

Research Article

Biological Analysis and Phytochemical Studies of The Exocarp Fruits Extracts of African Star Apple (*Chrysophyllum albidum* G. Don)

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Abstract: The exocarp of the fruit extracts of *Chrysophyllum albidum* was extracted using hexane and ethyl acetate. This study was carried out to investigate the phytochemicals and to also test for antimicrobial activity. These extracts were tested against some gram-negative organisms (*Escherichia coli*, *Klebsiella pneumonia*) and gram-positive organism (*Staphylococcus aureus*) using disc diffusion and micro broth dilution techniques with the disc concentration 60 µg/disc. The entire organisms were sensitive to ethyl acetate extract of the plate at 30 µg/disc concentration as follows; *E. coli* (9 mm), *Klebsiella spp.* (9 mm) and *Staphylococcus spp.* (11 mm). *E.coli* and *Staphylococcus spp.* were insensitive in the hexane extract and they were not inhibited. Except *Klebsiella spp.* that is sensitive at a disc concentration of 60 µg/disc with zone of inhibition diameter of 9mm. The result of phytochemical studies of both extracts show the presence of alkaloids, saponins, steroids, tannins and volatile oil but saponins and tannins are absent in the hexane extract.

Keywords: *Chrysophyllum albidum*, gram-negative organism, gram-positive organism, phytochemical, zone of inhibition.

INTRODUCTION

Mankind has deepened on both herbal and non-herbal traditional medicines for curative and prophylactic purpose [1]. Drugs such as the ones for the treatment of malaria a prevailing disease in tropical Africa: Chloroquine and more recently artemisinin have their origin from plants source- Cinchona bark and *Artemisia annua* [2]. Fruits are main source of minerals, fibre and vitamins which are inevitable for human health. *Chrysophyllum albidum* is a tropical ever green 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 has common names known as *agbalumo* (Yoruba), *udala* (Igbo), *agbaluba* (Hausa) and *eha* (Ebirá) 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 the presence of secondary metabolites and also reduced cost, relative lower incidence of adverse reactions compared to modern synthetic pharmaceuticals. African star apple is good for the treatment of fibroids as reported by Egunyomi [3]. *C.albidum* is widely used as an application to sprains, bruises and wounds in herbal medicine in Southern Nigeria. It is an important medicinal plant used as a remedy for yellow fever and malaria. 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. Also have great medicinal benefits which includes plasma cholesterol level reduction, rate of sugar uptake as well as its detoxifying action and effectiveness in diarrhea therapy [4]. The juice from the seed and root when applied on the fresh wounds, inhibited microbial growth of known wound contaminants and accelerates wound healing process [5]. The sweet fleshy fruits has an excellent source for vitamin C, iron, thickener or jam and flavor to diets and raw materials to some manufacturing industries such as resin [6]. In one of our earlier works on this plant, the free radical scavenging activity of the exocarp fruits extract was determined using 2, 2,- Diphenyl-1- picrylhydrazyl (DPPH) and ascorbic acid was used as standard. The plant could be employed as sources of natural antioxidant boosters and for the treatment of some oxidative stress disorders in which free radicals are implicated Orijajogun *et. al.* [7]. Therefore, the aim of this study is to investigate the phytochemical and antimicrobial studies of the exocarp fruit extract of the plant.

MATERIALS AND METHODS

Plant materials

The exocarp 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.

Processing of Plant Samples

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

Extraction of plant samples

The pulverized samples 60g were extracted with 250 ml of the following solvents: ethyl-acetate and hexane in soxhlet apparatus for 18 hrs. The extract was collected and concentrated with the aid of a Stuart Rota-vapor and kept in a refrigerator. The percentage yield of extracts: hexane 91.3% and ethyl-acetate 90.9%.

Phytochemical screening

The ethyl acetate extract of the exocarp fruits of *C.albidum* was screened for the presence of phytochemical constituents such as 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 the 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 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 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 Anthraquinones (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 precipitate of copper (I) oxide indicates the presence of carbohydrate.

Antimicrobial activity determination

The test organisms used were obtained from University of Abuja Teaching Hospital, Abuja, Nigeria. Activity of the crude, purified ethyl acetate and hexane extract from the exocarp fruits of *Chrysophyllum albidum* were tested on these gram positive bacteria: *Staphylococcus aureus* and gram negative bacteria: *Escherichia coli* and *Klebsiella pneumoniae*.

Microbial strains and growth

The species of microorganisms; *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli*, were used in this study. They were maintained on agar slant at 4 °C in the Biotechnology Advanced Laboratory of Sheda Science and Technology Complex (SHESTCO). These strains were sub-cultured on a fresh appropriate agar plate 24 hrs prior to any antimicrobial test. The identity of the tested microbial species was confirmed before use by culturing on the specific media followed by biochemical characterization.

Preparation of Stock Solution

Organic solvent extracts were dissolved in 1ml Dimethyl Sulphoxide (DMSO) while aqueous extract were dissolved in 1ml sterile distilled water (0.06g of extract was dissolved in 1ml of solvent). 0.5ml of extract was introduced into 50 sterile discs respectively in Bijour bottles to make 60µg/disc concentration. Half ml of DMSO was added into the remaining stock solution making 1ml, 0.5ml, and placed into bottle containing 50 filter paper disc and labeled 30 µg/disc, 0.5ml was taken and placed into another 0.5ml was taken and placed into another 50 filter paper discs and labeled 15µg/disc. Each disc has the capacity of adsorbing 0.01ml of solution; the procedure was used to prepare 15, 30, and 60µg/disc concentration. The bactericidal assay of the extracts was carried out using agar diffusion method. The agar plates were respectively streaked with broth culture of bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*).

Preparation of sensitivity Discs

Whatman No.1 filter paper discs of 6mm in diameter were punched and placed in Bijour bottle.

They were then sterilized using autoclaving at 121^oc for 15 minutes. The discs were then cooled.

Bioassay procedure

Standard Inocula of the isolate were swabbed on to the surface of prepared and solidified Mueller Hinton agar in separate petri- dishes. The prepared discs of the extracts and the standard antibiotic discs (Chloramphenicol) were placed onto the surface of the inoculated media at intervals. The plates were incubated

at 37^oC for 24 hours before observation for measurement of zones of inhibition (NCCLS, 2008). At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. Similar procedure was adopted for the chloramphenicol and the corresponding zone diameter was compared accordingly.

RESULTS AND DISCUSSION

Table1: Some phytochemical constituents of *C.albidum*

Test	EEE	HEE
Alkaloids	+	+
Saponins	+	-
Tanins	+	-
Steroids	+	+
Volatile Oil	+	-

Key: EEE-Ethyl acetate extract, HEE- Hexane extract, + = present, - = absent.

Table 2: Inhibitory activity of *C.albidum* extract against the test isolates.

Isolates	EEE(µg/disc)			HEE(µg/disc)			CH(30µg/disc)	DMSO(µg/disc)
	15	30	60	15	30	60		
<i>E. coli</i>	9	9	16	NI	NI	NI	24	NI
<i>Staphylococcus spp</i>	9	11	17	NI	NI	NI	26	NI
<i>Klebsiella spp</i>	NI	9	12	NI	NI	9	24	NI

Key: EEE-Ethyl acetate extract, HEE- Hexane extract, CH –Chloramphenicol, DMSO- Dimethyl sulphoxide, NI- Not inhibited

The result of extraction showed that higher yield of the extract was obtained with hexane than with ethyl acetate. Results of phytochemical screening showed the presence of some secondary metabolites which are alkaloids, saponins, tannins and steroids from Table 1 above. The ethyl acetate and hexane extract revealed the presence of alkaloids, steroids, saponins, tannins and volatile oil but in the hexane extract saponins, tannins and volatile oil were not found. It should be noted that steroidal compounds are of importance and interest in pharmacy due to sex hormones [9]. The results of the antimicrobial showed that the ethyl acetate exhibited a higher degree of antimicrobial activity as compared with hexane extract. This could be as a result of some bioactive substances present in the ethyl acetate extract as shown in Table 2. The plant extracts were active against the tested organisms with the ethyl acetate extract being active against *E. coli* (9 mm) and *Staphylococcus spp* (11 mm), *Klebsiella spp* (9 mm) while the hexane extract is being slightly active only against *Klebsiella spp*.

The results of the antimicrobial showed that the ethyl acetate was more active than the hexane extract. The extracts are less active than the standard antibiotic (Chloramphenicol) with minimum inhibition zone of diameter of 24mm at equal disc potency of 30 µg/disc. The minimum inhibitory concentration (MIC) the fruit extracts of *C albidum* against tested microbes

ranged from 9-17 mg/ml. This reveals that there is present of bioactive substances that are inhibitory to growth of common pathogens such as *Staphylococcus* or *streptococcus* which can cause meningitis, skin infection, a systemic inflammatory response producing shock, massive vasodilatation and death [10]. *C. albidum* has ability to inhibit the growth of *E. coli* which shows that this plant can be used in the treatment of gastroenteritis hemorrhagic colitis; hemolytic uremic syndrome that has been associated with *E. coli* [11].

CONCLUSION

The result of the phytochemical analysis shows the presence of some metabolites which confirms the usefulness of the plant in the treatment of various diseases. *C. albidum* has the ability to inhibit both gram positive and gram negative bacteria, there by serving as a potential antimicrobial agent.

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