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PHYTOCHEMICAL AND ANTIBACTERIAL INVESTIGATIONS ON THE EXTRACTS OF *CHRYSOPHYLLUM ALBIDUM* STEM BARK

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ABSTRACT

This research work is aimed at evaluating the phytochemicals, antibacterial potentials of the *Chrysophyllum albidum* stem bark extracts. The ethyl acetate and methanol extracts were obtained using soxhlet extraction method. The extracts obtained were screened for the presence of phytochemicals by standard methods. Agar diffusion and agar dilution methods were used to evaluate the preliminary antibacterial activity against *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Proteus mirabilis, Streptococcus mutans, Bacillus subtilis* and minimum inhibitory concentration (MIC) of the extracts The presence of terpenoids, steroids, alkaloids, flavonoids, tannins was found in the ethyl acetate extract with the absence of saponins and glycoside. Alkaloids, flavonoids, cardiac glycosides, steroids and terpenoids were all found in the methanol extract of the sample. At 100 mg/mL, methanol extract had IZD ranged from 08-20 mm and MIC 3.125 – 25 mg/mL against all the test organisms whereas the ethyl acetate extract had IZD ranged 09-11 mm and MIC 25 mg/mL against four of the test organisms. The antibacterial activity shown by the extracts may be attributed to the presence of phytochemicals identified in the extracts. This corroborates the folkloric uses of the plant part in the treatment of various infectious diseases.

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INTRODUCTION

Bacterial infections result in about 17 million deaths worldwide annually, mostly in children and the elderly [1]. The morbidity and mortality associated with bacterial infections have remained significant, despite advances in antimicrobial chemotherapy. Medicinal plants contain bioactive organic chemical compounds often referred to as phytochemicals, which play a defensive role against major chronic diseases in both host-metabolic or genetic dysfunctional disease and infectious disease, and found in grains, vegetables, fruits, and other plant

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products [2]. Modern medicine today utilizes active compounds isolated from higher plants, and about 80% of these active ingredients indicate a positive correlation between their modern therapeutic use and the traditional uses [3].

Chrysophyllum albidum also known as African star apple, is a medicinal plant used in various vegetation zone in Uganda, Nigeria, Niger, Cameroon and Cote d'Ivoire due to its pharmacological activities such as antioxidant, anti-diabetes, anti-plasmodial, anti-microbial and among others [4]. The roots, barks, fruit pulp and seeds of *C. albidum* have different medicinal uses [5]. The present study was undertaken in order to investigate the claim that the plant has antibacterial activity, since it is used in the treatment of bacterial infections. In our effort to further explore *Chrysophyllum albidum* for biologically important molecules, we screened for the phytochemical constituents.

MATERIALS AND METHODS

Materials

Collection of Plant Material

The stem bark of *C. albidum* was collected from its natural habitat at Umuekwe Village, Enugu-Agu, Ozalla in Nkanu West Local Government Area of Enugu State. The plant was identified and assigned voucher number 021/08 by the taxonomist in-charge of herbarium unit of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology (ESUT), Enugu State, Nigeria.

Test Organisms

Clinical isolates of *Salmonella typhi*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Proteus mirabilis, Streptococcus mutans* and *Bacillus subtilis* were obtained from the Department of Pharmaceutical Microbiology and Biotechnology Laboratory, ESUT, Enugu.

Methods

Preparation of Plant Material

The *C. albidum* stem bark was harvested and rinsed with tap running water to remove dust particles. The cleaned stem bark was cut into small particles and dried under shade for 21 days and pulverized using milling machine into coarse particle and made ready for extraction.

Extraction

The extracts were obtained using soxhlet extractor at temperature of 40^{o}C. The pulverized stem bark 30g was placed on the thimble and 300 ml each of ethyl acetate and methanol were used as extracting solvent. The extraction was allowed to continue for 5 hours. The mixture was removed and concentrated using rotary evaporator at 40°C to get the crude ethyl acetate and methanol extracts which were stored at 4°C pending further analysis.

Phytochemical Screening of Extracts

Standard phytochemical screening test was carried out to detect the presence of secondary metabolites to relate the antibacterial potential of *C. albidum* stem bark extract with the presence or absence of these active constituents. Thus, the test for alkaloids, saponins, flavonoids, phenols, steroid, anthraquinone, glycosides and tannins were performed using standard test procedures [6-7].

Antibacterial Study

Agar-well Diffusion

The antibacterial activity of the extracts *C. albidum* leaves were determined using a method earlier described with modifications [8]. All the extracts were reconstituted using 2-fold serial dilution to obtain the following concentrations; 100, 50, 25, 12.5, 6.25mg/ml, and 3.125 mg/ml using dimethyl sulphoxide (DMSO). A 0.1 ml of 1: 10,000 broth culture dilutions (equivalent to 10^6 cfu/ml) of fresh overnight culture of the test organisms; *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Streptococcus mutans, Klebsiella pneumoniae, Escherichia coli*, *Salmonella typhi* and *Bacillus subtilis*, were provided. The mixture of sterile nutrient agar was aseptically poured into sterile Petri dishes and allowed to set. Using a sterile cork-borer of 6 mm diameter, equidistant wells were made in the agar. Drops of the re-suspended, (2 ml per well) extracts with concentrations of 100 mg/ml to 3.125 mg/ml were introduced into the wells. A 50 mg/mL of Gentamicin was used as the standard drug. The plates were allowed to stand on the bench for an hour, to allow pre-diffusion of the extracts before incubation at 37 °C for 24 hours for the bacterial isolates. The inhibition zones diameter were measured in duplicates using a transparent metre rule to the nearest millimetre (mm).

Minimum Inhibitory Concentration (MIC)

The minimum inhibition concentrations were determined with some modifications for the bacteria that showed reasonable sensitivity to the test extracts using the broth dilution technique [9-10]. To measure the MIC values, various concentrations of the stock, from 0.8125 mg/ml to 50 mg/ml were

prepared in nutrient agar at 48°C and were assayed against the test bacteria. Plates were dried at room temperature for 30 min prior to inoculation. The minimum inhibitory concentration was defined as the lowest concentration able to inhibit any visible bacterial growth on the culture plates [11].

RESULTS

Extraction Yield

The result of the percentage yield of the methanol and ethyl acetate extracts showed that *C. albidum* stem bark contained more polar constituents as shown in Table 1.

Phytochemical Analysis

The Phytochemical screening of the methanol and ethyl acetate of extract of *C. albidum* stem bark indicated the presence of therapeutic bioactive constituents as shown in Table 2.

Antibacterial Activity

The inhibition zone diameter (IZD) in millimeter (mm) for methanol extract indicated that at 100 mg/ml IZD ranged from 08-25 mm against test organism except *E. facalis and E. coli*; At 25 mg/ml IZD ranged 08-20 against all test organisms except *E. facalis and E. coli* and at 3.125 mg/ml the IZD ranged 05-14 mm against all test organisms except *E. facalis and E. coli*. The effect of the extract on test organisms compared favorably with the standard antibiotic gentamycin at 10µg/mL as presented in Table 3. The results of the antibacterial activity of the methanol and ethyl acetate extracts of *C. albidum* stem bark showed that *Salmonella typhi*, *Pseudomonas aeruginosa and Klebsiella sp.* had MIC value at 3.125 mg/ml as the most susceptible among test organisms for methanol extract whereas most test organism showed resistance to ethyl acetate extract and only became susceptible at a high dose of 25 mg/ml expect *Salmonella typhi*, that had MIC value at 12.5 mg/ml as presented in Table 4.

Table 1: Percentage yield of methanol and ethyl acetate extracts of *C. albidum* stem bark

S/No	Extract	Yield (grams)	Percentage Yield (%)
1.	Methanol	12.89	42.98
2.	Ethyl Acetate	10.58	35.27

Table 2: Phytochemical screening of methanol and ethyl acetate extracts

Bioactive constituents	Methanol extract	Ethyl acetate extract
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	-
Saponins	+	-
Steroids	+	+
Glycosides	+	-
Terpenoids	+	+

Key: (+) present and (–) absent

Table 3: Inhibition Zone Diameter (IZD) (mm) of Methanol and Ethyl acetate Extracts

Test samples	Conc. (mg/mL)	Inhibition Zone Diameter (IZD) (mm)								
		S. Aureus	*E. faecalis*	*P. aeruginosa*	*P. mirabilis*	*S. pneumoniae*	*K. pneumoniae*	*E. coli*	*S. typhi*	*B. subtilis*
Ethyl Acetate Extract	100	11	-	12	-	-	-	-	10	09
	25	08	-	09	-	-	-		08	07
	3.125	07	-	-	-	-	-	-	08	-
Methanol Extract	100	20	-	14	25	15	13	-	08	17
	25	16	-	10	20	10	11	-	08	12
	3.125	14	-	08	18	07	07	-	05	08
Gent.	(10µg/mL)	20	30	19	24	10	20	15	15	30

S. aureus = Staphylococcus aureus, B. subtilis = Bacillus subtilis, S. typhi = Salmonella typhi, P. aeruginosa = Pseudomonas aeruginosa, K. pneumoniae = Klebsiella pneumoniae, E. faecalis= Enterococcus faecalis P. mirabilis =, E. coli = Escherichia coli, S. mutans.

Table 4: Minimum inhibitory concentrations (MICs) of the plant extracts

Plant extracts	Minimum Inhibitory Concentration (MIC mg/mL)								
	S. Aureus	*E. faecalis*	*P. aeruginosa*	*S. tyhpi*	*K. pneumoniae*	*B. subtilis*	*E. coli*	*P. mirabilis*	*S. mutan.*
Methanol	6.25	-	3.125	3.125	3.125	6.25	-	3.125	6.25
Ethyl Acetate	25		25	12.5	25	25		25	25

S. aureus = Staphylococcus aureus, B. subtilis = Bacillus subtilis, S. typhi = Salmonella typhi, P. aeruginosa = Pseudomonas aeruginosa, K. pneumoniae = Klebsiella pneumoniae, E. faecalis= Enterococcus faecalis P. mirabilis =, E. coli = Escherichia coli, S. mutans.

DISCUSSION

Recently, antibiotic resistance in some bacteria strains has emerged as a global threat to the treatment options for bacterial infections, and a subject of current research [12-13]. The antibacterial investigation on the extracts of *C. albidum* stem bark demonstrated varying effect on the test organisms Table 3 and 4. The ethyl acetate extract demonstrated antibacterial activity against some test organisms though not very significant when compared to the standard antibiotics (gentamycin) used as control while methanol extract exhibited significant activity. The IZD recorded showed that at 100mg/mL it ranged as 09-11 mm against *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis Salmonella typhi*, at 25 mg/ml it ranged as 07-09 mm against *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis Salmonella typhi* and at 3.125 it ranged 07-08 mm against *Staphylococcus aureus* and *Salmonella typhi*. The methanol extract of *C. albidum* stem bark exhibited antibacterial activity against seven out of the nine tested bacteria viz- *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis Salmonella typhi*, *Proteus mirabilis, and Streptococcus pneumonia.* This is similar to some previous report on the methanol extract of the leaf of *C. albidum* [15-16]. The phytochemical analysis of the methanol extract of *Chrysophyllum albidum* stem bark, revealed the presence alkaloid, flavonoid, tannins, saponins, steroids, glycosides and terpenoids while tannin, saponin and glycosides were found absent in the ethyl acetate extract of the plant stem bark (Table 2). This is in agreement with the previous report on *C. albidum* fruits which indicated the presence of flavonoids, steroids, alkaloids, tannin, anthraquinone and cardiac glycosides [14]. Phytochemicals are believed to possess antimicrobial properties and this was demonstrated by the methanol extract which had all the phytochemicals screened compared to the ethyl acetate extract. Our finding suggests that the methanol extract contains some bioactive metabolites responsible for the broad spectrum

activity against bacterial infections and so, corroborate the folkloric claims of the plant as remedies for the treatments of infectious diseases. The present study has demonstrated that the extracts of *Chrysophyllum albidum* stem bark possesses considerable antibacterial properties.

CONCLUSION

Our findings revealed that *C. albidum* is a rich source of phytoconstituents such as flavonoids, terpenoids, saponins, steroids, glycosides and alkaloids which has been reported to demonstrate antibacterial activity. The presence of these phytoconstituents could be responsible for the observed antibacterial activity demonstrated in the study on pathogenic organisms that causes ailments. Hence, it justifies the traditional uses of the plant part in treating ailments caused by infectious diseases.

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