Pure Appl. Biol., 11(2):386-396, June, 2022
http://dx.doi.org/10.19045/bspab.2022.110038

Research Article

Green synthesis of silver nanoparticles and assay of their antibacterial activity

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Citation
Uzma Batool, Nadia Jabbar, Sadaf Shaheen, Muhammad Shahid Rafique and Iqra Arooj. Green synthesis of silver nanoparticles and assay of their antibacterial activity. Pure and Applied Biology. Vol. 11, Issue 2, pp386-396. http://dx.doi.org/10.19045/bspab.2022.110038

Received: 17/04/2021 Revised: 19/06/2021 Accepted: 26/06/2021 Online First: 27/07/2021

Abstract

Nanoparticles have gained considerable attention in recent era among which, silver nanoparticles (AgNPs) have demonstrated exceptional characteristics in medical sciences, particularly. Use of plants for AgNP synthesis represents an eco-friendly, low cost and energy-efficient approach to combat microbial diseases and growing antibiotic resistance. The present study aimed at synthesizing AgNPs using extracts of *Aloe vera* peel as well as *Ranunculus paludosus* and *Matthiola incana* flowers and assessment of their antibacterial potential against clinically isolated multi-drug resistant (MDR) bacteria. The biocidal potential of crude extracts as well as synergistic action of crude extracts and selected antibiotics was determined by Kirby-Bauer disk diffusion method. Results showed that crude extracts alone or with selected antibiotics showed no antibacterial potential against MDR pathogens including *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. AgNPs were synthesized by addition of silver nitrate solution to crude extracts. UV-Visible spectroscopy analysis was done to confirm the synthesis of AgNPs while Fourier transform infrared (FTIR) spectroscopy analysis was performed to depict the functional groups associated with formation of AgNPs. Antimicrobial activity of synthesized AgNPs alone and their synergistic activity with selected antibiotics was then estimated. Results demonstrated the biocidal effectiveness of synthesized AgNPs against MDR pathogens which was further enhanced when used in combination with antibiotics as depicted by wide and clear zones of inhibition. These observations are suggestive of promising antibacterial activity of *Aloe vera* peel as well as flowers of *Ranunculus paludosus* and *Matthiola incana* which may be further validated by extensive research.

Keywords: Antibiotics; Biocidal action; Multidrug resistant pathogens

Introduction

Nanotechnology as an advance emerging field of science, deals in particles with large surface area and small size ranging from 1 to 100nm **[1]**. Several unique aspects are attributable towards the nanoparticles regarding their functionalities and effective structural properties which has made them attractive and apparent in the biological and biomedical sciences as well as various other disciplines of science including physics and chemistry **[2]**. Nanoparticles are categorized into various types depending on their morphological characteristics and physico-

chemical properties, of which size is the main consideration. Based on their nature, they can be polymeric, carbon-based, lipid-based, ceramic or metallic [3]. Among all of these, metallic nanoparticles including those employing zinc, silver, gold, magnesium dioxide, cadmium, titanium, iron oxide etc have been well known for their peculiar properties owing to size and shape [4]. Among all of the metallic nanoparticles reported to date, silver nanoparticles (AgNPs) display excellent properties with regard to physico-chemical as well as biological aspects [5]. These are widely considered as being one of the most capable metallic nanoparticles that have demonstrated good catalytic as well as conductance phenomena which have proven to be especially advantageous in the fields of photochemistry, biomedicine and agriculture [6].

For the synthesis of AgNPs, diverse methodologies involving chemical, physical and biological processes have been employed [7]. Use of expensive, hazardous and noxious materials in physical and chemical methods have made them unreliable for use [1]. Exploitation of naturally occurring living entities (plants, microbes, algae etc) for synthesizing AgNPs has proven helpful to prevail over these problems efficiently. Among these, biologically active units such as plants containing naturally occurring metabolites provide high reducing and capping capabilities upon which working potential and properties of synthesized nanoparticles rely, and so have been the best choice for silver nanofabrication [8, 9].

Various components of medicinally active plants including flowers, rhizome, peel, leaves, etc can be utilized as a source of extraction for silver nanofabrication [10]. Synthesis of AgNPs has previously been reported for numerous plants including, for instance, *Plumeria alba* (frangipani) flower extract, *Azadirachta indica* leaf extract, *Boerhaavia diffusa* whole plant extract as well as peel extracts of *Musa paradisiacal*, *Citrus sinensis* and *Eucalyptus hybrid* [7, 11, 12]. Research has established that *Aloe vera* as a medicinally active plant having multitask attributes regarding health care problems plays a pivotal role as an anti-inflammatory and anti-bacterial agent [13, 14]. *Ranunculus paludosus*, a perennial plant and *Matthiola incana*, an ornamental plant, also potentially possess potent biocidal activity which has never been tested earlier, to the best of our knowledge. Keeping in view the significance of these three plants, present study was carried out for fabrication of AgNPs from peel of *Aloe vera* and fresh flowers of *Ranunculus paludosus* and *Matthiola incana*. Furthermore, characterization and evaluation of their antibacterial potential against clinically isolated MDR pathogens was successfully achieved.

Materials and Methods

Preparation of plant extracts

Fresh *Aloe vera* plants ware collected from Madinah nursery located at Muzaffarabad Sher Shah Road, Multan while fresh flowers of *Ranunculus paludosus* and *Matthiola incana* were collected from Baaghbahara nursery near Northern By-pass, Multan. These samples were taken in sterile plastic bags and washed using double distilled water so as to remove contaminants or debris, if any. Leaves of *Aloe vera* were peeled off carefully to discard the gel portion subsequent to which peel was oven dried and converted to fine dry powder. 5g fine powder was mixed in 100ml of distilled water, ethanol and methanol in separate flasks, respectively. This solution was mixed by using shaking incubator at 37^{o}C and 120 rpm for 24 hours, and filtered later on. Petals of *Ranunculus paludosus* and *Matthiola incana* were dried at room temperature, converted into thin paste and then mixed with distilled water in ratios 1:3, 1:4 and 1:5 in separate flasks, respectively. Solution was then heated

at magnetic stirrer and later on cooled down at room temperature and filtered.

Selection of clinical isolates

Cultures of MDR pathogenic bacteria were obtained from Diagnostic Laboratory of Nishtar Hospital, Multan to evaluate the antimicrobial potential of synthesized AgNPs. These included *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli* and *Staphylococcus aureus* which were used for all sources of nanoparticles, in general. Additionally, antibacterial potential of *Aloe vera* was assessed against *Klebsiella pneumoniae* and that of *Ranunculus paludosus* and *Matthiola incana* was evaluated against *Salmonella enterica* and *Enterobacter* spp., specifically.

Biosynthesis of silver nanoparticles

For synthesizing AgNPs, silver nitrate solution was prepared by addition of 0.0102g of silver nitrate to 12ml of dist. water which was subsequently added to crude extracts of *Aloe vera* peel and flowers of *Ranunculus paludosus* and *Matthiola incana*. Change in color from green to dark brown (*Aloe vera* and *Ranunculus paludosus*) or dark purple (*Matthiola incana*) marked the synthesis of AgNPs which were stored at 2^{o}C until further use.

Characterization of silver nanoparticles

Reduction of Ag^{+} to Ag^{o} was observed by measuring the UV-isible spectrum within the range of 400-800nm at a resolution of 10nm using the UV-Visible spectrophotometer. Crude extracts were used as control. Fourier transform infrared (FTIR) spectroscopy was done to evaluate characteristic phytochemicals in each flower extract. The role of flower extracts as reducing and capping agents as well as presence of vital groups was confirmed by FTIR analysis in transmittance mode, over the range of 500-4000 wave number cm^{-1}.

Antibacterial activity of crude extracts and synthesized silver nanoparticles

For the estimation of antibacterial activity of crude extracts and synthesized AgNPs, Kirby-Bauer method was used. Wells in Müller-Hinton agar plates were filled with 20µg of crude extracts and synthesized nanoparticles of *Aloe vera* peel, and flowers of *Ranunculus paludosus* and *Matthiola incana* separately. Plates were then incubated at 37^{o}C for 24 hours.

Synergistic activity of silver nanoparticles with antibiotics

Synergistic activity of biosynthesized AgNPs was estimated with selected antibiotics including Ampicillin, (AMP, 10µg), Augmentin (AK, 30µg), Azithromycin (AZM, 15µg), Ciprofloxacin (CIP, 5µg), Amikacin (AMC, 30µg), Levofloxacin (LEV, 5µg), Novobiocin (NV, 5µg), Tetracycline (TE, 30µg) and Vancomycin (VA, 5µg). For this purpose, well diffusion method was employed. To each well, 20µl of AgNP solution was added, approximately and selected antibiotic disks were soaked in these wells. Plates were then incubated at 37^{o}C for 24 hours. All analyses were performed in triplicates to avoid false positive or negative results and to minimize human error.

Results

Fabrication of silver nanoparticles

Confirmation of AgNP synthesis from *Aloe vera* peel, as well as *Ranunculus paludosus* and *Matthiola incana* flowers was done by visual analysis separately. When colorless silver nitrate solution was added to greenish crude extract of *Aloe vera*, a brown colored suspension was produced while the light yellowish green crude extract of *Ranunculus paludosus* turned into dark brown suspension. Similarly, *Matthiola incana* floral extract changed from light purple to dark purple indicating the presence of AgNPs (Fig. 1).

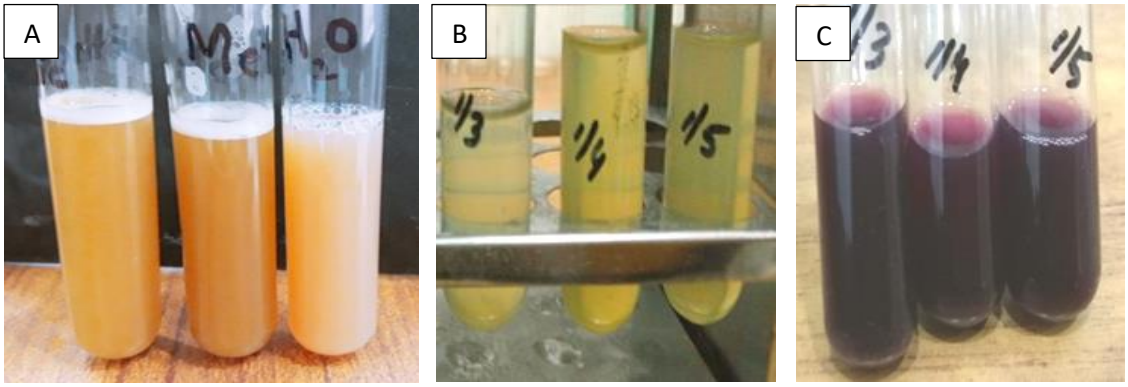

Figure 1. Visual analysis of nanoparticle synthesis for extracts of (A) *Aloe vera* peel (B*) *Ranunculus paludosus* flowers (C) *Matthiola incana* flowers

Spectroscopic analysis of silver nanoparticles

For UV-visible spectroscopy, both the diluted and concentrated solutions were used. Maximum peak was shown by diluted sample and absorbance for both flower extracts was between 1.0 and 1.5 in the range of 400-450nm, confirming the presence of AgNPs (Fig. 2). FTIR bands for *Matthiola incana* were observed in different regions including the most broadened absorbance band at 3442.22 cm^{-1} (Fig. 3). The peak detected at 3442.22 cm^{-1} represented O-H group because of stretching as well as deformation allocated to water adsorption onto the metal surface. Correspondingly, peaks observed at 876.6 cm^{-1}, 1063 cm^{-1}, 1130 cm^{-1}, 1454 cm^{-1} and 1644 cm^{-1}, indicated various functional groups such as thiols, secondary amines and carboxylic groups present in the synthesized nanoparticles. FTIR band for *Ranunculus paludosus* was observed at 3316.59 cm^{-1} that showed O-H group (Fig. 4). The peak observed at 1635.33 cm^{-1} indicated other functional groups.

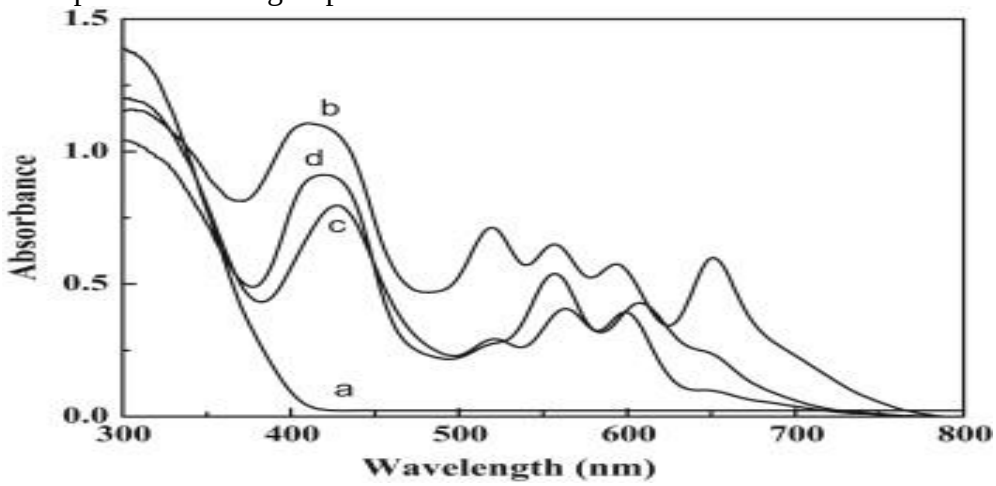

Figure 2. UV-Visible absorption spectrum for silver nanoparticles of (a) concentrated flower extract of *Matthiola incana* (b) diluted flower extract of *Ranunculus paludosus* (c) diluted flower extract of *Matthiola incana* (d) concentrated flower extract of *Ranunculus paludosus.*

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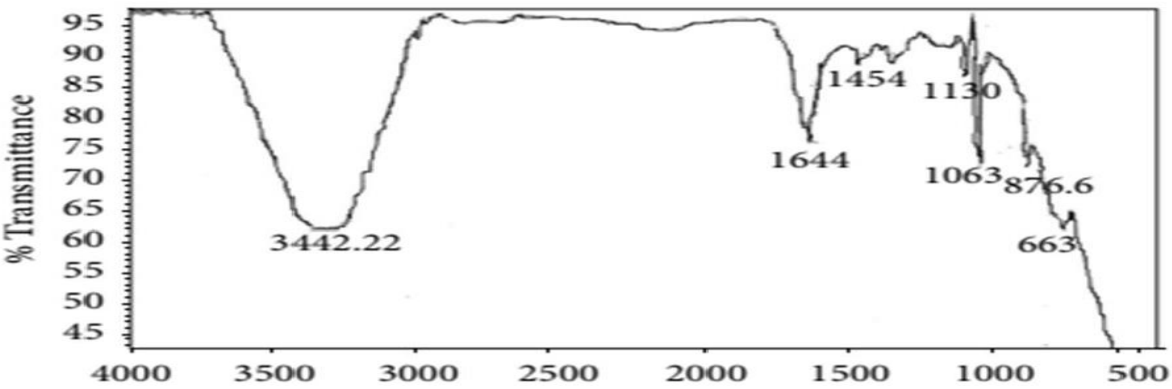

Figure 3. FTIR spectrum recorded for *Matthiola incana* showing the presence of phytochemicals

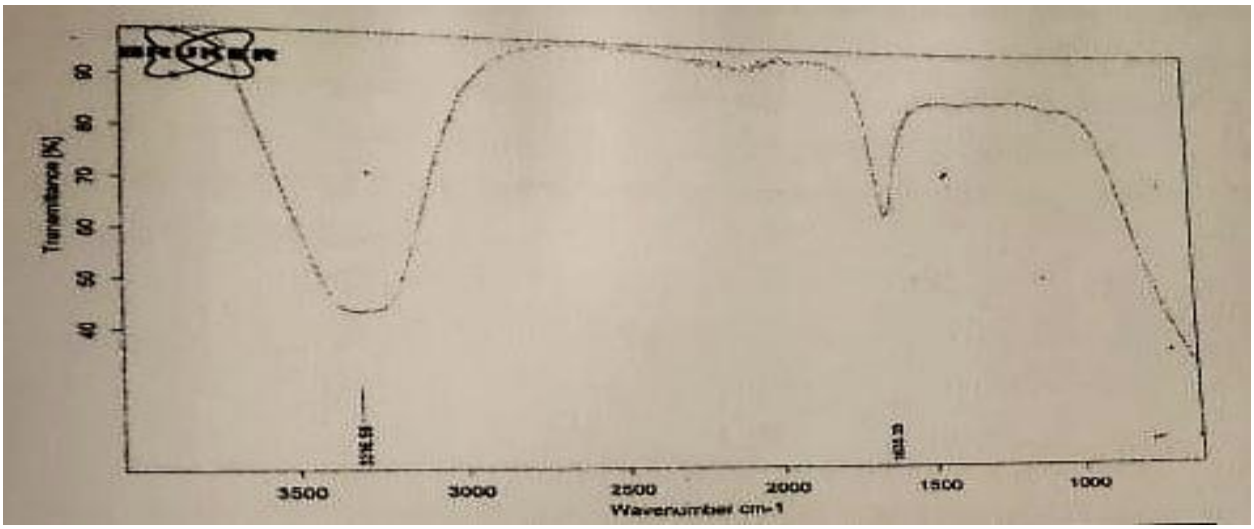

Figure 4. FTIR spectrum recorded for *Ranunculus paludosus* showing the presence of phytochemicals

Antibacterial activity of crude extracts and synthesized silver nanoparticles

After incubation, no zones of inhibition were observed for the crude extracts of either the *Aloe vera* peel or flowers of *Ranunculus paludosus* and *Matthiola incana*. Apparently, this indicated the low biocidal effect of crude extracts against MDR pathogenic bacteria. For *Aloe vera*, aqueous extract containing AgNPs exhibited antibacterial activity against *E. coli*, *Staph. aureus* and *Proteus vulgaris*, methanolic extract against *E. coli*, and ethanolic extract against all selected pathogens (Table 1). For *Ranunculus paludosus* and *Matthiola incana*, the most concentrated extract proved to be the most effective and results are included only for the nanoparticles synthesized from it. As shown in (Table 2), AgNPs synthesized from *Ranunculus paludosus* floral extract were the most effective against *P. aeruginosa* and *Proteus vulgaris* while those produced using *Matthiola incana* floral extract proved to be the most efficacious against *P. aeruginosa* and *Salmonella enterica*.

Table 1. Mean zones of inhibition produced by synthesized silver nanoparticles of *Aloe vera* peel

Test Organism / Extract	No antibiotic (mm)	AMC (mm)	AUG (mm)	TE (mm)	AZM (mm)	AMP (mm)	CIP (mm)	NV (mm)	LEV (mm)	VA (mm)
E. coli										
Aqueous	12	13	13	20	20	13	17	--	--	--
Methanolic	12	--	20	--	--	--	19	16	19	14
Ethanolic	10	---	20	17	17	10	14	--	--	--
Staph. aureus										
Aqueous	12	13	13	20	20	13	17			
Methanolic	--	--	20	--	--	--	10	16	18	10
Ethanolic	8	12	Clear	Clear	Clear	15mm	Clear	--	--	--
P. aeruginosa										
Aqueous	--	13	12	23	15	11	21	--	--	--
Methanolic	--	--	20	--	--	--	19	16	19	14
Ethanolic	10	12	20	16	14	12	15	--	--	--
K. pneumoniae										
Aqueous	--	13	10	23	15	11	21	--	--	--
Methanolic	--	--	20	--	--	--	19	20	19	19
Ethanolic	15	10	24	13	15	10	15	--	--	--
Proteus vulgaris										
Aqueous	12	13	15	20	20	13	17	--	--	--
Methanolic	--	--	14	--	--	--	19	10	15	10
Ethanolic	12	10	24	18	10	10	19	--	--	--

AMC=Amikacin, AUG=Augmentin, AMP=Ampicillin, TE=Tetracycline, AZM=Azithromycin, AMP=Ampicillin, CIP=Ciprofloxacin, NV=Novobiocin, LEV=Levofloxacin, VA=Vancomycin

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Table 2. Mean zones of inhibition produced by synthesized silver nanoparticles of *Matthiola incana* and *Ranunculus paludosus* floral extracts

Test Organism / Extract	No Antibiotic (mm)	AMC (mm)	AUG (mm)	TE (mm)	AZM (mm)	AMP (mm)	CIP (mm)	NV (mm)	LEV (mm)	VA (mm)
E. coli										
Matthiola incana	9.9	24	19	26	24	23	26	22	24	19
Ranunculus paludosus	7.9	22	18	18	19	26	19	18	18	18
Staph. aureus										
Matthiola incana	8.2	16	22	25	12	26	16	16	16	22
Ranunculus paludosus	8.9	17	18	20	20	22	16	17	24	22
P. aeruginosa										
Matthiola incana	10.3	19	16	18	16	24	18	24	22	16
Ranunculus paludosus	10.5	22	18	20	16	24	24	26	22	16
Enterobacter spp.										
Matthiola incana	9.1	22	22	18	18	24	20	22	24	22
Ranunculus paludosus	8.7	22	16	18	16	24	16	20	18	18
Salmonella enterica										
Matthiola incana	10.2	22	24	23	20	26	21	22	16	19
Ranunculus paludosus	10.2	16	20	22	24	26	19	19	18	19
Proteus vulgaris										
Matthiola incana	8.5	22	20	19	18	24	18	19	18	19
Ranunculus paludosus	10.5	24	18	19	19	24	19	18	18	19

AMC=Amikacin, AUG=Augmentin, AMP=Ampicillin, TE=Tetracycline, AZM=Azithromycin, AMP=Ampicillin, CIP=Ciprofloxacin, NV=Novobiocin, LEV=Levofloxacin, VA=Vancomycin

Synergistic activity of silver nanoparticles with antibiotics

In case of *Aloe vera* peel, AgNPs synthesized from ethanolic extract in synergy with antibiotics AUG, TE, AZM and CIP demonstrated the strongest antibacterial activity against *Staph. aureus* by clearing its growth completely (Table 1). Against *E. coli*, aqueous NPs with TE and AZM, as well as methanolic and ethanolic extracts with AUG were the most effective. Ethanolic NPs in combination with AUG produced the best results against *K. pneumoniae* as well as *Proteus vulgaris*. Aqueous NPs with TE demonstrated the highest activity against *P. aeruginosa*. In terms of floral extracts, it was observed that the antibacterial potential of *Ranunculus paludosus and Matthiola incana* was markedly enhanced in the presence of antibiotics (Table 2). Specifically, *Matthiola incana* proved to possess the most robust activity against *E. coli* in combination with TE and CIP and against *Staph. aureus* as well as *Salmonella enterica* in concert with AMP. Likewise, *Ranunculus paludosus* was described as being the most effective against *E. coli* as well as *Salmonella enterica* in combination with AMP, and against *P. aeruginosa* in synergy with NV.

Discussion

Antibiotic overuse and misuse has intensified the proliferation of MDR pathogens which are now considered an emerging global epidemic to combat which, innovative approaches are obligatory. Many metals, such as silver (Ag), copper (Cu), and zinc (Zn), have long been used to combat bacteria even before the use of antibiotics became widespread [15]. Ag salts have been well recognized for their antimicrobial properties since antiquity, and Ag was once thought to be the most popular antibacterial and antifungal agent. AgNPs have recently been shown to have strong antibacterial activity against several pathogens, including *Salmonella*, *Pseudomonas*, and *Staphylococcus* species [16].

The current study aimed at assessing the antibacterial potential of *Aloe vera* peel extract as well as floral extracts of *Ranunculus paludosus and Matthiola incana* to accomplish which, AgNPs were synthesized. The color of crude extract of *Aloe vera* peel changed from light green to dark brown when silver nitrate solution was added to it indicating the reduction of silver nitrate into AgNPs. Previously, Chaudhary *et al* reported color change from green to yellow while synthesizing zinc oxide nanoparticles using *Aloe vera* peel [1]. Upon addition of silver nitrate solution, light yellowish green crude extract of *Ranunculus paludosus* turned into dark brown suspension and that of *Matthiola incana* turned dark purple from light purple. In another report, Rashid *et al* described color change from light yellow to dark brown during the synthesis of AgNPs from *Balantidium ciliate* [17].

To confirm the formation of AgNPs from flower extracts, UV-Visible and FTIR spectroscopy analyses were performed. Due to surface plasmon resonance (SPR), a color change was observed during the formation of AgNPs for a longer reaction time which was the characteristic feature of silver giving maximum absorbance at 425 nm [18]. Through the action of phytochemicals present in plant extracts, formation of AgNPs occurred by the reduction of silver ions into metallic silver (Ag°). Color variation can be attributed to variation in particle size and shape [19]. The more intensified color change with time reflects more formation of AgNPs which validated their synthesis. Higher absorbance was observed in case of diluted sample of *Ranunculus paludosus* which is in accordance with previous studies [20]. SPR absorption band occurs due to presence of free electrons present in metals being the reason for combined vibration of

metallic nanoparticles' electrons in resonance with a light wave [21].

Infrared spectrum comprises two main regions: fingerprint region and functional group region. The organic compounds give absorption spectra usually in the region of functional group while metals generally give absorption band in the fingerprint region [22]. Formerly, FTIR analysis of nanoparticles synthesized from leaf extracts of *Hagenia abyssinica* revealed peaks similar to our observations showing the presence of various functional groups such as –OH, -COOH, and –CN groups of secondary amines [23]. Our results were in accordance with this study as manifested by different peaks indicating the presence of functional groups.

The AgNPs of *Aloe vera* demonstrated the largest inhibition zones against *Staph. aureus* and the smallest inhibition zones against *Proteus vulgaris*. The synthesized AgNPs showed larger zone of inhibition against *Staph. aureus* than gram-negative bacteria possibly due to differences in their cell wall composition. For floral extracts, the highest concentration produced the most remarkable results against all isolates because concentration of extract is a critical determinant of optimal yield of AgNPs due to effect on availability of biomolecules [24]. These observations reinforced that synthesized AgNPs possess excellent antibacterial activity [25]. Antimicrobial activity of synthesized AgNPs from *Aloe fleurentiniorum* extract against *Staph. aureus* and *E. coli* has also been reported previously [26]. On the contrary, decrease in biocidal action of zinc oxide nanoparticles against pathogenic bacteria when combined with antibiotics has been reported due to disruption of antibiotic structural features by zinc oxide nanoparticles [1]. Synergistic activity of AgNPs with antibiotics against pathogens including *Staph. aureus*, *E. coli* and *P. aeruginosa* by means of another plant source (*Murrayakoenigii* spp.) has also been described previously [12]. AgNPs have been reported to possess the potential to arrest the multiplication cycle of many of the microbes [27]. Together, these findings emphasize the fact that the efficacy of antibiotics can be improved enormously by combining them with nanofabricated silver particles from biological sources.

Conclusion

A critical requirement in the discipline of nanotechnology is development of dependable as well as eco-friendly processes to synthesize nanoparticles. Here, antibacterial efficacy of silver nanoparticles synthesized using a simple, biological approach involving reduction of silver nitrate solution has been reported. Biologically synthesized silver nanoparticles possess the potential to be of enormous usage in medical sciences due to their potent antimicrobial potential as evidenced by persuasive antibacterial potential of *Aloe vera*, *Ranunculus paludosus and Matthiola incana*. In the present era of growing antibiotic resistance, this bio-friendly and economical approach seems highly promising necessitating further research in this area.

Authors' contributions

Conceived and designed the experiments: U Batool, N Jabbar & I Arooj, Performed the experiments: U Batool & N Jabbar, Analyzed the data: U Batool, N Jabbar & I Arooj, Contributed materials/ analysis/ tools: MS Rafique & S Shaheen, Wrote the paper: U Batool, N Jabbar, I Arooj & S Shaheen.

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