

## IN VITRO ANTIBACTERIAL ACTIVITY OF LEAF EXTRACT FROM *CHRYSOPHYLLUM ALBIDUM* ON SOME BACTERIAL ISOLATES

<sup>1</sup>\*Omojoyegbe, R. T., <sup>2</sup>Fasogbon, A. O., <sup>1</sup>Daodu, A. A.

<sup>1</sup>Department of Biological Sciences, College of Basic and Applied Sciences, Samuel Adegboyega University, Ogwa, Edo State.

<sup>2</sup>Department of Microbiology, Faculty of Life Sciences, Bayero University, Kano.

\*Corresponding author: [ojotomilolaruth@gmail.com](mailto:ojotomilolaruth@gmail.com) , [tojo@sau.edu.ng](mailto:tojo@sau.edu.ng); +234 7030386275

**Abstract** - *Chrysophyllum albidum* G. is a tropical plant and commonly found in Nigeria. It belongs to the sapotaceae family and used in folklore for the treatment of yellow fever, malaria, diarrhoea, vaginal and dermatological infections. The study was aimed at investigating the phytochemical composition and antibacterial activity of methanol: sterile distilled water crude extracts from the leaves of *Chrysophyllum albidum* in the ratio 3:2 on some bacterial isolates. The phytochemical analysis of the crude extract of *Chrysophyllum albidum* revealed the presence of alkaloids, flavonoids, steroids, saponins, Cardiac glycosides and phlobalamins. The antimicrobial activity of the crude extract at a concentration of 30mg/ml, 35mg/ml and 40mg/ml was determined against some bacterial isolates using agar-diffusion method. The extract showed apparent antimicrobial activity in all concentrations against all bacterial isolates tested except *Klebsiella pneumoniae*, Methicillin resistant *Staphylococcus aureus* and *Escherichia coli* (Ek 58). The zone of inhibition exhibited by the extract ranged from 8 mm to 18 mm, 10 mm to 20 mm and 10 mm to 20 mm for concentrations of 30 mg/ml, 35 mg/ml and 40 mg/ml respectively. Streptomycin, which was used as a control also inhibited the growth of the bacterial isolates except *Bacillus stearothersophilus* (NCIB 822), *Bacillus polymyxa* (LIO), *Pseudomonas fluorescens* (NCIB 3756). The minimum inhibitory concentration (MIC) exhibited by the crude extract of *Chrysophyllum albidum* against the bacterial isolates ranged from 1.093mg/ml to 35mg/ml. The minimum bactericidal concentration of the crude extract ranged from 8.75 mg/ml to 35 mg/ml. The result obtained from this study revealed the importance of *Chrysophyllum albidum* which has a good potential as an antibacterial agent and further research is therefore suggested to isolate, characterize and elucidate bioactive compounds from *C. albidum*.

**Keywords:** *Chrysophyllum albidum*, methanol,

### INTRODUCTION

Human beings have been using plants as source of food, shelter and medicine for decades. Plant materials are important resources to combat serious diseases in the world. The use of medicinal plants plays vital roles to cover the basic health needs in the developing countries (Edeoga *et al.*, 2005). More than a tenth of the plant species (over 50 000 species) are used in pharmaceutical and cosmetic products. However, the distribution of medicinal plants across the world is not uniform (Huang, 2011; Rafieian-Kopaei, 2012), and medicinal herbs are mainly collected from the wildlife population. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al.*, 2008). The medicinal value of these plants lies on some chemical substances that produce a definite physiological action on the human body and these chemical

substances are called phytochemicals. These are non-nutritive chemicals that have protective or disease preventive property (Subhashini *et al.*, 2010). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Edeoga *et al.*, 2005).

In Africa, traditional healers have for centuries been the main provider of primary health care (Kala *et al.*, 2004). Traditional medicine continues to provide health coverage for over 80% of the world population, especially in the developing countries (Enerijiofi and Isola, 2019). The use of traditional medicine for treatment of various diseases have been practiced for generations and a large number of populations in the world use traditional medicine for their day to day healthcare needs (Hamza *et al.*, 2006).

*Chrysophyllum albidum* belongs to the family sapotaceae. It is commonly known as Agbalumo in Yoruba, Udala in Igbo and Agbaluba in Hausa language. This plant is also referred to as “The white star apple” (Idowu *et al.*, 2006). *Chrysophyllum albidum* is a popular tropical fruit tree and widely distributed in the low land rain forest zones (Madubuike and Ogbonnaya, 2003). In Nigeria this plant is distributed within the southwestern part of the country (Oyebade *et al.*, 2011). Nigerians have been using *Chrysophyllum albidum* leaves for series of infection in years back when scientific evidence for its antimicrobial effect was not detected. Studies have been carried out on the seed, Leaves, bark as well as its fruit and root that show the antimicrobial effectiveness on different diseases. For example, the fruit of *Chrysophyllum albidum* has been found to have a very high content of ascorbic acid with 1000 to 3,300mg of ascorbic acid per 100g of edible fruit or about 100 times that of oranges and 10 times that of guava or cashew (Amusa *et al.*, 2003). It was also reported as an excellent source of vitamins, irons, flavours to diet (Adisa, 2000). The fruit are not usually harvested from the trees but left to drop naturally to the forest floor where they were picked up (Amusa *et al.*, 2003). *Chrysophyllum albidum* is rich in natural antioxidants which promote health (Burits and Bucar, 2002). This plant is highly rich in flavonoids, steroids, glycosides and saponins and thus serves as a source of anti-inflammatory, anti-spasmodic, as well as possesses diuretic properties (Savithramma *et al.*, 2011).

The extracts from seeds and roots of *C. albidum* effectively arrested bleeding from fresh wounds and also promote wound healing (Okoli and Okere, 2010). The bark is used for the treatment of yellow

fever and malaria, while the leaf is used as an emollient and for the treatment of skin eruption, stomachache and diarrhea (Adisa, 2000; Idowu *et al.*, 2006). The leaf has antiplatelet and hypoglycemic properties (Adebayo *et al.*, 2010). The root bark has anti-fertility effects (Onyeka *et al.*, 2012a). Stem bark extracts has antiplasmodial (Adewoye *et al.*, 2010) and antimicrobial effects (Adewoye *et al.*, 2011). Leaves extract from *C. albidium* exhibited antibacterial activities against *S. aureus*, *Escherichia coli*, *Salmonella typhi* and *Shigella* species (Duyilemi and Lawal, 2009). Stem bark extract from *C. albidium* exhibited bactericidal activities against Vancomycin resistant *S. aureus* (Akinpelu *et al.*, 2016). Seeds and root extracts obtained from *C. albidium* exhibited anti-inflammatory, anti-diarrhoeal and anti-haemorrhoidal properties (Savithramma *et al.*, 2011).

In addition, its seeds are a source of oil, which is used for diverse purposes (Ugbogu and Akukwe, 2008). The fruits also contain 90% anacardic acid, which is used industrially in protecting wood and as source of resin, while several other components of the tree including the roots and leaves are used as a remedy for yellow fever and malaria (Duyilemi and Lawal 2009). Onyeka *et al* (2013) demonstrated hypoglycaemic, antioxidant and hepatoprotective activities of root bark extract of *C. albidium* in alloxaninduced diabetic rats.

There is a constant need for new and effective therapeutic agents. Many plant species have been utilized for traditional medicine but it is to establish the scientific basis for the therapeutic actions of these plants as these may serve as the source for the development of more effective drugs. Therefore intensive work should be done to search for more antibacterial agents of natural origin to combat the effect of multiple resistances to the available antibiotics by some pathogens. This present study is therefore carried out to investigate the antibacterial and phytochemical screening of *Chrysopyllum albidum* leaves using methanol extract against some bacterial isolates.

## MATERIALS AND METHODS

### Microorganisms Used

Microorganisms used in this study were obtained from culture collections of Prof. D. A. Akinpelu, Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The bacterial

isolates used include typed cultures of National Collection of Industrial Bacteriology (NCIB) and locally isolated organisms (LIO). These bacterial isolates used include:

**Gram- positive:** *Bacillus stearothermophilus* (NCIB 822), *Bacillus polymyxa* (LIO), *Bacillus cereus* (NCIB 6349), *Clostridium sporogenes* (NCIB 532), Methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus* (NCIB 8588), *Staphylococcus aureus* and *Streptococcus pneumoniae*.

**Gram- negative:** *Escherichia coli* (NCIB 86), *Escherichia coli* (EK 58), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* (NCIB 3756), *Shigella* (S15).

### **Preparation of plant materials and extraction**

Fresh Leaves of *Chrysophyllum albidum* used for this study was collected at Obafemi Awolowo University, Ile-Ife. (7.4667° N, 4.5667° E), Osun State, Nigeria in June, 2016. The plant sample was identified in Herbarium of the Department of Botany, Obafemi Awolowo University, Ile Ife, Osun State, Nigeria. Voucher specimen of the plant sample was prepared (Voucher Number IFE-17419) and deposited for reference purposes. The plant sample was air dried until a constant weight was achieved. The dried leaf was ground into powder using a mill, the powdered material was stored in an air-tight container for further use.

Exactly 350 g of the powdered sample was extracted with methanol/sterile distilled water in ratio 3:2 (v/v) for four days with regular agitation. The supernatant collected was later filtered into a clean sterile dried conical flask. The filtrate was concentrated *in vacuo* using rotary evaporator to remove the solvents (Igbinosa *et al.*, 2009) and later lyophilized to obtain crude extract of the plant. The yield was noted and has dark brown colour.

### **Phytochemical screening of the crude extract**

The screening was done using the methods of Trease and Evans, (2002) and Enerijiofi and Isola, (2019) to test for the presence of secondary metabolites such as tannins, saponins, cardiac glycosides xanthoproteins, triterpenes, alkaloids, flavonoids, anthraquinones phlobalamins and steroids.

**Sensitivity testing of *Chrysophyllum albidum* extract on bacterial isolates**

The sensitivity testing of the plant extract was determined using agar-well diffusion method as described by Akinpelu and Kolawole (2004). The bacterial isolates were first grown in nutrient broth for 18 h before use. About 0.2 ml of the standardized test isolates ( $10^6$  cfu/ml of 0.5 McFarland standards) were then subcultured on to Mueller-Hinton agar (Lab M). The medium was allowed to set and wells were then bored into the agar medium using a sterile 6 mm cork borer. The wells were therefore carefully filled up with prepared solution of the extract at a concentration of 30 mg/ml, 35 mg/ml and 40 mg/ml. The plates were allowed to stand on the laboratory bench for about 1-2 h to allow proper inflow of the solution into the medium before incubating the plate in an incubator at 37° C for 24h. The plates were later observed for the zones of inhibition. The effects of the extract on bacterial isolates were compared with standard antibiotic at a concentration of 1mg/ml. The standard antibiotic was used as a positive control.

**Determination of minimum inhibitory concentrations (MICs) of the methanolic leaf extract of *Chrysophyllum albidum* on bacterial isolates**

The MICs of the extract was determined using the method described by Akinpelu and Kolawole (2004). Two-fold serial dilution of the extract was prepared and 2 ml of different concentrations of the solution was added to 18 ml of pre-sterilized molten nutrient agar at 40°C to give final concentrations regimes of 0.547 to 35.0 mg/ml. The medium was then poured into sterile Petri dishes and allowed to set. The surfaces of the media were allowed to dry before streaking with 18 h old standardized bacterial cultures. The plates were later incubated at 37°C for up to 72 h after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented the growth of the bacteria.

**Determination of Minimum Bactericidal Concentration (MBC)**

The MBC of the crude extract was determined by a modification of the method of Spence and Spencer (2004). Samples were taken from plates with no visible growth in the MIC assay and sub-cultured onto freshly prepared nutrient agar plates and later incubated at 37°C for 48 hours. The lowest concentration

of the extract that did not show any growth on a new set of plates was taken as the minimum bactericidal concentration of the extract.

## RESULTS AND DISCUSSION

Phytochemical screening of the extract showed the presence of alkaloids, flavonoids, saponins, steroids, phlobalamins and cardiac glycosides (Table 1). Table 2 shows the sensitivity pattern of each of the bacterial isolates to the crude extract obtained from leaves of *Chrysophyllum albidum* at a concentration of 30 mg/ml, 35 mg/ml and 40 mg/ml. Out of fourteen bacterial isolates tested, eleven were susceptible to the antibacterial activity of the plant extract. The zones of inhibition exhibited by the extract against the isolates ranged from 8 mm to 18 mm, 10 mm to 20 mm and 10 mm to 20 mm at a concentration of 30 mg/ml, 35 mg/ml and 40 mg/ml respectively. *Pseudomonas aeruginosa* showed the least diameter of zone of inhibition of 8 mm at 30 mg/ml, the highest zone of inhibition (20 mm) was exhibited by *Bacillus cereus* at 35 mg/ml and 40 mg/ml. The extract was not active against isolates of *Klebsiella pneumonia*, Methicillin resistant *Staphylococcus aureus* and *Escherichia coli* (Ek 58). Streptomycin used as positive control inhibited 11 out of 14 test isolates. The zones of inhibition exhibited by streptomycin against the test isolates ranged between 10 and 26 mm. (Table 2)

### **The Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) of leaves extract of *Chrysophyllum albidum***

The MIC and MBC of the extract of *Chrysophyllum albidum* against the test isolates were determined and the results obtained were recorded in table 3. The MIC of the crude extract exhibited against the bacterial isolates ranges between (0.781 to 35 mg/ml) while the MBC exhibited by the crude extract ranges between (6.25 to 35 mg/ml).

**Table 1: Result of the phytochemical screening of the leaves extract of *Chrysophyllum albidum***

CHEMICAL TEST	RESULT
Saponins	Positive
Tannins	Negative
Alkaloids	Positive
Flavonoids	Positive
Triterpenes	Negative
Anthraquinones	Negative
Steroids	Positive
Phlobalamins	Positive
Cardiac glycosides	Positive
Xanthoproteins	Negative

**Table 2: Susceptibility patterns exhibited by the bacterial isolates against crude extract of the leaves of *Chrysophyllum albidum* at different concentrations and streptomycin**

Microorganisms	Zone of inhibition (mm)			Streptomycin (1mg/ml)
	<i>Chrysophyllum albidum</i> (30mg/ml)	(35mg/ml)	(40mg/ml)	
<i>Bacillus stearothermophilus</i> (NCIB 822)	12	14	14	0
<i>Bacillus polymyxa</i> (LIO)	16	18	14	0
<i>Bacillus cereus</i> (NCIB 6349)	18	20	20	26
<i>Clostridium sporogenes</i> (NCIB 532)	16	16	18	16
<i>Staphylococcus aureus</i> (NCIB)	14	14	16	18
<i>Escherichia coli</i> (NCIB 86)	14	18	18	18
<i>Pseudomonas aeruginosa</i>	8	10	10	16
<i>Klebsiella pneumonia</i>	0	0	0	10
<i>Streptococcus pneumonia</i>	10	12	12	20
<i>Pseudomonas flourescens</i> (NCIB 3756)	12	10	10	0
<i>Staphylococcus aureus</i>	10	10	10	15
<i>Shigella</i> (S15)	11	11	13	18
MRSA	0	0	0	16
<i>Escherichia coli</i> (EK 58)	0	0	0	16

**KEY**

NCIB = National Collection for Industrial Bacteria

LIO = Locally Isolated Organisms

0 = Resistant

**Table 3: The minimum inhibitory concentration and maximum bactericidal concentration exhibited by the leaf extract of *Chrysophyllum albidum* against susceptible bacterial isolates.**

Microorganisms	MIC mg/ml	MBC mg/ml
<i>Bacillus stearothermophilus</i> (NCIB 822)	35	35
<i>Bacillus polymyxa</i> (LIO)	35	35
<i>Bacillus cereus</i> (NCIB 3756)	6.25	12.5
<i>Clostridium sporogenes</i> (NCIB 532)	6.25	6.25
<i>Escherichia coli</i> (NCIB 86)	35	35
<i>Pseudomonas aeruginosa</i>	12.5	12.5
<i>Pseudomonas flourescens</i> (NCIB 3756)	35	35
<i>Shigella sp</i> (S15)	6.25	35
<i>Staphylococcus aureus</i>	35	35
<i>Staphylococcus aureus</i> (NCIB 8588)	0.781	6.25
<i>Streptococcus pneumonia</i>	35	35

The phytochemical screening of the extract from the leaves of *Chrysophyllum albidum* revealed the presence of alkaloids, flavonoids, steroids, saponins, Cardiac glycosides and phlobalamins. These phytochemical compounds are known to be biologically active and possess antimicrobial activities and thus aid the antimicrobial activities of *Chrysophyllum albidum*. Flavonoids are naturally occurring in plants and are thought to have positive effects on human health (Montoro *et al.*, 2005). *In vivo* studies have shown that flavonoids have anti-allergic, anti-inflammatory, anti-microbial (Cushnie and Lamb, 2005), anti-cancer and anti-diarrheal activities (Schuier *et al.*, 2005).

Alkaloid is also one of the phytochemical compounds observed in the leave extract of *Chrysophyllum albidum*. Alkaloids have been associated with medicinal uses for centuries. Alkaloids possess anti-inflammatory, anti-asthmatic, and anti-anaphylactic properties with consequences of altered immunological status *in vivo* (Ganguly and Sainis, 2001). The presence of Alkaloids in leaves of this plant may be responsible for its effects as antimalaria, analgesic properties and its use in treatment of stomach disorder. This is consistent with the past works of (Okwu, 2001; Okwu and Josiah, 2006).

The presence of these phytochemicals in the investigated plant may contribute to the effects of plants as remedy for various diseases such as vaginal disorders, malaria, yellow fever and so on and so forth. These results suggest that the presence of potent antibacterial activity of the leaves extracts of the *Chrysophyllum albidum* against the bacteria isolates might be due to naturally occurring bioactive phytochemicals present in the plant materials.

The antibacterial activity of the leaf extract of *Chrysophyllum albidum* was investigated against fourteen bacterial isolates comprising of both Gram-positive and Gram-negative bacteria. The result of this investigation revealed that the extract at a final concentrations of 30mg/ml, 35mg/ml and 40mg/ml exhibited *in vivo* antimicrobial activities against eleven out of the fourteen bacterial isolates (Table 1). The organisms inhibited include *Bacillus stearothermophilus*, *Bacillus polymyxa*, *Bacillus cereus*, *Clostridium sporogenes*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Shigella* which cut across gram-positive and gram-negative organisms. Hence the extract exhibited a broad spectrum activity. Ajetunmobi and Towolawi (2014) reported that the activities of *Chrysophyllum albidum* extract appeared to be broad

spectrum since the the extract inhibited both gram – positive and gram – negative bacteria which coincide with this study.

In the antibacterial sensitivity test, methanolic extract of *Chrysophyllum albidum* exhibited the most outstanding antibacterial activity against *Bacillus cereus* with an inhibiting effect of 20.0 mm at 35 mg/ml and 40 mg/ml concentrations (Table 1) which was higher than others at the same concentration, while *Pseudomonas aeruginosa* showed the least zone of inhibition 8 mm at 30 mg/ml.

The minimum inhibitory concentration (MIC) exhibited by the crude extract of *Chrysophyllum albidum* against the bacterial isolates ranged from 0.781mg/ml to 35mg/ml. The lowest MIC exhibited by the extract was against *Staphylococcus aureus* (NCIB 8588) while the highest MIC was against *Bacillus stearothermophilus* (NCIB 822), *Escherichia coli*, *Bacillus polymyxa*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Streptococcus pneumonia* and *Shigella* sp. The highest minimum bactericidal concentration exhibited by the crude extract of *Chrysophyllum albidum* was 35 mg/ml and was exhibited against *Pseudomonas aeruginosa*, *Shigella* sp, *Staphylococcus aureus*, *Bacillus stearothermophilus*, *Escherichia coli*, *Bacillus polymyxa* and *Pseudomonas fluorescens*. On the other hand, the lowest minimum bactericidal concentration was 6.25 mg/ml and was exhibited against *Staphylococcus aureus* (NCIB 8588), *Bacillus cereus* and *Clostridium sporogenes*.

## CONCLUSION

The results revealed the presence of phytochemicals which are medicinally active constituents in the *Chrysophyllum albidum* leaf extracts studied. The leaf extract of *Chrysophyllum albidum* also showed varying degrees of antibacterial activity on the bacteria isolates tested. This study indicated that the extracts of leaf of *Chrysophyllum albidum* have good potential as antibacterial and further provide a rationale for the use of the leaf extract of this plant in traditional medicine practice in Nigeria for treatment. The plant extracts could therefore be seen as a potential source for useful drug. The continued traditional medicinal use of these plants is therefore encouraged. The findings could also be of

commercial interest to both pharmaceutical companies and research institute in the production of new drugs.

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