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ANALYSIS OF ANTIMICROBIAL POTENTIAL OF SILVER NANOPARTICLES SYNTHESIZED BY LEAF EXTRACT OF *MORINGA OLEIFERA*

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Abstract

In the present study silver (Ag) nanoparticles synthesized from extract taken from *Moringa oleifera leaves,* were characterized and examined for antimicrobial properties. Color change, UV-visible spectroscopy and Fourier transform infrared (FTIR) techniques were exercised for characterization of Ag nanoparticles. Antimicrobial potential of Ag nanoparticles was analyzed using disc diffusion method against clinically important Gram-positive bacteria (*Geobacillus, Staphylococcus aureus, Bacillus subtilis*), Gram negative bacteria *(Klebsiella pneumonia, Psuedomonas aeruginosa, Escherichia coli)* and a fungal species (*Candida albicans*). The antimicrobial activity was assayed by size of growth inhibition zone developed by Ag nanoparticles, and its dilutions 3:1, 1:1, in comparison to the inhibition activity exhibited by positive control (amoxicillin /fluconazole) and negative control (deionized water) against the tested strains of bacteria and fungus. The largest growth inhibition zone (17.00±0.577) was observed on staphylococcus aureus among Gram-positive bacteria, while in Gram-negative bacteria, the largest inhibition zone (17.66±1.2) was obtained on *Pseudomonas aerginosa.* The results revealed that there is insignificant difference among zone of inhibition developed by pure silver nanoparticles solution and its dilutions (17.00±0.577, 16.00±0.577 & 15.66±0.881). Moreover, inhibition zone developed by positive control and nanoparticle solution (18.66±1.20 & 17.00±0.577) were insignificantly different. But the zone of inhibition on *Candida albicans* developed by nanoparticles is significantly small as compared to positive control (17.66±0.33 & 12.66±0.33). Taken together, findings of this study revealed that Ag nanoparticles showed the most significant inhibition activity (p=0.00) against the examined strains of Gram-positive and Gram-negative bacteria but this activity is poor on *C. albicans* as exhibited by inhibitory zone.

Key words: Antimicrobial activity, Silver nanoparticles, *Moringa oleifera*, Gram-positive bacteria, Gram-negative bacteria, *Candida albicans*

Introduction

Clinically, resistant microbes are a severe concern to human health and even cause deaths (Anon., 2020). Due to excessive and misuse of drugs/antibiotics, microbes have attained tremendous resistance among them. This evolving resistance in these pathogens is harder to treat and requires more expensive options. Nanoparticles (NPs) have novel properties of interaction with plants, animal cells and microorganisms (Siddiqi & Hasan, 2017). Metallic nanoparticles such as Au (gold), Ag (silver), Pb (lead), Cu (copper), FeO (iron oxides), ZnO (zinc oxides) and a variety of metal oxides are being exploited for treatment in medical science (Aritonang *et al*., 2017). Ag nanoparticles had antimicrobial properties against microbes and parasites. Ag+ particles caused cells to pass by blocking bacterial cell wall. This multitude of activities are associated with antimicrobial components of Ag nanoparticles (Vazquez-Munoz, 2017). Ag nanoparticles have diverse applications in agriculture, textiles, nanomedicines, drug delivery and cure of many disorders as Ag nanoparticles have large surface area to volume ratio (Slepicka, 2020).

Among the different metallic NPs, silver nanoparticles (AgNPs) have enormous applications in the medical and biotechnological fields (Vinayagam *et al*., 2021). Different substance approaches are being utilized for the combination of nanoparticles. Silver nanoparticles, through imaginative synthetic methodology, are incorporated by trisodium citerate without utilizing any covering and settling specialist (Barbinta *et al*., 2020) and their characterization has been studied using X-ray diffraction (XRD), energy-dispersive X-ray spectroscopy (EDX), and field-emission scanning electron microscopy (FE-SEM) analysis. These techniques are used to study the crystallography, microstructure, crystal defects and morphology of variety of nanoparticles like $Bi_xZn_{1-x}O$ nanoparticles (Hussanien *et al*., 2019). Similarly, ZnO and Rb-doped ZnO nanoparticles, RbxZn1−xO-NPs are synthesized by sol–gel technology. The technique is very effective as it controls the crystallization, crystal imperfections, and morphological features ZnO nanoparticles (Hussanien *et al*., 2019).

Recently, Green synthesis of NPs has gained a lot of attention due to its sustainability, and ecofriendly nature. Bacteria, algae, fungi, yeast and plant extract are used as reducing agents for green synthesis of Ag nanoparticles (Singh *et al*., 2021). In green synthesis of Ag nanoparticles plant parts like leaves, root and fruits are used. The role of plants in biosynthesis of Ag NPs is to provide secondary metabolite bioactive composites that act as reducing/capping agents (Kang *et al*., 2013). To evaluate efficacy, Ag NPs were applied against different strains of microbes. Results revealed that NPs synthesized by green method were more efficient compared with silver metal ions (Benakashani *et al*., 2017). Ag NPs prepared using polymers from plants had high antimicrobial activity for Gram-negative and Gram-positive bacteria. These NPs interfere with cell membrane of bacteria and destroy it (Sharma *et al*., 2007). *Moringa oleifera* because of its outstanding medicinal and nutritional use has obtained interest of researchers. *Moringa oleifera,* on the basis of its medicinal value, has been proposed as model plant for fabricating nanoparticles. The aim of present study is to use *Moringa oleifera* to prepare of Ag nanoparticles which will be subsequently characterized for their size and presence. The authors in this research have

estimated the antimicrobial capability of Ag nanoparticles manufactured by *Moringa oleifera* against different obsessive Gram-positive, and Gram-negative types of microscopic organisms and C. albicans. Also, development hindrances under nanoparticle treatment will be looked at among microorganisms and growths.

Material and Methods

The leaves of *M. oleifera* were collected from the university vicinity, cleaned and washed properly. Leaves were dried under the fan at a room temperature.

Synthesis of Ag NPs: 10 g leaves suspended in 100 ml of deionized water were boiled at 80°C on hot plate for 10 minutes; cooled and filtered twice by Whatman filter paper. The leaf extract was poured drop-wise in a 1 mM silver nitrate solution (≥ 99.0 % purity Merck). When color of the silver nitrate solution transformed from colorless to dark color (brown), this signaled formation of Ag NPs. The extract was filtered via Whatman No.1 filter paper and stored at 25°C until use.

The preparation of Ag nanoparticles was followed by UV-Visible spectrophotometer (Thermoscientific Genees S10) with a resolution of one nanometer. Peaks obtained between 200 to 350 nm using absorption spectrum confirmed the presence of Ag NPs in sample.

To recognize the various phyto-constituents present in the *Moringa oliefera* extract and the biomolecules capped on the synthesized NPs, evaluation was made by Fourier transform infrared rays, FITR (Thermoscientific Nicolate S5). Therein the sample was lyophilized with the help of a freeze-drying machine which is then subjected to FTIR and the spectrum was noted between 4000-400 cm^{-1}.

Antimicrobial assay of Ag NPs: For antimicrobial evaluation of Ag NPs, various bacterial strains such as *Staphylococcus aureus*, *Geobacillus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and a pathogenic fungus, *Candida albicans* were subjected to NPs treatments. Original solution of Ag NPs, its dilutions like 1:1 (both Ag NPs and water in equal proportion) and 3:1 (3 parts Ag NPs: 1 part water), along with positive and negative control were tested against bacterial and fungal species.

Amoxicillin and fluconazole antibiotics were used as positive control and deionized water as negative control to assess the antimicrobial activity of nanoparticles against bacteria and fungus. Moreover, the petri plates were kept in incubator at 37°C for 24 hrs. Zone of inhibition was measured with a scale. Experiment was treated in replicate for better results. Further, the zone of growth inhibitions at different concentrations of Ag NPs for various bacterial strains was compared by a one-way ANOVA, Tukey's Test using SPSS software version 16.

Results

Figure 1A shows the absorption spectrum peaks for silver nanoparticles between 200 to 350 nm which suggested the presence of Ag NPs in the sample.

The FTIR technique was employed to analyze the presence of different phyto constituents and bio-functional groups used for reduction and capping of nanoparticles. In FTIR spectrum of nanoparticles, peaks at 700.12 cm^{-1}, 850.30 cm^{-1} and 900 cm^{-1} represented the presence of C-Cl stretch. The peaks of absorption band at 1267.92 cm^{-1}, 1231.9 cm^{-1}, 1135.41 cm^{-1}, 1168.0 cm^{-1} and 1192 cm^{-1} indicated the stretch of aliphatic amines. While the peak of absorption bands at 1430.50 cm^{-1} showed alkane groups. The peak at 1520.90 cm^{-1} represented N-O asymmetric stretching of nitro compounds and 1750 cm^{-1} indicated C=O bonds of aldehyde groups. The FTIR spectrum of nanoparticles showed stretching in peaks at 2859 cm^{-1}, 2922 cm^{-1} and 3000 cm^{-1} for C-H bond of alkanes (Fig. 1B).

Antimicrobial activity of Ag NPs: The antimicrobial activity of silver nanoparticles results showed that bacterial growth for Gram positive bacteria is inhibited in positive control, Ag NPs solution and its dilutions (3:1 and 1:1) as noted by the presence of large zones of growth inhibition around discs. Negative control group did not show any inhibition zone. Statistical analysis using one-way anova (SPSS/16 software) showed that groups shown by superscript "a", "b", and "c" are significantly different ($p=0$) from one another. The results obtained using silver nanoparticle solution and its dilutions (3:1 and 1:1) had no significant difference (Fig. 2A and Table 1).

Table 1. Growth of Zone inhibition under Ag NPs treatments against gram positive bacteria, gram negative bacteria and *Candida albican*.

Microbes	Zone of inhibition				
	Negative control	Positive control	Silver nanoparticles	Silver NPs dilutions (3:1)	Silver NPs dilutions (1:1)
Gram +ve bacteria *Geobacillus*	0.0 ± 0.000^{a}	17.3 ± 1.45^{c}	13 ± 0.577^{b}	13 ± 0.66^{b}	12 ± 0.00^{b}
Bacillus subtilis	0.0 ± 0.00^{a}	21.33 ± 1.76^{c}	16.66 ± 0.666^{b}	16.66 ± 0.88^{b}	16.3 ± 0.66^{b}
Staphylococcus aureus	0.0 ± 0.00^{a}	18.66 ± 1.20^{b}	17.00 ± 0.577^{b}	16.00 ± 0.577^{b}	15.66 ± 0.881^{b}
Gram -ve bacteria *Escherichia coli*	0.0 ± 0.00^{a}	21.66 ± 1.20^{c}	16.33 ± 0.88^{b}	16.33 ± 0.88^{b}	16.00 ± 1.00^{b}
Klebseilla pneumonia	0.0 ± 0.00^{a}	19.66 ± 1.33^{c}	16.66 ± 0.333^{bc}	16.66 ± 0.66^{bc}	15.66 ± 0.88^{b}
Psuedomonas aerginosa	0.0 ± 0.00^{a}	21.00 ± 1.52^{c}	17.66 ± 1.20^{bc}	16.33 ± 0.66^{bc}	16.00 ± 1.00^{b}
Fungus species *Candida albican*	0.0 ± 0.00^{a}	17.66 ± 0.33^{d}	12.66 ± 0.33^{c}	11.66 ± 0.033^{bc}	11.00 ± 0.00^{b}

Note: The zone of inhibitions is shown with ± SE. The values in columns with different subscripts are significantly different from one another. The mean values were calculated from 3 replicates

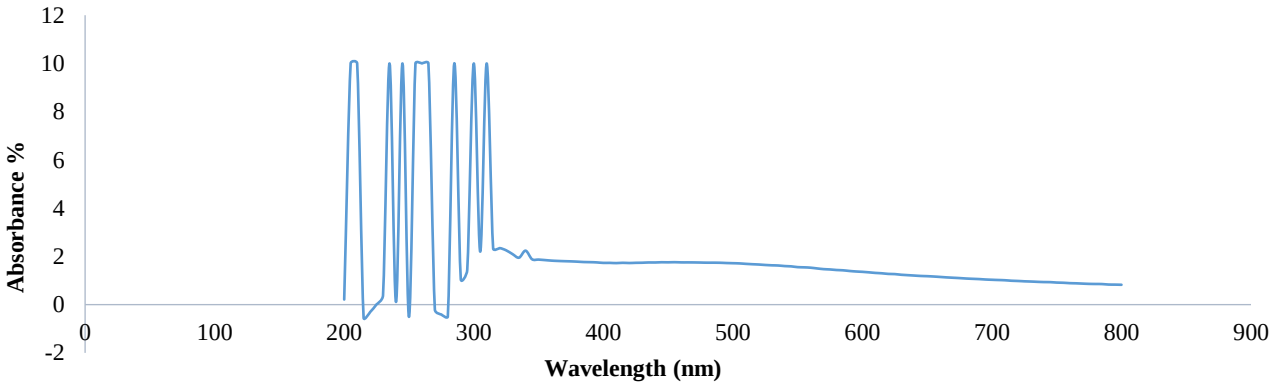

Fig. 1A. A shows absorbance spectrum of Ag NPs in solution fabricated by *Moringa olifera* leaves.

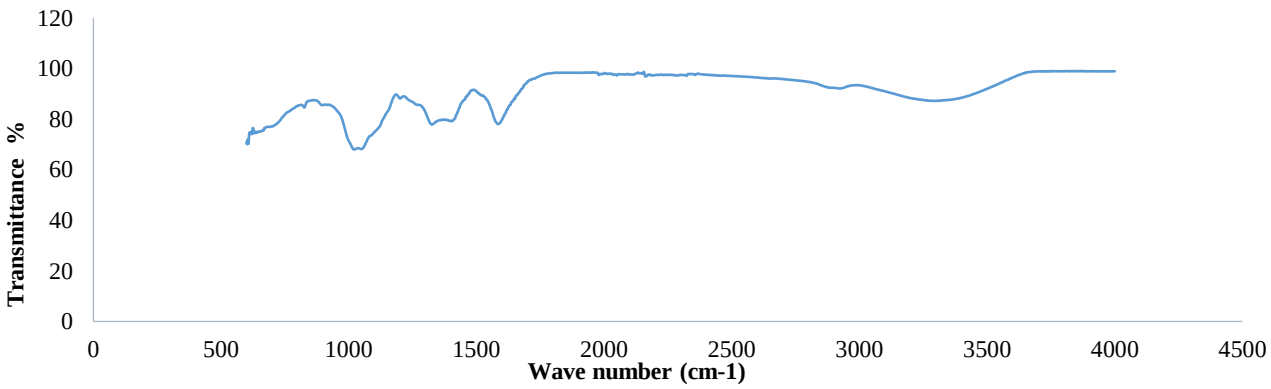

Fig. 1B. B Shows Absorption spectrum peaks that indicating the presence of different functional groups in Ag NPs solution.

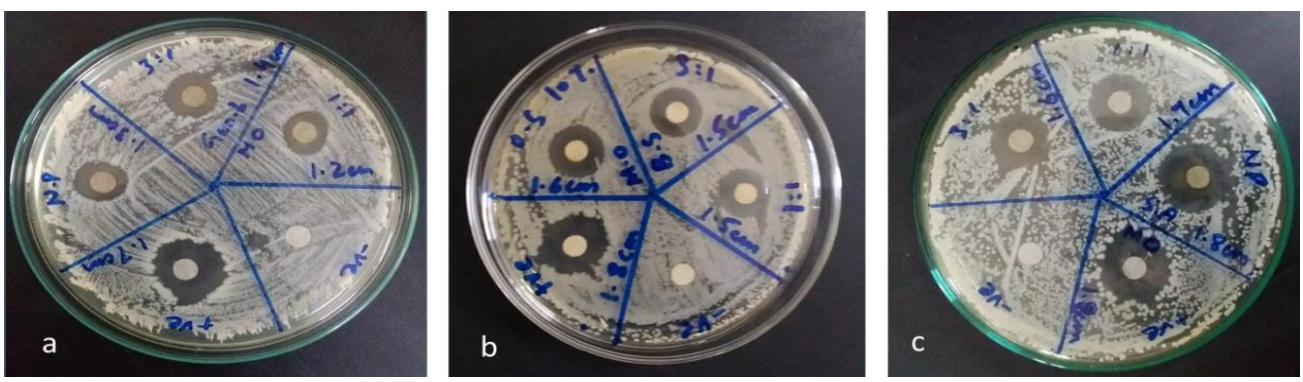

Fig. 2A. Gram positive bacterial species, *Geobacillus(a)*, *Bacillus subtilus (b) and staphylococcus aureus* (c) cultured on agar medium were subjected to Ag NPs, dilutions of N.P 3:1 and 1:1 along with negative control and positive control. The positive control, N.Ps and its dilutions showed significant growth inhibition zone.

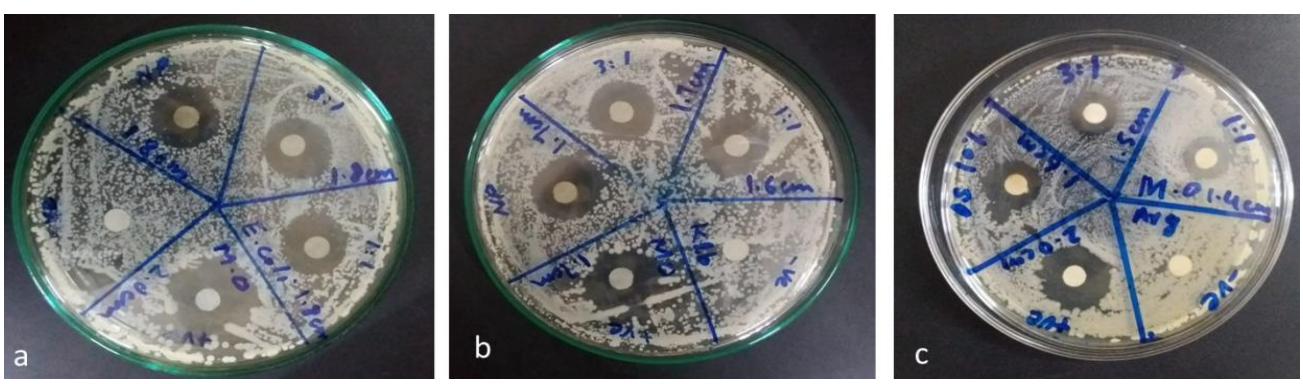

Fig. 2B. Gram Negative bacterial species, *Escherichia coli, Klebseila pneumonia, Psuedomonas auroginosa* cultured on agar medium were subjected to Ag NPs, dilutions of N.P 3:1 and 1:1 along with negative control and positive control. The positive control, N.Ps and its dilutions showed significant growth inhibition zones.

Fig. 2C. *Candida allbicans* cultured on agar medium was subjected to Ag NPs, dilutions of N.P 3:1 and 1:1 along with negative control and positive control. The positive control, N.Ps and its dilutions showed significant growth inhibition zones.

Figure 2B and Table1shows the effect of Ag- NPs solution and its dilutions, 3:1 and 1:1 on Gram negative bacteria such as *Escherichia coli*, *Klebseila pneumonia, Psuedomonas auroginosa.* The largest zone of inhibition (17.66±1.2) was obtained on *Pseudomonas aerginosa* and the results of inhibitory zones developed by dilutions of nanoparticles (3:1 and 1:1) are very close to each other (16.33±0.66 & 16.00±1.00).

Figure 2C and Table 1 shows the inhibitory effect of silver nanoparticles and its dilutions on *Candida albicans.* The zones of growth inhibition are comparable between the pure nanoparticle solution and its dilutions (12.66±0.33, 11.66±0.33 & 11±0.00).

Discussion

Nanoparticles (NPs) are considered the most effective carriers of phyto-constituents, drug moieties and functional groups as capping and reducing agents. NPs are used as therapeutic agents against diverse infections and various cancers. Conventional therapies cure infections with inherent side effect(s) (Khatoon *et al*., 2017). The increasing use of antibiotics against infections has affected the economic, physical and psychological position of the patients. Alternative drug therapies are required to effectively treat the infections with no or minimum side effects.

Green synthesis method for Ag nanoparticles was employed as the most economical and environment friendly method for control of microbial infections (Amin *et al*., 2012; Mukaratirwa-Muchanyereyi *et al*., 2022). In the current review, *Moringa oleifera* leaves were utilized as a diminishing or settling specialists for manufacture of Ag NPs. Leaf confine was mixed with arrangement of AgNO3 and thereafter, its tone transformed from no variety to dull brown, demonstrating manufacture of Ag nanoparticles. Change in color is the sign of nanoparticle synthesis as reported by Marslin *et al*., (2018). Similar approach was used by Mukaratirwa-Muchanyereyi *et al*., 2022 to synthesize silver nanoparticles from *Erythrina abyssinica*. The further confirmation regarding presence of NPs was made by UV-visible spectrophotometric analysis that showed absorption spectrum between 200 to 350 nm for the NP solution. The results of absorption spectrum are agreeing well with the literature reports (Nyoni *et al*., 2019). The characterization and various functional groups on the surface of synthesized NPs was substantiated by FTIR spectrum at a wavelength region between 500-4000 cm^{-1} (Mukaratirwa-Muchanyereyi *et al.,* 2022). The characterization methodology coincided with reports of Khadka *et al*., (2020) and Mukaratirwa-Muchanyereyi *et al*., (2022) who showed the presence of bio-functional groups at a transmittance region between 4000-500 cm^{-1}. The C-H stretching at 2859 cm^{-1}, 2922 cm^{-1} and 3000 cm^{-1} is due to stretching vibration of C-H bond of alkanes (Cascione *et al*., 2022). Indication for various functional groups present in *Moringa* extract for the formation of Ag NPs is revealed by peaks at different regions in FTIR graph (Amin *et al*., 2012; Khatoon *et al*., 2017).

In recent study silver nanoparticles are utilized as antimicrobial agents against Gram positive, Gram-negative microscopic organisms and against *C. albicans* which is in accordance with different reports referenced in literature (Khatoon *et al*., 2017; Mukaratirwa-Muchanyereyi *et al*., 2022). The disc diffusion method was used for assessment of Ag nanoparticles potential. Green synthesized Ag NPs exhibit antimicrobial effect equivalent to positive control groups in all selected microbes. Though, the zone of growth inhibition exhibited by the NPs and their dilutions were significantly comparable to antibiotic used as positive control group (17.00±0.577, 16.00±0.577 & 15.66±0.881). In comparison with the standard medicine, the green synthesized nanoparticles have ability to be utilized as drug against various Gram positive (*Geobacillus, Staphylococcus aureus, Bacillus subtilis*), and Gram-negative bacterial strains (*Klebsiella pneumonia, Psuedomonas aeruginosa, Escherichia coli*). The Ag nanoparticles exhibited a substantial antimicrobial activity due to the high surface area to volume ratio that enables them to bind with bacterial cell wall to produce effective inhibitory activity (Mukaratirwa-Muchanyereyi *et al*., 2022) which results from spillage of amino corrosive and protein. It is accounted for that Ag nanoparticles hinder the elements of different chemicals which eventually prompts the passing of microbes (Varadavenkatesan *et al*., 2021).

Results of this study confirmed with Artinang *et al*., (2019) who prepared different concentrations of Ag nanoparticles using green synthesis method and studied its antimicrobial character. The shape and size dependent antimicrobial potential of Ag NPs were checked against two species of Gram-negative bacteria, *Pseudomonas aeruginosa* and *Escherichia coli* by Amin *et al*., (2012). Our findings about zone of growth inhibition (17.00±0.577) of green synthesized Ag nanoparticles against Gram positive bacteria is in line with zone of inhibition obtained by silver nanoparticles against

salmonella (16.9 ± 0.15 mm) (Mukaratirwa-Muchanyereyi *et al*., 2022). Similarly, the Ag and gold NPs moderated from rhizome extract from *C. longa* showed conspicuous bactericidal effect on *B. subtilis* and *E. coli* (Sharma *et al*., 2020). The findings of the present study are further supported by the results of Benakshani *et al*., (2017) who used *Lepidium draba* to synthesize Ag NPs and characterized the synthesized NPs by FTIR spectroscopy, X-ray diffraction method and TEM. Our results of antimicrobial activity of Ag NPs and its dilutions against Gram negative and GXram positive bacteria are similar to their report.

The antifungal effect of Ag NPs as revealed in this study is corroborated by the results of Xia *et al*., (2014). They assessed the anti-fungal effect of Ag NPs on *T. asahii* (pathogenic fungi). Similarly, green synthesized Ag NPs exhibited excellent antimicrobial activity against bacteria and fungi (*Candida* sp.) (Mallman *et al*., 2015). Our findings about Ag NPs are also supported by findings of Rozykulyyeva *et al*., (2020). They reported antibacterial action versus two Gram negative and two Gram positive bacterial species. Their results showed that Ag NPs are more effective against *S. aureus* and *P. aeurginosa* than other strains of bacteria and thus corroborated our results. Similarly, our results of silver nanoparticle's antimicrobial effects are substantiated by the reports of Gomathi *et al*., (2019) who checked the antimicrobial effect against Gram positive, *S. aureus* and Gram negative, *E. coli*.

Conclusion

Taken together, it was concluded that UV-Vis spectra absorbed by green synthesized silver nanoparticles lies between 200 to 350 nm confirm the existence of NPs in solution. Silver nanoparticles have shown significant antimicrobial effect against Gram-positive, Gram-negative bacteria and *Candida albican*. The zone of inhibition exhibited by pure nanoparticle solution is very close to the inhibition zones produced by nanoparticle dilutions (17.00±0.577, 16.00±0.577 & 15.66±0.881). Moreover, the inhibitory zone produced by nanoparticles is very close in diameter to the zone of inhibition produced by positive control. The antifungal activity of silver nanoparticles is not effective if compared with the positive control. It is therefore recommended that green synthesized silver nanoparticles can be used as antimicrobial agents by conducting further in-vivo and in-vitro studies to evaluate dosage and duration frequency.

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