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Antimicrobial Activities of *Chrysophyllum Albidium* Seed Oil Extract On Pathogenic *Staphylococcus Aureus Isolates*

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Abstract: Antimicrobial resistance in *Staphylococcus aureus* has continued to rise and has become a general medical problem. Thus the point of this study was to use the *Chrysophyllum albidum* seed extract as an antibiotic against pathogenic *staphylococcus aureus*. The antimicrobial impact of *Chrysophyllum albidium* seed oil on pathogenic *Staphylococcus aureus* from various sources were explored utilizing agar well dissemination strategy. The oil was separated utilizing the soxhlet extraction strategy with n-Hexane as the solvent. A 24hrs Culture isolate of *Staphylococcus aureus* was gotten from chosen research facilities. They were tried against various concentration of the oil (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml). The first isolate, yielded the following zones of inhibition (24.6 mm, 21.5 mm, 19.0 mm, and 16.0 mm) respectively. Similarly, the second *Staphylococcus aureus* yielded the following zones of inhibition (24.6 mm, 21.3 mm, 18.3 mm and 15.0 mm) individually. The last *Staphylococcus aureus* produced the following zones of inhibition; (22.0 mm, 20.6 mm, 17.6 mm and 14.6 mm) separately. They were observed to be significantly sensitive when contrasted against the zone of inhibition of the control (ciprofloxacin) utilized. The minimum inhibitory concentration was accomplished at a mean value of 125 mg/ml, while the mean minimum bactericidal concentration was achieved at 250 mg/ml. Hence oil from seeds of *Chrysophyllum albidum* can be an elective therapeutic agent utilized for treatment of infections caused by *Staphylococcus aureus*.

Keywords: Antimicrobial, *Chrysophyllum albidium*, Extract, Isolate, *Staphylococcus aureus*.

INTRODUCTION

Staphylococcus aureus (S. aureus) is a Gram positive, cocci-shaped bacterium regularly found in the upper respiratory tract and on the skin. It is part of the microbiota (normal Flora) of the body. *Staphylococcus aureus* typically act as a commensal of the human microbiota it can likewise turn into an opportunistic microorganism being a typical reason for skin infections including abscesses, respiratory diseases, for example, sinusitis and food poisoning [1]. Pathogenic strains regularly advance diseases by creating virulence factors like powerful protein toxins, and the release of a cell-surface protein that cleaves and inactivates antibodies. A key natural property of *S. aureus* is the capacity to asymptomatically colonize individuals. Roughly 30% of people are asymptomatic nasal carriers of *S. aureus* [2]. *S. aureus* carriers are presumed to be at elevated risk of infection and they are seen to be a significant wellspring of spread of *S. aureus* strains among people. The significant method of transmission of *S. aureus* is by direct contact; skin-to-skin contact with tainted individuals, contact with contaminated items and surfaces also plays a role in transmission. Different host factors, including loss of the ordinary skin barrier, presence of illnesses, for example, diabetes and acquired immunodeficiency syndrome, or diseases that affect neutrophils are considered major risk factors [3]. Diseases brought about by antibiotic-resistant strains of *S. aureus* continue to rise worldwide. The general number of staphylococcal infection is expanding in numerous nations in both hospitals and local area settings [4]. The rise of anti-microbial resistant strains of *Staphylococcus aureus* for example, methicillin-resistant *Staphylococcus aureus* (MRSA) is an issue in public health. The development of protection from numerous antibioics by *S. aureus* has included obtaining of determinants by horizontal gene transfer of hereditary components [5]. Resistance additionally can arise by changes that modify the biding site of antibiotics and by increasing expression of endogenous efflux pumps. It is extremely evident that *Staphylococcus aureus* alongside different microorganisms has an uncommon capacity to foster protection from any antibiotic they are exposed to. This was first uncovered by the securing of β-lactamase on 'penicillinase plasmids' and the resulting reaction to β-lactamase stable derivatives by obtaining SCCmec components by MRSA [6]. Since the last part of the 1980s, there has been a problem in the release of new classes of antimicrobial medications that can be used as curative agents of staphylococcal diseases, the last recorded was the lipopeptide daptomycin in 1987 [7]. Regardless of much innovative work, no immunization for *Staphylococcus aureus* has been supported [8]. It is therefore paramount to discover alternatives for relieving diseases brought about by *Staphylococcus aureus*.

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Chrysophyllum albidum otherwise called Africa Star Apple (udara) is a therapeutic plant belonging to the Sapotaceae family which has up to 800 species and make up practically 50% of the order [9]. *Chrysophyllum* is a class of around 70-80 species of tropical trees that develops quickly to 10-20m or more in size. The genus is usually seen in tropical parts of the world, with the best number of species in Northern South America. *Chrysophyllum albidum* is a prevailing shelter tree of lowland mixed rain forest sometimes riverine [10]. Tannins, Flavonoids, terpenoids, proteins, sugars and tars are the phytochemicals that have been detected for in *Chrysophyllum albidum* [11,12,13]. It has antioxidant properties which work by removing free radicals, diminishing lipid perioxidation and expanding the endogenous blood antioxidant enzymes level [14]. The leaf of *Chrysophyllum albidum* was documented to contain alkaloids cardiac glycoside, anthraquinone flavonoids, terpenoids and steroids which are valuable substances that have medicinal and physiological effects [15]. The seed of African Star apple contains generally little quantity of oil which contains medium chain fatly acids (MCFAs). This agrees with later reports on MCFAs having significant antimicrobial effect on *Streptococci* and *Staphylococci* [16, 17,18]. The seeds, when grinded and the solvent squeezed out, uncovers yellowish clear oil which is made up of almost 100% fatty substances with free unsaturated fats making up to 0.2%. It likewise contains 48% Lautic acid, 70% Caprylic acid and 6% capric acid; these constituents make up the antimicrobial properties of *Chrysophyllum albidum* seed [15]. Along these lines, the point of this research was to use the *Chrysophyllum albidum* seed extract as an anti-microbial against pathogenic *staphylococcus aureus.*

Aims of the Study

- To evaluate the antimicrobial activity of oil extracts of *Chrysophyllum albidium* seed on pathogenic *Staphylococcus aureus*
- To establish the minimum inhibitory concentration of the oil extracts on the isolated
- To establish the minimum bactericidal concentration of the oil extracts on the isolated.

MATERIALS AND METHODS

***Chrysophyllum albidum* Collection and Preparation of plant extracts**

Fresh fruits of *chrysophyllum albidum* were purchased from some local markets at Owerri, Imo State. The seeds were manually removed from the fruit pulp, washed and allowed to dry at room temperature, the outer coat of the seeds were removed. The seeds were allowed to air dry at room temperature for two weeks, the seeds were grinded into powder using electric blender [17].

Figure 1: Chrisophyllum albidum fruit and seed

Extraction of *Chrysophyllum albidum* Oil

The oil was extracted using the soxhlet extractor with n-Hexane as the solvent. 30grams of the dried and grounded seeds was soaked in n-hexane and mounted in the thimble of the soxhlet extractor and allowed to extract for 2 hours. Thirty six milligrams oil was recovered, the physiochemical properties were assessed and recorded before it was stored in sterile capped bottle and labeled [18].

Test Organism

Staphylococcus aureus isolates were obtained from the Microbiology Laboratory Abia State University Uturu, General Hospital Medical Laboratory Okigwe and Immanuel Diagnostic Laboratory Umuokpara okigwe imo state, Nigeria and stored in a nutrient agar slant labelled A, B and C. The isolates were confirmed using Gram-Stain, Catalase and Coagulase test.

Standardization of Inoculum

Test organism was sub-cultured onto fresh plates of Mueller Hinton agar for 24 hours at 37°c. Colonies from the sub-cultured organism was suspended in sterile normal saline to a turbidity matching 0.5 mcfarland standard containing 1x 10^4 cfu/ml of the bacteria isolates.

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Antimicrobial Assay

The standardized inoculums were seeded on prepared Mueller Hinton agar uniformly by using a sterile swab to roll over the entire plate surface. Wells of 5mm in diameter and about 2cm apart were made in the culture media with sterile cork-borer. Then 1ml of concentrated *Chrysophyllum albidum* oil extract was serially diluted using 2ml of distilled water with five test tubes, where the first test tube contained 500mg/ml of the oil extract, test tube 2 contained 250mg/ml, test tube 3 contained 125mg/ml, test tube 4 contained 62.5mg/ml and test tube 5 contained 31.25mg/ml of the *Chrysophyllum albidum* oil extract. The diluted *Chrysophyllum albidum* oil extract ranging from 500mg/ml to 62.5mg/ml were poured in different wells. Ciprofloxacin was used as the positive control while water was used as negative control on another media plate which was seeded with the standardized inoculum. The plates were incubated at 37^{o}c for 24hrs. The zone of inhibition around the wells were measured in millimeter by using a ruler, the control plates of the organisms were also measured in millimeter [21].

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration was carried out using the micro-broth dilution method. The MIC was determined for each bacterium. Two-fold serial dilutions of the extracts were done. The cultures were incubated at 37°C for 24hrs. 0.1 ml of the standard inoculums of the microorganisms was transfered into different concentrations of the serially diluted extract and the test tubes were incubated at 37°C for 24hrs. The least concentration that yielded 100% inhibition was utilized to determine MIC values.

MINIMIUM BACTERICIDAL CONCENTRATION (MBC)

From the test tubes showing clear appearance, a culture streak was made on nutrient media on Petri dishes to evaluate and ascertain the actual concentration that eliminated the microorganisms. This was shown by the appearance or disappearance of growth.

Concentration (MIC):

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

RESULTS

This study investigated the antimicrobial activity of the seed oil extract of *Chrysophyllum albidum* on *staphylococcus aureus*. The *staphylococcus aureu* isolates obtained from some laboratories was confirmed with the aid of Laboratory guide for microbiology by Onyeagba [20]. A Yellow, Circular, Smooth raised Colony was observed on Muller Hinton agar. The Gram stain showed a cocci shaped organism in chains with purple background indicating a positive Gram reaction. Production of gas bubbles in the test tube indicates a positive catalase reaction. Coagulation was detected after the coagulase test was carried out. The isolates were also found to be oxidase negative, citrate positive, non-motile and indole negative. There was no hydrogen sulphide gas produced, TSI showed alkaline slant and acidic butt. Various concentration of the seed oil extract of *chrysophyllum albidum* used as an antimicrobial agent in an agar well method showed different zones of inhibition measured in millimeter.

Table 1. Contains the physicochemical properties of the extracted oil which includes the colour, odour texture as well as the values of refractive index, solidification point, specific gravity and state at room temperature.

Table 1. Physicochemical Properties of the Extracted Oil

S/N	Characteristics	Analysed Value
1	Refractive index	1.4672 at 31^{o}C
2	Odour	Agreeable
3	Colour	Yellow
4	Texture	Viscous
5	Solidification point	-2^{o}c
6	State at 28^{o}c	Liquid
7	Specific gravity	0.89

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Table 2.0 contains the Biochemical characteristics of the obtained Staphylococcus aureus, the results confirmed the isolated organisms to be pathogenic Staphylococcus aureus.

Table 2. Biochemical Characteristics of the Obtained Staphylococcus Aureus

Isolate	Gram Reaction	Motility	Catalase	Oxidase	Coagulase	Citrate	Indole	TSI	H_2S	Organism Identified
A	+	–	+	–	+	+	–	K/A	–	Staphylo-
B	+	_	+	_	+	+	_	K/A	_	Coccus
C	+	_	+	_	+	+	_	K/A	_	Aureus

KEY: K= Alkaline; A = Acidic; + = Positive; – = Negative

The table contains the zones of inhibition recorded with regards to the various concentrations of the oil extracts.

Table 3. Zones of Inhibition of Chrysophyllum Oil on Staphylococcus Aureus Isolate

Clinical Isolates Of Staphylococcus Aureus From	500mg/Ml $\bar{x} \pm SD$	250mg/Ml $\bar{x} \pm SD$	125 Mg/Ml $\bar{x} \pm SD$	62.5 Mg/Ml $\bar{x} \pm SD$	Sensitivity
A	24.6 ±0.34	21.5 ±0.23	19.0 ±0.14	16.0 ±0.15	S
B	24.6 ±0.25	21.3 ±0.14	18.3 ±0.13	15.0 ±0.15	S
C	22.0 ±0.13	20.6 ±0.03	17.6 ±0.14	14.6 ±0.16	S

KEY: $\bar{x}$= mean; SD = Standard deviation; mg/ml = milligram per mil; S = Sensitive

Table 4.0 contains the values of the minimum inhibitory concentrations and minimum Bactericidal concentrations of the oil extracts on the isolated

Table 4. Minimum Inhibitory Concentration (Mic)/Minimium Bactericidal Concentration (Mbc) Of Clinical Isolates

Clinical Isolates of Staphylococcus Aureus	MIC mg/ml	MBC mg/ml
A	125	500
B	125	250
C	125	250

KEY: MIC – Minimum Inhibitory Concentration; MBC – Minimum Bacteriocidal Concentration; Mg/ml – Milligram per Mil

DISCUSSION

The cultural morphology and biochemical reactions of the obtained isolates confirmed that they are *Staphylococcus aureus* [20]. The different concentration of the *Chrysophyllum albidum* oil ranging from 500mg/ml to 62.5mg/ml used against *Staphylococcus aureus* from different laboratories gave different values of zones of inhibition which proved to be significant when compared with the values of the control. This shows that *Chrysophyllum albidum* oil inhibits the growth of *staphylococcus aureus*. This findings disagrees with the work of George *et al.*, 2018 who recorded that petroleum ether seed extract of *Chrysophyllum albidum* were not sensitive to *Staphylococcus aureus* [22]. However, the study corroborates with the study of Samuel *et al.*, 2016 on Phytochemical and Antibacterial Properties of Ethanolic Seed Extracts of *Chrysophyllum albidum* [23]. The phytochemical components of the extract may have caused the significant sensitivity on *S.* aureus. This agrees with previous reports on phytochemical component of *Chrysophyllum albidum* [24,25,26]. According to Okoli & Okere (2011) [27] the extracts of the seeds and roots of Africa star apple have anti-inflammatory, antidiarrheal and anti-hemorrhoidial qualities thus there use in home remedies and herbal treatments of certain diseases.

A comparative research was carried out by Adeleye *et al.* (2016), on the extraction of the phytochemical from *Chrysophyllum albidum* fruit. The phytochemical tests showed the presence of flavonoids, alkaloids, tannis, steroids, anthraquinone and cardiac glycoside. The antimicrobial properties of the extract were examined on *Escherichia coli*, *staphylococcus aureus*, *klebsiella pneumonia* and *candida albicans*. The

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extracts of the seed cotyledons was successful in the treatment of *candida albicans*, while the root extracts inhibited *pseudomonas aeruginosa*, *Escherichia coli*, *staphylococcus aureus*, C. tetani, Bacillus subtilis [28]. However, from our study, the minimum inhibitory concentration of *staphylococcus aureus* A was established at 125mg/ml while the minimum bactericidal concentration was gotten at 500mg/ml. For *staphylococcus aureu* B, MIC was at 125mg/ml while MBC was at 250 mg/ml; while for *Staphylococcus aureus* C, MIC was achieved at 125mg/ml and MBC at 250mg/ml. Therefore oil from seeds of *Chrysophyllum albidum* can be an alternative source of antimicrobial oil. The result of this study shows that the extracts from the seed of *Chrysophyllum albiduim* used in traditional medicine, validates the claim that extracts from different parts of the plant have been used to cure different infections caused by *Staphylococcus aureus*. Further researches should be conducted to appraise the significant ability of the extracts as antibacterial against *Staphylococcus aureus.*

CONCLUSION

In the face of increasing antimicrobial resistance of *Staphylococcus aureus* and scarcity of sources of unsaturated oils, oil extracts of *chrysophyllum albidum* seeds have shown greater sustained antibacterial effect on *staphylococcus aureus.*

Recommendations

Efforts should be geared towards commercializing the extraction of these natures gift and harnessing it into oils for food, pharmaceuticals, skincare products, Aromatherapies, and industrial lubricants.

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