Antimicrobial and antioxidant activity of Silver, Gold and Silver-Gold Alloy Nanoparticles phytosynthesized using extract of Opuntia ficus-indica

Abstract: Aqueous extract of Opuntia ficus-indica (OF) was employed to reduce salt solutions of silver nitrate, gold chloride and silver nitrate/gold chloride to OFAgNPs, OFAuNPs, and OFAg-AuNPs respectively. Characterization of the particles was carried out using different standard methods, and their antimicrobial and antioxidant activities were also evaluated. The UV-vis spectroscopy showed silver, gold and silver-gold alloys nanoparticles with surface plasmon resonance at 462, 545 and 539 nm, respectively. The significant FTIR peaks: OFAgNPs (3300 and 1635 cm$^{-1}$), OFAuNPs (3288 and 1635 cm$^{-1}$) and OFAg-AuNPs (3307 and 1637 cm$^{-1}$) for synthesized nanoparticles pointed to protein as both capping and stabilizing agent. Generally, particles were spherical with size range of 27-38 nm, 11-28 nm and 15-54 nm for silver, gold and silver-gold alloys nanoparticles respectively. OFAgNPs gave highest antagonistic effects against tested bacteria (35-82%), followed by OFAg-AuNPs (12-75%) and AuNPs (11-48%). The fungal inhibition of 44-70% for OFAgNPs, 42-79% for OFAuNPs and 52-92% for OFAg-AuNPs were obtained. DPPH scavenging activities were obtained as follows: OFAgNPs (52.25–57.50%), OFAuNPs (37.07-59.07%) and OFAg-AuNPs (37.07-63.00%) at tested concentrations of 20–100 µg/ml, which were dose-dependent in comparison with standard BHA (42.57–91.27%) and ascorbic acid (52.13–84.20%). The bleaching inhibition assay of ABTS showed activities of 41.33–61.83% (OFAgNPs), 27.37-50.60% (OFAuNPs) and 32.83-44.33% (OFAg-AuNPs). The synthesized nanoparticles demonstrated an excellent antioxidant and antimicrobial activity. The current study, to
the best of our knowledge, is the first time to use *Opuntia ficus-indica* to synthesize silver, gold and silver-gold alloys nanoparticles.

**Keywords:** *Opuntia ficus-indica*; Nanoparticles; Antibacterial activity; Antifungal activity; Antioxidant activity

## 1 Introduction

Metallic nanoparticles (MNPs) are attractive to the scientific community because of their exciting properties and importance in diverse fields; including medicine, chemistry, electronics and engineering [1–3]. Different methods (physical, chemical and biological) can be employed to synthesize MNPs. While some conditions, such as high usage of energy and toxic chemicals, may be required in physical and chemical methods [4], biological methods rely on the use of living organisms and their metabolites at benign condition, which are less expensive and eco-friendly in nature [5, 6]. Previous reports on phytosynthesis of nanoparticles from plant sources include the following; *Azadirachta indica* [7], *Glicidica sepium* Jacq [8], *Carica papaya* [9], *Opuntia ficus-indica* [10], *Lippia citriodora* [11], *Murraya koenigii* [12], *Citrus sinensis* [13], *Saururus chinesis* [14], *Eucalyptus* hybrid [15], *Ocimium sanctum* [16], and *Coriandrum sativum* [17].

*Opuntia ficus-indica* is a member of Cactaceae (cactus family), with 1,600 species from about 97 genera. While the origin of species has been traced to Mexico [18], it is also found in other continents of the world including Africa. Height of *O. ficus-indica* is always around 8m with shallow roots. The diameter of the plant over the ground can spread up to 40 m, with thick and succulent stems called cladodes [19, 20]. Osuna-Martínez *et al.* [21] reported the ability of *O. ficus-indica* in reducing the risks of different human diseases and pharmacological activities in terms of antioxidant activity, oxidative damage protection, radical scavenging activities, lipid peroxidation reduction and improved glutathione (GSH) levels.

Although several studies have been conducted to synthesize AgNPs, AuNPs and Ag-AuNPs using different biological sources, there are only few reports on the use of *O. ficus-indica* to synthesize AgNPs [22] and AuNPs [23] with proven antibacterial activity [10]. Therefore, in order to expand the application horizon of this medicinal plant in nanobiotechnology, the current work explores the phytosynthesis of silver, gold and silver-gold alloy nanoparticles using *O. ficus-indica* aqueous extract, and demonstration of their biomedical applications as antibacterial, antifungal and antioxidant nano-agents.

## 2 Experiments

### 2.1 Extraction of *O. ficus-indica* extract

*O. ficus-indica* was collected from Station Road area, Offa, Kwara State, Nigeria (location: 8.1491°N, 4.7207°E) and its identity was confirmed and authenticated. The spines were removed, washed and dried in an oven at 40°C and then pulverized using electric blender for 10 s. Pulverized (10 g) of *O. ficus-indica* was macerated in 150 ml of deionized water in a dark cupboard at ambient condition (30 ± 2°C) and allowed to stay for 24 h in tight closed container. The mixture was filtered, centrifuged for 20 min at 4000 rpm [24] and the supernatant was collected for use.

### 2.2 Synthesis and characterization of Ag, Au and Ag-AuNPs using *Opuntia ficus-indica* (OF) extract

Ag, Au and Ag-AuNPs mediated by *O. ficus-indica* extract was carried out following the approach demonstrated by Lateef *et al.* [25]. The reaction vessels containing 1 ml each of the extract in 24 ml of 1 mM silver nitrate (AgNO₃) and chloroauric acid (HAuCl₄) solutions to reduce the silver and gold ions were separately carried out, while that of alloy was prepared by the mixture of 1mM AgNO₃ and 1mM AuCl₄ in ratio 4:1 (v/v), respectively [26]. Materials used were held at ambient condition (30 ± 2°C) for colour change due to formation of nanoparticles. Changes in colour of the experimental set-ups were monitored, while UV-visible spectrophotometer (Cecil) was used to obtain absorption spectral of the reaction mixtures to determine the surface plasmon resonance of the synthesized nanoparticles. Fourier Transform Infrared (FTIR) spectroscopy was carried out on solid dried nanoparticles obtained after centrifuged at 10,000 rpm for 20 min, with powdery form used for FTIR measurement using KBr pellets. Scanning electron microscopic (SEM) images and elemental composition of the samples were obtained using A LYRA3 TESCAN FESEM coupled with energy dispersive X-ray (EDX) (Oxford), and working at 20.0 kV. Morphological characterization of nanoparticles was carried out by using drops of coated purified nanoparticles on a glass slide, allowed to dry and applied the films. JEM-400 TEM (JEOL, Peabody, Massachussets, USA) working at 20 kV was used to obtain Transmission electron microscopy (TEM) images. The grain size, crystalline nature, shape and alignment of the synthesized nanoparticles were also identified using X-
antimicrobial and antioxidant activity of silver, gold and silver-gold alloy nanoparticles

2.3 antibacterial assay of OFAgNPs, OFAuNPs and OFAg-AuNPs

Antibacterial properties of the synthesized OFAgNPs, OFAuNPs and OFAg-AuNPs were evaluated using Prasannaraj and Venkatachalam [27] liquid culture method. Clinical isolates of Escherichia coli (stool), Escherichia coli (ATCC 25922), Bacillus subtilis (ATCC 6633), Listeria monocytogenes (ATCC 19111), Klebsiella pneumonia (Urine), Proteus vulgaris (Abdomen), Pseudomonas aeruginosa (ATCC 27853), Staphylococcus aureus (ATCC 29523), Staphylococcus aureus (Ear), and Streptococcus pyogenes (Sputum) were used as test organisms. Peptone water was used for the growth of bacterial isolates by inoculating with 1 ml of 1 \times 10^6 cfu/ml of test organism. For the experiments, 1 ml of gradient concentrations of nanoparticles (20-80 µg/ml) was added to 8 ml of peptone water already inoculated with 1 ml of the test bacterium and incubated at 37 °C for 24 h. Each experiment set-up was replicated thrice and the control contained the same mixture without addition of nanoparticles. The UV-vis spectrophotometer was used to obtain optical density of the cultures at 600 nm. Estimation of percentage growth inhibition was done using the formula:

\[
\text{Bacterial growth inhibition} = \frac{\text{Control} \times 100}{\text{ControlOD} - \text{Test sample OD}}
\]

where OD is the optical density

2.5 DPPH free radical-scavenging assay of OFAgNPs, OFAuNPs and OFAg-AuNPs

Free radical scavenging activity was determined following the methods of Öztürk et al. [29] and Adebayo et al. [24] by using 2, 2-diphenyl-1-picrylhydrazyl, DPPH (Sigma-Aldrich, USA). To about 4 ml of methanolic DPPH (0.1 mM), was added 1 ml of graded concentrations of each of OFAgNPs, OFAuNPs and OFAg-AuNPs. The reaction mixtures were incubated in the dark for 30 min at room temperature with blank made up of 1 ml of methanol in 4 ml of methanolic DPPH. At the end of the experiment, the absorbance was read at 517 nm under the same conditions, while percentage inhibition of DPPH was calculated [10, 30].

\[
\text{DPPH inhibition} = \frac{A(\text{blank}) - A(\text{sample})}{A(\text{blank})} \times 100;
\]

where A is the absorbance reading

Ascorbic acid and BHA were used as standard antioxidant compounds.

2.6 ABTS radical cation decolourisation assay of OFAgNPs, OFAuNPs and OFAg-AuNPs

A modified spectrophotometric method was employed for ABTS** radical cation decolourisation assay [29]. The ABTS** radical cation was generated by the reaction between 7 mM ABTS in water with 2.45 mM potassium persulfate. ABTS** generated was stored in the dark for 13 h at room temperature before use. The ABTS** solution was first adjusted to the absorbance of 0.710 ± 0.020 at 734 nm with 99% ethanol in the ratio 65.67 to 1 (ethanol: reagent). Addition of 160 µL of ABTS** solution to 40 µL (0.0-5 mg/mL) of nanoparticles in ethanol produced oxidized form of ABTS** at different concentrations. The absorbance was later measured after 10 min, while percentage inhibition for each concentration was calculated in relation to the control (ethanol). ABTS** scavenging capabili-
Figure 1: Biogenic formation of OFAgNPs, OFAuNPs and OFAg-AuNPs and their applications

3 Results and its discussion

3.1 Biogenic formation of OFAgNPs, OFAuNPs and OFAg-AuNPs

The formation of AgNPs, AuNPs and Ag-AuNPs was mediated under ambient condition (30±2°C) by the extract of *O. ficus-indica* within a period of 30, 45 and 60 min, with the development of a dark brown, dark green and pinkish colour, respectively (Figure 1). The colour inten-
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3.1 Optical properties of biosynthesized OFAgNPs, OFAuNPs and OFAg-AuNPs

The absorption spectra of biosynthesized OFAgNPs, OFAuNPs and OFAg-AuNPs with a concentration of 3000 Å to 1635 Å, respectively, are presented in Figure 2. FTIR absorption spectra revealed the presence of strong peaks at 3300–3288 cm⁻¹ for OF-AgNPs, 3466 cm⁻¹ for OF-AuNPs, and 420 cm⁻¹ for OF-Ag-AuNPs. These peaks are indicative of the presence of proteins, lipids, and nucleic acids. The XRD spectra [Figure 4(C)] showed prominent peaks at 2θ = 39.05, 44.5, 65.09 and 78.44° which correspond to (111), (200), (220) and (311) for OFAgNPs; 37.49, 47.06, 63.98° which corresponds to (111) and (200) for OF-AuNPs; and 33.08 and 47.03° which correspond to (111) and (200) for OF-Ag-AuNPs, which could be indexed to face centered cubic (fcc) structure of Au and Ag NPs.

3.2 Antibacterial activities of biosynthesized OFAgNPs, OFAuNPs and OFAg-AuNPs

The antibacterial activities of biosynthesized OFAgNPs, OFAuNPs and OFAg-AuNPs were tested against ten clinical bacterial isolates at different concentrations of 20-80 µg/ml. AgNPs showed maximum inhibition of 82% against E. coli (ATCC 25922), and 77% against P. vulgaris (Abdomen) was observed. However, the least activities of 35% against P. vulgaris (Abdomen) was observed at 20 µg/ml (Table 1).
Figure 3: FTIR spectra of the biosynthesised OF-AgNPs (A), OF-AuNPs (B) and OF-Ag-AuNPs (C) using Opuntia ficus-indica extract
Figure 4: Structural Characterization of OFAgNPs; (A) Transmission electron micrograph (TEM), (B) Selected area electron diffraction pattern (SAED), (C) X-ray diffraction (XRD) and (D) Energy dispersive x-ray signal (EDX)

Table 1: Antibacterial Activities (%) of Biosynthesized OFAgNPs at different concentrations

| Isolates                                | Concentration | µg/ml | 60 | 80 |
|-----------------------------------------|---------------|-------|----|----|
| Escherichia coli (stool)                | 61            | 68    | 68 | 77 |
| *Escherichia coli* (ATCC 25922)         | 59            | 63    | 66 | 82 |
| Bacillus subtilis (ATCC 6633)           | 61            | 72    | 77 | 78 |
| Klebsiella pneumoniae (Urine)           | 56            | 59    | 68 | 78 |
| Listeria monocytogenes (ATCC 19111)    | 41            | 65    | 72 | 74 |
| Proteus vulgaris (Abdomen)              | 35            | 61    | 63 | 65 |
| Pseudomonas aeruginosa (ATCC 27853)     | 51            | 51    | 63 | 70 |
| *Staphylococcus aureus* (ATCC 25923)   | 62            | 66    | 67 | 68 |
| *Staphylococcus aureus* (Ear)           | 49            | 55    | 55 | 74 |
| *Streptococcus pyogenes* (Sputum)       | 54            | 62    | 62 | 65 |

Each value is an average of three readings.
Figure 5: Structural Characterization of OFAuNPs; (A) Transmission electron micrograph (TEM), (B) Selected area electron diffraction pattern (SAED), (C) X-ray diffraction (XRD) and (D) Energy dispersive x-ray signal (EDX)

Table 2: Antibacterial inhibitory (%) of Biosynthesized OFAuNPs at different concentrations

| Isolates                          | Concentration | µg/ml |
|----------------------------------|---------------|-------|
|                                  | 20            | 40    | 60   | 80   |
| *Escherichia coli* (stool)       | 12            | 14    | 15   | 19   |
| *Escherichia coli* (ATCC 25922)  | 13            | 16    | 19   | 38   |
| *Bacillus subtilis* (ATCC 6633)  | 14            | 27    | 34   | 39   |
| *Klebsiella pneumoniae* (Urine)  | 11            | 15    | 18   | 26   |
| *Listeria monocytogenes* (ATCC 19111) | 12        | 18    | 31   | 33   |
| *Proteus vulgaris* (Abdomen)     | 11            | 12    | 17   | 20   |
| *Pseudomonas aeruginosa* (ATCC 27853) | 12        | 17    | 20   | 48   |
| *Staphylococcus aureus* (ATCC 25923) | 17        | 18    | 19   | 24   |
| *Staphylococcus aureus* (Ear)    | 11            | 14    | 16   | 17   |
| *Streptococcus pyogenes* (Sputum) | 11            | 12    | 23   | 33   |

Each value is an average of three readings.
In AuNPs, 48% was the highest inhibition activities obtained against *P. aeruginosa* (ATCC 27853), followed by 39% against *B. subtilis* (ATCC 6633), 38% against *E. coli* (ATCC 25922) at 80 µg/ml, and the least value of 11% against, *K. pneumoniae* (Urine), *P. vulgaris*, *S. pyogenes* (sputum) and *S. aureus* at 20 µg/ml (Table 2). Highest inhibition percentage of 75% against *B. subtilis* (ATCC 6633), followed by 72% against *E. coli* (stool), and 71% against *E. coli* (ATCC 25922) were obtained at 80µg/ml, while 60 µg/ml gave 65% against *P. vulgaris* (Abdomen), 63% against *S. aureus* (Ear) and 60% against *L. monocytogenes* (ATCC 19111). The least inhibitory antibacterial percentage in Ag-AuNPs was 12% against *S. aureus* (Ear) at 20 µg/ml (Table 3). AgNPs showed consistent higher inhibition percentage compared to AuNPs and Ag-AuNPs. Generally, the antibacterial efficacy of synthesized nanoparticles in this work was best recorded at concentrations of 80 µg/ml against the tested bacteria, and tested concentrations of 20–100 µg/ml of synthesized nanoparticles were dose-dependent. Nanoparticle concentration and property of microbial species are factors which could affect the antibacterial activity. AgNPs antibacterial activity tested was reported to be more effective against *E. coli* than against *S. aureus*, while lower nanoparticle concentrations inhibi-
Table 3: Antibacterial Activities (%) of Biosynthesized OFAg-AuNPs at different concentrations.

| Isolates                        | Concentration µg/ml |
|---------------------------------|---------------------|
|                                 | 20                  |
|                                 | 40                  |
|                                 | 60                  |
|                                 | 80                  |
| Escherichia coli (stool)        | 58                  |
|                                 | 56                  |
|                                 | 56                  |
|                                 | 72                  |
| Escherichia coli (ATCC 25922)   | 44                  |
|                                 | 48                  |
|                                 | 68                  |
|                                 | 71                  |
| Bacillus subtilis (ATCC 6633)   | 55                  |
|                                 | 55                  |
|                                 | 59                  |
|                                 | 75                  |
| Klebsiella pneumoniae (Urine)   | 28                  |
|                                 | 31                  |
|                                 | 44                  |
|                                 | 51                  |
| Listeria monocytogenes (ATCC 19111) | 53              |
|                                 | 55                  |
|                                 | 60                  |
|                                 | 64                  |
| Proteus vulgaris (Abdomen)      | 50                  |
|                                 | 53                  |
|                                 | 65                  |
|                                 | 66                  |
| Pseudomonas aeruginosa (ATCC 27853) | 26              |
|                                 | 44                  |
|                                 | 48                  |
|                                 | 64                  |
| Staphylococcus aureus (ATCC 25923) | 18              |
|                                 | 48                  |
|                                 | 51                  |
|                                 | 60                  |
| Staphylococcus aureus (Ear)     | 12                  |
|                                 | 59                  |
|                                 | 63                  |
|                                 | 66                  |
| Streptococcus pyogenes (Sputum) | 13                  |
|                                 | 47                  |
|                                 | 55                  |
|                                 | 59                  |

Each value is an average of three readings.

Table 4: Antifungal Activities of Biosynthesized OFAgNPs, OFAuNPs and OFAg-AuNPs at 200 µg/ml

| Isolates            | % Inhibition of OFAgNPs | % Inhibition of OFAuNPs | % Inhibition of OFAg-AuNPs |
|---------------------|--------------------------|-------------------------|---------------------------|
| Aspergillus niger   | 57                       | 59                      | 52                        |
| Aspergillus fumigatus | 70                    | 79                      | 60                        |
| Fusarium solani     | 62                       | 64                      | 57                        |
| Aspergillus flavus  | 64                       | 59                      | 92                        |
| Candida albicans    | 44                       | 42                      | 92                        |

Each value is an average of three readings.

Dose-dependent antimicrobial activity and bactericidal activity of AgNPs at different concentrations has been reported by Pazos-Ortiz et al. [48] and Gurunathan et al. [49] respectively. Katas et al. [50] reported the highest zone of inhibition of 16 mm against P. aeruginosa and lowest value of 11.2 mm against S. aureus by gold nanoparticles synthesized from Lignosus rhinocerotis and Chitosan. Au nanoparticles synthesized by co-precipitation protocol through the reduction of Gold Chloride hydrate (HAuCl₄) with Sodium citrate tribasic dehydrate reported to have higher inhibition percentage of 75%, 85% and 95% against Corynebacterium pseudotuberculosis which corresponds to 5 min, 10 min and 20 min exposure respectively [51]. Sreelakshmi et al. [52] compared AuNPs and AgNPs of 10 nm, with MIC results showed honey capped AgNP exhibited superior antimicrobial activity, while AuNPs revealed passable activity against tested strains. Consistency in percentage inhibitory activities obtained from AgNPs showed the efficacy and effectiveness of the monometallic to bimetallic nanoparticles. In this regard, the strength of the biosynthesized nanoparticles could be rated thus: AgNPs>Ag-AuNPs>AuNPs. Different authors have reported similar antibacterial activities of biosynthesized nanoparticles [25, 26, 53]. However, AgNPs and Ag-AuNPs in the current work demonstrated outstanding antibacterial activities against clinical bacteria. The high percentages growth-inhibitions obtained in this study could be as a result of even distribution and interaction of bacterial cells, which directly infiltrate into bacterial cell membrane cultured by nanoparticles in the broth culture (54). Antibacterial tube technique allows direct contact between nanoparticles and microbial cells, and physical barrier between nanoparticles and bacterial cell occurs in the disc and well diffusion techniques [55, 56].

3.3 Antifungal assay of biosynthesized OFAgNPs, OFAuNPs and OFAg-AuNPs

Biosynthesized OFAgNPs, OFAuNPs and OFAg-AuNPs showed a very strong antifungal effect on the growth of Aspergillus niger, Aspergillus fumigatus, Fusarium solani, Aspergillus flavus and Candida albicans (Figure 7). The OFAgNPs produced inhibitions of 44, 57, 62, 64 and 70% against C. albicans, A. niger, F. solani, A. flavus and A. fumigatus, respectively. The OFAuNPs (200 µg/ml) produced inhibitions of 42, 59, 59, 64 and 79% against C. albicans, A. niger,
Figure 7: Antifungal activities of synthesized OFAgNPs, OFAuNPs and OFAg-AuNPs

Table 5: Radical scavenging activities (%) of synthesized OFAgNPs, OFAuNPs and OFAg-AuNPs using DPPH

| Concentration (µg/ml) | 20  | 40  | 60  | 80  | 100 |
|----------------------|-----|-----|-----|-----|-----|
| OFAgNPs              | 52.25±0.79 | 52.07±0.23 | 56.93±0.09 | 57.50±0.32 | 57.43±0.29 |
| OFAuNPs              | 37.07±0.43 | 54.03±0.15 | 54.07±0.21 | 56.0±0.12 | 59.37±0.59 |
| OFAg-AuNPs           | 37.80±0.26 | 58.00±0.09 | 60.77±0.38 | 60.10±0.25 | 63.07±0.43 |
| BHA                  | 42.63±0.41 | 54.57±0.61 | 78.33±0.49 | 91.27±0.16 | ND |
| ABS                  | 52.13±0.18 | 58.93±0.32 | 63.63±0.18 | 84.20±0.41 | ND |

ND: not determined.
AuNPs demonstrated similar antifungal property against Ag-AuNPs exhibited excellent antifungal property of 92%.

Table 6: ABTS bleaching activities (%) of biosynthesized OFAgNPs, OFAuNPs and OFAg-AuNPs

| Concentration (µg/ml) | 20   | 40   | 60   | 80   | 100   |
|----------------------|------|------|------|------|-------|
| OFAgNPs              | 41.33±0.57 | 48.40±0.42 | 52.87±0.39 | 57.23±0.44 | 61.83±0.68 |
| OFAuNPs              | 27.60±0.29 | 30.47±0.43 | 35.80±0.12 | 43.43±0.54 | 50.37±0.38 |
| OFAg-AuNPs           | 32.83±0.26 | 37.50±0.44 | 41.40±0.44 | 44.47±0.23 | 49.33±0.59 |
| BHA                  | 42.63±0.14 | 54.57±0.16 | 78.3±0.49  | 91.27±0.16 | ND     |
| ABS                  | 52.13±0.18 | 58.93±0.32 | 63.63±0.18 | 84.20±0.14 | ND     |

ND: not determined.

A. flavus, F. solani and A. fumigatus respectively, while OFAg-AuNPs showed 52, 57, 60, 92 and 92% against A. niger, F. solani, A. fumigatus, A. flavus and C. albicans respectively. Biosynthesized OFAgNPs displayed satisfactory antifungal activity with growth inhibitions of 64% and 70% against A. flavus and A. fumigatus respectively. OFAuNPs displayed a better antifungal activity with growth inhibitions of 64% and 79% against F. solani, A. fumigatus, while Ag-AuNPs exhibited excellent antifungal property of 92% inhibitions against A. flavus and C. albicans. Interestingly at same concentration of 200 µg/ml, OF-AgNPs and OF-AuNPs demonstrated similar antifungal property against A. flavus and F. solani, respectively yielding the same inhibition value of 64%.

The study has revealed that bimetallic synthesized nanoparticles (OFAg-AuNPs) could be better than the monometallic nanoparticles, however both revealed satisfactory antifungal properties. The current study showed better antifungal activity of bimetallic Ag-AuNPs at 200 µg/ml over previous work. Elegbede et al. [56] reported highest antifungal activity of86 and 85% against A. flavus NEAg-AuNPs (from xylanases of Aspergillus niger NE) and TEAg-AuNPs (from xylanases Trichoderma longibrachiatum-TE) respectively, while 92% inhibitions against A. flavus was reported in this work. Abkhoo and Panjehkeh [57] reported 24% growth reduction in Fusarium oxysporum at 5000 ppm concentration of AgNPs after 24h incubation, which is far lesser than what was obtained in this study against Fusarium species, while AgNPs from Psidium guajava said to inhibit the growth of Saccharomyces cerevisiae, A. niger and R. oryzae at concentration of 300 µg/ml. Ajitha et al. [58] and Francis et al. [59] reported 11 mm and 12 mm growth inhibition zones by Elephantopus scaber extract mediated AgNPs against A. flavus and A. penicilloides respectively. Green synthesized AgNPs has reported to have high antifungal activity with MIC ≤ 1.68 µg ml⁻¹ against Candida albicans, C. glabrata and C. tropicalis [47]. AuNPs antifungal potency had been connected to the acidification of intracellular environment by inhibition of H⁺-ATPase in the yeast cells [60]. Antifungal inhibitory potential of nanoparticles could be associated with the initiation of cell wall which attack an event that mars and weaken fungal spore wall, leading to leakage of cytoplasmic contents and finally cell death. Also, the size of particles enhances the easy penetration of metallic nanoparticles into the fungal cell wall, resulting into the cascade events that lead to fungal cells death [56].

3.4 DPPH free radical-scavenging and ABTS radical cation decolourisation assay of OFAgNPs, OFAuNPs and OFAg-AuNPs

The biosynthesized OF-AgNPs displayed satisfactory DPPH-scavenging activities with inhibitions of 52.25–57.50%, OF-AuNPs displayed inhibition of 37.07–59.37%, while OF-Ag-AuNPs showed inhibition of 38.80–63.07% that were dose-dependent at investigated concentrations (20–100 µg/ml) (Table 5). However, ascorbic acid and butylatedhydroxyanisole (BHA) used as standards displayed inhibitions of 52.13–84.2% and 42.57–91.27%, respectively, at the same concentrations. Bleaching inhibition assay by Azino-bisdiammonium salt (ABTS) showed activities of 41.33–61.83% for the OFAgNPs, 27.60–50.37% for the OFAuNPs and 32.83–49.33% for the OFAg-AuNPs obtained at 20–100 µg/ml (Table 6). The scavenging properties of the OFAgNPs, OFAuNPs, OF-Ag-AuNPs in the present study are related to those previously reported. Enterococcus species biofabricated AuNPs displayed a little bit higher value of DPPH-scavenging activities of 51.47% at concentration of 40 µg/ml compared to the current study [61]. Suganthy et al. [62] gave DPPH-free radicals scavenging activities of Terminalia arjuna mediated AgNPs and AgNPs as 88.13% and 80.06% respectively, which is also higher than the current work. Ahmad et al. [63] reported the DPPH-scavenging activities of 50.50% at concentration of 25 µg/ml by metallic Ag-AuNPs Trapanatans peel mediated. The satisfactory ABTS scavenging activity may thus associate with biomolecules present in AgNPs, AuNPs and Ag-AuNPs. The functional groups of the biomolecules serv-
ing as bioreductant adhering to the surface of the particles could be responsible for the free radical scavenging activity of the biosynthesized AgNPs [64]. Improved surface area of activity is the role of nanoparticles in the antioxidant activity, which activate the catalytic action of the biomolecules borne on the particles [61]. Hence protein and other molecules on the synthesized nanoparticles as revealed by FTIR and discussed earlier could suffice for the antioxidant activities. However, the particles in this study displayed lower free radicals scavenging activities compared to some previous studies at evaluated concentrations, which indicated mild free radicals scavenging in biomedical applications.

4 Conclusion

*Opuntia ficus-indica* extract was used as reducing agent to biosynthesized OFAgNPs, OFAuNPs and OFAg-AuNPs under benign conditions. The face-centred cubic structure nanoparticles were mainly spherical in shape. The FTIR analysis revealed that different biomolecules such as protein, amine (N-H) and hydroxyl (O-H) groups accounted for stabilisation and capping of the particles. The synthesized nanoparticles demonstrated outstanding antibacterial and antifungal properties. The particles also displayed varying degrees of free radical scavenging activity (DPPH assay), ABTS bleaching inhibition assays. The study further established the relevance of *O. ficus-indica* in green nanobiotechnology, and for the first time, its exploitation for the synthesis gold and silver-gold alloy nanoparticles for biomedical applications.

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