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# *In vitro* Evaluation of Antibacterial and Antifungal Activities of *Chrysophyllum albidum* Stems Bark

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author AAO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MOO and KOA managed the analyses of the study. Authors EOA and ASO managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

The aim of this study was to evaluate the antibacterial and antifungi activities of ethanolic and methanolic extracts from *Chrysophyllum albidum* stem bark. The crude extracts were screened against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella*, *Candida albicans* and *Aspergillus niger* at different concentrations (3.125 mg/mL, 6.25 mg/mL, 12.5 mg/mL, 25 mg/mL, 50 mg/mL and 100 mg/mL) using the agar well diffusion technique. The ethanolic extracts had stronger

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inhibitory effects on test organisms than the methanolic extracts. The antimicrobial activity observed with the ethanolic extract ranged between 10 and 21.5 mm with no detectable activity on *Aspergillus niger*. Stronger antimicrobial activity was observed with the methanolic crude extracts at all concentrations with all test organisms. The result of this study is indicative that *C. albidum* stem bark extracts can be used in the treatment of infections.

*Keywords: Chrysophyllum albidum; extracts; antimicrobial; antifungal.*

## 1. INTRODUCTION

Before the arrival of synthetic medicine, man relied solely on the healing properties of medicinal plants [1]. Despite various researches conducted from chemistry and biotechnology in bringing forth synthetic drugs, plants are still the only healing provider to human. The benefits deduced from using medicine derived from plants are that they are comparatively safer than the synthetic alternative by offering significant therapeutic benefits and more affordable treatment [2,3]. Plants bring out numerous organic compounds which are used significantly in the treatment of different ailments. Herbal remedies have played a crucial role in the treatment of ailments from ancient to recent times. Although these subjects lost their dominance in 20th century owing to the advent of modern synthetic treatments, there is a restored interest today in medicinal plants usage as natural products for the generation of semi-synthetic derivatives [4]. According to World Health Organization (WHO), more than 80% of the World's populations rely on traditional medicine [5]. Medicinal plants are therefore the "anchor" of traditional medicine, which implies that more than 3.3 billion people in the under-developed countries use medicinal plants on a regular basis [6]. They, therefore, serve as a promising alternative or additive control means due to their anti-microbial activity, non-phytotoxicity, systemicity as well as biodegradability [7].

*Chrysophyllum albidum* (Sapotaceae) often called "white star apple" or "mululu" is a tropical forest tree found in different ecozones in Nigeria, Uganda, Niger republic, Cameroon and Cote d" Ivoire [8]. The decoctions of its leaves are used as emollients and for the treatment of skin problems, diarrhoea and stomach disorder which are due to infections and inflammatory reactions [9]. The high content of saponin in *C. albidum* leaves and roots vindicates the use of the extracts in controlling coronary heart disease and reduce blood cholesterol as reported by Aletor [10]. *C. albidum* has also been reported to serve as anti-inflammatory, antispasmodic, antianalgesic and diuretic because of its high flavonoids, steroids and glycosides content [11]. The phenolic compounds in *C. albidum* may be responsible for the therapeutic, antiseptic, antifungal or antibacterial properties of the plant; this is also responsible for the bacteriostatic and fungistatic activity [12,13]. Phytochemicals such as Tannins, flavonoids, terpenoids, proteins, carbohydates and resins have been reported in *C. albidum* [14]. Eleagnine, tetrahydro- 2 - methylharman as well as skatole have been isolated from this plant and eleagnine was the primary compound accountable for its antimicrobial activity [15]. Antimicrobial features of substances are worthy tools used to curb the unwanted microorganisms particularly the treatment of infectious diseases. The active constituents usually interact with the growth and metabolism of microorganisms in a negative manner [16]. Bacterial infections are among the crucial infectious diseases as they; among several health problems, account for more than 41% of the world's disease load. In spite of the advancements recorded in the discovery of antibacterial agents, there are still urgent needs to find new antibacterial agents due to increasing multidrug resistant bacteria [17] which are a result of the accumulation of diverse antibiotic resistance mechanisms inside the same strain [18]. Although, the pharmacological companies have produced a number of new antibiotics in time past, yet drug resistance has grown. This situation has propelled researchers towards herbal products, in search of development of better-quality drugs with improved antibacterial, antifungal, and antiviral activities. The occurrence of drug resistant microbial strains calls for the studies of synergistic effects of antibiotics in combination with plant's derivatives to improve the antimicrobial cocktail with a broader spectrum of activity and reduce the adverse side effects of antimicrobial agents. This study, therefore, sought to investigate the *in vitro* antibacterial and antifungal activities of the methanolic and ethanolic extracts of *C. albidum* stem bark.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Preparation of Plant Materials

Fresh stem bark peelings of *C. albidum* were collected at a local farm in the suburbs of Ado Ekiti, Nigeria. The plant was identified and authenticated in the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria and a voucher specimen was deposited accordingly at the herbarium of the Department. They were washed and then air dried for 3 weeks. The stem barks were then reduced to fine powder with the aid of a mechanical blender and stored in an air-tight container until further use.

### 2.2 Collection of Clinical Isolates

The clinical test isolates used in this study are *Staphylococcus* sp., *Streptococcus* sp., *Escherichia coli* (Pathogenic), *Klebsiella* sp., *Candida albicans* and *Aspergillus niger.* Pure cultures of bacterial, yeast and the filamentous fungal isolates were sourced from the Microbiology Laboratory, Department of Microbiology, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria.

### 2.3 Preparation of the Ethanolic and Methanolic Extracts

Cold extraction was used in performing the extraction from the stem bark. Extracts were produced using ethanol and methanol as solvents. The filtrate was concentrated using rotary evaporator and then weighed after the solvent had been removed.

### 2.4 Standardization of Microbial Inocula

Bacterial and fungal isolates were sub-cultured onto freshly prepared Nutrient agar and Potato Dextrose agar plates and incubated for 24 h at 37°C and 5 days at room temperature respectively. A portion of the streaked bacterial colonies and a small inoculum from the lawn of fungal growth was transferred into McCartney bottles containing 1 ml of sterile distilled water. Vortexing was carried out and the turbidity was adjusted to match 0.5 Mc Farland standards (106 CFU/ml and 106 spores /ml).

### 2.5 Determination of Antimicrobial Activity

The agar-well diffusion assay as described by Vollekova et al. [19] was used to ascertain the inhibitory effects of the respective stem bark extracts on the test isolates. The tests were carried out using a stock concentration of 100 mg/ml. Mueller-Hinton agar plates were seeded with 0.1 ml of standardized bacterial and fungal cultures. The microbial lawn was done using a sterile glass rod and the seeded plates were allowed to dry. A sterile cork borer was used to punch 2 equidistant holes in the middle of the labelled inoculated agar plates and filled with 0.2 ml of the same concentrations of the two extracts. Following the diffusion of the extracts into the agar at room temperature, the bored agar plates were incubated at 37°C for 24 h for bacteria isolates while those with the filamentous fungal cultures were kept at room temperature for 3 to 5 days and observations made at the end of the incubation period. The antibacterial activity of the stem bark extracts was assessed by an inhibition zone surrounding the well while the antifungal activity was measured after 3 to 5 days incubation at room temperature using a meter rule. The mean zones of inhibition were measured and expressed in millimeters. For the positive control, a standard antibiotic (Gentamycin) (6.25 mg/ml) was used for comparison while for the negative control; DMSO (25% v/v) was used.

### 2.6 Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the methanolic and ethanolic extracts of *C. albidum* was determined by the broth dilution method [20]. The plant extracts were prepared to the highest concentration of 100 mg/ml in 25% of DMSO and serial double dilutions were made to give concentrations ranging from 50 mg / ml to 3.125 mg/ ml (from earlier studies). 1 ml of Nutrient broth and 1 ml of each stem bark extract were put into different test-tubes according to the varied concentrations. 0.2 ml of the standardized microbial cultures was inoculated into the labelled tubes containing the diluted extracts and the Nutrient Broth. The tubes were incubated at 37°C for 24 h for bacteria and fungi. The least concentration of the extract which inhibited the growth of the inoculum was considered as the minimum inhibitory concentration.

## 3. RESULTS

Results showed that as the concentrations increased, there was a corresponding increase in the zones of inhibitions. Tables 1 to 3 show the zones of inhibition of the organisms due to the

antimicrobial activities of the ethanol and methanol extracts of *C. albidum*. The zones of inhibition for all the test isolates using ethanolic stem bark extract of *C. albidum* ranged from 10 mm to 21.5 mm while it ranged from 9 to 11.5 mm for methanolic extract for the entire test isolates (Tables 1 and 2) with *A. niger* showing resistance at all concentrations.

Table 3 shows the mean values for the antimicrobial activity of ethanolic and methanolic stem bark extract of *C. albidum* ranging from 12 to 16.8 mm for the former and 9.9 to 11 mm for the latter extract with *A. niger* showing resistance in the mean values obtained and *Klebsiella* spp showing resistance for the methanolic extract. The minimum inhibitory concentration of the extracts against the test isolates was shown by Tables 4 and 5.

**Table 1. Antimicrobial activity of ethanolic stem bark extract of *Chrisophylum albidum***

| Organism | Diameter of zone of Inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | **100** | **50** | **25** | **12.5** | **6.25** | **3.125(mg/ml)** |
| *Staphylococcus* spp | 13 | 12 | 11 | R | R | R |
| *Streptococcus* spp | 14 | 14 | 12 | 11 | R | R |
| *E. coli* (pathogenic) | 16 | 14.5 | 11.3 | 11 | 9 | R |
| *Klebsiella* spp | 20 | 18.5 | 12 | R | R | R |
| *C. albicans* | 21.5 | 20 | 18.7 | 12 | 11 | 10 |
| *A. niger* | R | R | R | R | R | R |

*R= Resistant*

**Table 2. Antimicrobial activity of methanolic stem bark extract of *Chrisophylum albidum***

| Organism | Diameter of zone of Inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | **100** | **50** | **25** | **12.5** | **6.25** | **3.125(mg/ml)** |
| *Staphylococcus* spp | 11 | R | R | R | R | R |
| *Streptococcus* spp | 11 | 10 | R | R | R | R |
| *E. coli* (pathogenic) | 11.8 | 11.5 | R | R | R | R |
| *Klebsiella* spp | R | R | R | R | R | R |
| *C. albicans* | 10.8 | 10.5 | 10.2 | 10 | 9 | 9 |
| *A. niger* | R | R | R | R | R | R |

*R= Resistant*

**Table 3. Mean values for the antimicrobial activity of ethanolic and methanolic stem bark extracts of *C. albidum***

| Organism | Diameter of zone of Inhibition (mm) | |
|---|---|---|
| | **Ethanolic extract** | **Methanolic extract** |
| *Staphylococcus* spp | 12 | 11 |
| *Streptococcus* spp | 12.75 | 10.5 |
| *E. coli* (pathogenic) | 12.36 | 10.75 |
| *Klebsiella* spp | 16.80 | R |
| *C. albicans* | 15.53 | 9.9 |
| *A. niger* | R | R |

*R= Resistant*

**Table 4. Minimum inhibitory concentration of ethanolic and methanolic stem bark extract of *C. albidum***

| Organism | Minimum inhibitory concentration (mg/ml) | |
|---|---|---|
| | **Ethanolic extract** | **Methanolic extract** |
| *Streptococcus* spp | 25 | R |
| *E. coli* (pathogenic) | 25 | R |
| *Klebsiella* spp | 50 | R |
| *C. albicans* | 100 | 100 |

*R= Resistant*

**Table 5. Minimum inhibitory concentration of ethanolic and methanolic stem bark extract of *C. albidum***

| Organism | Diameter of zone of Inhibition (mm) | |
|---|---|---|
| | Ethanolic extract | Methanolic extract |
| *Streptococcus* spp | 25 | R |
| *E. coli* (pathogenic) | 25 | R |
| *Klebsiella* spp | 50 | R |
| *C. albicans* | 100 | 100 |
| *Staphylococcus* spp | 25 | R |

*R= Resistant*

## 4. DISCUSSION

Antimicrobial features of substances are suitable tools in restraining unwanted microorganisms particularly in the treatment of infective diseases. The natural products sequestered from plants used in traditional medicine, which have strong antiplasmodial activity *in vitro*, express prospective sources for new anti-malarial drugs [21]. Hence, the interaction of these active constituents with growth, development and metabolism of microorganism is unfavourable [16]. The alcoholic extracts from the stem bark of *C. albidum* were seen to have strong antibacterial activity. The antimicrobial activities of the plant stem extract were due to the presence of tannins. This is consistent with the work of Anie et al. [22]. The antimicrobial activity of the methanol and ethanol stem bark extracts of *C.albidum* were reported in this study (Tables 1 to 3). The result of the antimicrobial screening indicated that the test isolates were susceptible to methanol and ethanol extracts of different plants. This reveals that some of the antimicrobial compounds in the investigated plants might be polar. The zones of inhibition of growth of the microorganism are a function of the relative antibacterial and antifungal activity of the extracts. The MIC of the ethanol and methanol stem bark extracts of *C. albidum* plants against the test microorganisms ranged from 25 to 100 mg/ml. The effect of the plant extract on the MIC for the test microorganisms differs extensively in the degree of their susceptibility [23]. A low MIC level is associated with plant extracts with high antimicrobial activity while plant extracts with low antimicrobial activity is known to have high MIC levels. The antimicrobial activities of the stem bark extract were as a result of the presence of plant secondary metabolites [24] which is responsible for most of their therapeutic activities [25]. Also, the plants containing these metabolites (alkaloids, flavonoids, tannin, saponins, etc) usually exhibits stronger antimicrobial properties than others [26]. The presence of these phytochemicals in the investigated plant may have contributed to their effect as a remedy for various diseases. This suggests that the presence of potent antibacterial activity of the stem bark extracts of the investigated plants against the bacteria might be due to naturally occurring bioactive phytochemicals present in the plant materials. The high degree of antimicrobial activity of some of the plants seems to confirm the folk therapy of infections and traditional therapeutic claims of these herbs. In furtherance, there was a proportionate increase in the zones of inhibition as concentrations increased (Tables 1 and 2). The inhibitory zones elaborated by the test isolates exposed to *C. albidum* ranged from for 9.9 mm *C albicans* spp. to 16.8 mm for *Klebsiella* spp. (Table 3). The observed antimicrobial activity of the extracts might also be dependent on both the concentration as well as the nature of the extraction solvent used. Comparatively, the *C. albidum* ethanolic extract exhibited a greater antifungal activity against the fungal isolates than the *methanolic* extract (Tables 1 to 3). The highest MIC values were displayed by the ethanolic extract at 25 mg/ml against *Streptococcus*, *E. coli* and *C. albicans* (Table 4). *C. albicans* and *E. coli* exhibited the lowest MIC reading at 100 mg/ml. *Streptococcus*, *E. coli* and *Klebsiella* showed resistance against methanolic extract, while *A. niger* showed resistance against both ethanolic and methanolic extracts (Tables 3 and 4).

In the same vein, the results obtained from previous studies on *C. albidum* revealed that the inhibitory zones elaborated by the test isolates exposed to *C. albidum* ethanolic root extract ranged from 8 ± 0.06 mm for *S. aureus* to 18 ± 0.03 mm for *E. coli*. Also, the inhibitory zones displayed by the test isolates exposed to *C. albidum* chloroform root extract ranged from 10.7 ± 0.05 mm for *S. aureus* to 26 ± 0.02 mm for *E. coli*, with *A. niger* showing resistance to both extracts [27]. Comparatively, the ethanolic

extract of *C. albidum* from this present study was more effective than the ethanolic root extract against the test isolates used. The methanolic extract from this study had the same effect as the petroleum and chloroform spirit root bark extracts from the previous study against the fungal isolates [27].

## 5. CONCLUSION

The ethanolic extract of *C. albidum* was comparatively more potent against the test isolates than the methanolic extract based on the MIC results. All the respective extracts exhibited a greater antibacterial activity in comparison with the antifungal attributes. The presence of bioactive antimicrobial compounds in the investigated alcoholic extracts of the medicinal plants shows the possibility of obtaining potentially valuable antimicrobial phytochemicals from these plants. The results got from this study support the use of this plant parts in the traditional treatment of diseases in Nigeria. The results of this finding could be very helpful to pharmaceutical industries in the development of new antimicrobial drugs in order to address unmet therapeutic needs. Such screening of various natural organic compounds and identification of active agents is the need of the hour to savage life and provide good health to humanity. There is therefore a need for further studies on the plant parts in order to isolate, identify, characterize and elucidate the structure of antimicrobial bioactive compounds.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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