narraX

Short Communication

Antibacterial activity of *Chrysophyllum albidium* seed oil extract on pathogenic *Staphylococcus aureus*

Martin C. Onuigbo[1*], Chimere Y. Ukegbu[2] and Kelechukwu C. Uzoigwe[2]

[1]Department of Microbiology, College of Natural and Applied Sciences, Gregory University, Uturu, Nigeria; [2]Department of Biochemistry, College of Natural and Applied Sciences, Gregory University, Uturu, Nigeria

*Corresponding author: martinsemerem@gmail.com

Abstract

Antimicrobial resistance in *Staphylococcus aureus* has continued to rise and has become a general medical problem. Thus, the objective of this study was to use the *Chrysophyllum albidum* seed extract as an antibiotic against pathogenic *Staphylococcus aureus*. The antimicrobial impact of *Chrysophyllum albidum* 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. The oil extract was then prepared in various concentrations (62.5–500 mg/mL) and tested against three different pathogenic isolates of *Staphylococcus aureus*. At the highest concentration (500 mg/mL), the oil extract yielded 22–24.6 mm inhibition zones. Meanwhile, at the lowest concentration (62.5 mg/mL), the inhibition zones achieved were 14.6–16 mm. The minimum inhibitory concentration was 125 mg/mL, while the mean minimum bactericidal concentration was 250 mg/mL. In conclusion, our data suggested that the oil from seeds of *Chrysophyllum albidum* has antibacterial activities against *Staphylococcus aureus* and this needs to be further studied.

Keywords: Africa star apple, antimicrobial, extract, isolate, *Staphylococcus aureus*

Introduction

Staphylococcus aureus is an opportunistic bacterium and commonly present in the upper respiratory tract and on the skin. The bacteria is a normal microbiota in the body but it could cause cutaneous infections which leads to skin abscesses. It also could infect the respiratory system resulting of pneumonia, bronchitis and sinusitis [1]. Pathogenic strains of *S. aureus* can cause diseases by producing virulence factors such as potent protein toxins and a cell-surface protein that could break and render antibodies inactive. One of the notable features of *S. aureus* is its ability to colonize individuals without causing any symptoms. It is estimated that around 30% of world's population carry *S. aureus* in their nasal passages without exhibiting any symptoms [2]. These carriers are believed to be at a higher risk of infection, and are considered a significant source of *S. aureus* transmission among individuals. Direct contact with infected individuals, as well as contact with contaminated surfaces and objects, are the major modes of transmission for *S. aureus*. Skin-to-skin contact with infected individuals also contributes to the spread of this bacterium. Some host factors such as loss of skin barrier, presence of illnesses, such as diabetes and acquired immunodeficiency syndrome, or diseases that affect neutrophils are considered major risk factors [3].

Received: April 4, 2023 - Accepted: April 29, 2023 - Published online: April 30, 2023

Staphylococcal infections are becoming more prevalent in many countries, both in hospital settings and in the community [4]. The rise of anti-microbial resistant strains of *S. aureus* for example, methicillin-resistant *S. aureus* (MRSA) is an issue in public health. The increasing resistance of *S. aureus* to multiple antibiotics is attributed to the acquisition of genetic elements through horizontal gene transfer [5]. Furthermore, resistance to antibiotics in *S. aureus* can also result from changes that alter the binding site of antibiotics and from the upregulation of endogenous efflux pumps [5]. It is well established that *S. aureus*, like other microorganisms, has a remarkable ability to develop resistance to antibiotics. This was first demonstrated by the acquisition of β-lactamase on 'penicillinase plasmids', and the subsequent response to β-lactamase-resistant derivatives through the acquisition of SCCmec elements by MRSA [6]. Since the late 1980s, there has been a challenge in the development of new classes of antimicrobial drugs that can effectively treat staphylococcal infections. The most recent class to be introduced was the lipopeptide daptomycin in 1987 [7]. Despite extensive research and development efforts, there is currently no approved vaccine for *S. aureus* [8]. It is therefore paramount to discover alternatives for relieving diseases brought about by *S. aureus*.

Chrysophyllum albidum, also known as Africa star apple or *udara*, is a medicinal plant that belongs to family *Sapotaceae* constituted by almost 800 species and represents nearly 50% of the order [9]. *C. albidum* is an abundant shade tree in lowland mixed rainforests, including riverine areas [10]. The plant has been found to contain a variety of phytochemicals including tannins, flavonoids, terpenoids, proteins, sugars, and resins [11,12,13]. It possesses antioxidant properties with free radicals scavenging, reducing lipid peroxidation, and increasing the levels of endogenous blood antioxidant enzymes as its main mechanisms [14]. Its leaf has been reported to contain alkaloids cardiac glycoside, anthraquinone flavonoids, terpenoids, as well as steroids which are valuable substances that have medicinal and physiological effects [15]. The seeds of *C. albidum* contain little quantity of oil which comprised of medium chain fatly acids (MCFAs). MCFAs have significant antimicrobial effects on *Streptococci* and *Staphylococci* [16, 17,18]. The seeds, when grinded and the solvent squeezed out, produce yellowish clear oil which is made up of almost 100% fatty substances with free unsaturated fats making up to 0.2% [15]. It likewise contains 48% lautic acid, 70% caprylic acid, and 6% capric acid; these constituents contribute to antimicrobial properties of *C. albidum* seed [15]. The objective of this study was to investigate the antimicrobial potential of oil extracted from *C. albidum* seeds against pathogenic strains of *S. aureus*. The study also aimed to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the oil extract against *S. aureus*.

Methods

Collection and preparation of *Chrysophyllum albidum* seed extract

Fresh fruits of *C. albidum* were purchased from some local markets at Owerri, Imo State, Nigeria. The seeds were manually removed from the fruit pulp, washed and allowed to dry at 20 to 25°C, for 48 h before the outer coat of the seeds were removed. The photographed images of the fresh fruits and the separated seeds have been presented in **Figure 1**. The seeds were allowed to air-dry at room temperature for two weeks and were ground into powder using an electric crusher to produce seed powder [17].

Extraction of *Chrysophyllum albidum* oil

The oil was extracted from the *C. albidum* seeds using a Soxhlet extractor with n-hexane. Briefly, 30 g *C. albidum* seed powder was soaked in n-hexane and mounted in the thimble of the Soxhlet extractor and allowed to extract for 2 h. A total of mg of oil was recovered, where the physiochemical properties were assessed and recorded before it was stored in a sterile capped bottle and labeled [18].

Bacterial specimen

The pathogenic *S. aureus* isolates were obtained from the Microbiology Laboratory, Abia State University Uturu, General Hospital Medical Laboratory Okigwe and Immanuel Diagnostic Laboratory Umuokpara Okigwe state, Nigeria and stored in a nutrient agar slant. The isolates

were labelled A, B and C, respectively. The isolates were confirmed using Gram-Stain, catalase, and coagulase test.

(a) **(b)**

Figure 1. Photographed images of *Chrysophyllum albidum* fruits (a) and its seeds (b)

Standardization of inoculum

The test bacteria were sub-cultured onto fresh plates of Mueller Hinton agar and incubated for 24 h at 37°C. Colonies from the sub-cultured bacteria were then suspended in sterile normal saline to a turbidity matching 0.5 McFarland, which contained $1x10^4$ CFU/mL of the bacterial isolates.

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 5 mm in diameter and 2 cm apart were made in the culture media with a sterile cork-borer. Then 1 mL of concentrated *C. albidum* oil extract was serially diluted using 2 mL of distilled water with four test tubes (i.e., each tube containing 500 mg/mL, 250 mg/mL, 125 mg/mL and 62.5 mg/mL of the *C. albidum* oil extract). Ciprofloxacin was used as the positive control while water was used as negative control. The plates were incubated at 37°C for 24 h. After incubation, the zone of inhibition was measured in millimeters. The control plates of the organisms were also measured in millimeters for comparison [21].

Determination of minimum inhibitory concentration (MIC)

MIC was measured 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 24 h. As much as 0.1 mL of the standard inoculums of the microorganisms was transferred into different concentrations of the serially diluted extract and the test tubes were incubated at 37°C for 24 h. The least concentration that yielded 100% inhibition was utilized to determine MIC values.

Determination of minimum bactericidal concentration (MBC)

From the test tubes showing a 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, following the suggestion from previous study [19]. The concentration range of the extract used in this analysis was between $1x10^5$ and $1x10^6$ CFU/mL.

Results

Physicochemical properties of the extracted *C. albidum* seed oil, including color, odor, texture, refractive index, solidification point, specific gravity and state at room temperature are presented in **Table 1**. The oil had sweet smelling odor and was yellow in color with a refractive index of 1.4672 (at 31°C). It had viscous texture and appeared as liquid at room temperature (28°C). The specific gravity of the oil was 0.89 kg/m^3 and can be solidified at -2°C.

Table 2. Physicochemical properties of the extracted *Chrysophyllum albidum* seed oil

Characteristics	Value or remark
Refractive index	1.4672 at 31°C
Odor	Sweet smelling
Color	Yellow
Texture	Viscous
Solidification point	-2°C
State at 28°C	Liquid
Specific gravity	0.89 kg/m^3

The biochemical characteristics of the isolates are presented in **Table 2**. It was found that all isolates were positive for catalase and citrate tests, while they were negative for motility, oxidase, indole, and H_2S tests. In the triple sugar iron test, the samples were positive for dextrose fermentation only (alkaline/acidic). Identification of the isolates revealed them as pathogenic *S. aureus*. The *S. aureus* isolates obtained from some laboratories was confirmed with the aid of Laboratory Guide for Microbiology [20].

Further, antibacterial activities of *C. albidum* seed oil were confirmed on these three isolates, with inhibition zone ranged from 14.6±0.16 mm (isolate C) to 24.6±0.34 mm (isolate A) (**Figure 2**). The inhibition zones against all isolates are dependent on the seed oil concentration (**Figure 2**). MICs of *C. albidum* seed oil against all isolates were 125 mg/mL. Meanwhile, the MBCs obtained for isolate A, B, and C were 500 mg/mL, 250 mg/mL, and 250 mg/mL, respectively.

Table 2. Biochemical characteristics of the isolates

Characteristics	Bacterial isolate		
	Microbiology Laboratory, Abia State University (Isolate A)	General Hospital Medical Laboratory Okigwe (Isolate B)	Immanuel Diagnostic Laboratory (Isolate C)
Motility	Negative	Negative	Negative
Catalase	Positive	Positive	Positive
Oxidase	Negative	Negative	Negative
Citrate	Positive	Positive	Positive
Indole	Negative	Negative	Negative
Triple sugar iron	Alkaline/acidic	Alkaline/acidic	Alkaline/acidic
H_2S	Negative	Negative	Negative
Identification	*S. aureus*	*S. aureus*	*S. aureus*

Discussion

The cultural morphology and biochemical reactions of the obtained isolates confirmed that they were *S. aureus* [20]. The different concentrations of the *C. albidum* oil ranging from 62.5 to 500 mg/mL were used against *S. aureus* isolates, where the inhibition zones were larger than that of control (ciprofloxacin). This suggests that *C. albidum* oil could inhibit the growth of *S. aureus*. These findings are not in line with that reported by previously where they found that petroleum ether extract of *C. albidum* seed could not inhibit the growth of *S. aureus* [22]. However, the present study is in agreement with the study of Samuel *et al.* [23]. The phytochemical components of the extracted oil may be responsible for the antibacterial activity against *S. aureus* [23]. Previously, phytochemical components of *C. albidum* have been identified and evaluated for their role in inhibiting the bacterial growth [24,25,26]. According to the previous study [27], the extracts of the seeds and roots of *C. albidum* have anti-inflammatory, antidiarrheal and anti-hemorrhoidal activities, suggesting its potential use as home remedies and herbal treatments.

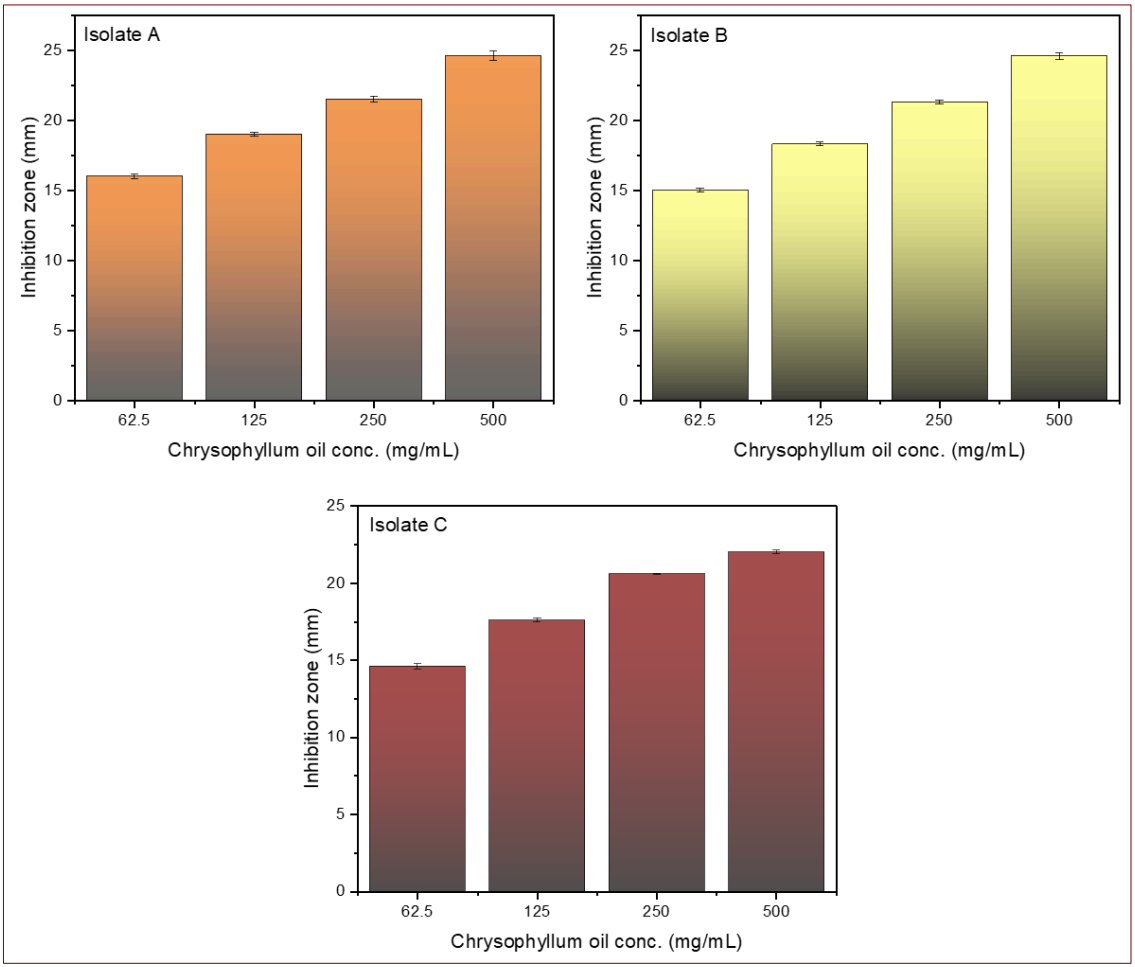

Figure 2. Effect of *Chrysophyllum albidum* oil on various clinical isolates of *Staphylococcus aureus*. All isolates are considered sensitive to the *C. albidum* oil.

Comparative study was carried out previously on the extraction of the phytochemical from *C. albidum* fruit [28]. The phytochemical tests indicated the presence of flavonoids, alkaloids, tannins, steroids, anthraquinone, as well as cardiac glycoside [28]. The antimicrobial properties of the extract were examined on *Escherichia coli, S. aureus, Klebsiella pneumonia*, along with *Candida albicans* [28]. The extract of the seed cotyledons was active in inhibiting *C. albicans,* while the root extract was active against *Pseudomonas aeruginosa, E. coli, S. aureus*, *Clostridium tetani,* and *Bacillus subtilis* [28]. In this present study, the MIC against the isolate A was found to be 125 mg/mL, while its MBC was 500 mg/mL. In the case of isolate B, the MIC and MBC were 125 mg/mL and 250 mg/mL, respectively. Similarly, for the isolate C, the MIC and MBC were achieved at 125 mg/mL and 250 mg/mL, respectively. These results suggest that the extract from the seed of *C. albidum* is potentially to be used to treat pathogenic *S. aureus*. Nonetheless, as the limitations, this research was unable to identify the components responsible for the antibacterial activities and elucidate their mechanisms of actions.

Conclusions

In the face of increasing antimicrobial resistance of *S. aureus* and scarcity of sources of unsaturated oils, oil extracts of *C. albidum* seeds have been evidenced to be effective against *S. aureus*. However, further studies are important to be conducted to identify the components responsible for the antibacterial activities and elucidate their mechanisms of actions.

Ethics approval

Not applicable

Acknowledgments

Authors wish to thank the Founder of Gregory University Uturu for given the opportunity to lecture and serve as Head of Microbiology Department.

Conflict of interest

All the authors declare that there are no conflicts of interest.

Funding

This study received no external funding.

Underlying data

All data underlying the results are available from the corresponding author upon reasonable request.

How to cite

Onuigbo MC, Ukegbu CY and Uzoigwe KC. Antibacterial activity of *Chrysophyllum albidium* seed oil extract on pathogenic *Staphylococcus aureus*. Narra X 2023; 1 (1): e77 – http://doi.org/10.52225/narrax.v1i1.77.

References

1. Tong SY, Davis JS, Eichenberger E, *et al. Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 2015;28(3):603–61.
2. Miller LG and Diep BA. Clinical practice: colonization, fomites, and virulence: rethinking the pathogenesis of community-associated methicillin-resistant *Staphylococcus aureus* infection. Clin Infect Dis 2008;46:752–60.
3. Deurenberg RH. Cross-border dissemination of methicillin-resistant *Staphylococcus aureus*, Euregio Meuse-Rhin region. Emerg Infect Dis 2009;15:727–34.
4. Hersh AL, Chambers HF, Maselli JH, *et al.* National trends in ambulatory visits and antibiotic prescribing for skin and soft-tissue infections. Arch Intern Med 2008;168:1585–91.
5. JensenSO, LyonBR. Genetics of antimicrobial resistance in *Staphylococcus aureus*. Future Microbiol 2009;4:565–82.
6. NesmeJ, Simonet P. The soil resistome: a critical review on antibiotic resistance origins, ecology and dissemination potential in telluric bacteria. Environ Microbiol 2015; 17:913-30.
7. Silver L.L. Challenges of antibacterial discovery. Clin Microbiol Rev 2011;24:71–109.
8. Zorzet A. Overcoming scientific and structural bottlenecks in antibacterial discovery and development. Ups J Med Sci 2014;119:170–5.
9. Houessou LG, Lougbegnon TO, Gbesso FG, *et al.* Ethno-botanical study of the African star apple (*Chrysophyllum albidum* G. Don) in the Southern Benin (West Africa). J Ethnobiol Ethnomed 2012;8(1):1-10.
10. Ologunagba MO, Azubuike CP, Silva BO, *et al.* Characterization of *Chrysophyllum albidum* (family: Sapotaceae) endosperm seed gum for potential application as pharmaceutical excipient. Trop J Nat Prod Res 2017;1(5):217–222.
11. Edem DO, Eka OU and Ifon ET. Chemical evaluation of the value of the fruit of African Star Apple (Chrysophyllum albidum). Food Chem 2017;14:303–311.
12. Esomosele IC and Komolafe TO, Characterization of oil and chemical analysis of the seed of Chrysophyllum albidum. J Plant Foods Hum Nutr 2009;46:361–365.
13. Kamba AS and Hassan LG. Phytochemical Screening and antimicrobial activities of African Star Apple (*Chysophyllum albidum*) leaves, stem, against some pathogenic microorganisms. Int J Pharm Front Res 2016;1(2):119–129.
14. Asare IK, Abenaa AO, Duah, *et al.* Nutritional and phytochemical constituents of the African Star Apple. Ann Food Sci Technol 2015;16:138-46.
15. Idowu TO, Iwelawa EO and Ogundaini, AO. Biochemical and behavioural effects of eleagnine from *Chysophyllum albidum*. J Biol Sci 2016;6:1029–1031.

16. Ugboko HU, Nwinyi OC, Oranusi SU, *et al.* Antimicrobial importance of medicinal plants in Nigeria. Sci World J. 2020;2020:7059323.
17. Nwadinigwe CA. Nutritional value and mineral contents of *Chrysophyllum albidum* fruit. J Sci Food Agric 1982;33(3):283-286.
18. Ajoyi IA and Ifedi EN. Chemical and Preliminary Toxicology Evaluation of *Chrysophyllum albidum* seed. J Environ Sci Toxicol Food Tech 2015;9(6):59–67.
19. Cane HPCA, Musman M, Yahya M, *et al.* Phytochemical screening and antibacterial activity of ethnomedicinal plants from Gayo Lues Highland, Indonesia. J Pharm Pharmacogn Res 2023;11(1):117-128.
20. Forbes BA, Hall GS, Miller MB, *et al.* Practical guidance for clinical microbiology laboratories: mycobacteria. Clin Microbiol Rev 2018;31(2):e00038-17.
21. Carey RB, Bhattacharyya S, Kehl SC, *et al.* Practical guidance for clinical microbiology laboratories: implementing a quality management system in the medical microbiology laboratory. Clin Microbiol Rev 2018;31(3):e00062-17.
22. George OA, Adenipekun EO, Fasogbon SA, *et al.* Antimicrobial activities of *Chrysophyllum albidum* leaves, fruit and seed extract. Am J Biomed Sci 2018;10(1):24-48.
23. Samuel IO, Raphael CM, Kolawole OA, *et al.* A Phytochemical and antibacterial properties of ethanolic seed extracts of *Chrysophyllum albidum*. Orient J Phys Sci 2016;1(1&2):5-9.
24. Akaneme FI. Identification and preliminary photochemical analysis of herbs that can arrest threatened miscarriage in orba and Nsukka towns of Enugu State. Afr J Biotechnol 2008;7(1):6-11.
25. Bonjar S. Evaluation of antibacterial properties of some medicinal plants used in Iran J. Eittinopharmacol 2004;94(2-3):301–305.
26. Duyilemi OP and Lawal IO. Antibacterial activity and phytochemical screening of *Chrysophyllum albidum* leaves. Asian J food Agro-Ind 2019;2:75–79.
27. Okoli BJ and Okere OS. Antimicrobial activity of the phytochemical constituents of *Chrysophyllum albidum*. J Res Nat Dev 2010;8(1):1035-1037.
28. Adeleye S, Orji C and Akaluka C. Phytochemisty and antimicrobial property of fruits of *Chysophyllen albidum* against Selected clinical isolates. Int Lett Nat Sci. 2016;55:44–51.