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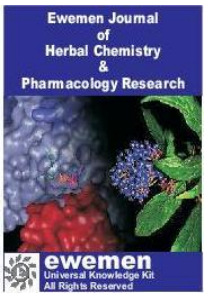

Original Research

PHYTOCHEMICAL, PROXIMATE PHARMACOGNOSTIC ANALYSES AND THIN LAYER CHROMATOGRAPHY OF *CHRYSOPHYLLUM ALBIDUM* SEED

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ABSTRACT

The seed of *Chrysophyllum albidum* has been recognized to be one of the numerous plant seed in Africa with ethnomedicinal value. It was successively extracted with hexane, ethyl acetate and methanol solvents using the soxhlet extractor. Phytochemical screening carried on the seed extracts showed the presence of steroids in the hexane and ethyl acetate extract, carbohydrates, saponins, steroids, tannins, alkaloids and flavonoids in the methanol extract, and alkaloids, flavonoids and anthraquinone water extract. Proximate analysis of *C. albidum* seed revealed 12% moisture content, 88% total solid, 1.8% total ash, and water and alcohol extractive value of 11.67 % and 7.08% respectively. Thin Layer Chromatography of the extracts using hexane/Ethyl acetate (9:1), hexane/Chloroform (2:8) and ethyl acetate/methanol (2:3) solvent system for the hexane, ethyl acetate and methanol extracts respectively revealed separation profiles of 4, 2 and 4 spots respectively on the TLC chromatograms. The result shows the rich phyto-constituents of the seed and its suggested suitability for nutritive feed formulation. The values obtained could also be used as reference to identify adulteration of the seed samples.

Keywords: *Chrysophyllum albidum*, seed, phytochemical, proximate pharmacognosy, thin layer chromatography, medicinal plant.

INTRODUCTION

Plants has been used for the treatment of diseases all over the world, before the advent of modern clinical drug and are known to contain substance that can be used for therapeutic purpose or precursor for the synthesis of useful drugs and dietary supplements. According to the World Health Organization, more than 80% of the world's population mostly in poor and less developed countries depend on traditional

plant-based medicines for their primary health needs (Egharevba *et al.*, 2015*a,b*). Medicinal plants are nature's gift to human beings to lead a disease-free and Healthy life. It plays a vital role in preserving our health. Nigeria is one of the most medico-culturally diverse countries in the world, where the medicinal plant sector is part of a time-honored tradition that is respected even today (Egharevba *et al.*, 2015*a,b*).

Despite technologic advances, the drug discovery process is facing a major innovation deficit that is adversely affecting the pharmaceutics industry (Dahanaukar *et al*., 2000). Drug discovery based on Ethno-pharmacology and use of natural products keep gaining momentum in the current world order where the poor remains at the receiving end due to the problem of affordability and accessibility (Patwardhan *et al*., 2004). It is also a known fact that many modern drugs have their origin in ethno-pharmacology and this has fueled research in the direction of ethnomedicine and natural product research. Hence, medicinal plant like *C. albidum* (Sapotaceae), present interesting focus as reservoir of bioactive compounds that may provide leads in drug discovery process.

Chrysophyllum albidum (Sapotaceae) is a forest fruit tree described by the Scottish botanist George Don. They are commonly found throughout tropical Africa (GRIN, 2013). The plant is commonly found in the Central, Eastern and Western African region (Amusa *et al.,* 2003), and are widely distributed in Nigeria, Uganda, Niger, Cameroun and Cote d'voire (Adewusi, 1997). Its common name is the white star apple and it is closely related to the Africa star apple which is also common throughout West Africa. The fruit is widely eaten and may serve as astringent. It is known locally in Nigeria as *Agbalumo* (Yoruba), *Udara* (Igbo) and *Agwaluma* (Hausa).

The seed of *Chrysophyllum abidum* are shiny (1-1.5 x 2cm), arranged in a star-shaped pattern in the yellow pulp. The flattened seeds (Figure 1) are bony, light brown and hard (Orwa *et al.*, 2009). Though there are reports from different regions of the world on the use of the plant as dietary supplement for animal feeds, the veracity of local variety has not been well studied. The seed had been reportedly used for intestinal worms and haemorrhoids and are rich in linoleic (38.4%) and oleic (29.6%) acids and could be used in free fatty acid production (Houessou *et al.,* 2012; Ajewole and Adeyeye, 1990). In Western Nigeria, ointments of the seed cotyledons are used in the treatment of vaginal and dermatological infections (Adebayo *et al.,* 2011; Egunyomi *et al*., 2005).

Figure 1: *Chrysophyllum albidum* Seeds

The seed had also been reported to contain flavonoids which are potent water soluble super antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer and anti-allergic activity as well as inhibits tumor growth (stauth, 1993; Cushine & Lamb, 2005). *Chrysophyllum albidum* seed had been reported to exhibits haemostatic, antimicrobial and wound healing activity (Faleyimi *et al.,* 2008). Lotito *et al.* (2006) suggested that the presence of some photochemical may contribute to the wound healing activity by suppressing inflammatory reaction of injured tissues.

Methanolic extract of the seed had been reported to contain eleagnine (1,2,3,4-tetrahydro-1-methyl-β-carboline), tetrahydro-2-methylharman (1,2,3,4-tetrahydro-1,2-dimethyl-β-carboline) and skatole (3-methylindole). The alkaloid eleagnine (Figure 2) had been reported to possess antimicrobial activities against *Candida albicans* (MIC 62.5 mg/ml) and *Candida pseudotropicalis* (MIC 250 mg/ml) (Idowu *et al.,* 2006). Eleagnine had been found to exhibit anti-inflammatory and antioxidant activity. These pharmacological effects of eleagnine may suggest it to be one of the constituents of *C. albidum* responsible for the observed pharmacological activities in ethno-medical use as antimicrobial agent (Ajetunmobi and Towolawi, 2014; Idowu *et al.,* 2006). Enzo (2007) also reported high tannins content in the seed cotyledon (77.06 mg/100g), and suggested that the tannins were responsible for the anti-diarrheal activity of the seed. Skatole is one of the many

compounds that are attracted to males of various species of the orchid bees. Skatole had also been found to cause pulmonary edema in goats, sheep, rats, and some strains of mice. It appears to selectively target club cells, which are the major site of cytochrome p450 enzymes in lungs. These enzymes converts skatole to a reactive intermediate, 3-methyleneindolenine, which damages cells by forming protein adducts.

The ethanolic extract of *Chrysophyllum albidum* seed cotyledon has been reported to be of potential benefit in alternative medicine in the treatment of diabetes mellitus (Olorunnisola *et al.,* 2008). Ajayi and Ifedi (2015), reported that the seed flour of *C. albidum* contain many mineral components and found values as high as 5100.00 mg/kg Potassium, 2100.00 mg/kg Magnesium, 1960.00 mg/kg Calcium, 210.00 mg/kg Sodium, 47.20 mg/kg Iron, 24.20 mg/kg Manganese, 12.90 mg/kg Cupper and 6.70 mg/kg Zinc. Other heavy metal such as Nickel, Chromium and Lead were found to be absent. Ajayi and Ifedi (2015), suggested that the low Na/K ratio (0.04) obtained may be partly responsible or helpful in observed high blood pressure reducing activity of the seed (Ajayi and Ifedi, 2015).

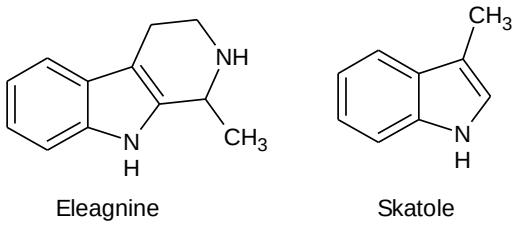

Figure 2: Chemical structures of Eleagnine and Skatole

The stem bark and seed cotyledons of *C. albidum* had also been reported to be widely used in livestock and poultry feeds. These parts are had been reported to be rich in secondary metabolites such as saponins, tannins, flavonoids and alkaloids (Okoli and Okere, 2010; Egunjobi 1969). Saponins are used as dietary supplements, expectorant and anti-inflammatory agent (Xu *et al.,* 1996; Marjan and Hossein, 2008). Skene *et al.* (2006) also reported that saponin is use as adjuvant in vaccines. The aim of this study is to analyze the *C. albidum* seed from North-Central Nigeria for the presence of metabolite and establish its chromatographic profile which could be used as reference for adulterated samples, provide lead(s) in drug discovery process, and also suggests its suitability as dietary supplement in the formulation of livestock and poultry feed.

MATERIALS AND METHODS

All reagent used were of analytical grade and obtained from Zayo-Sigma. The seeds of *C. albidum* were collect from IBB Market, Suleja Local Government Area, Niger State, Nigeria, on 22nd March 2015. The seed was identified by a Botanist/ Taxonomist at the Department of Medicinal Plant Research and Traditional Medicine, NIPRD, Abuja.

Plant Preparation

The seeds were washed and air-dried at ambient temperature for 3 weeks, and thereafter crushed to increase its extractive surface area using a mortar and pestle. The crushed dried material was kept in air-tight cellophane until required.

Extraction:

A portion (300 g) of the crushed material was extracted successively with hexane, ethyl acetate and methanol using a Soxhlet extractor. The liquid extracts were separately concentrated with a rotary evaporator and left to air-dry. The dried extracts were kept in a sealed air-tight container until required.

Phytochemical Screening:

Phytochemical and proximate pharmacognostic analyses were carried out using standard methods (Sofowora, 2008 and Evans, 2002).

TLC Chromatography:

Thin Layer Chromatography (TLC) was done on a normal phase (K_5) glass plate and 10% sulfuric acid in ethanol was used as spray reagent. Hexane/ethyl acetate (9:1), hexane/chloroform (2:8), and ethyl acetate/methanol (2:3) were used as solvent systems for hexane, ethyl acetate and methanol extracts respectively.

RESULT AND DISCUSSION

The results of phytochemical and proximate pharmacognostic analyses are as depicted in Tables 1

and 2 respectively. The results of phytochemical screening of hexane and ethyl acetate extracts revealed the presence of sterols only, while the methanol extracts indicated the presence of saponins, tannins, carbohydrates, alkaloids, flavoniods, and steroids. Although anthraquinone was absent in the methanol extract, the crude material/aqueous extract revealed the presence of anthraquinones. The result of phytochemical screening compare relatively well with the result of obtained by Okoli and Okere (2010), on the water extract. The slight difference in the phytochemical profiles of the seed could be as a result of different ecological location of the plants investigated or the seasons the plants were collected.

Table 1: Results of Phytochemical Screening of the seed of *C. albidum*

Parameters	Crude sample	Hexane extract	Ethyl acetate extract	Methanol extract
Saponins	+	-	-	+
Tannins	+	-	-	+
Flavonoid	+	-	-	+
Carbohydrates	+	-	-	+
Sterol	+	+	+	+
Alkaloid	+	-	-	+
Anthraquinone	+	-	-	-

Key: Present = (+), Absent = (-)

Table 2: Results for Proximate pharmacognostic analysis of the seed of *C. albidum*

Parameter (%)	MC	WEV	AEV	TAV	TS
Values	12.00	11.67	7.08	0.45	88

MC is moisture content; WEV is water extractive value; AEV is alcohol extractive value; TAV is total ash value; TS is total solid

Table 3: Results of TLC of the seed extracts of *C. albidum*

Hexane Extract		Ethyl acetate Extract		Methanol Extract	
DS (cm)	R_f	DS (cm)	R_f	DS (cm)	R_f
1.0 cm	0.14	5.7cm	0.74	1.6cm	0.22
2.4cm	0.33	6.5cm	0.84	2.7cm	0.36
4.2cm	0.58			4.0cm	0.56
5.1cm	0.71			6.7cm	0.91

DS = distance moved by spot from origin; R_f = Retention factor

The close similarity in the chemical profile between this work and that of Okoli and Okere may indicate that the plant maintains a high degree of chemical profile integrity regardless of its ecological location. The phytochemical screening on the seed thus showed rich chemical components like alkaloids, flavonoids, tannins, saponins, sterols, carbohydrates, and anthraquinone, which have been known to exhibit various pharmacological activities (Sofowara, 1993). The antimicrobial activity of this plant could be traced to the tannins, saponins, flavonoids, alkaloids and most especially anthraquinone present in the plant (Idowu *et al.,* 2006). The presence of these metabolites suggests that the local species might be of great importance in phytomedicines development. For instance, alkaloids, tannins, flavonoids and saponins which have been reported to exhibit anti-inflammatory, anti-microbial, anti-oxidant, antinociceptic, anti-diarrheal and anti-allergic activities, are believed to be responsible for biological activities reported by earlier worker (Cushine & Lamb, 2005; Emzo, 2007; Idowu *et al*., 2006).

Tannins for example act as iron depriver and interact with specific proteins and enzymes in microbial cells through hydrogen bonding of the polar regions and hydrophobic interactions of non-polar regions, forming tannin-protein complexes (McRae and Kennedy, 2011; Scalbert, 1991; Hagerman, 1989). Herbs that have tannins in them are astringent in nature and are used for treating intestinal disorder such as diarrhea and dysentery (Okoli and Okere, 2010) thus exhibiting antimicrobial activity. Li *et al.* (2003) revealed the biological activities of tannins and observed that tannins have remarkable activity in cancer treatment and anticancer. Thus, suggesting that *Chrysophyllum albidum* could be a possible source of important bioactive molecule for treatment and prevention of cancer. In addition to its antimicriobial and anticancer activity, tannins functions as a stable and potent antioxidant (Trease and Evans, 1983). Flavonoids are also potent water soluble super antioxidant and free scavengers which prevent oxidative cell damage, and thus have strong anticancer and antitumor activities (Stauth, 1993). The anticancer activity of flavonoids had been established by many workers (Cushine and Lamb, 2005).

The presence of carbohydrate in the *Chrysophyllum albidum* seed can also suggest of its usage in dietary supplement since carbohydrate plays a significant

role as the preferred energy or fuel for muscle contraction and biologic work. Carbohydrates decrease the breakdown of protein and lipids in the body and increase energy level. Thus, carbohydrate is an essential component in dietary fiber and in formulation of animal feeds (Ajayi and Ifedi, 2015; Trowell, 1972).

Alkaloids had been associated with medicinal uses for centuries. One of the most common biological properties of alkaloids is their toxicity against foreign organisms. The antinociceptic, antimicrobial, anti-inflammatory and antioxidant activity of this seed has been reported to be as a result of the eleagnine, an alkaloid isolated from the seed (Idowu *et al*., 2003). The activities of eleagnine could therefore support the ethnomedicinal and ethnobacterial use of the *Chrysophyllum albidum* seed. Furthermore, alkaloid is one of the largest groups of phytochemicals in plants which has been utilized in the development of powerful analgesics (Raffauf, 1996). It is also a known active class of compounds of most anti-malarial and psycho-active drugs (Evans, 2002).

Saponins had been associated with numerous pharmacological properties (Estrada *et al*., 2000). Saponins are considered a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effects in medicinal plants (Lui and Henkel, 2002). It had also been reported to produce inhibitory effect on inflammation (Just *et al*., 1998). The results of our study revealed that the seed of *Chrysophyllum albidum* contains anthraquinone, and other studies had shown that anthraquinone aids in digestion, reduces inflammation in arthritis patients, and also inhibits the growth of cancer cell. Although anthraquinones speed up digestion, it can cause discoloration of intestine in some patients which may be upsetting for some people (Taiwo *et al.,* 2013). In the management of arthritis, anthraquinones are applied in the form of aloe vera supplements. Most anthraquinone that are used for medicinal purposes are found to be naturally occurring plants such as *C. albidum*. Okwu (2001), reported that the presence of sterol in the seed of *Chrysophyllum albidum* suggests that the seed can serve as a source of lead in drug discovery due to its relationship with sex hormones. Anabolic sterols are synthetic hormones that can boost the body's ability to produce muscle and prevent muscle breakdown. It is not unlikely that these bioactive compounds found in the seed of *Chrysophyllum albidum* are the reasons for the wide activities it exhibits. Thus *C. albidum* seed may be of good economic value if exploited medicinally.

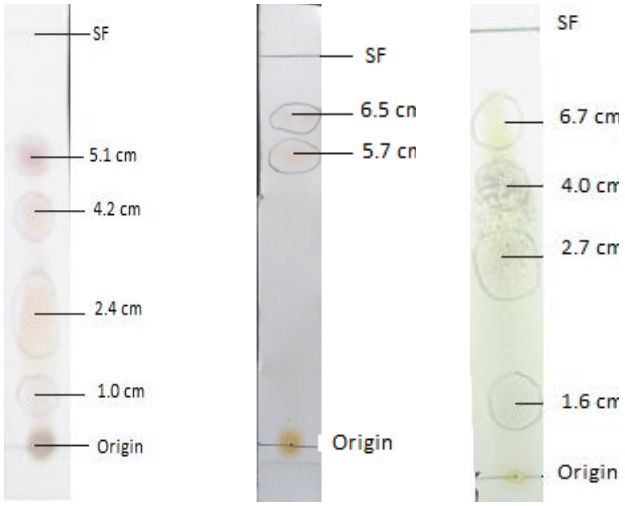

Figure 3: TLC chromatogram of the Hexane, ethyl acetate and methanol extracts (SF means solvent front)

The results of thin layer chromatography (TLC) on normal phase (K_5) glass plates are as shown in Table 3 and Figure 3. The TLC conducted on the Hexane, Ethyl acetate and Methanol extract using Hexane: Ethyl acetate (9:1), Hexane: Chlorofoam (2:8), Ethyl acetate and methanol (2:3) as the solvent system used respectively revealed distinctively four spots, two spots and four spots on the chromatogram respectively. The TLC profile could serve as a tool for standardization of the seed samples.

Pharmacognostic analysis showed moisture content of 12% which is within the official ranges of 8-14% for vegetable drugs (Prohp *et al.,* 2011; Adeshina *et al*., 2008*a,b*; African pharmacopeia, 1986), and suggests that the plant could be store for a relatively long duration without fear of microbial attack when stored under good condition, due to low moisture retention of the dried seed. This has good implication for the shelf-life of a well packaged dried product (Egharevba *et al*., 2015*c*). The total ash value of 0.45% suggests that the amount of inorganic substance present in the seed is low and therefore the seed may not be a good source of mineral elements as dietary supplement. The alcohol and

water extractive value of 7.09% and 11.67% respectively, suggests that water would be a better solvent of extraction than alcohol. These values could serve as standards for *C. albidum* seed samples and used for detecting adulterated dry samples (Egharevba *et al.,* 2015*d*).

CONCLUSION

The analysis carried out on this seed shows that *Chrysophyllum albidum* is rich in secondary metabolites and may be a good source of dietary supplements for animal feeds. The seed could also be explored as potential drug leads for phytomedicine development. Due to its rich phytochemical profile, it is recommended, that further studies and analysis be done to isolate and identify the active compound(s).

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