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## **Antimicrobial Activities of *Chrysophyllum Albidum* Leaves, Fruits and Seeds**

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### **Abstract**

**Background:** *Chrysophyllum albidum* is a medicinal plant that belongs to the Sapotaceae family. This study was carried out to determine the antimicrobial activities of the leaves, seeds and fruits of *Chrysophyllum albidum*.

**Materials and Methods:** The fruits, leaves and seeds of *Chrysophyllum albidum* were extracted with distilled water, Seaman's Schnapps, Methanol and Petroleum ether using cold extraction. These extracts were tested on various organisms. Antimicrobial activities of the various extracts were determined using the agar well diffusion technique to detect the Minimum Inhibitory Concentration (MIC).

**Results and Discussion:** Methicillin Resistant *Staphylococcus aureus* was inhibited by all the extracts at MIC between 32µg/ml to 512µg/ml except Seaman's Schnapps leaves and petroleum ether seed extract. The fruit extracts had a high antimicrobial activity against all the tested organisms. The extracts from Seaman's Schnapps had little activity while the aqueous extracts had MIC ranging from 64- 512µg/ml on most of the organisms tested. The statistical analysis of the extracts indicated P-value of 0.122 for Aqueous extracts, which was statistically insignificant compared to 0.003 for Methanolic extracts and 0.001 for Seaman's Schnapps extracts in the inhibition pattern of the extracts. Aqueous extracts showed more inhibitory activity than Methanolic and Seaman's Schnapps extracts.

Conclusion: *Chrysophyllum albidum* possess antimicrobial properties that can also be developed as antimicrobial for Multidrug resistant (MDR) organisms. *Chrysophyllum albidum* is therefore recommended to be developed into new antimicrobials in readily available forms for use in Medical Microbiology Laboratory.

**Keywords:** *Chrysophyllum albidum*;Antimicrobial; MIC;Extracts.

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## 1. Introduction

*Chrysophyllum albidum* is a medicinal plant that belongs to the Sapotaceae family which has up to 800 species and make up almost half of the order Ericales [1]. *Chrysophyllum* is a genus of about 70-80 species of tropical trees growing rapidly to 10-20m or more in height. The generic name is derived from the Greek word *Chrysos* meaning 'gold' and *Phyllos* meaning 'leaf' [2]. The genus is native to tropical regions throughout the world, with the greatest number of species in northern South America. *Chrysophyllum albidum* is a dominant canopy tree of lowland mixed rain forest, sometimes riverine. It is widely distributed from West Africa to the Sudan with an eastern limit in Kakamega forest, Kenya [2]. *Chrysophyllum albidum*, is distributed throughout the Southern part of Nigeria where it is called 'Agbalumo' (Yoruba) ; 'Udara' (Igbo), while in the Northern Nigeria, it is called 'Khada'(Hausa) [3,4]. Tannins, flavonoids, terpenoids, protein, carbohydrates, and resins are the phytochemicals that have been reported in *Chrysophyllum albidum* [5]. *Chrysophyllum albidum* has antioxidant properties by scavenging free radicals, decreasing lipid peroxidation and increasing the endogenous blood antioxidant enzymes level [6]. The leaves of *Chrysophyllum albidum* was shown to reveal the presence of Alkaloids, Cardiac glycoside, Anthraquinones, Flavonoids, Terpenoids, and Steroids, which are useful substances that have medicinal and physiological activities. Vitamin D which is also believed to contain have also been linked with breast cancer [7-8].

In recent times, there have been an increased antibiotic resistant strains of clinically important pathogens, which has led to the emergence of new bacterial strains that are multi-resistant [9,10,11]. Therefore, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially

active ingredients that can serve as a source for the synthesis of new antimicrobial drugs [12].

Medicinal plants use date back to several centuries in Africa and other regions of the world. It is about the first form of traditional medicine known and reported, and the practice is still popular [13, 4]. The traditional preparations comprise medicinal plants, minerals and organic matter. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparation for therapy [14], however the search for agent to cure infectious disease began long before people were aware of the existence of microbes. These early attempts used natural substances, usually native plants or their extracts and many of these herbal remedies proved successful [15]. A number of studies have been done on the advantages or potency of herbs as cure for example, capsule Artimin from plant, and is a vegetarian capsule and dietary supplement that helps maintain the physiological well being of joints. It contains extracts of *Salix alba* (willow), *Harpagophytum procumbens* (*Harpagus*), *Boswellia serrata* (*Boswellia*), *Malpighia punicifolia* (*Acerola*) and *Uncaria tomentosa* (*Uncaria*) [16, 17]. Therefore, more plants have to be checked for antimicrobial properties, so the need for this study.

Several workers have carried out the in vitro effects of extracts from plants with claimed medicinal use. Several experimental approaches are used. Usually water is used for extraction just as is used traditionally [18]. Other solvents used for extraction apart from water include chloroform, petroleum ether, ethanol, methanol, acetone etc. These extracts were tested against a wide range of microorganisms and their efficacy results well documented. It is not at all times however that such result confirms the claims of the tested herbs [19].

There is paucity of data on the antimicrobial screening of *Chrysophyllum albidum* leaves, fruits

and seed against multidrug resistant organisms, including Extended Spectrum Beta Lactamase producers and Methicilin Resistant Staphylococcus aureus. This study investigated the antimicrobial properties of Chrysophyllum albidum leaves, fruits and seed against multidrug resistant organisms.

## 2. Materials and Methods

### 2.1 Area of Study

This study was carried out at Department of Medical Microbiology, College of Medicine University of Lagos, Lagos State, Nigeria.

### 2.2 Ethical Consideration

Approval for this research work was obtained from the Research Ethics Committee of College of Medicine University of Lagos, Lagos State, Nigeria

### 2.3 Collection and Preparation of Plant Extract

The plants was identified and authenticated at the herbarium unit and Pharmacognosy Laboratory, College of Medicine, University of Lagos. The leaves and fruits of Chrysophyllum albidum (Agbalumo-Yoruba, Udara-Igbo and Khada-Hausa) were purchased at the Mushin Market in Lagos, Nigeria. The seed were obtained from the fruit pulp. (Plate 1 and 2)



Plate 1 Chrysophyllum albidum Leaves



Plate 2 Chrysophyllum albidum Fruits

### 2.4 Processing of Plant Materials

The fresh leaves and fruits were properly washed in tap water and the seed were removed manually and washed in tap water. The plant leaves, fruits and seeds were allowed to dry for two weeks. The plants materials were pulverized into powder using an electric blender.

### 2.5 Extraction of Plant Materials

Distilled water, Seaman's Schnapps, Methanol and petroleum ether were used for the extraction of plant extracts. 100 grams of the dried and grounded leaves were suspended in 500ml of distilled water, Seaman's Schnapps and Methanol for extraction. 100 grams of the dried and grounded fruits were suspended in 500ml of Distilled water, Seaman's Schnapps and Methanol for extraction while 80 grams of the dried and grounded seed was soaked in distilled water and Petroleum ether. This was left to soak at room temperature for 72hours with agitation at intervals. The extracts were filtered with Whatman filter paper No. 42 (125mm). The filtrate were concentrated using the rotary evaporator at 450C. The concentrated extracts from each solvent were taken for lyophilization at the Department of Biochemistry, College of Medicine, University of Lagos. Each solid extract/ paste obtained after lyophilization was reconstituted in their respective solvents to obtain a stock solution of 512µg/ml [25, 26]. The stock solutions were stored in sterile capped bottles and labeled as follows:

1. Aqueous Leave Extract (ALE)
2. Aqueous Fruit Extract (AFE)
3. Aqueous Seed Extract (ASE)
4. Seaman's Schnapps Leave Extract (SSLE)
5. Seaman's Schnapps Fruit Extract (SSFE)
6. Methanolic Leave Extract (MLE)
7. Methanolic Fruit Extract (MFE)
8. Petroleum ether Seed Extract (PSE)

The stock solutions were stored at 4 0C -80C.

### 2.6 Test Organisms

The organisms used were Shigella flexneri, Klebsiella pneumoniae (ESBL), Pseudomonas aeruginosa (MDR), Escherichia coli, Staphylococcus aureus(MRSA), Enterococcus faecalis and Candida albicans. The test organisms were obtained from the research laboratory of Medical Microbiology and Parasitology of College

of Medicine, University of Lagos and Lagos University Teaching Hospital Laboratory.

## 2.7 Control Organisms

Control strains of *Staphylococcus aureus* ATTC 25923 and *Escherichia coli* ATTC 25922 were used and tested along with the test organisms as control.

## 2.8 Identification of Microorganisms

Microorganisms collected were authenticated for identification by Analytical Profile index 20E which is a standardized identification system for Enterobacteriaceae and other non-fastidious, Gram-negative rods.

The Analytical profile index 20E (API 20E, Biomerieux) strip consists of 20 micro tubes containing dehydrated substrates. These strips are inoculated with a 0.5 McFarland standard bacterial suspension that reconstitutes the media. They were then incubated at 37°C overnight. The reactions are recorded and the identification is obtained by referring to the Analytical profile index identification software/flow chart.

## 2.9 Reading the Strip

Identification of the organisms is made by looking up the seven /nine digit profile in the Analytical Profile Index (API20E). The index is divided into two sections, the seven digit profiles read after 18-24 hours of incubation and the nine digit profiles read after 36-48 hours of incubation. The seven digit profile was used in this experiment. The flow chart was followed in the identification of the organisms.

## 2.10 Phenotypic Detection of extended spectrum beta-lactamase

The presence of Extended Spectrum Beta-Lactamase (ESBL) was detected by the double disc synergy test (DDST). A 0.5 McFarland Standard suspension of the test organism was inoculated on Mueller-Hinton agar. A disc containing 30 µg Amoxicillin plus clavulanic acid disc was placed centrally on the plate (Plate 5). Disc containing Cefotaxime (30 µg), Cefotaxime (30 µg) and Ceftriaxone (30 µg) were placed round the Amoxicillin plus Clavulanic acid disc at a distance of 15 mm [10]. The plates were incubated overnight at

37°C for 24 hours. Isolates that exhibited a distinct shape/size with an increased zone of inhibition towards Amoxicillin plus Clavulanate disc were considered as Extended spectrum beta-lactamase producers [10].

## 2.11 Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA)

The presence of MRSA was detected using the modified Kirby-Bauer method (Bauer, et al., 1966). Isolated colonies from an 18 hours culture was used to prepare a direct inoculum equivalent to a 0.5 McFarland Standard. Suspension of the test organism was inoculated on Mueller Hinton Agar using a sterile swab. An Oxacillin disc containing (1 µg) and Cefoxitin (30 µg) were placed on the agar. The Mueller Hinton Agar on which Oxacillin discs was applied were supplemented with 2% Sodium Chloride (NaCl) to enhance the detection of MRSA and incubated aerobically for 24 hours at 37°C while the one on which Cefoxitin was placed was not supplemented with sodium chloride (NaCl) but incubated aerobically at 37°C for 24 hours (Plate 6). The strains were reported as sensitive, or resistant, to Oxacillin/ Cefoxitin with inhibition zone diameter equal or more than 13 mm and less than or equal to 10 mm respectively [27].

## 2.12 Standardization of Inoculum

Test organisms were sub-cultured onto fresh plates of Mueller Hinton agar and Sabouraud Dextrose Agar for 18-24 hours at 37°C for bacteria and fungi respectively. Colonies from these were suspended in sterile normal saline to a turbidity matching 0.5 McFarland standard containing 1 x 10<sup>8</sup> cfu/ml for bacteria and fungi respectively [25].

## 2.13 Antimicrobial Assay

Suspension of the microorganisms were made in sterile normal saline and adjusted to 0.5 McFarland standards. The resulting suspension contains 1 x 10<sup>8</sup> cfu/ml. Each labeled medium Plate were uniformly seeded with a test organism by means of sterile swab rolled in the suspension and streaked on the plate surface. Well of 5 mm in diameter and about 2 cm apart were punched in the culture media with sterile cork-borer. Then 10 µl of the concentrations of the *Chrysophyllum albidum* extracts ranging from 4 µg/ml to 512 µg/ml were

placed in each well [28, 26]. Ciprofloxacin, Distilled water, Seaman's Schnapps, methanol and petroleum ether solvents were placed in a well on each plate seeded with test organisms as controls. Each plate was kept in the refrigerator at 40C for 1 hour to allow the extracts to diffuse into the culture medium while the immediate growth of the organism was stopped from taking place [25]. These plates were then incubated at 370C for 24 hours. The zones of inhibition around the wells were measured in millimeter.

### 2.14 Reading of Results

A ruler was placed across the well and zone of inhibition was measured in millimeter. This was recorded as positive bioactivity. Control strains of organism were set up and measured as well.

### 2.15 Antifungal Assay

Suspension of the *Candida albicans* were made in sterile normal saline and adjusted to 0.5 McFarland standards. The resulting suspension contains 1 x10<sup>8</sup>cfu/ml. Each labeled medium Plates were uniformly seeded with the *Candida albicans*

by means of sterile swab rolled in the suspension and streaked on the plate surface. Well of 5mm in diameter and about 2cm apart were punched in the culture media with sterile cork-borer. The concentrations of the extracts ranging from 4µg/ml to 512µg/ml were dropped in each well to fullness [28, 26]. Each plate was kept in the refrigerator at 40C for 1 hour to allow the extracts to diffuse into the culture medium while the immediate growth of the organism was stopped from taking place. [25]. These plates are then incubated at 370C for 48 hours. The zones of inhibition around the wells were measured in millimeter.

### 2.16 Determination of minimum inhibitory concentration (MIC)

To measure the MIC values, various concentrations of the stock, 512µg/ml, 256µg/ml, 128µg/ml, 64µg/ml, 32µg/ml, 16µg/ml, 8µg/ml, and 4µg/ml were assayed against the test bacteria. The minimum inhibitory concentration was defined as the lowest concentration able to inhibit any visible bacterial growth on the culture plates [29, 28, 26].

**Table 1 Zone of inhibition (mm) of organisms to aqueous leave extract (ale) of *chrysoyllum albidum***

TEST ORGANISMS	CONCENTRATION (µg/ml)										
	512	256	128	64	32	16	8	4	CIP	H <sub>2</sub> O	MIC
S.aureus ATCC 25923	14	11	10	-	-	-	-	-	15	-	128
S. aureus	18	17	-	-	-	-	-	-	15	-	256
S.aureus(MRSA)	15	12	-	-	-	-	-	-	-	-	256
E.coli ATCC 25922	11	8	-	-	-	-	-	-	30	-	256
E.coli (ESBL)	12	10	-	-	-	-	-	-	29	-	256
Shigella flexneri	11	8	-	-	-	-	-	-	-	-	256
E.feacalis	12	10	-	-	-	-	-	-	-	-	256
Klebsiella pneumonia	15	10	-	-	-	-	-	-	-	-	256
Pseudomonas aeruginosa(MDR)	16	8	-	-	-	-	-	-	-	-	256
Candida albicans	10	-	-	-	-	-	-	-	-	-	512

#### Key

H<sub>2</sub>O = Water

MRSA = Methicillin resistant *Staphylococcus aureus*

- = No activity
- CIP = Ciprofloxacin control
- MIC = Minimum Inhibitory Concentration
- ALE = Aqueous leave extract.
- MDR = Multi drug resistance

**Table 2 Zone of inhibition (mm) of organisms to aqueous fruit extract (afe) of chrysophyllum albidum**

TEST ORGANISMS	CONCENTRATION (µg/ml)										
	512	256	128	64	32	16	8	4	CIP	H <sub>2</sub> O	MIC
S.aureus ATCC 25923	16	14	-	-	-	-	-	-	16	-	256
S. aureus	31	24	20	11	-	-	-	-	24	-	64
S.aureus(MRSA)	30	23	16	14	-	-	-	-	15	-	64
E.coli ATCC 25922	-	-	-	-	-	-	-	-	28	-	-
E.coli (ESBL)	-	-	-	-	-	-	-	-	20	-	-
Shigella flexneri	-	-	-	-	-	-	-	-	-	-	-
E.feacalis	-	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumonia	16	10	-	-	-	-	-	-	-	-	256
Pseudomonas aeruginosa(MDR)	15	13	11	10	-	-	-	-	-	-	64
Candida albicans	14	13	8	-	-	-	-	-	-	-	128

**Key**

- H<sub>2</sub>O = Water
- MRSA = Methicillin resistant *Staphylococcus aureus*
- = No activity
- CIP = Ciprofloxacin control
- MIC = Minimum Inhibitory Concentration
- AFE = Aqueous fruit extract
- MDR = Multi drug resistance

**3. Results**

The results in Table I showed that all the tested organisms were inhibited by the aqueous leave extracts of *Chrysophyllum albidum* with Minimum Inhibitory Concentration (MIC) ranging between of 128- 512µg/ml. *S. aureus* ATCC 25923 had the lowest MIC.

Ciprofloxacin was used as control antibiotics for all tests carried out as well as the solvents used for extraction.

The aqueous fruit extract inhibited six organisms with MIC range between 64-256µg/ml (Table2 and Plate 3).

*Escherichia coli* ATCC 25922, *Shigella flexneri*, ESBL *Escherichia coli* and *Enterococcus faecalis* were resistant to the aqueous fruit extract of *Chrysophyllum albidum*. Methicillin Resistant *Staphylococcus aureus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* had the lowest MIC of 64µg/ml respectively (Table 2)

The aqueous seed extract of *Chrysophyllum albidum* showed inhibition on all the organisms tested except *Pseudomonas aeruginosa* and *Enterococcus faecalis* which were resistant. The

MIC range is between 128-512 µg/ml. Methicillin Resistant *Staphylococcus aureus* had the lowest MIC of 128 µg/ml (Table 3).



**Plate 3 Showing the zone of inhibition of Methicillin Resistant *Staphylococcus aureus* to the aqueous leaf, fruit and seed extracts of *Chrysophyllum albidum***

**Table 3 Zone of inhibition (mm) of organisms to aqueous seed extract (ase) of *chrysophyllum albidum***

TEST ORGANISMS	CONCENTRATION (µg/ml)										
	512	256	128	64	32	16	8	4	CIP	H <sub>2</sub> O	MIC
S.aureus ATCC 25923	18	13	-	-	-	-	-	-	18	-	256
S. aureus	16	15	-	-	-	-	-	-	24	-	256
S.aureus(MRSA)	22	12	11	-	-	-	-	-	14	-	128
E.coli ATCC 25922	14	-	-	-	-	-	-	-	40	-	512
E.coli (ESBL)	6	-	-	-	-	-	-	-	15	-	512
Shigella flexneri	15	10	-	-	-	-	-	-	35	-	256
E.feacalis	-	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumonia	13	-	-	-	-	-	-	-	-	-	512
Pseudomonas aeruginosa(MDR)	-	-	-	-	-	-	-	-	-	-	-
Candida albicans	16	12	-	-	-	-	-	-	-	-	256

**Key**

- H<sub>2</sub>O = Water
- MRSA = Methicillin resistant *Staphylococcus aureus*
- = No activity
- CIP = Ciprofloxacin control
- MIC = Minimum Inhibitory Concentration
- ASE = Aqueous seed extract
- MDR = Multi drug resistance

The results in Table 4 showed that *Pseudomonas aeruginosa* had (MIC) of 64µg/ml with the Seaman's Schnapps leaf extract (Plate 4).

*Shigella flexneri* was sensitive with MIC of 256µg/ml while other organisms were resistant to the Seaman's Schnapps leaf extract (Table 4).

The Seaman's Schnapps fruit extract exhibited antimicrobial activity against all the organisms. The MIC range is between 32-512µg/ml. *S.aureus* (MRSA), *E.coli* (ESBL), *Pseudomonas aeruginosa* (MDR) and *Candida albicans* had MIC of 64µg/ml

respectively. The control strains *S.aureus* ATCC 25923, *S.aureus*, and *E.faecalis* had MIC of 512µg/ml. The lowest MIC was exhibited by *Klebsiella pneumonia* with MIC of 32µg/ml (Table 5).

The methanolic leaf extract of *Chrysophyllum albidum* exhibited antimicrobial activities against *S.aureus* (MRSA), *Pseudomonas aeruginosa* and *Shigella flexneri* which had MIC of 512µg/ml respectively (Table 6).

**Table 4 Zones of inhibition (mm) of organisms to seaman's schnapps leaf extract (ssle) of chrysophyllum albidum**

TEST ORGANISMS	CONCENTRATION (µg/ml)									CIP	SS	MIC
	512	256	128	64	32	16	8	4				
<i>S.aureus</i> ATCC 25923	-	-	-	-	-	-	-	-	-	20	-	--
<i>S. aureus</i>	-	-	-	-	-	-	-	-	-	17	-	-
<i>S.aureus</i> (MRSA)	-	-	-	-	-	-	-	-	-	-	-	-
<i>E.coli</i> ATCC 25922	-	-	-	-	-	-	-	-	-	31	-	-
<i>E.coli</i> (ESBL)	-	-	-	-	-	-	-	-	-	-	-	-
<i>Shigella flexneri</i>	12	10	-	-	-	-	-	-	-	35	-	256
<i>E.feacalis</i>	-	-	-	-	-	-	-	-	-	8	-	-
<i>Klebsiella pneumonia</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i> (MDR)	15	13	16	15	-	-	-	-	-	20	-	64
<i>Candida albicans</i>	-	-	-	-	-	-	-	-	-	-	-	-

**Key**

SS = Seaman's Schnapps

MRSA = Methicillin resistant *Staphylococcus aureus*

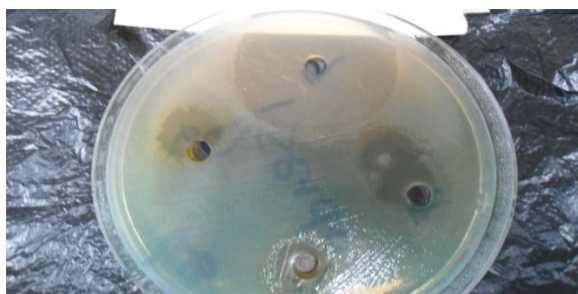
- = NO activity

CIP = Ciprofloxacin control

MIC = Minimum Inhibitory Concentration

MDR = Multi drug resistance





**Plate 4 Showing the zone of inhibition of *Pseudomonas aeruginosa* to the Seaman's schnapps leave extracts of *Chrysophyllum albidum***

**Table 5 Zones of inhibition (mm) Of organisms to seaman's schnapps fruit extract (ssfe) of chrysophyllum albidum**

TEST ORGANISMS	CONCENTRATION ( $\mu\text{g/ml}$ )										
	512	256	128	64	32	16	8	4	CIP	SS	MIC
S.aureus ATCC 25923	15	-	-	-	-	-	-	-	24	-	512
S. aureus	16	-	-	-	-	-	-	-	-	-	512
S.aureus(MRSA)	15	14	12	10	-	-	-	-	-	-	64
E.coli ATCC 25922	14	12	11	-	-	-	-	-	23	7	128
E.coli (ESBL)	14	13	12	10	-	-	-	-	36	-	64
Shigella flexneri	16	9	7	-	-	-	-	-	34	-	128
E.feacalis	15	-	-	-	-	-	-	-	23	-	512
Klebsiella pneumonia	15	10	9	7	6	-	-	-	-	-	32
Pseudomonas aeruginosa(MDR)	17	16	14	11	-	-	-	-	32	-	64
Candida albicans	13	12	10	9	-	-	-	-	-	-	64

**Key**

SS = seaman's schnapps

MRSA = methicillin resistant *Staphylococcus aureus*

- = NO activity

CIP = Ciprofloxacin control

MIC = Minimum Inhibitory Concentration

MDR = Multi drug resistance

The methanolic fruit extract exhibited antimicrobial activity against all the tested organisms with MIC range between 16-256µg/ml of *C.albidum*.

*Staphylococcus aureus* had the lowest minimum inhibitory concentration of 16mg/ml. *S.aureus* (MRSA) and *Shigella flexneri* both had MIC of 32µg/ml. *E.coli*, *E.faecalis*, *K.pneumoniae* were susceptible at MIC of 64µg/ml respectively. *E.coli* ATCC 25922 and *Pseudomonas*

*aeruginosa* both showed MIC of 128µg/ml respectively. *Staphylococcus aureus* ATCC 25923 and *Candida albicans* showed MIC of 256µg/ml (Table7).

*Enterococcus faecalis* with MIC of 8µg/ml showed high sensitivity to the seed oil extract followed by *Shigella flexneri* with MIC of 64µg/ml. Other organisms were resistant to the seed oil extract. (Table 8)

**Table 6 Zones of inhibition (mm) of organisms to methanolic leaf extract (mle) of *chrysophyllum albidum***

TEST ORGANISMS	CONCENTRATION (µg/ml)										
	512	256	128	64	32	16	8	4	CIP	METH	MIC
<i>S.aureus</i> ATCC 25923	-	-	-	-	-	-	-	-	-	-	-
<i>S. aureus</i>	-	-	-	-	-	-	-	-	20	-	-
<i>S.aureus</i> (MRSA)	6	-	-	-	-	-	-	-	-	-	512
<i>E.coli</i> ATCC 25922	-	-	-	-	-	-	-	-	14	-	-
<i>E.coli</i> (ESBL)	-	-	-	-	-	-	-	-	35	-	-
<i>Shigella flexneri</i>	8	-	-	-	-	-	-	-	17	-	512
<i>E.feacalis</i>	-	-	-	-	-	-	-	-	18	-	-
<i>Klebsiella pneumonia</i>	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i> (MDR)	10	-	-	-	-	-	-	-	25	6	512
<i>Candida albicans</i>	-	-	-	-	-	-	-	-	-	-	-

**Key**

- METH. = Methanol
- MRSA = Methicillin resistant *staphylococcus aureus*
- = No activity
- CIP = Ciprofloxacin control
- MIC = Minimum Inhibitory Concentration
- MDR = Multi drug resistance

**Table 7 Zones of inhibition (mm) of organisms to methanolic fruit extract (mfe) of chrysophyllum albidum**

TEST ORGANISMS	CONCENTRATION (µg/ml)										
	512	256	128	64	32	16	8	4	CIP	METH.	MIC
S.aureus ATCC 25923	16	13	-	-	-	-	-	-	27	-	256
S. aureus	22	20	16	10	8	7	-	-	-	10	16
S.aureus(MRSA)	21	16	15	10	9	-	-	-	-	-	32
E.coli ATCC 25922	17	14	10	-	-	-	-	-	37	-	128
E.coli (ESBL)	19	17	10	7	-	-	-	-	32	-	64
Shigella flexneri	20	15	13	12	11	-	-	-	33	-	32
E.feacalis	16	11	10	8	-	-	-	-	20	-	64
Klebsiella pneumonia	15	14	11	10	-	-	-	-	-	-	64
Pseudomonas aeruginosa(MDR)	14	13	8	-	-	-	-	-	25	7	128
Candida albicans	12	10	-	-	-	-	-	-	-	-	256

**Key**

METH.= Methanol

MRSA = Methicillin resistant *Staphylococcus aureus*

- = No activity

CIP = Ciprofloxacin control

MIC = Minimum Inhibitory Concentration

MDR = Multi drug resistance

**Table 8 Zones of inhibition (mm) of organisms to petroleum ether seed extract (pse) of chrysophyllum albidum**

TEST ORGANISMS	CONCENTRATION (µg/ml)										
	512	256	128	64	32	16	8	4	CIP	PET	MIC
S.aureus ATCC 25923	-	-	-	-	-	-	-	-	27	-	-
S. aureus	-	-	-	-	-	-	-	-	-	-	-
S.aureus(MRSA)	-	-	-	-	-	-	-	-	-	-	-
E.coli ATCC 25922	-	-	-	-	-	-	-	-	38	-	-
E.coli (ESBL)	-	-	-	-	-	-	-	-	-	16	-
Shigella flexneri	17	15	12	10	-	-	-	-	33	-	64
E.feacalis	25	24	23	20	18	17	15	-	20	-	8
Klebsiella pneumonia	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa(MDR)	-	-	-	-	-	-	-	-	37	-	-
Candida albicans	-	-	-	-	-	-	-	-	-	-	-

## Key

- PET. = Petroleum ether.  
MRSA = Methicillin resistant *Staphylococcus aureus*  
- = No activity  
CIP = Ciprofloxacin control  
MIC = Minimum Inhibitory Concentration  
MDR = Multi drug resistance

## 4. Discussion

The organisms used included *Shigella flexneri*, *Klebsiella pneumoniae* (ESBL), *Pseudomonas aeruginosa* (MDR- multi drug resistant), *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, Methicillin Resistant *Staphylococcus aureus*, *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* and these gave a wider range of comparison of antimicrobial properties among several bacteria unlike other medicinal plants studies where only four to six organisms were used [20, 22].

A research reported that water and methanolic extracts of *Chrysophyllum albidum* leaves used against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Shigella* species suggested that water and methanolic extracts from the leaves may contain active agent(s), which make them susceptible to the tested organisms [20]. Another study reported also that ethanolic extract of *Chrysophyllum albidum* leaves and stem used against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella* species, *Bacillus subtilis*, and *Candida albicans* were susceptible to plant ethanolic extract [21][7].

Another research also reported that the aqueous extracts of the leave, stem, root and seed of *Chrysophyllum albidum* was used against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Clostridium tetani* and *Candida albicans*. It was reported that *Chrysophyllum albidum* stem and root extracts inhibited *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Clostridium tetani* and *Candida albicans* [22]. The seed cotyledon only showed inhibition on *Candida albicans*, while the leaves did not inhibit

any of these pathogens. Antimicrobial resistance is resistance of a microorganism to an antimicrobial drug to which it was originally sensitive. Resistant organisms (bacteria, fungi, viruses and some parasites) are able to stop the antimicrobial medicines from working, such as antibiotics, antifungals, antivirals and anti-malarials, so that standard treatments become ineffective and infections persists thereby increasing risk of spread to others [23]. It is the ability of a microorganism to withstand the effects of an antibiotic [24].

Of note is the activity of *Chrysophyllum albidum* aqueous leave extract which inhibited all multi drug resistant organisms which was similar to a report by [25], who reported that the lime oil (neat) showed a high antimicrobial activity against all organisms tested which include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

The aqueous seed extract of *Chrysophyllum albidum* inhibited all the organisms tested except *Enterococcus faecalis* and *Pseudomonas aeruginosa* which were resistant. Methicillin Resistant *Staphylococcus aureus* had the lowest MIC of 128 µg/ml.

The aqueous fruit extract of *Chrysophyllum albidum* did not inhibit *Escherichia coli* ATCC 25922, ESBL *Escherichia coli*, *Shigella flexneri* and *Enterococcus faecalis*.

However, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and Methicillin Resistant *Staphylococcus aureus* had MIC of 64 µg/ml respectively. This is similar to the study by [30], in which it was reported that the aqueous extract of lemon grass showed inhibition to *Escherichia coli* ATCC 25922, *Shigella flexneri*, *Escherichia coli*, and *Salmonella paratyphi*.

Notable in this study also was activity of Seaman's Schnapps leaves extract on *Shigella flexneri* and *Pseudomonas aeruginosa* (MDR)

which had MIC of 256µg/ml and 64µg/ml respectively showing the possibility of the extract as an agent that can be used in treating infections caused by *Pseudomonas aeruginosa*. This is similar to the report by [21] where *Pseudomonas aeruginosa*, *S.aureus*, *E.coli*, and *Klebsiella* species were susceptible to the ethanolic leaf extract of *C.albidum*. Unlike the work by [22], who reported that the ethanolic leaf extract showed no sensitivity to *Pseudomonas aeruginosa*. This may be due to the time of harvest of plants and age of plants [25]. Methicilin Resistant *Staphylococcus aureus* (MRSA), *Shigella flexneri* and *Pseudomonas aeruginosa* were the only organisms susceptible to the methanolic leaf extract. This supports the report of [20] that *S.aureus*, *E.coli*, *S.typhi* and *Shigella* species were susceptible to aqueous extract and methanolic leaf extract of *Chrysophyllum albidum* unlike the work by [26] on *Albrus precatorius* methanolic extract that showed no inhibition to *Pseudomonas aeruginosa*. This may be as a result of the difference in concentration and solvent for the extracts or time of collection or age of plant according to [31]. The minimum inhibitory concentration (MIC) of *Chrysophyllum albidum* Seaman's Schnapps fruit extracts varied with respect to the test organisms. At 32µg/ml the growth of *Klebsiella Pneumoniae* was inhibited. At 64µg/ml concentration, *S.aureus* (MRSA), *E.coli* (ESBL), *Pseudomonas aeruginosa* and *Candida albicans* were inhibited. At 512µg/ml, *S.aureus* (ATCC 25923), *S.aureus*, and *Enterococcus feacalis* were inhibited. *Staphylococcus aureus* was inhibited by the methanolic fruit extract which is similar to the work done by [26] on methanolic extract of *Abrus precatorius* (L) fabaceae in which it was reported that *Staphylococcus aureus* was the most sensitive organism. [22] reported that only *Candida albicans* showed inhibition to the aqueous seed extract of *C. albidum*, however, this study showed inhibition of *S.aureus*, *S.aureus* (MRSA) and *S.aureus* ATCC 25923, *E.coli*, ESBL *E.coli*, *Shigella flexneri*, *Klebsiella pneumonia* and *Candida albicans* to the seed extract of *Chrysophyllum albidum*. This can be due to the age of leaves, time of harvesting and handling after harvesting [32]. Petroleum ether seed extract of *Chrysophyllum albidum* did not inhibit *S.aureus*, *S.aureus* (MRSA) and *S.aureus* ATCC 25923, although, it inhibited *Shigella flexneri* and

*Enterococcus faecalis* showing the oil of *C. albidum* has antimicrobial properties on some organisms. However, this is in contrast to [26], where they reported that the seed oil extract of *Abrus precatorius* (L) Fabaceae was the most potent and had greater zones of inhibition against most of the *Staphylococcus* species used. The aqueous leaves extract in comparison to the aqueous fruit extract showed that the aqueous fruit extract had more antimicrobial potency than the leaves extract. Though the aqueous leaves extract inhibited all the tested organisms with MIC range between 128-512µg/ml, aqueous fruit extract with MIC range between 64-256µg/ml, was more potent in antimicrobial activities. This probably was due to time of harvesting and the use since plants from vegetative parts are less effective in therapy [33].

Aqueous leaves inhibited all the tested organisms when compared with Seaman's Schnapps leaves extract which inhibited only *Pseudomonas aeruginosa* and *Shigella flexneri* which may be due to the solvents inability to extract the necessary ingredients or due to drug interaction [33]. Aqueous leaves extract of *Chrysophyllum albidum* inhibited all the tested organisms and has more active antimicrobial activities than Methanolic leaves extract which inhibited MRSA, *Shigella flexneri* and *Pseudomonas aeruginosa* different from the study by [26] who reported that methanolic extract of *Abrus precatorius*(L) Fabaceae was more potent than Aqueous leave extract.

It is interesting to note that the Aqueous leaves extract and the Methanolic fruit extract both inhibited all the tested organisms, however, Methanolic fruit extract exhibited more antimicrobial potency against the tested organisms similar to the study by [26]. The methanolic leave and Petroleum ether seed extract of *Chrysophyllum albidum* has no antibacterial effect on *Staphylococcus aureus* and *Escherichia coli* which was in contrast to the methanolic seed extract of *Daucus carota* which showed antibacterial effect on the organisms as reported by [34]. The aqueous seed extract of *Chrysophyllum albidum* inhibited *S.aureus*, MRSA, *E.coli*, *Shigella flexneri*, *Klebsiella pneumonia* and *Candida albicans* which were similar to the studies by [35] who reported that the ethanolic and methanolic seed extracts of *Mangifera indica* L (Mango) inhibited these

organisms except *Klebsiella pneumonia* which was resistant.

The aqueous leave extract of *Chrysophyllum albidum* had antibacterial effect on *S.aureus*, *E.coli*, *Shigella flexneri* and *Pseudomonas aeruginosa* unlike studies by [36] in which it was reported that the crude leaf extract of *Mangifera indica* Linn had no activity on these bacteria.

The methanolic crude extract of the fruit and leaves of *Momordica charantia* (Bitter melon) inhibited *S.aureus*, *E.coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans* as reported by [37], which is similar to the result obtained with methanolic fruit extract of *Chrysophyllum albidum* in this study.[38] reported that the aqueous extracts of Carrot, Amla and Pomegranate inhibited *S.aureus*, *E.coli*, *Pseudomonas aeruginosa* and *Shigella flexneri* which is similar to the result obtained with the aqueous leave extract of *Chrysophyllum albidum* in this study.

The result was different with the aqueous extract of Honey which inhibited *Escherichia coli* and *Shigella flexneri*. Aloe vera extract inhibited *Escherichia coli*, *Pseudomonas aeruginosa* and *Shigella flexneri* [38].

The methanolic fruit extract of *Chrysophyllum albidum* inhibited all the organisms used in this study which was similar to the study by [39], who reported that the methanolic extract of *Ocimum sanctum*, *Azadirachita indica* and *Phyllanthus amarus* inhibited *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

The lowest Minimum Inhibitory Concentration of 8µg/ml was exhibited by the petroleum seed extract against *Enterococcus faecalis*, which was closely followed by methanolic fruit extract against *Staphylococcus aureus* while the Seaman's Schinapps fruit extract which had the lowest MIC of 32µg/ml against *Klebsiella pneumoniae* which was similar to the study by [40], who reported that all the organisms used including *Klebsiella pneumonia* were susceptible to the ethanolic extract of Unripe banana (*Musa sapientum* L).

The aqueous leaves extract shows more antimicrobial activities than the Seaman's Schinapps and methanolic leaves extracts. However, methanolic fruit extract was the best for the extraction of the active ingredients in

*Chrysophyllum albidum* because it inhibited all the tested organisms.

Methicillin Resistant *Staphylococcus aureus* is known to be of clinical importance due to its resistance pattern. The aqueous and methanolic leave extracts of *Chrysophyllum albidum* inhibited MRSA which was significant, except Seaman's Schnapps leaves and Petroleum ether seed extracts which showed resistant to the organism. A study by [26] showed that methanolic extract of *Abrus precatorius* (L) Fabaceae was more potent than aqueous leave extract.

Multi-drug resistance in Gram negative organisms is becoming a global threat and most available drugs are not active [9]. *Chrysophyllum albidum* had activity on these organisms and unlike studies by [36], which had no activity on bacteria isolates.

## 5. Conclusion

This study revealed that *Chrysophyllum albidum* leaves, fruits and seed extracts are active against Multidrug Resistant (MDR) organisms. Aqueous extract of *Chrysophyllum albidum* possess antimicrobial properties. *Chrysophyllum albidum* is therefore recommended to be developed into new antimicrobials in readily available forms for use in Medical Microbiology Laboratory.

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