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Phytochemical and Antibacterial Properties of Ethanolic Seed Extracts of *Chrysophyllum albidum* (African Star Apple)

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ABSTRACT

Phytochemical and antibacterial properties of ethanolic extract of the seeds of African Star Apple (*Chrysophyllum albidum*) were investigated. The phytochemical result revealed the presence of saponins, carbohydrates, flavonoids, quinones, cardiac glycosides, fatty acids and terpenoids. The antibacterial activity was studied using agar well diffusion method at different concentrations against six pathogenic bacterial strains, three Gram-positive (*Staphylococcus aureus, Micrococcus varians and Bacillus cereus*) and three Gram-negative (Escherichia coli, Pseudomonas aeruginosa and Proteus vulgaris). Significant inhibitory activities were exhibited by the ethanolic seed extracts for all test organisms except *Bacillus cereus*. Zone of inhibition of the crude ethanolic extract was correlated with that of a standard antibiotic Gentamicin, for antibacterial activity. The results indicated a notable inhibition of the bacterial growth.

Key-words: *Chrysophyllum albidum*, Seed Extract, Cotyledon, Phytochemicals, Antibacterial.

INTRODUCTION

Many people in developing countries depend on plants and herbs and concoctions derived from plants and herbs for the treatment of ailments.[1] Several studies in vitro have revealed that secondary metabolites of plant origin function as antimicrobial agents.[2]

Extracts from different parts, including the bark, leaves, roots and seeds of *C. albidum* have been used for the treatment of different ailments, such as yellow fever, malaria, certain skin diseases, stomach ache, and diarrhoea, vaginal and infertility problems as well as dermatological and urinary related infections. The extracts have also found use as liniments and in stopping microbial growth in open wounds.[3-7] The extracts of the leaves and fruits using different solvent of varying polarity have shown antimicrobial and antioxidant properties in vitro and in vivo.[8-10]

Other studies relating to extracts from different parts of the plant show that ethanolic extracts from the plant significantly reduced blood glucose levels and hepatic lipids at higher dose concentrations except HDL-cholesterol, which was found to increase significantly in diabetic rats.[11]

The extracts were also found to reduce platelet concentration,[12] as well as cause reduction in serum levels.[13] These results point to the fact that extracts from this plant have antiplatelet, hypolipidemic, hypoglycemic and antioxidant properties.[14] An alkaloid, Eleagnine that is known for its anti-nociceptive, anti-inflammatory and antioxidant properties has recently been identified as a component of the seed extract.[10] The compounds from the extracts that are responsible for these activities are not known, therefore, it is necessary to identify the components of the seed extract. In this research work we have extracted the oil from the seed of C albidum in ethanol with the aim of identifying the components of the extract by GCMS and LCMS and identifying the specific active components. In this report we present a preliminary account of the phytochemical and antibacterial studies carried out on the ethanolic extract of the seed of *C. albidum* in order to assess the content of the extract and the efficacy of its components.

MATERIALS AND METHODS

Seed Collection and Extraction

Fresh and healthy fruits of the plant *Chrysophyllum albidum* were collected during its fruiting season, between January and April 2016, from various parts of Canaan land, Ota, Ogun State, Nigeria and the seeds removed. Each seed was broken up to yield cotyledon and seed coat, both of which were ground separately and used in the extraction. Both cotyledon and seed coat were extracted in the same way.

The ground powder (200 g) of each part of the seed was extracted using 1000 mL of ethanol in a Soxhlet extractor for 72 h. The ethanol extract was concentrated using rotary evaporator. The dried extract yielded a dark brown viscous residue (43.38 g) which was kept in a refrigerator for further analysis.

Preliminary Phytochemical Screening

The phytochemical screening was performed according to the AOAC standards.[15]

Test Microorganisms and Growth Media

The following six pure clinical microbes *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Proteus vulgaris and Micrococcus varians* were incubated for 24 hours at 37°C on nutrient agar. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C and maintained at 4°C.

Susceptibility Test

Antibacterial activities of the ethanolic extracts were examined against six pathogenic strains, *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Proteus vulgaris and Micrococcus varians* by the agar disk diffusion method. The dissolved extracts in dimethyl sulfoxide were filtered with sintered glass filter and stored at 4°C. Stock solutions of the test extracts at different concentrations were prepared using acetone/water (1:1) mixture as solvent. The plates were incubated at 37°C for 24 h. The sensitivity test was done in triplicate and the mean zone of inhibition was taken. The zones of growth inhibition were measured following 18 to 24 h incubation at 37°C. Microorganism susceptibility to the seed extracts were measured based on the sizes of inhibitory zones (including the diameter of disk) on the agar surface over the disks. Control experiments were carried out using Gentamicin as standard drug.

Determination of Minimum Inhibitory Concentration (MIC)

MIC values were determined by the macro-broth dilution technique. The minimum inhibitory concentration was determined for each bacterium, that is, *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Proteus vulgaris and Micrococcus varians* using broth dilution method. Two-fold serial dilutions of the extracts were prepared in concentrations of 50, 25, 12.5, 6.25, 3.125 and 1.563 mg/mL. The cultures were incubated at 37°C for 24 h, with shaking. Having obtained different concentration of the compounds in the broth, 0.1 mL of the standard inoculums of the micro-organisms in the normal saline was then inoculated into different concentration in the test tubes and the test tubes were incubated at 37°C for 24 h. The least concentrations that that induced 100% inhibition were used to determine MIC values.

RESULTS

Preliminary Phytochemical Screening

The ethanolic crude extracts of *Chrysophyllum albidum* seed were found to contain saponins, carbohydrates, flavonoids, quinones, cardiac glycosides, fatty acids and terpenoids as presented in Table 1.

Table 1: Phytochemical Analysis of Ethanolic Crude Extracts from *Chrysophyllum albidum*

	Cotyledon	Seed Coat
Saponins	+	–
Carbohydrates	+	+
Flavonoids	+	–
Quinones	+	–
Cardiac Glycosides	+	+
Terpenoids	+	–
Fatty Acids	+	+

Antibacterial Activity

Tables 2 and 3 show the results of the antibacterial screening and the minimum inhibitory concentrations of the crude extracts of *Chrysophyllum albidum*.

Table 2: Antibacterial susceptibility test of extracts of *Chrysophyllum albidum* Seed

	Cotyledon	Seed Coat	Gentamicin (Control)
	Zones of Inhibition (mm)		
Staphylococcus aureus	17	18	16
Escherichia coli	16	–	18
Bacillus cereus	–	–	8
Pseudomonas aeruginosa	13	18	Resistance
Proteus vulgaris	13	14	18
Micrococcus varians	12	–	11

Table 3: Minimum Inhibitory Concentration

	Cotyledon	Seed Coat
	Minimum Inhibitory Concentration (mg/mL)	
Staphylococcus aureus	12.50	25.00
Escherichia coli	6.50	–
Bacillus cereus	–	–
Pseudomonas aeruginosa	50.00	6.25
Proteus vulgaris	12.50	6.25
Micrococcus varians	50.00	–

– Represents No Activity

DISCUSSION

In the screening test, ethanolic extracts from the seed of *Chrysophyllum albidum* were found to be effective against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris and Micrococcus varians*. The results as shown in table 2 suggest that the crude extracts obtained from the seed of *C. albidum* showed strong activity against most of the test bacterial strains when compared with Gentamicin standard.

A closer look at the figures in Table 3 shows that apart from *Staphylococcus aureus* the measured MIC was lower for the seed coat extract than the cotyledon extract. This could be an indication that the seed coat extract possesses more active and potent components. Our result supports that previously presented by Imaga and Urua[16] antibacterial activity of ethanolic and water extracts of *C. albidum*.

Similar phytochemicals as obtained in Table 2 have also been reported by Imaga and Urua[8] like saponins, terpenoids, cardiac glycosides, quinones, flavonoids, fatty acids and carbohydrates with biological activities and beneficial therapeutic index is revealed in this contemporary investigation.

In this research we have shown that the extracts from the seed of *C. albidum* (African Star Apple), has secondary metabolites such as saponins, terpenoids, cardiac glycosides, quinones, flavonoid, acids and carbohydrates which may contribute to pharmacognosy.

These metabolites are available to humans, animals and higher plants, as they help to protect against infectious diseases. The antibacterial activity study which showed that the extracts from the seed were active against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris and Micrococcus varians* suggests that the seed extract contain components that can be used as antibacterial agents. This study, although the extracts from the seed are not usually used in traditional medicine, substantiates the claim that extracts from various parts of the plant have been used in the traditional medicine to cure various contagious diseases caused by the microbes; therefore seed extracts should be considered along with other parts of the tree. Additional studies should be carried out to better appraise the potential capability of the crude extracts as antibacterial agents. More studies, towards the isolation and structural elucidation of the antibacterial effective components from the plant have commenced.

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