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The Preliminary Chemical Constituents and Free Radical Scavanging Activities of The Exocarp of The Fruit Extract of African Star Apple (*Chrysophyllum albidum G. Don*)

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ABSTRACT

This study was carried out to investigate the phytochemical and free radical scavenging activities of the exocarp of the fruit extract of *C. albidum*. The phytochemical screening of the ethyl acetate exocarp extract of the plant revealed the presence of tannins, steroids, alkaloids, saponins, resins, balsam, triterpeniods and volatile oil. The free radical scavenging activity of the exocarp extract was determined using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid was used as reference standard. The plant could therefore be employed as sources of natural antioxidant boosters and for the treatment of some oxidative stress disorders in which free radicals are implicated. The result obtained supports the ethno medicinal application of *C.albidum*.

KEY WORDS: Free radicals, *Crysopyllum albidum*, 2, 2-diphenyl-1-picrylhydrazyl radical, ascorbic acid.

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INTRODUCTION

The use of plants by people for the treatment of diseases and ailments has being in practice for a long time. Alternative medicine has however played a great role especially as numbers of modern day medicine have their origin from plants. Drugs such as the ones for the treatment of malaria a prevailing disease in tropical Africa: choloroquine and more recently artemisinin have their origin from plant sources-cinchona bark and Artemisia annua[1]. *C. albidum* is a tropical evergreen edible fruit tree. It belongs to the family of Sapotaceae and it is common throughout the tropical Central, East and West Africa regions for its sweet edible fruits and various ethno-medical uses.

African star apple (*C. albidum*) has common names known as *agbalumo* (Yoruba), *udala* (Igbo), *agbaluba* (Hausa) and *eha* (Ebira) in the local languages in Nigeria. The tree is about 8-36 m in height, the fruit is seasonal (December –April). Medicinal plants are used as sources of therapeutic agents due to their high properties; these includes among others reduced cost, relative lower incidence of adverse reactions compared to modern synthetic pharmaceuticals. *C. albidum* is good for the treatment of fibroids as reported by Egunyomi [2]. It is an important medicinal plant used as a remedy for yellow fever and malaria. The leaves are oval, green above, densely golden pubescent below from which the genus is named. 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. The fruits can be fermented and distilled for the production of wine and spirits. Freshly harvested African star apple fruits contain crude protein content of 8.75%, carbohydrate content of 29.6% and moisture content of 42.1% as reported by Amusa [3]. The fleshy and juicy fruits have potentials as an ingredient of soft drinks and can be fermented for wine or other alcohol production[4].Its 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[5]. In fact, the effect of DPPH free radical scavenging activity on the fractions of petroleum ether, ethanol, buthanol, ethyl acetate and water extracts of the leaves was determined. The ethyl acetate fraction was purified in column chromatography to obtain myricetin rhamnoside which also exhibited an excellent radical scavenging activity compared with the standard or positive control as studied byAdebayo[6]. Therefore the aim of this study is to investigate the chemical constituents and the antioxidant activities of the exocarp of the fruit of *C. albidum*.

MATERIAL AND METHODS

Chrysophyllum albidum fruits

The fruits of *Chrysophyllum albidum* were collected from Ekpedo market in Akoko Edo local Government Edo state, Nigeria during the month of February 2013.The plant was authenticated at the herbarium unit of the Department of Biological Science, Ahmadu Bello University Zaria, Nigeria, where voucher specimen was deposited.

Chemicals and reagents.

All the chemicals and reagents used in this study were of analytical grade and were products of British Drug House Laboratory,England**.**

Figure 1.*Chrysophyllum albidum* fruits.

Processing of plant samples.

The ripe fruits were washed and the exocarps were manually removed then rinsed in sterile distilled water and were air- dried. The samples were pulverized using 240V 4L blender (Thomas Scientific Swedes born, U.K).

Extraction of plant samples

The pulverized sample 60g was extracted with 250 ml of solvent ethyl-acetate in soxhlet apparatus for 18 h.The extract was collected and concentrated with the aid of a Stuart Rota-vapor and kept in a refrigerator. The percentage yield of extract is 90.9% also the free radical scavenging activities test was carried out of the scavenging effect on 2, 2-Diphenyl-1-picrylhydrazyl (DPPH).

Phytochemical screening

The ethyl acetate extract of the Exocarp of C.albidum was screened for the presence of phytochemical constituents such alkaloids, tannins, terpenoids, flavonoids, balsams, saponins, sterols, cardiac glycosides and volatile oil.

Test for flavonoids (shinoda test)

A little amount of magnesium powder and a few drops of concentrated HCL were added to 3 ml of methanolic extract. A red or intense red coloration indicates the presence of flavonones.

Test for tannins

About 0.5g of extract was stirred with about 10mls of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2ml of the filtrate. The occurrence of a blue –black, green or blue green precipitate indicates the presence of tannins.

Test for cardiac glycoside-(Keller- Killani test)

0.2g of the methanolic extract was dissolved in 2 ml of glacial acetic acid containing 1 drop of ferric chloride solution followed by the addition of 1 ml of concentrated sulphuric acid.A brown ring at the interface confirmed the presence of cardiac glycosides.

Test for saponins

0.2g of methanolic extract was boiled with 5ml of distilled water and filtered. To the filtrate, about 3ml of distilled water was further added and shaken vigorously. Frothing which persists on warming was taken as evidence for the presence of saponins.

Test for Antraquinones (Borntrager's Test)

About 0.2g of extract was shaken with 4 ml of benzene and then filtered 0.5 ml of 1% ammonia solution was then added to the filtrate and there after shaken. Appearance of a pink, red or violet colour in the ammonical (lower phase) was taken as the presence of free anthraquinones.

Test for Carbohydrate (Fehling's Test for Reducing Sugar)

5 ml of mixture of equal volume of Fehling's solution A and B was added to 2ml of test extract in a test tube. The resultant mixture was boiled for 2mins. A brick red ppt of copper(i) oxide indicates the presence of carbohydrate.

Antioxidant activity

Scavenging effect on (DPPH)

The free radical scavenging activity of the exocarp of the fruit extract was evaluated by assessing its discoloration of 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH) using a UV-Visible Spectrophotometer at 517nm.Radical scavenging activity was measured by a slightly a modified method of Brand – Williams et al(1995).The following concentration of the extract were tested 0.05,0.1,0.2,0.5,1.0,2.0, and 5.0 mg/ml. Vitamin C (Ascorbic acid) was used as the antioxidant standard at concentrations 0.05,0.1,0.2,0.5,1.0,2.0,and 5.0mg/ml.1 ml of the extract was placed in a test tube and 3ml of methanol was added, followed by 0.5 ml of 1 mM DPPH in methanol and thereafter the decrease in adsorption was measured on a UV-Visible Spectrophotometer 10 minutes later.

A blank solution was prepared containing the same amount of methanol and DPPH.The radical scavenging activity (RSA) was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{A-B}{A} \times 100$$

Where A = the absorption of the blank sample (without extract); B = the absorption of the extract.

RESULTS AND DISCUSSION

Table 1: Phytochemical screening of the exocarp of the fruit of *Chrysophyllum albidum*

Test	exocarp of fruit
Steroids	+
Tannins	+
Alkaloids	+
Flavonoids	-
Cardiac glycoside	+
Phlobatannins	-
Saponins	+
Terpenoids	-
Volatile oil	+

(+) Present; (-) absent

The results in table 1 above shows the phytochemical analysis of the ethyl acetate extract of the exocarp of C. *albidum* fruit was found to contain saponins, alkaloids, cardiac glycosides ,tannins, balsams, steroids and volatile oil, while it showed absence of phlobatannins , terpenoids and flavonoids

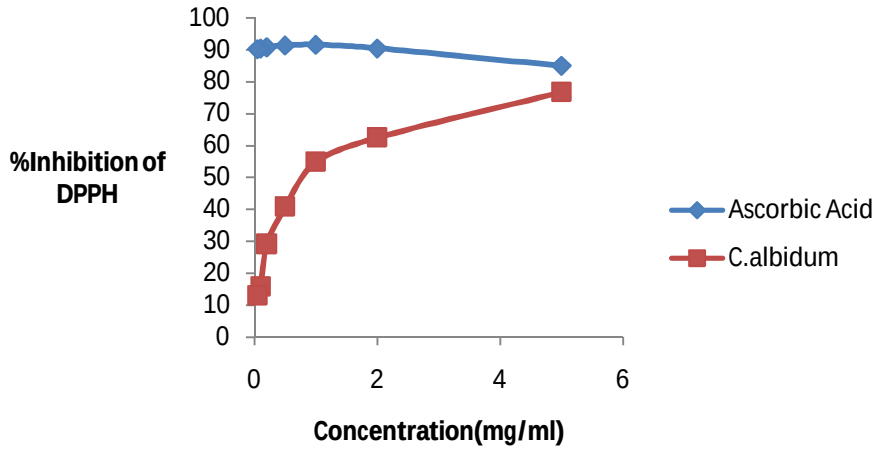

Figure 2: Antioxidant activity curve for ethyl acetate exocarp fruit extract of *C.albidum*

Table 2.Antioxidant activity of vitamin C (ascorbic acid)

Concentration (mg/ml)	Inhibition (%)
5.0	85.00
2.0	90.41
1.0	91.50
0.5	91.32
0.2	90.77
0.1	90.47
0.05	90.19

Table 3. Free radical scavenging activity of the exocarp of the fruit extract of C. *albidum*

Concentration (mg/ml)	Inhibition (%)
5.0	76.68
2.0	62.46
1.0	54.89
0.5	40.85
0.2	29.11
0.1	15.74
0.05	13.02

DISCUSSION

The phytochemical analysis of the ethyl acetate extract of the exocarp of C. *albidum* fruit revealed the presence of secondary metabolite such as alkaloids,saponins,steroids, cardiac glycosides ,tannins, balsams, anthraquinones and volatile oil, while flavonoids, phlobatannins and terpenoids were not found. Studies have revealed that the polyphenolic content in the plants are associated with their antioxidant activities and good potentials as anti-inflammatory, anti-diarrheal and anti-hemorrhoidal compound [7].Hence, the presence of cardiac glycoside in C. *albidum* makes it to be effective in congestive heart failure as reported by Aboaba[8].The presence of these metabolite confirm the usefulness of the plant in the treatment of various diseases[9]. The plant exhibited potent antioxidant activity. The present of phenolic compound like tannins could be responsible for the free radical scavenging effect.

The DPPH test provides information on the reactivity of the test compound with a stable free radical. It gives a strong absorption band at 517nm in visible region. When the odd electron become paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution decolourized as the colour changes from deep violet to light yellow. The degree of reduction in absorbance measurement is an indicative of the radical scavenging (antioxidant) power of the extract. The exocarp fruit extract of C.*albidum* lends to be as potent as Vitamin C (ascorbic acid) with maximum inhibition of 76.68% at a concentration of 5.0mg/ml. It is a concentration- dependent because concentration of the extract increases with the percentage of inhibition. Therefore, the antioxidant activity of the extract is more potent in comparison with vitamin C.

CONCLUSION

The free radical scavenging activity of *C labium* exocarp fruit extract showed that there is increase in antioxidant activity of the extract with concentration. It also indicated a promising sources of potential antioxidant .Thus, it a good source of natural antioxidant which acts against oxidative stress and also efficient as preventive agent for some diseases as heart disease and cancer.

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