

Green synthesis of silver nanoparticles from peel extract of *Chrysophyllum albidum* fruit and their antimicrobial synergistic potentials and biofilm inhibition properties

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Abstract Current methods for green synthesis of metal nanoparticles often require continuous harvesting of fresh bio-materials for every synthesis cycle. Practices and procedures that economize bio-materials need to be employed if green synthesis could become a sustainable and eco-friendly method for synthesizing metal nanoparticles. This study explores Chrysophyllum albidum peels (mostly regarded as waste) to prepare silver nanoparticles (Alb-AgNPs). The technique employed in the synthesis allows repeated use of the peels, thus, reducing the heavy dependence on bio-materials. The optical and structural properties of the Alb-AgNPs were studied with Scanning electron microscope, Fourier transform infrared spectrometer, UV-Vis spectrophotometer and powder X-ray diffractometer. The antimicrobial properties of the Alb-AgNPs were studied with selected microorganisms namely; S. aureus, E. coli, K. pneumoniae, B. subtilis, S. mutans, P. aeruginosa, S. typhi, and Candida albicans. High inhibitory activity against the microorganisms were exhibited with MICs ranging from 15.62 to 1000 µg/mL. Again, the

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Alb-AgNPs showed the ability to enhance the efficacy of standard antimicrobial agents. The results of the combined interaction with standard antibacterial and antifungal agents ranged from synergistic to antagonistic effects against the tested microorganisms. In addition, the *Alb-AgNPs* could serve as a biofilm inhibitor with the highest percent inhibition of about 92% against methicillin-resistant *Staphylococcus aureus*. The results from this study thus provide access to the simple, sustainable, economic and ecofriendly synthesis of silver nanoparticles with efficient antimicrobial properties as drug candidates as a means of overcoming the prevailing antibiotic resistance menaces.

Keywords Chrysophyllum albidum · Silver nanoparticles · Biofilm inhibition · Synergistic effect · Antimicrobial properties · Green synthesis

Introduction

The rise in concerns about the health implications of metal nanoparticles (MNPs) has necessitated the search for methods that aid the production of MNPs which are benign to the environment. The green method for synthesizing MNPs offers the possibility of producing nanoparticles through routes that reduce or eliminate the use of dangerous, costly and toxic chemicals (Shankar et al. 2003; Philip 2009; Bankar et al. 2010; Vanaja and Annadurai 2013;

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Mo et al. 2015; Skiba and Vorobyova 2019). This approach uses biochemicals in extracts of plants, bacteria, fungi, algae, yeast etc., for the preparation of MNPs (Gajbhiye et al. 2009; Khanna et al. 2019; Gloria Martin and Vergara Padilla 2020; Shu et al. 2020; Yusefi et al. 2021; Bahrulolum et al. 2021). Plant products, when used to prepare MNPs furnish the nanoparticles with numerous properties. Plants extracts are mostly non-toxic and possess little or no environmental toxicity, thus serving as a benign reducing and stabilizing agent for preparing nanoparticles (Zhang et al. 2020).

Metal nanoparticles prepared by the green approach, especially that of silver, have been extensively studied for their ability to disrupt and damage bacteria and fungi cells to inhibit growth (Anees Ahmad et al. 2020). Silver nanoparticles prepared biogenically with extracts from Centella asiatica, B. diffusa, P endlicherianum, Solanum tricobatum, Adathoda vasica, Ocimum tenuiflorum, Syzygium cumini etc., have shown enhanced efficacy in preventing the growth of bacteria (Brayner 2008; Vijay Kumar et al. 2014; Latha et al. 2016; Şeker Karatoprak et al. 2017). This efficacy spans from the ability to inhibit and kill bacteria cells, synergistically increase the potency of antibiotic or antifungal agents, and inhibit the formation of microorganism biofilms (Fayaz et al. 2010; Sadeghi-Kiakhani et al. 2022).

Green synthesis of MNPs requires continuous harvesting of natural plant products which can place gruesome stress on biodiversity if demands for biosynthesized MNPs continue to rise. The review study by Siddiqi et al. (2018) revealed that plant parts such as leaves, fruits and seeds are mainly used for the green synthesis of MNPs. These plant parts are crucial in other respects, in that most of the fruits used in green synthesis of nanoparticles are 'super foods' for human consumption and are already in high demand (Sabine 2017). On the other hand, plant leaves are indispensable in photosynthetic processes, which are crucial in the fight against global warming (Tkemaladze and Makhashvili 2016). Therefore, it is not an overstatement that the current most subscribed practice, and used plant parts, may not be sustainable in the near future if green synthesis becomes the household method for nanoparticle synthesis. As a result, studies have focused on using other plant parts of less demand and considered 'non-essential' or 'waste' to prepare MNPs. Plants products such as orange peels (Skiba and Vorobyova 2019), banana peel (Bankar et al. 2010), avocado peels (Villanueva-Ibáñez et al. 2015), rice husk (Lieu et al. 2018), corn husk (Villanueva-Ibáñez et al. 2015) etc., have been explored as potential sources of bio reductants for the preparation of MNPs.

In this study, we explore plant extracts obtained from the peels of Chrysophyllum albidum fruit as potent bio-reducing and stabilizing agents for synthesizing silver nanoparticles. Chrysophyllum albidum, also known as African star fruit, is mainly grown in tropical regions. Healthwise, it contains an adequate amount of carbohydrates, protein, fats, oil, and vitamins (Asare et al. 2015). It has anti-inflammatory properties, and the high amount of pectin, polyphenols and vitamin C make it a potent plant for detoxification (Folasade et al. 2019). Previously, efforts have gone into synthesizing silver nanoparticles from seed and leaf extracts of Chrysophyllum albidum for catalytic applications and investigating α - amylase interaction, respectively. These silver nanoparticles were attained through elaborate processes of pulverization, heating and microwave irradiation. In the present study, dried peels of Chrysophyllum albidum fruit were swirled with deionized water and used to prepare silver nanoparticles. The peels could then be reused in a subsequent synthesis, making the approach simple, easy and economical. The nanoparticles were studied for their bactericidal, fungicidal, synergistic and biofilm inhibition effects. The results from this study showed that the peels of Chrysophyllum albidum can serve as an effective bio-reductant source for green synthesis of silver nanoparticles for antibacterial applications in the combat against antimicrobial resistance.

Experimental

Chemicals

Silver nitrate (AgNO₃, Merck, \geq 99%) was used as a precursor in the synthesis of silver nanoparticles. *Chrysophyllum albidum* fruit was purchased from the local market. Hypochlorite solution was used to disinfect the *Chrysophyllum albidum* fruit peel before use. Methanol (Sigma Aldrich, analytical grade), Mueller-Hinton Broth (Oxoid, USA), MTT (3-(4,5dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide, 0.1%, w/v, Sigma Aldrich), Phosphate Buffered Saline (PBS, Sigma Aldrich, analytical grade), McFarland standard (barium chloride and sulphuric acid) were used to study the antimicrobial properties of the prepared silver nanoparticles.

Biosynthesis of silver nanoparticles using peel extract of Chrysophyllum albidum fruit (Alb-AgNPs)

Preparation of Chrysophyllum albidum peel extract

The outer layer of *Chrysophyllum albidum* fruit was peeled off, washed, and then dried in an oven at 80 °C for 4 h. About 1 g of the *Chrysophyllum albidum* peel was measured and disinfected with hypochlorite solution. Deionized water was finally used to wash the peels severally. About 40 mL of deionized water was added to the peels and swirled for less than a minute. The water was decanted through filter paper, and the filtrate (extract) was stored for further use. It is noteworthy that the peels can be reused through the outlined procedure to attain fresh extracts.

Synthesis of silver nanoparticles using peel extracts of Chrysophyllum albidum

About 1 mL of 0.01 M AgNO₃ was added to 40 mL of extract solution and exposed to the sunlight for 5 min. The formation of the *Chrysophyllum albidum* stabilized silver nanoparticle (*Alb-AgNPs*) was observed as a colour change from pale yellow to dark red. The *Alb-AgNPs* were purified severally by centrifugation and redispersed in deionized water for further use.

Antimicrobial properties of Alb-AgNPs

Test organisms

The antimicrobial properties of the *Alb-AgNPs* were tested against eight different microorganisms, namely, Methicillin resistant *Staphylococcus aureus* (NCTC12493), *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae* (*NCTC 13,440*), *Bacillus subtilis* (ATCC 10,004), *Streptococcus mutans* (ATCC 700,610), *Pseudomonas aeruginosa* (ATCC 4853), *Salmonella typhi* (ATCC14028), and *Candida albicans* (ATCC 90,028). These organisms were selected based on their implications in microbial infections. Next, these microorganisms were sub-cultured for

24 h before the experiment in a nutrient agar at 37 °C. A prepared inoculum of these strain cultures was then adjusted to obtain a final concentration of 10^5 CFU/ mL using a 0.5 McFarland standard.

Determination of minimum inhibitory and bacteri/ fungi-cidal concentrations (MIC and MBC/MFC) of Alb-AgNPs

The minimal inhibitory concentration (MIC) was performed using a microdilution broth susceptibility assay (Clinical and Laboratory Standards Institute, 2011). Two-fold serial dilutions of the Alb-AgNPs ranging from 500 to 0.198 µg/mL in methanol were prepared in Mueller-Hinton Broth (MHB;100 µL) in a 96-well microtiter plate. Microbial suspensions were prepared from each test strain freshly grown in Mueller Hinton broth (approximately 10⁵ CFU/mL), and 100 µL of these individual suspensions were added to each well. In all cases on each column, one well was designated as positive control inoculated with each test microorganism and the sterile broth plus methanol (diluent) as the negative control without organism in another well (12). After incubation at 37 °C for 24/48 h, microbial growths were recorded using MTT (i.e., 3-(4,5- dimethylthiazole-2- yl)-2,5-diphenyltetrazolium bromide, 0.1%, w/v). MICs of the various Alb-AgNPs samples were denoted as the lowest concentrations at which no colour change (from yellow to purple) was observed. Afterwards, cultures were seeded in Mueller-Hinton Agar (MHA) medium and incubated for 24 h at 37 °C to determine the minimum bacteri/fungi-cidal concentration (MBC/ MFC) which gives the lowest concentration of the Alb-AgNPs sample that kills test organisms. All experiments were performed in triplicate (Nester et al. 2004).

Evaluation of synergistic effects of the Alb-AgNPs sample and antibiotics

Combinatory effects between the *Alb-AgNPs* and antibiotics were carried out using the checkerboard test against the strains of test microbes with slight modification according to the protocol reported by Khodavandi et al. (2010 and Nascimento Da Silva et al. (2013). Briefly, solutions with different proportions of *Alb-AgNPs* : drug (final volume of 200 μ L) were prepared from twice MIC solutions of each

test sample ($2 \times MIC$) and the individual antibiotics (1 mg/mL), and the antibacterial activity was tested as described for MIC determination. The Fractional Inhibitory Concentration index (FICI) was calculated according to Eq. (1);

$$FICI = \left(\frac{MICA + S}{MICA}\right) + \left(\frac{MICS + A}{MICS}\right) \tag{1}$$

where *MICA* + *S* is the minimal inhibitory concentration of antibiotic in combination with *Alb-AgNPs* sample, *MICS* + *A* is minimum inhibitory concentration of *Alb-AgNPs* sample in combination with antibiotic. *MICA* and *MICS* are the minimum inhibitory concentrations of antibiotic and Alb-AgNPs, respectively. Results were categorized as synergistic if FICI was ≤ 0.5 , partial synergistic if FICI was $^{>} 0.5$ and <1, additive if FICI was =1, no difference if FICI was $^{>} 1$ and ≤ 4 , antagonistic if FICI was $^{>} 4.0$.

Determination of antibiofilm activity of Alb-AgNPs

The activity of the Alb-AgNPs against the microbial biofilms was examined using the 96-well microtiter plate of microbial biofilm formation and susceptibility testing (Pierce et al. 2010) with slight modification. Briefly, Mueller Hinton broth (50 µL) were added to each well in a flat-bottom 96-well microplate; each of the Alb-AgNPs samples (50 µL) was then serially diluted to arrive at 10 different concentrations ranging from 500 to 0.197 µg/mL. Subsequently, 50 µL of the microbial suspension at a concentration of 2×10^6 cells/mL were added to wells of columns 1-11, and the microtiter plates were incubated for 24 h at 37 °C. After this period, the liquid was carefully pipetted without touching the biofilm. They were then washed with PBS (100 μ L) twice to remove planktonic and non-adherent cells. The postprocessing to quantify the metabolic activity after the antimicrobial treatment was checked by XTT (Sigma Aldrich) reduction assay as previously described by (Pierce et al. 2008) with slight modifications. Finally, plates were read by spectrophotometry at 490 nm in a microtiter plate reader. Each of the procedures was repeated thrice. The biofilm inhibition potential of each of the *Alb-AgNPs* samples to reduce the optical density compared to the negative control was noted as the biofilm inhibitory activity;

% biofilm inhibition =

$$\left(\text{optical density (OD)of control} - \frac{\text{OD of treatment}}{\text{OD of control}}\right) \times 100$$
(2)

Characterization

The morphology of the *Alb-AgNPs* was observed with Hitachi S-4800 FE-SEM (field emission scanning electron microscope). Shimadzu UV – 1800 UV-VIS Spectrophotometer was used to measure the plasmonic absorption of the Alb-AgNPs. The FTIR spectrum of the *Alb-AgNPs* were acquired with PerkinElmer FT-IR spectrometer. The PXRD pattern of the *Alb-AgNPs* was acquired with PANalytical Empyrean X-ray Diffractometer.

Results and discussion

Synthesis of *Chrysophyllum albidum* peel extract stabilized silver nanoparticles (Alb-AgNPs)

In the preparation of the *Alb-AgNPs*, the extract and AgNO₃ mixture was exposed to sunlight for about 5 min leading to a complete reduction of Ag^+ to Ag^0 and the subsequent formation of silver nanoparticles. The formation of the Alb-AgNPs was observed as a colour change from pale yellow to dark red (Fig. 1). After the dark red colour was attained, no visible colour change was observed when the reaction was allowed to proceed for an hour. In the absence of sunlight, the Alb-AgNPs still form under normal room conditions; however, the reaction occurs in about 4 h. Sunlight, thus, speeds up the reduction of silver ions leading to the formation of the Alb-AgNPs (Ahmed et al. 2015; Nguyen 2020). The faster reaction under sunlight might be because electron transfer from the phytochemicals in the extract to the Ag⁺ is faster under sunlight exposure compared to normal room conditions.

The FTIR spectrum of the *Alb-AgNPs* presented in Fig. 2a reveals the functional group of the phytochemicals in the extract solution, which were responsible for reducing silver ions and stabilizing the nanoparticles. As shown in Fig. 2(a), a number of bands were recorded. Prominent bands were observed around 3268 cm^{-1} , 2970 cm^{-1} and 2921 cm^{-1} , 1736 cm^{-1} , 1620 cm^{-1} , 1365 cm^{-1} , 1216 cm^{-1} , and 1042 cm^{-1} . These bands can be assigned to O–H stretch, -C-H



Fig. 2 a FTIR spectrum of Alb-AgNPs, b Scanning electron microscope image of Alb-AgNPs, c EDS spectrum of Alb-AgNPs.

stretch, -C=O stretch, aromatic -C=C- stretch, -C-H stretch of alkene, -C-N stretch of aliphatic amine and -C-O stretch, respectively (Krithiga et al. 2015). It presupposes that molecules with these functional groups in the extract solution may have been responsible for the reduction of the Ag⁺ and stabilization of the *Alb-AgNPs* (Huang et al. 2007). Studies have revealed the presence of phytochemicals such as flavonoids, phenolic compounds and vitamin C in the peels of *Chrysophyllum albidum* (Folasade et al. 2019). These phytochemicals may have been responsible for the reduction and stabilization of the *Alb-AgNPs*.

Figure 2b shows the Scanning electron microscope image of the *Alb-AgNPs*. The size of the particles ranged between 28 and 90 nm and was primarily quasi-spherical. Occasionally, nanoparticles greater than 100 nm were observed. Similar polydisperse silver nanoparticles resulting from green synthesis have been observed in the study reported by Jelin et al. (2015). The EDS spectrum of the *Alb-AgNPs* confirms the presence of silver metal in the composite nanostructure (Fig. 2c). The plasmonic absorption of the *Alb-AgNPs* was observed around 434 nm in the visible region of the electromagnetic spectrum (Fig. 3a).

The X-ray diffraction spectrum of the *Alb-AgNPs* is presented in Fig. 3b; the diffraction pattern observed around 38° , 44° , 64° , 78° and 82° can be attributed to the (111), (200), (220), (311) and (322) diffraction planes of face-centred cubic (FCC) structure silver nanoparticles (Krithiga et al. 2015).

Antibacterial properties of Alb-AgNPs

Minimum inhibitory and bacteri/fungi-cidal concentrations (MIC and MBC) of Alb-AgNPs

Nanoparticles interact strongly with microbial surfaces primarily due to their high surface-volume ratio and size. This strong interaction facilitates the antimicrobial actions of the metal nanoparticles. In the present study, the antimicrobial properties of silver nanoparticles stabilized with extracts from *Chrysophyllum albidum* were studied against a broad range of microorganisms. These microorganisms comprised

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gram-negative and gram-positive bacteria, as well as fungus. Table 1 presents the MIC values of the *Alb-AgNPs* against the selected microorganisms. The synthesized silver nanoparticles (*Alb-AgNPs*) have the potency to inhibit and destroy microbial cells. The lowest MIC value of 15.62 µg/mL was recorded for *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *C. albicans*.

Further insight into the antimicrobial efficacy of the *Alb-AgNPs* was gained when it was compared to $AgNO_3$ of the same Ag concentration. As illustrated in Fig. 4a, the effectiveness of the *Alb-AgNPs* was

greater than silver nitrate of similar Ag concentration. Except for *S. mutans*, *B. subtilis* and *S. typhi*, the MIC values of *Alb-AgNPs*, was lower than that of AgNO₃. Moreover, the lowest MIC value AgNO₃ could attain against the selected microorganism was 62.5 μ g/mL, threefold higher than the 15.62 μ g/MI for *Alb-AgNPs*.

Analysis of the antimicrobial efficacy in Fig. 4b reveals that the *Alb-AgNPs* is more sensitive to gram-negative than gram-positive bacteria. Except for *S. typhi*, all the gram-negative bacteria showed higher susceptibility towards the *Alb-AgNPs* than



Table 1MIC and MBC ofAlb-AgNPs against selectedmicroorganisms

Test Organism	Alb-AgNPs					
	MIC (µg/mL)	MBC (µg/mL)	MIC/MBC	Interpretation		
E. coli (ATCC 25922)	15.62	125	8	Bacteriostatic		
K. pneumoniae (NCTC 13440)	15.62	125	8	Bacteriostatic		
MRSA (NCTC 12493)	125	125	1	Bactericidal		
S. mutans (ATCC 700610)	250	250	1	Bactericidal		
B. subtilis (ATCC 10004)	1000	1000	1	Bactericidal		
S. typhi (ATCC 14028)	1000	1000	1	Bactericidal		
P. aeruginosa (ATCC 4853)	15.62	1000	64	Bacteriostatic		
Candida albicans (ATCC 90028)	15.62	62.5	4	Fungistatic		

their gram-positive counterparts. This variation can be attributed to the differences in the cell wall structures of both gram-negative and gram-positive bacteria.

The MBC/MIC ratio presented in Table 1 clearly shows the efficacy of the Alb-AgNPs against the selected microorganisms. Interpretation of the MBC/ MIC values shows that the Alb-AgNPs are bactericidal against MRSA, S. mutans, B. subtilis and S. typhi, bacteriostatic towards E. coli, K. pneumoniae and P. aeruginosa, and fungistatic against Candida albicans. The antimicrobial properties of silver nanoparticles have extensively been studied, and although there has not yet been any definitive mechanism for the effect, studies have suggested various plausible mechanisms. It is reported that the antimicrobial activities might be attributed to the release of Ag⁺ ions into the microbial medium (Siddiqi et al. 2018). The positively charged silver ions can interact with negatively charged molecules in microbial cells through electrostatic interactions. This interaction can occur, for instance, when silver ions bind to sulfur-containing proteins in the cytoplasm or cell wall of the microbe (Hsueh et al. 2015; Helmlinger et al. 2016; Siddiqi et al. 2018). The strong adherence significantly increases the permeability of the Ag⁺ into the internal structures of the microbe resulting in disruption and damage to the microbial cell. Studies have revealed that when the free Ag⁺ enters the microbe's cells, it produces reactive oxygen species (ROS), which are responsible for the disruption and damage of the microbe (Siddiqi et al. 2018). This damage may arise from deoxyribonucleic acid (DNA) alteration, which affects replication, cell propagation, etc., or hinder the manufacturing of ribosomal components (Anees Ahmad et al. 2020).

Synergistic effect of the Alb-AgNPs

The synergistic effect of the *Alb-AgNPs* was studied in combination with antibiotic and antifungal agents using a checkerboard microdilution method. The effects were evaluated by determining the Fractional Inhibitory Concentration index (FICI) (Eq. 1); the results are presented in Table 2. The Alb-AgNPs was investigated in combination with tetracycline (TET) and ciprofloxacin (CIP) against bacteria strains. The Alb-AgNPs in combination with TET (Alb-AgNPs + TET) showed synergistic effect against K. pneumoniae, S. mutans and B. subtilis. Partial synergy was demonstrated against MRSA and P. aeruginosa, whereas against E. coli and S. typhi, the effect was antagonistic. This shows that the Alb-AgNPs can enhance the efficacy of standard antibiotics. Studies have reported that biosynthesized AgNPs in combination tetracycline have a synergistic effect. Aabed and Mohammed (2021) observed that AgNPs prepared biogenically with (A) hierochuntica plants in combination with TET shows synergistic effect against MRSA and E. coli. Masoud Hussein et al. (2019) also reported that biosynthesized AgNPs in combination with TET show synergistic effect against K. pneumoniae. These results are in agreement with that reported in the present study. The Alb-AgNPs, when combined with ciprofloxacin (CIP), showed synergistic effect against MRSA and P. aeruginosa. Partial synergy was observed for K. pneumoniae, and antagonistic effect was displayed against E. coli and S. typhi. The effect was additive and indifference for B subtilis and S. mutans, respectively. The synergism of the Alb-AgNPs + CIP towards MRSA and P. aeruginosa also agrees with the study by Aabed and Mohammed (2021)

Table 2FICI of the Alb-
AgNPs in combination
with tetracycline and
ciprofloxacin

Text Organism	FIC Index Alb-AgNPs + Tetracycline	Interpretation	FIC Index Alb-AgNPs + Ciprofloxacin	Interpretation
E. coli (ATCC 25,922)	4.25	Antagonism	127.67	Antagonism
K. pneumoniae (NCTC 13,440)	0.14	Synergy	0.78	Partial synergy
MRSA (NCTC 12,493)	0.56	Partial synergy	0.02	Synergy
S. mutans (ATCC 700,610)	0.08	Synergy	1.5	Indifference
B. subtilis (ATCC 10,004	0.07	Synergy	1.13	Additive
S. typhi (ATCC 14,028)	32.39	Antagonism	127.8	Antagonism
P. aeruginosa (ATCC 4853)	0.62	Partial synergy	0.14	Synergy

The Alb-AgNPs were also investigated for their ability to enhance the efficacy of standard antifungal agents as shown in Table 3. To study this, the Alb-AgNPs were tested in combination with fluconazole, ketoconazole and Nystatin, against Candida albicans. The Alb-AgNPs, in combination with fluconazole showed an additive effect. The effect was also additive in combination with ketoconazole, and with nystatin, an antagonistic effect was observed. The potency of the *Alb-AgNPs* to enhance the efficacy of antibiotics and antifungal agents has been attributed to the strong interaction of AgNPs with certain components in antibiotics. It is reported that AgNPs form complexes with antibiotics molecules, which then bind to the bacterium. The Ag^+ in the complex is released to create high silver ions concentration, killing the bacterial or fungal strains (Fayaz et al. 2010).

Biofilm inhibition properties of the Alb-AgNPs

Studies have shown that biofilm-forming microbes are responsible for many infectious diseases (Joo and Otto 2012). Pathogenically important microbes such as the gram-positive Methicillin resistant S. aureus biofilms are responsible for many nosocomial infections (Joo and Otto 2012). The biosynthesized Alb-AgNPs were studied for their ability to inhibit biofilm formation of gram-negative and gram-positive bacteria, namely Staphylococcus aureus, Salmonella typhi, Streptococcus mutans and Bacillus subtilis, as well as, the fungus Candida albicans (Fig. 5). For all the test organisms, the amount of biofilm formation was found to decrease with increasing Alb-AgNPs concentration. The inhibition in biofilm formation by Alb-AgNPs was dramatic against MRSA. At a 250 µg/ mL concentration, over 90% of S. aureus biofilm formation was inhibited. This result is comparable to the study reported by Goswami et al. (2015), where about 89% inhibition was observed for biosynthesized AgNPs using tea leaves. The Alb-AgNPs were also able to inhibit the biofilm formation of *Bacillus* subtilis; similar to the case of *MRSA*, biofilm formation was decreased as *Alb-AgNPs* concentration was increased. At the highest concentration (250 μ g/mL), the maximum inhibition was about 54%. This result is consistent with the study by Rodríguez-Serrano et al. (2020), which observed inhibition of about 50% when AgNPs synthesized with *A. tubingensis* fungus were used against *B. subtilis* biofilm.

The antibiofilm inhibition properties of the Alb-AgNPs against S. mutans, as presented in Table 4, show that biofilm formation decreased with increasing concentration. At a 250 µg/mL concentration, biofilm formation was inhibited for about 57%. Biofilm formation inhibition with AgNPs against S. mutans has also been observed by Pipattanachat et al. (2021) with graphene oxide-coated silver nanoparticles. Against S. typhi, the Alb-AgNPs also showed the antibiofilm formation of about 83% at the highest concentration of 250 µg/mL. Balakrishnan et al. (2020) also observed this enhanced antibiofilm formation. The Alb-AgNPs also showed enhanced activity against Candida albicans biofilm formation. A decrease in biofilm formation when Alb-AgNPs concentrations were increased was also observed. As presented in Table 4, at a concentration of 250 µg/mL, about 88% of Candida albicans biofilm was inhibited. The enhanced inhibition of the Alb-AgNPs against Candida albicans biofilm agree with the study reported by Lara et al. (2015).

A comparison between the sensitivity of the *Alb-AgNPs* toward the individual microorganisms reveals that the as-prepared nanoparticles are active against both gram-negative and gram-positive bacteria, as well as fungus (Fig. 5f). However, the sensitivity towards each microorganism differs. For the bacteria strains, the *Alb-AgNPs* were more active against inhibiting *MRSA* biofilms, followed by *S. typhi*, then *B. subtilis* and finally, *S. mutans*. Several factors can be attributed to the variation in sensitivity. It has

Table 3 Synergistic effect of Alb-AgNPs against fluconazole, ketoconazole and nystatin antifungal agents

Text Organism	FIC Index Alb- AgNPs + Flu- conazole	Interpretation	FIC Index Alb- AgNPs + Keto- conazole	Interpretation	FIC Index Alb-AgNPs +Nystatin	Interpretation
Candida albicans (ATCC 90,028)	1.12	Additive	1.12	Additive	8.12	Antagonism

Fig. 5 a-e plots of optical density (OD) against concentration of *Alb-AgNPs*, indicative of the amount of biofilm formed by the different microorganisms, **f** comparison of % biofilm inhibition by the different concentration of *Alb-AgNPs* against different microorganisms



 Table 4
 Percentage (%) biofilm inhibition of the Alb-AgNPs against microorganisms

Test Organ- ism	% Biofilm inhibition at various concentrations								
	1.96 (µg/mL)	3.91 (µg/mL)	7.81 (µg/mL)	15.63 (μg/ mL)	31.25 (μg/ mL)	62.5 (µg/mL)	125 (µg/mL)	250 (µg/mL)	
MRSA	58.32 ± 3.7	68.40 ± 2.4	70.75 ± 2.6	77.42 ± 2.6	82.44 ± 0.9	88.73±1.7	90.86 ± 2.1	92.46±1.6	
S. mutans	23.17 ± 2.0	24.89 ± 0.1	27.51 ± 0.1	28.92 ± 0.4	30.7 ± 1.9	37.8 ± 0.4	39.2 ± 0.5	57.8 ± 0.4	
B. subtills	28.8 ± 2.4	28.9 ± 2.4	29.3 ± 2.5	29.6 ± 2.5	39.1 ± 3.0	42.9 ± 0.1	53.8 ± 2.8	54.8 ± 2.1	
S. typhi	10.1 ± 13.1	21.1 ± 10.4	48.8 ± 11.6	53.2 ± 8.5	59.9 ± 7.9	62.9 ± 7.7	68.2 ± 10.2	83.1 ± 7.7	
C. albicans	32.6 ± 0.3	40.3 ± 0.4	44.9 ± 0.1	46.1 ± 1.0	59.0 ± 0.1	71.4 ± 1.1	77.7 ± 1.1	87.5 ± 1.0	

been observed that the strength of biofilms may differ for different microorganisms due to variations in cell properties. Biofilm formation may depend on cell surface hydrophobicity, extracellular appendages such as flagella, and extracellular polymeric substances. As these properties differ from cell to cell, biofilm formation's strength may also vary (Right et al. 2021). Several studies have reported the mechanisms underlying silver nanoparticles' inhibition of bacteria biofilms. Biofilms are generally strong extracellular matrix due to the strong cell-cell adhesion of bacteria that prevent drug permeation (Joo and Otto 2012). Thus, the ability of silver nanoparticles to enter the biofilm matrix has been attributed to the potency of silver nanoparticles to destabilize bacteria cell walls (Barapatre et al. 2016).

Conclusion

This study has demonstrated that the peel extract of Chrysophyllum albidum fruit can be an effective bioreductant for the green synthesis of silver nanoparticles (Alb-AgNPs) and a drug candidate for exploration in the quest against antibiotic resistance fight. The Alb-AgNPs were characterized with FTIR, UV-Vis, SEM and PXRD. The ability of the Alb-AgNPs to inhibit bacterial growth, synergically enhance the efficacy of antibacterial or antifungal agents, and prevent biofilm formation were studied. The Alb-AgNPs displayed an enhanced ability to inhibit microbial growth with MICs as low as 15.62 µg/mL against E. coli, K. pneumoniae, P. aeruginosa and Candida albicans. The synergy between the Alb-AgNPs and standard antibacterial and antifungal agents was also observed. In addition, the Alb-AgNPs demonstrated over 90% ability to inhibit biofilm formation. The results from this study suggest that Chrysophyllum albidum peels can serve as an efficient bio product for sustainable green synthesis of silver nanoparticles, and the nanoparticles thus obtained can also provide access to cheap and eco-friendly antimicrobial agents.

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Declarations

Conflict of Interest The authors declare that there is no competing interest.

Research involving humans and animals rights This research does not involve humans and animals.

Informed consent All authors authorize the publication of this manuscript.

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