ANTIMICROBIAL ACTIVITY OF THE PHYTOCHEMICAL CONSTITUENTS OF *CHRYSOPHYLLUM ALBIDUM* G.DON_HOLL. (AFRICAN STAR APPLE) PLANT

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Abstract

The antimicrobial activity of alkaloids, tannins, saponins, flavonoids, phenol and cardic glycoside present in the various parts of Chrysophyllum albidium plant were investigated. These phytochemical were determined quantitatively and tested againststaphylococcus aureus, B. subtilis, pseudomonas aeruginosa, E. coli, C. tetani, and the fungus; candida albicans. Most the plant parts were found to contain alkaloids, tannins, phenols and flavonoids except for the absence of cardic glycosides in the root, tannins in leaves, and phenol in seed. The significance of the plant parts in traditional medicine and the importance of the distribution of these phytochemicals were discussed with respect to the role of these plant parts in ethno-medicine in Nigeria.

Keywords: Medicinal plants, ethno-medicine, photochemical constituents.

Introduction

The *Chrysophyllum albidium* G.Don_Holl.(*Sapotaceae*) tree is common throughout the tropical Central, East and West Africa regions for its sweet edible fruits and various ethno-medical uses (Dalziel,1937,Amusa *et al*.,2003). *Chrysophyllum albidum*fruits(known as African star apple) are widely eaten in southern Nigeria. The fruit is seasonal (December-March), when ripe, ovoid to sub-globose, pointed at the apex, and up to 6 cm long and *5* cm in diameter. 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 which are not usually eaten. The seed-coats are hard, bony, shiny, and dark brown, and when broken reveal white-coloured cotyledons. The fruit has immense economic potential, especially following the report that jams that could compete with raspberry jams and jellies could be made from it (Okafor, 1975). The fleshy fruit pulp is suitable for jams and is eaten especially as snack by both young and old (Okafor, 1975, Amusa *et.al*., 2003) .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) It is reported as an excellent source of vitamins, irons, flavours to diets

(Nwadinigwe, 1982; Adisa, 2000). In addition, its seeds are a source of oil, which is used for diverse purposes. The fruits also contain 90% anacadic acid, which is used industrially in protecting wood and as source of resin, while several other components of the tree including the roots and leaves are used for medicinal purposes (Adewusi, 1997). The Bark is used as a remedy for yellow fever and malaria, while the leaves are used as emollients and for the treatment of skin eruptions, diarrhea and stomach-ache, which are as a result of infections and inflammatory reactions (Adewusi, 1997).

It is rich sources of natural antioxidants have been established to promote health by acting against oxidative stress related disease such infections as; diabetics, cancer and coronary heart diseases (Burits & Bucar, 2002). Studies have shown a diminished risk of chronic diseases in populations consuming diets high in fruits and vegetables and it has been suggested that antioxidants found in large quantities in fruits and vegetables may be responsible for this protective effect (Halliwell, 1994). Generally, food antioxidants act as reducing agents, reversing oxidation by donating electrons and hydrogen ions. Much attention has been focused on natural antioxidants and some antioxidants isolated from natural sources with high activity have been reported by Parasakthy *et al*. (1996).

Materials and methods

Collection and identification of plants

The healthy plant parts of *Chrysophyllum albidum* were collected from uncultivated and cultivated farmlands, respectively located at Western parts of Nigeria. The two plant samples were identified and authenticated by comparison with corresponding herbarium specimens. The plant parts were thoroughly washed with water and air dried at room temperature. Each sample was grind into coarse powder using a Thomas-Willey milling machine. The aqueous extract of each sample was prepared by soaking 100 g of dried powdered samples in 200 ml of distilled water for 12 h. The extracts were filtered using Whatman filter paper No 42 (125 mm).

Collection of microorganisms

The slants of different microorganisms were obtained from a laboratory stock at the Department of Microbiology University of Ibadan. These microorganism are *staphylococcus aureus, B. subtilis, C. tetani,, pseudomonas aeruginosa, E. coli, and the fungus, candida albicans.*

Phytochemical screening

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Test for alkaloids: 1cm3 of 1 % HCl was added to 3cm3 of each extract in a test tube. Each extract treated with a few drop of Meyer's reagent. A creamy white precipitate was observed indicating the presence of alkaloids

Test for tannins: About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for saponin: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for flavonoids: 2 cm3 of extract was heated with 10cm3 of ethyl acetate on water bath cooled. The layers were allow to separate and the colour of the NH3 layer noted (red colouration formed)

Test for terpenoids (Salkowski test): 5 ml of each extract was mixed in 2 ml of chloroform, and concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for cardiac glycosides: 10cm3 of 50% H2S04 was heated in boiling water for 5 min. 10cm3 of Fehlings solution (5cm3 of each solution A and B) was added and boiled. A brick red precipitate indicating presence of glycoside was observed.

Quantitative determination of the phytochemical constituents Preparation of fat free sample 2g of the sample were defatted with 100ml of diethyl ether using a soxhlet apparatus for 2 h.

Determination of total phenols by spectrophotometric method

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. This was measured at 505 nm.

Alkaloid determination using Harborne (1973) method

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 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to

the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Tannin determination by Van-Burden and Robinson (1981) method

500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in amechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Saponin determination

The method used was that of Obadoni and Ochuko (2001). The samples were ground and 20 g of each were put into a conical flask and 100 cm3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating 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 combined n butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

Flavonoid determination by the method of Bohm and Kocipai- Abyazan (1994)
10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol 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 into dryness over a water bath and weighed to a constant weight.

Total phenol determination

Total phenol content was determined according to McDonald's method using Folin-Ciocalteau reagent (Gallic acid as a standard) (Kolawole *et al*,2006).

Results

The present study carried out on the plant parts revealed the presence of medicinally active constituents Tables 1.

Table 1**:** Qualitative analysis of the phytochemical of *Chrysophyllum albidum* plant parts.

C.albidum plant parts	Alkaloids	Tannin	Saponin	Phenol	Cardic glycoside	Flavonoid
Seed	+	+	+	-	+	+
Root	+	+	+	+	-	+
Leave	+	-	+	+	+	+
Stem slash	+	+	+	+	+	+

Presence of constituent = + Absence of constituent = -

Alkaloids, tannins, and flavonoids were present in all the plants. Phenols, anthraquinone, tannins and cardic glycosides were absent in seed, leaveand roots respectively. (Table 1)

Quantitative estimation of the crude chemical constituents in the plant plants studied is summarized in Table 2. Seed cotyledoncontained the highest percentage crude yield of alkaloids (21.23 mg/100g-1), while the leavescontained the lowest yield of alkaloid(6.40 mg/100g-1) but the highest yield of Cardic glycoside (40.20 mg/100g-1). Phenolswere obtained in all the plant parts except in the seed cotyledon but the yields recorded wereminimal (2.03 mg/100g-1 -6.40 mg/100g-1).

Discussion

The phytochemical screening and quantitative estimation of the crude yields of chemical constituents of the plant parts studied were rich in alkaloids, flavonoids, tannins, cardic glycosides and saponins. They were known to show medicinal activity as well as exhibiting physiological activity (Sofowara, 1993).

Table 2: Phytochemical constituents of *Chrysophyllum albidum* plant parts investigated expressed as mg/100g-1.

Phytochemicals	Stem slash	Seed cotyledon	Leaves	Roots
Alkaloids	18.30±0.16b	21.23±1.50c	6.40±0.28c	14.04±0.17e
Phenols	3.56±0.20a	-	2.03±0.17e	6.40±1.00a
Tannins	9.12±0.11c	77.06±40b	-	12.00±0.28c
Flavonoid	14.30±20a	45.80±2.00e	15.30±0.10d	15.15±0.17e
Saponins	14.04±0.22c	7.06±0.01c	18.30±1.10b	42.40±3.00a
Cardic glycoside	15.15±0.16b	2.60±0.07b	40.20±2.50d	-

Figures are mean ±SD. Figures bearing different alphabets differ significantly ($P < 0.05$): N=3

The presence of tannins in seed cotyledon leaves and stem slashhave also been reported by other researchers, and this plant parts has anti-inflammatory effect which help control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders (Dharmananda, 2001,Hayashi *et al*., 1993). Seed cotyledoncontains high tannins and flavonoid and this conforms with the report of Idowu *et al*, (2003) and Faleyimu *et al*, (2008). The latter also observed that some of the *Sapotaceae* species including *Chrysophyllum delevoyi* are used for treating fibroid when grind, mix with water and potash or alcohol and potash. They are also used in the treatment of gonorrhea and hay fever (Burkill, 1994, Gill, 1992).
Both stem slash and seed cotyledonpossess very high levels of alkaloids and flavonoids, and the latter show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity (Cushnie & Lamb, 2005). They are also widely employed in livestock and poultry feed (Egunjobi, 1969).

The seed cotyledon phyto-constituents were dominated by flavonoids and tannins (45.40 and 77.06 mg/100g-1 respectively), followed by leaves and roots, which contained 40.20 and 42.40 mg/100g-1 of cardic glycoside and saponins respectively. The high tannin content of this plant may be responsible for the ethno-medicinal usage (Adewusi, 1997). These alkaloids may be toxic chemical element in the seed cotyledon, used as a remedy for fever ,while the stem slash is used as emollients and for the treatment of skin eruptions, diarrhea and stomachache, which is as a result of infections and inflammatory reactions as stated by Adisa (2000). This confirms the efficacy seed against vaginal infections and dermatological infections (Idowu *et al*, 2003) and also its activity against *Candida albicans and C. pseudotropicalis*. This further explains the therapeutic and medicinal properties of *Chrysophyllum albidium* and supported the use of this plant as an external application for skin eruptions diseases.

The saponin content was very high on the roots (42.40 mg/100g-1), followed by the leaves, which contained (18.30 mg/100g-1) of saponin. The stem slash and seed cotyledon contained 14.04 mg/100g-1 and 7.06 mg/100g-1of saponin respectively. The high saponin content of *Chrysophyllum albidium* leaves and roots justifies the use of the extracts to control human cardiovascular disease and reduce blood cholesterol as documented by Aletor (1993). The value of tannins was high on the seed cotyledon (77.06 mg/100g-1) while the leaves and roots contained 28.00 mg/100g-1 and 12.00 mg/100g-1 of tannins respectively. It has been observed that tannins are responsible for anti-diarrheal activity (Enzo,2007) and saponins used as dietary supplements, expectorant and anti-inflammatory agent (Xu R et al,1996 & Marjan et al,2008).

Evaluation of the potentials of *Chrysophyllum albidium* in wound care showed that the cotyledon extract exhibited haemostatic, antimicrobial and wound healing activities (Faleyimu *et al,* 2008). The cotyledon extract mixed with shear butter oil arrested bleeding from fresh wounds by reducing bleeding and blood clothing time. The haemostatic effects of the extract may be due to increase in the coagulation process with the consequent reduction in clothing time as well as vasoconstriction which are necessary in limiting

blood loss from damaged vessels. The phytochemicals may contribute to the wound healing activity by suppressing inflammatory reaction involved by injured tissues (Lotito et.al 2006).

The value of flavonoids was very high in the seed cotyledon (45.80 mg/100g-1), followed by the leaves which contains 15.30 mg/100g-1, the roots contained 15.15 mg/100g-1of flavnoids while the stem slash had 14.30 mg/100g-1 of flavonoids. 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). A study has shown that flavonoids can inhibit the development of fluids that result in diarrhea by targeting the intestinal cystic fibrosis trans-membrane conductance regulator (Schuier et.al, 2005).

Flavonoids in intestinal tracts lower the risk of heart disease (Okwu, 2004). The biological functions of flavonoiods include protection against allergies, inflammatory, free radical scavenging, platelets aggregation, microbes, ulcers, hepatoxins, viruses and tumors (Okwu & Omodamiro, 2005, Okwu & Emenike,2006).This accounts for the natural antioxidants by acting against oxidative stress related disease such infections as; diabetics, cancer and coronary heart diseases (Burits & Bucar, 2002).Hence, people that are prone to such infection can feed on *Chrysophyllum albidium* fruit as source of natural antioxidants

The phenolic content range from 2.03 mg/100g-1 obtained from the leaves to 6.40 mg/100g-1 found in the root. The stem slash and seeds contained 3.56 mg/100g-1 and 2.78 mg/100g-1 of phenols respectively. The presence of phenolic compounds in the plant parts indicates that *C. albidium* contain antimicrobial agents.

The antimicrobial activity of *Chrysophyllum albidium* extract showed potent inhibition on some microorganisms. *Chrysophyllum albidium* root extracts successfully inhibited *P. aeruginosa, E. coli, S. aureus, C. tetani, B. subtilis, and C. albicans.* The stem slash also showed potent inhibition on these microorganisms. The seed cotyledon only showed inhibition on *C. albicans*, while the leaves did not inhibit any of these pathogens. Moreover, *Chrysophyllum albidium* stem slash extracts also showed the highest activity against *B. subtilis* (Table 3). The minimum inhibitory concentration (MIC) of *Chrysophyllum albidium* seed extract was 25-50mg/ml while root extracts showed minimum inhibition concentration activity at 12.5–50mg/ml. The tested organism therefore showed a higher sensitivity to *Chrysophyllum albidium* seed extracts then the extracts of the root (Table 4 and 5), *P. aeruginosa, E. coli, S. aureus, C. tetani, and B. subtilis* are human commensels and have been found to be responsible for the infections on wound (Ijeh & Omodamiro, 2006, Okwu & Morah 2007). These findings supported the use of *Chrysophyllum albidium* seed and root extracts in herbal medicine (Adewusi, 1997). Also, the antibacterial activity of *Chrysophyllum albidium*supported its use as an antiseptic after birth (Nwadinigwe, 1982). Inhibition was observed with extracts of *Chrysophyllum albidium* seed, roots and stem slash on *C. albicans* (Table 3). The inhibition property of the extracts resides mainly in the pytochemicals contained in the plants (Okwu & Morah, 2007). Pytochemicals analysis of the extracts

indicated the presence of typical plant constituents such as alkaloids, saponins, tannins and phenolic compounds. The phenolic compounds in*Chrysophyllum albidium* may be responsible for the therapeutic, antiseptic, antifungal or bacterial properties of the plant. This agreed with the findings of Adewusi,(1997) who reported that latex or exudates from *Chrysophyllum albidium* has antimicrobial properties against *staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Streptococus pyogenes and Candida albicans.* The observed inhibiting role on these microorganisms explains the reason behind the utilization of the seeds and roots extract in traditional medicine as anti-cancer agent and wound healing activity (Faleyimu *et al*, 2008).). The mechanism of inhibitory action of these phytochemicals on micro-organisms may be due to the impairment of variety of enzyme systems, including those involved in energy production, interference with the integrity of the cell membrane and structural component synthesis (Huang & Chung, 2003, Okwu & Morah, 2007).

Phenolic compounds are considered to be bacteriostatic and fungistatic (Okwu & Iroabuchi, 2001, Okwu & Morah, 2007). These compounds caused swelling of hyphal tips, plasma seeping around hypae, leaking of plasma, cell wall distortion, abnormal branching or fusion of hypae and consequently wrinkling of hypae surface (Huang & Chung, 2003, Okwu & Morah, 2007).

Table 3**:** Zone of inhibition (mm) of the crude extracts of *Chrysophyllum albidium* plant parts using streptomycin and clotrimazole as positive control

Test organism	Extract mg/100g			0.05mg/ml		
	Stem slash	Root	Leave	Seed cotyledon	Clotrimazole	Streptomycin
Staphylococcus aureus,	12.0±0.10	10.0±0.10	-	-	-	23.0±0.01
B. subtilis	16.0±0.01	14.0±0.10	-	-	-	23.0±0.10
Pseudomonas aeruginosa,	12.0±0.20	13.0±0.10	-	-	-	36.0±0.11
E. coli,	10.0±0.20	11.0±0.10	-	-	-	22.0±0.10
C. tetani,	10.0±0.10	11.0±0.10	-	-	-	23.0±0.01
Candida albicans.	15.0±0.10	10.0±0.11	-	15.0±0.10	18.0±0.10	-

The antimicrobial activities of phytochemicals are further evidenced by their active role in plant disease resistance (Amadioha & Obi, 1998, Ijeh & Omodamiro, 2006, Okwu & Morah, 2007) and antioxidant activity.

The plant is widely used as an application to sprains, bruises and wounds in herbal medicine in southern Nigeria. The seeds and roots extracts of *Chrysophyllum albidium* effectively arrested bleeding from fresh wounds, inhibited microbial growth of known wound contaminants and accelerates wound healing process.

Table 4**:** Minimum inhibitory concentration (mg ml-1) of the crude extracts of *Chrysophyllum albidium* root.

Test organism	Concentration of the extract 100 mg/ml				MIC mg/ml
	100	50	25	12.5	
	Zone of inhibition (mm)				
Staphylococcus aureus,	12	6	2	-	25
B. subtilis	16	8	3	-	25
Pseudomonas aeruginosa,	13	6	2	-	25
E. coli,	11	4	-	-	50
C. tetani,	11	4	-	-	50
Candida albicans	10	4	-	-	50

Table 5: Minimum inhibitory concentration (mg ml-1) of the crude extracts of *Chrysophyllum albidium* seed cotyledon.

Test organism	Concentration of the extract 100 mg/ml				MIC mg/ml
	100	50	25	12.5	
	Zone of inhibition (mm)				
Staphylococcus aureus,	15	8	2	-	25
B. subtilis	16	8	3	2	12.5
Pseudomonas aeruginosa,	14	8	3	2	12.5
E. coli,	10	4	-	-	50
C. tetani,	12	5	-	-	50
Candida albicans.	15	7	2	-	25

Conclusion

The results of this study indicate that the extracts of the seeds and roots of *Chrysophyllum albidium* have good potentials as anti-inflammatory, anti-diarrheal and anti-hemorrhoidal compound and further provide a rationale for the use of the seed and root extracts of this plant in traditional medicine practice in Nigeria.

The plant parts studied here have also been seen as a potential source of useful drugs. Further studies are going on these plants in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds. The antimicrobial activities of these plants for the treatments of the diseases as claimed by traditional healers are also being comprehensively investigated.

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