* was resistant to *Pseudomonas aeruginosa* , this was corroborated by the similar report of seed extract of C. *albidum* as reported by George et al., [8]. The activity of the oil against *Klebsiella pneumonia* at a concentration as low as 10 mg/mL is especially noteworthy as this organism is notorious for its intrinsic resistance to most standard antibacterial agents.

The soap of the ash-leaves shows the highest antimicrobial activities because it's MIC is very close to the standard, (0.67 is the closest to 0.125 of Streptomycin). It possesses activity against *Escherichia coli* and *Klebsiella pneumonia* at a concentration as low as 5 mg/mL. This could largely be as a result of the phytochemicals present in the leaves of the plant. The study by [22] on the methanol leaf extracts of *C. albidum* revealed the presence of flavonoids, phenols, glycosides, terpenoids, saponins, steroids and alkaloids. [23] also revealed the presence of alkaloids, flavonoids, saponins and tannins in *Chrysophyllum albidum*. According to [24], leaf extracts of *Chrysophyllum albidum* contains anthraquinone, tannin and cardiac glycoside and with no traces of reducing sugars, saponin and alkaloids. These may also be present in the seeds and responsible for the antimicrobial activities.

3.2. Chemical Composition

The proximate study of C. *albidum s*eed cotyledons as shown in table 4 reveals the composition of nutrients classified in percentages.

Table 4. Proximate Composition of C. *albidum* Seeds

Parameter	Moisture content	Ash content	Crude Fibre	Fat/oil	Protein	Carbohydrates
Value (%)	14.98 ± 0.01	1.90 ±0.00	2.09 ±0.00	2.38 ±0.00	12.14 ± 0.00	67.65 ± 0.00

The percentage moisture content of the seed obtained from the analysis as shown in table 4 is 14.98%, this value is above 9.0% and 9.39% reported by [25, 26]. The value is meaningfully lesser than 24.17% as found by [27]. The moisture content of any food is the amount of the water in of the food. The level of moisture present in a food is a determinant of the texture because the more organize the endosperm structure, the lesser the moisture content. The reduced moisture content of the seed helps to improve its storage firmness by stopping mould from growing and reducing moisture depending on biochemical reactions [28].

The percentage ash content of *Chrysophyllum albidum* seed cotyledons which was obtained from the proximate analysis as shown in table 4 is 1.90%. This is higher than 1.40% as reported by [23] but slightly lesser than 2.62% and 3.80% reported by [25, 26] consistently. The ash content shows the presence of carbon compounds and inorganic component in the form of salt and oxides in the seed of *Chrysophyllum albidum*. It also helps to decide the mineral components available in the sample.

The percentage carbohydrate observed from the proximate analysis of the seed cotyledons was 67.65 % as displayed in table 4. The value is significantly higher than 60.39% previously reported by [27] but lower than 70.60% and 71.40% respectively reported [25, 26]. These analysis shows that the *C. albidum* seed can serve as food and can also give energy as it is a very good source of carbohydrate. The percentage of crude fibre content of the seed cotyledons discovered after the analysis as shown in table 4 is 2.09%. The value is greater than 1.36% reported by [27] but a bit lesser than 2.80% and 2.96% reported by [25, 26]. The presence of crude fibre helps to improve the rigidity of the seed. It can also be used in removing insoluble particles from a solution which holds heavy particles. Fibres have been reported to prevent heart diseases, diabetes, and colon cancer, fibre also adds to treatment of digestive disorder and constipation [29]. Fibres decrease the absorption of cholesterol from the gut, delays the digestion and conversion of starch to simple sugars, and also functions in the protection against cardiovascular disease, colorectal cancer and obesity. The fibre like substances mostly the gums and protein when swallowed in addition to a diet have been reported to reduce postprandial blood glucose levels. Taking in 5-10 g of soluble fibre daily from a diverse source have been established to lessen serum cholesterol by 5-10%. This suggests that the fruit skin having the highest fibre portion could be successfully exploited in handling diabetes mellitus, colorectal cancers and weight reduction in the individual as adjunct therapy.

The percentage protein observed from the analysis of C. *albidum* seed cotyledons was 12.14%. This value is meaningfully higher than 4.50% and 2.45% previously reported by [25, 27] correspondingly. But significantly lower than 13.14% stated by [26]. Protein is an indispensable nutrient which works for repairing worn out tissues and cells.

The percentage lipid discovered from the proximate analysis was 2.38%, this is expressively above 0.82% and inferior to 7.80% as described by [26, 27] respectively. Fat is an imperative foundation of energy shields, guard internal tissues and enhances cell processes. It also increases transport of fat-soluble vitamins; it's also an exceptional source of energy. The differences in the percentage composition of the proximate analysis may be attributed to various species and sizes of the seed. Other climatic and environmental factors could have also contributed to these general findings.

4. CONCLUSION

The broad-spectrum activities of the seed oil against strains of organisms responsible for many infectious diseases together with the favourable physicochemical properties as reported for this oil reinforces its tremendous potential for cosmetic and pharmaceutical industries.

The proximate analysis showed that *Chrysophyllum albidum* seed cotyledons are good source of nutrients; hence they can be good snack or fruit for consumption for all. Its high carbohydrates content makes an excellent source of energy; the low moisture content of the seed cotyledons enhances its storage stability by inhibiting mould growth, the ash contents in the seed show an indication of the presence of carbon compounds and inorganic component in the form of salt and oxides in the seed. The fibre content in the seed decreases the absorption of cholesterol from the gut, delays the digestion and conversion of starch to simple sugars, and also functions in the protection against cardiovascular disease, colorectal cancer and obesity. The protein content present in the seed serves as an essential nutrient for repair of worn out tissues and provide support for cells and the fat content in the seed serves as an important source of energy, it insulates and protects internal tissues and plays a role in essential cell processes.

This study has affirmed that *Chrysopyllum albidum* seeds could be put to productive use in the food, cosmetics and pharmaceutical industries rather than constituting a menace in the environment. However, further research on toxicological assessment of the seed's cotyledon should be done before use as food supplement.

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