doi.org/10.36721/PJPS.2021.34.3.REG.995-1001.1

Antimicrobial and antioxidant activities of silver nanoparticles synthesized by novel biogenic method using mixed reductants

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Abstract: A novel method, for the synthesis of silver nanoparticles that are eco-friendly by means of mixed reductants method, has been developed. The combined extract of *Mentha viridis* plant and *Prunus domestica* gum were used as reducing agents for the synthesis of silver nanoparticles of the size less than 40 nm in diameter. The effect of time and concentration on the formation of silver nanoparticles were also monitored. The silver nanoparticles formed were verified by surface Plasmon spectra using single and double beam UV-Vis spectrophotometer. The XRD technique and scanning electron microscopy were performed to analyze the crystalline structure, crystallite size and morphology. The synthesized silver nanoparticles were tested against different bacterial and fungus strains. The silver nanoparticles showed good inhibition in antimicrobial study and low MIC for bacterial strains. The antioxidant assay was performed to check the scavenging activity. In DPPH, the silver nanoparticles showed good scavenging activity and were found close to that of ascorbic acid.

Keywords: *Mentha viridis, Prunus domestica* gum, nanoparticles, antimicrobial, antioxidant.

INTRODUCTION

The medicinal plants for the treatment of various diseases are the traditional trends across the globe. *Mentha* is a medicinal plant that belongs to family *Labiatae*, with common name of mint. *Mentha* specifically is used in folk medicine for treatment of digestive, analgesic and liver complaints (Mahboubi, 2018, Salehi *et al*., 2018). *Prunus domestica* L. (family Rosaceae) is a shrubby, deciduous, small tree cultivated at high altitude (Ashirova, *et al*., 2018). There is a huge scope of natural gums as a novel natural polymer for the development of different drug delivery systems. Natural gums are widely used for various applications in pharmacy and medicine. Gums are water-soluble polysaccharides, which contain galactose, arabinose, rhamnose, uronic acids, galacturonic acid, protein, Ca and Mg as major structural ingredients as well as, glucose, xylose, mannose, protein, and fat as minor constituents (Islam *et al.,* 2017).

Recently, researchers have shown the use of nanotechnology in the field of metallurgy, surface enhanced Raman scattering and various biological applications (Lu *et al*., 2014) and is a fast growing field. According to the published data, novel properties and high performance of nanoparticles are not only due to the small size as compared to the bulk, but also their unvarying size distribution (Qu *et al*., 2014). The widespread use of nanoparticles, especially metallic nanoparticles, in many fields of knowledge, is due to the fact that they have important distinct characteristics compare to those of bulk metals e.g., silver nanoparticles offer high performance and low cost in catalysis for many types of value added chemical reactions provide the cheap and easy resources for catalysis (De lima *et al*., 2014). The synthesis of silver nanoparticles is evergreen research of modern era in which bio mediated experimental process is more imperative and significant (Islam *et al*., 2017). For example, silver and silver salts are supposed to be excellent hygienic and curative agents because of their broad-spectrum antimicrobial activity and limited microbial resistance, which generates the oxidative stress and destruct the DNA, as well as interact with the cell walls (Song *et al*., 2013). In this study, we evaluated mixed reductants synthesized/stabilized loaded silver nanoparticles of the *Mentha viridis* plant extracts and *Prunus domestica* gum, for *in vitro* antimicrobial and antioxidant activities, to introduce new and safe bioactive nanomedicines (Qu *et al*., 2014).

MATERIALS AND METHODS

Mentha viridis plant was collected from Botanical Garden, Islamia College Peshawar. *Prunus domestica* gum were collected from Platoona, Mardan district of Khyber Pakhtukhwa, Pakistan, identified by a plant taxonomist Prof. Dr. Naveed Akhtar and voucher specimen were submitted at herbarium Islamia college university Peshawar.

Materials

Silver nitrate ($AgNO_3$), 99.8-100 %, activated carbon, hydrochloric acid, 37-38 %, ethanol, 96-98 % pure were purchased from BDH Laboratory Supplies Poole, BH 15 1TD, England. Sodium borohydride, 96 % pure, was purchased from Sigma-Aldrich BATCH NO. 24886165

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and sodium chloride, 99.9-100 % pure, potassium chloride, 99-100 %, sodium hydroxide, 98 % pure, and methanol, 99.9 % pure, were purchased from Merck KGaA, 54271 Darmstadt, Germany, and was used as such without further purification.

Preparation of leaf extracts

The *Mentha viridis* plant was washed with tap water followed by deionized double distilled water. A total 20 % of *Mentha viridis* plant extracts were prepared by boiling the weighted *Mentha viridis* plant in deionized double distilled water at 80 ºC for 10 minutes. Similarly, 1g of *Pronus domestica* plant gum extract was obtained, washed with tap water, followed by stirring with deionized double distilled water at 80 ºC for 10 minutes. The extracts were filtered by using the whatman filter paper no.1 and refrigerated at 4 ºC for 24 hours.

Synthesis of silver nanoparticles via green method

Accurately weighed 1.0 mmol of silver nitrate was dissolved in 90 ml of deionized doubled distilled water. Solution was prepared in volumetric flask and placed on magnetic stirrer under constant slow stirring at room temperature. 2 mL of *Minta veridus* plant extract was taken and diluted to a volume of 10 ml. The extract was added drop wise to the silver nitrate solution. After few seconds, the color of the solution changed to blackish yellow. After the complete addition of the extracts, 1.5 ml of *Pronus domestica* plant gum extract was added to the solution at once and stirred for next 30 minutes at 60 ºC. The color of the solution changed to dark yellow. The solution was kept in incubator for 24 hours followed by centrifugation at 4500 rpm for 15 minutes (Janani *et al*., 2014, Veige *et al*., 2016). The sample was then kept for drying in vacuum oven at 50 ºC. After complete drying, the sample was washed with ethanol and dried again in vacuum oven at 50 ºC.

Characterization of silver nanoparticles

UV-Vis spectroscopy

The UV-Vis spectroscopy measurements were recorded using JASCO double beam UV-Vis spectrophotometer (Japan) and UV-Vis single beam spectrophotometer (model) with 1cm quartz cells (Mecha, & Pillay., 2014).

Fourier transform infrared spectroscopy (FTIR)

The Fourier transform infrared spectra were recorded in the range of 4000-400 cm^{-1} with Fourier transform infrared spectrophotometer (Palker Elmer), (Zaman *et al*., 2019, Islam *et al*., 2017).

X-ray diffraction studies

The XRD spectra were recorded by powder X-ray diffractometer JDX-3532 JEOL (Japan). The operating voltage were 40 kV with 20 mA current, 1.54 A° X-rays and Cu Ka radiation using powder sample on a glass substrate (Kumari *et al.,* 2015).

Scanning Electronic Microscopy (SEM)

Morphological investigations were carried out by using colloidal sample drop on copper grid followed by vacuum drying at room temperature and gold coating with auto fine coater. The samples were subjected to analysis using scanning electronic microscope JEOL Japan-JSM 5910.

Antimicrobial assay

Antibacterial activity

The synthesized samples of silver nanoparticles were tested for inhibition against five bacterial strains *Methicillin-resistant Staphylococcus aureus* (MRSA), *Vancomycin-resistant Staphylococcus aureus* (VRSA), *Methicillin-sensitive Staphylococcus aureus* (MSSA), *Pseudomonas aeruginosa* (PS) and *Proteus vulgaris* (PV) using disc diffusion method (Hashim *et al*., 2018, Khan *et al*., 2017). Impinum was used for positive control. The bacterial strains were inoculated in sterile nutrient broth and incubated at 37 ºC for 24 hours. The synthesized silver nanoparticles sample and control impinum were subjected to bacterial colony on agar plates and incubated for 24 hours at 37 ºC. The percent zone of inhibition was calculated by measuring the inhibition zone around the control and silver nanoparticles sample on nutrient broth.

Antifungal activity

The antifungal activity was tested using five fungal strains *Candida albicans* (CA), *Fusarium oxysporum* (FO), *Penicillium chrysogenum* (PC), *Aspergillus parasiticus* (AP) and *Trichoderma harzianum* (TH). The fungal species were obtained from the Center for Biotechnology, University of Peshawar. For positive control, Fluconazole and for negative control, Dimethyl sulfoxide (DMSO) were used (Torres-Martinez *et al*. 2019, Satti *et al*., 2019). The sample and control were studied for antifungal activity using sabaraud dextrose agar media in test tubes.

Antioxidant assay

Antioxidant assay were carried out by dissolving 0.01 g of silver nanoparticles sample in 1 ml of methanol. DPPH solution was prepared by dissolving 0.98 ml of DPPH in 500 ml of methanol. The ascorbic acid and DPPH solution were used as reference with the same concentrations as for silver nanoparticles and DPPH (Salehi *et al*., 2018, Michalska *et al*., 2017, Deveci *et al*., 2018). The stock solution was prepared and different concentrations from solution were monitored using UV-Vis spectrophotometer.

STATISTICAL ANALYSIS

All data related to bioassays are the averages of triplicate analyses. Data were recorded as mean ± S.D. Significant differences between means were determined by SPSS 20 software.

RESULTS

UV-Vis spectroscopy

The reduction of Ag+ ions was monitored by UV-Vis double beam spectrophotometer. Different concentrations of sample showed absorbance maxima between 425-440 nm fig. 1 (Padil *et al*., 2016)

X-ray diffraction studies

X-ray diffraction pattern was recorded for the green synthesized silver nanoparticles shown in fig. 2 with different Braggs reflections. The Braggs reflections were appeared at 2 theta 20-50 degree (Kumari *et al.,* 2015, Mohan *et al*., 2014, Rekha *et al*., 2015).

Scanning Electronic Microscopy (SEM)

Fig. 3 shows the SEM images of silver nanoparticles drop coated and dried powder analysis on copper grid with different magnifications, which shows the average particles size of less than 30 nm as shown in fig. 4. The surface morphology of silver nanoparticles synthesized by green mixed reductants method shows that the *Pronus domestica* gum and *Mentha viridis* plant extracts act as reducing agent as well as stabilize the silver nanoparticles (Ananth *et al*., 2014).

Fourier transform infrared spectroscopy (FTIR)

FTIR measurement was performed to identify the biomolecules and capping agents responsible for the reduction of silver ions and stabilization. All these characteristic peaks obtained were confirmed through literature (Zaman *et al*., 2019). Mixed reductants were found to act as both reducing silver ions and stabilizing the silver nanoparticles.

Table 1: MIC and MBC of silver nanoparticles

S. No.	Bacterial strains	MIC (mg/ml)	MBC (mg/ml)
1	VRSA	0.50	2.25
2	MRSA	0.25	2.25
3	PS	0.50	2.00
4	MSSA	0.50	2.25
5	PV	0.50	1.50

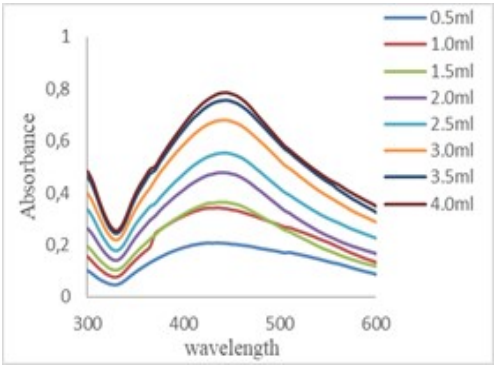

Fig. 1: UV-Vis spectra of silver nanoparticles

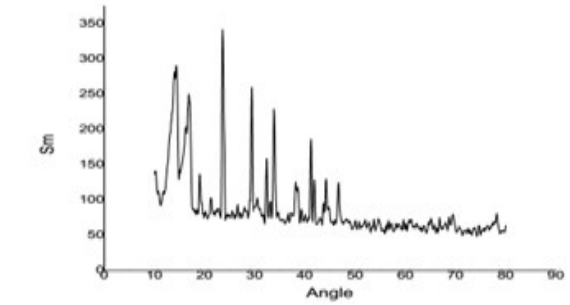

Fig. 2: XRD spectra of silver nanoparticles

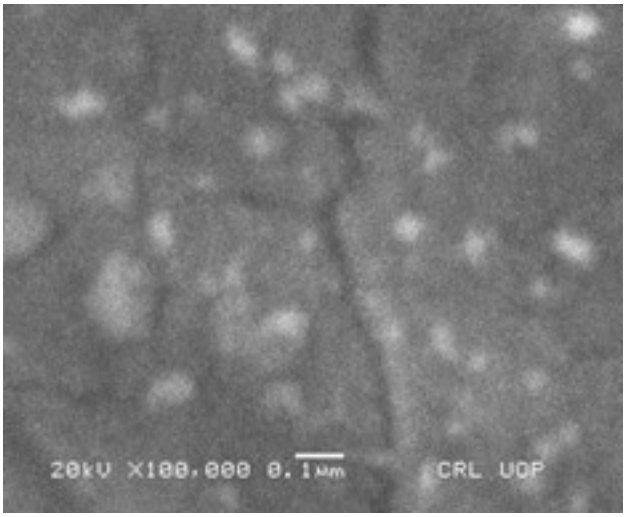

Fig. 3: SEM image of silver nanoparticles

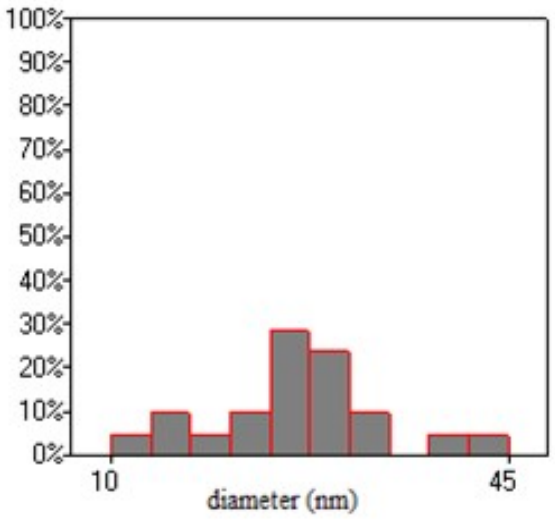

Fig. 4: Size distribution of silver nanoparticles.

Antimicrobial assay

Antibacterial activity

The readings of the tested bacterial strains on agar plates were made for a period of 24 hours. The antimicrobial activity of silver nanoparticles synthesized by *prunus domestica* gum and *mentha viridis* plant extracts were studied using MRSA, VRSA, MSSA, PV and PA after 24

hours of incubation (Mariselvam *et al*., 2014). The diameter of zone of inhibition around the samples was measured. The inhibitory effect was noted by the formation of clear circular zones of inhibition around the disks. The control zone of inhibition and the inhibitory zone were measured for all the five microbial species tested. Using formulas, the percent zone of inhibition was determined as shown in fig. 5. For MRSA, the size of the inhibition was between 16-17 mm. The diameter of zone of inhibition of VRSA was between 19-20 mm (Islam *et al*., 2017, Zaman *et al*., 2019). For MSSA, *Protius volgurus* and *Psodomonas*, the diameter of zone of inhibition were 9 mm, 12 mm and 14 mm, respectively.

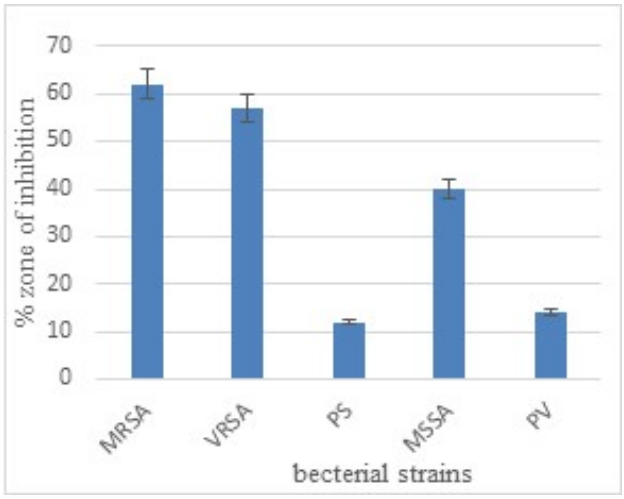

Fig. 5: Antibacterial activity of silver nanoparticles

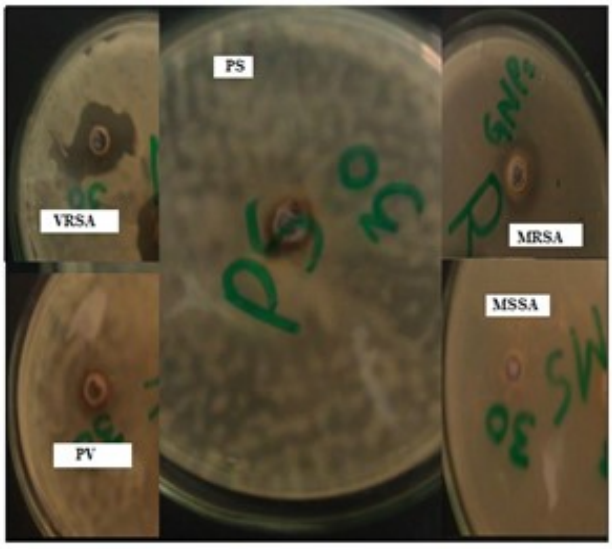

Fig. 6: Antibacterial activity inhibition zone.

Minimum inhibitory concentration (MIC) and ***minimum bactericidal concentration*** (MBC)

The minimum inhibitory concentration was determined by checking the turbidity of inoculum in tubes. The minimum MIC favors a high degree of antibacterial effectiveness (Shahbazi *et al*., 2015). In the present study, the minimum inhibitory concentration was determined for MRSA, VRSA, *Pseudomonas*, *Protius volgurus* and MSSA. The minimum concentration that inhibit according to MIC rules was 0.50 mg/ml for VRSA, 0.25 mg/ml for MRSA, 0.25 mg/ml for pseudomonas, 0.50 mg/ml for MSSA and 0.50 mg/ml for *Protius vulguros,* as shown in fig. 6. The minimum bactericidal concentration (MBC) was determined by streaking the colony on the plates from the dilutions of MIC as done experimentally and reported in activity section (Islam *et al*., 2017).

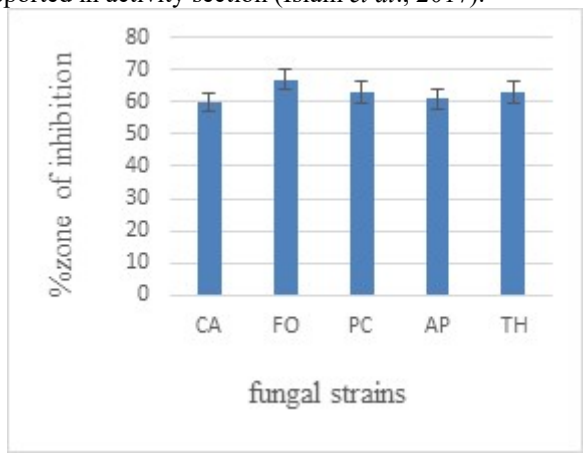

Fig. 7: Antifungal activity of silver nanoparticles

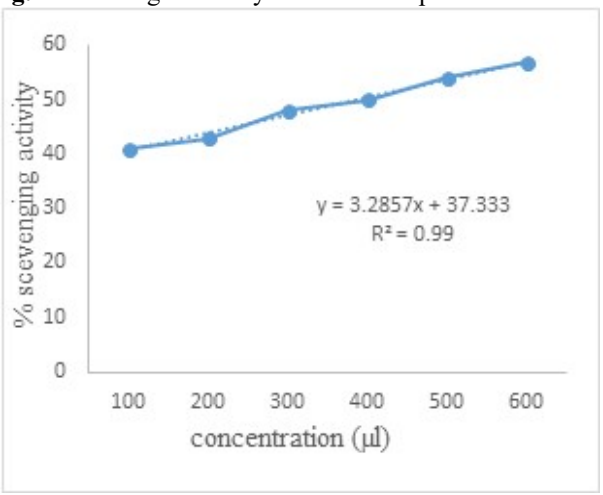

Fig. 8: Antioxidant activity of silver nanoparticles

Antifungal activity

The length of the slants and growth of fungal species were measured after 7 days of incubation. Among the five fungal species, *Candida albicans* showed low sensitivity fig. 7. as compared to the *Fusarium oxysporum*, *Penicillium chrysogenum*, *Aspergillus parasiticus* and *Trichoderma harzianum* (Gunasekaran *et al*., 2019, Mariselvam *et al*., 2014).

Antioxidant assay

The yield of antioxidant capacity of silver nanoparticles in DPPH showed that the silver nanoparticles have effective antioxidant activity (Salehi *et al*., 2018, Michalska *et al*., 2017) as shown in fig. 8.

DISCUSSION

The absorbance maxima between 425-440 nm in UV-Spectrum, fig. 1, clearly suggest the formation of silver nanoparticles. The broadening of the XRD peaks in fig. 2, are due to the crystalline nature of silver nanoparticles. Using debye Scherrer equation and statistical analysis, the average crystallite size was calculated 16 nm. (Zaman *et al*., 2019) reported the morphology and dimension of the *Acacia* tree gum nanoparticles *via* XRD analysis proposed that such size of nanoparticles are suitable as nano carriers targeted drugs. The reflections appeared at 2 theta 23.61, 29.36, 33.82, 41.30 are attributed to (210), (220), (311) and (400) planes according to (JCPD# 75-0033) which shows the crystalline planes of face centered cubic structure of silver nanoparticles crystallite **(**Akinluwade *et al*., 2017). Scanning Electronic Microscopy (SEM) images in figs. 3 and 4 shows that the particles do not seem to be penetrating into the inner surface of the green extract support (Zaman *et al*., 2019), which clarify that the particles are attached to the surface through van der walls forces.

The intense peak in Fourier transform infrared spectroscopy (FTIR) appeared in *mentha viridis* plant extracts at 3311 cm^{-1} is due to -OH stretching vibration, and peaks at 1568 cm^{-1}, 1398 cm^{-1} and 1099 cm^{-1} are suggested belong to the characteristics peaks of flavonoids (Islam *et al*., 2017, Zuas *et al.,* 2014). For *Prunus domestica* gum the intense peaks appeared at 3310 cm^{-1}, 1780 cm^{-1}, 1020 cm^{-1} and 623 cm^{-1} are due to the -OH groups, stretching vibrations, aromatic C=O stretching vibration, C-O groups, valence vibrations of uronic acids and C-C-O, C-O-C groups symmetric, asymmetric vibrations, respectively (Kumari *et al.,* 2015). For the synthesized silver nanoparticles, a downshift has been observed in the frequency from 1398 cm^{-1} to 1346 cm^{-1}. From the present study it has been observed that the water soluble phenolic compounds such as flavonoids and alkaloids are responsible for the reduction of silver ions to silver nanoparticles (Hashim *et al*., 2018, Michalska *et al*., 2017, Padil *et al*., 2016). From antibacterial activity results, it can be inferred that the MRSA, VRSA and MSSA shows highest sensitivity against silver nanoparticles as shown in figs. 5, 6. The MSSA shows moderate sensitivity towards the silver nanoparticles. The remaining microbial species *Protius volgurus* and *Psodomonas* shows low sensitivity against silver nanoparticles as compared with the other species tested (Kumari *et al*., 2015). The highest MICs dilution of silver nanoparticles having 99 % of inhibition was taken as MBC for silver nanoparticles synthesized via green methods as shown in table 1. The appearance of colonies was checked on media. Some of the plates showed reduced growth while some plates have no growth in the whole inoculation. The MBC for green synthesized silver nanoparticles was 2.25 mg/ml for VRSA, 2.25 mg/ml for MRSA, 2.00 mg/ml for pseudomonas, 2.25 mg/ml for MSSA and 1.50 mg/ml for *Protius volgurus*. The comparison with literature suggest that *Prunus domestica* gum and *Mentha viridis* plant extracts showed excellent inhibition against the tested bacterial strains (Gunasekaran *et al*., 2019, Zaman *et al*., 2019). In Antifungal activity results, the Fusarium oxysporum were more sensitive to silver nanoparticles. Silver nanoparticles showed significant activity against all five fungal species tested. The positive control fluconazole showed no growth in all five fungal strains. The results in fig. 7 show that the fungal species were more sensitive to silver nanoparticles, while the antioxidant assay in fig. 8 shows that with increasing concentrations, the scavenging activity of silver nanoparticles also increased due to the lowering absorbance (Hashim *et al*., 2018). The results are compared with the ascorbic acid (Michalska *et al*., 2017) showed that the silver nanoparticles scavenging activity were closed in effectiveness to ascorbic acid scavenging activity (Deveci *et al*., 2018, Hashim *et al*., 2018, Michalska *et al*., 2017).

Very limited literature has been cited on the synthesis of nanparticles using mixed reductants. Our study shows that the *Prunus domestica* gum and *Metha* plant extracts mutually blended silver nanoparticles possessed excellent antimicrobial properties. Islam *et al*. 2017 investigated the three antibacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*) against aqueous extract of gold and silver nanoparticles by using *P. domestica* gum as reducing agent. However, we report five bacterial strains including *Methicillin-resistant Staphylococcus aureus* (MRSA), *Vancomycin-resistant Staphylococcus aureus* (VRSA), *Methicillin-sensitive Staphylococcus aureus* (MSSA), *Protius volgurus* and *Psodomonas*. Torres-Martinez *et al*. 2019 tested antifungal activivty of *Metha* specie extract and showed low antifungal activity, while our results shows significant activities against all five fungal strains *Candida albicans*, *Fusarium oxysporum*, *Penicillium chrysogenum*, *Aspergillus parasiticus* and *Trichoderma harzianum*. While much literatures is available for antioxidant activities of *Prunus domestica* gum and *Metha* species separately, which may be due the presence of phenolics, flavonoids and vitamin C (ascorbic acid) in a considerable amount, (Torres-Martínez *et al*. 2019, Salehi *et al*., 2018, Hashim *et al*., 2018, Islam *et al*. 2017), but in comparison, our mixed reductants silver nanoparticles showed better performance in all antimicrobial and antioxidant tests.

CONCLUSION

In the present study, silver nanoparticles were synthesized using plants biomixed reductants. The antimicrobial assays were carried out using five bacterial strains MRSA, VRSA, MSSA, *Protius volgurus* and

Psodomonas and five fungal strains *Candida albicans*, *Fusarium oxysporum, Penicillium chrysogenum, Aspergillus parasiticus* and *Trichoderma harzianum*. The tested microorganisms were inhibited many folds shows that these nanoparticles exhibit excellent antifungal and antibacterial efficiency. Nano silver synthesized is found to be good scavenger as compared to that of ascorbic acid. The nanoparticles were confirmed by UV-Visible spectroscopy. The XRD analysis confirms face centered cubic structure of silver nanoparticles with crystallite size of about 16 nm. The SEM analyses were performed to study the morphology and particles size. The present work is believed to be helpful in various discoveries of medicine, medical devices, and antioxidant system.

ACKNOWLEDGMENTS

This project was supported by the Department of Chemistry, Islamia College Peshawar. The authors' thanks Center for biotechnology, University of Peshawar for conducting the antimicrobial and antioxidant assay studies.

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