Abstract
Green synthesis of metallic particles has grasped the attention of researchers due to its non-toxicity and low cost. In the present work, silver nanoparticles synthesized by using Ocimum, aloe vera, ficus and papaya leaves extract through a seed-mediated growth approach. Each of the seed solutions was prepared by reducing silver nitrate using leaf extract. In addition, growth solution of larger molarity was prepared by reducing silver nitrate by bio reducing agent. Nanoparticles were grown to a reasonable size by adding a small amount of seed solution to the growth solution. The anti-bacterial activity of the prepared nanoparticles was analyzed by Agar well method for gram-positive bacteria (Staphylococcus aureus). The nanoparticles were also characterized by UV-visible spectroscopy and Fourier transform-Infrared (FTIR) spectroscopic analysis. Among all the bio-reducing agents, the, overall, strongest activity was shown by the Aloe vera leaves extract. The UV-Visible spectroscopy showed the absorptions in the range of 220-430 cm corresponding to the plasmon absorbance of the AgNPs. FTIR spectroscopic analysis confirmed the presence of possible proteins acting as reducing and stabilizing agents for nanoparticles.

Introduction
Nanotechnology deals with the materials having a size range from 1 to 100 nm. Various kinds of nanoparticles including gold, silver, platinum and palladium have been synthesized by physical, biological and chemical methods in the past. The chemical technique is the most common but the employ of poisonous chemicals during nanoparticles’ synthesis produces highly toxic by-products [1]. Another method to synthesized nanoparticles is seed-mediated growth approach. This method was introduced in 2001 [2]. It is very easy and cheap method and it is also solution based, so no need to maintain pH [3]. In green synthesis, there is the use of biological materials like plants for synthesis of silver nanoparticles (AgNPs) offers numerous benefits of eco-friendliness, low toxicity, cost-effective and easily scaled up for large-scale synthesis [4]. Silver nanoparticles have unique properties [5]. The silver nanoparticles exhibit excellent bacterial action against both gram-positive and gram-negative bacteria. The antimicrobial activity of AgNPs is due to the formation of pores in the cell wall of bacteria that leads to the leakage of cellular content. The AgNPs shows an effective antifungal activity against the fungal species. The cytoprotective properties of silver are well known and are used for the prevention of HIV interaction to the host cell. It is also used to prevent infection after surgery and acting as anti-HIV agents. The AgNPs inhibits the binding of the virus to host cells [6].

Silver nanoparticles (AgNPs) are widely used in medical devices which include wound dressing, catheters and bone cement [7]. AgNPs are used in topical ointments as well as creams that are used to prevent wounds and infection of burns [1]. The AgNPs can be employed for purification in water filtering apparatus which may be due to its enhanced antimicrobial nature [8]. The different shapes of silver nanoparticles are incorporated in biosensors for...
sensing different interactions [9].

In the present research work, very first-time silver nanoparticles (AgNPs) were green synthesized by using a seed-mediated growth approach. For green synthesis, we used four different leaves named as Ocimum sanctum, aloe vera, ficus and papaya to get extracts which were used as a reducing agent (natural/bio-reducing agent) as well as a stabilizer for nanoparticles.

**Method and materials**

The silver nitrate AgNO₃ solution was used as a precursor, the leaf extracts from Ocimum (tulsi), Aloe vera, Ficus (fig) and Papaya were used as reducing agent, which also served as stabilizing agent.

**Preparation of extracts**

**Preparation of Ocimum extract**

Twenty grams of fresh Ocimum leaves collected from the botanical garden of Lahore College For Women University (LCWU) and Washed thoroughly with distilled water three times to remove dust particles. The leaves were chopped using mortar and pestle. The finely chopped Ocimum leaves added into 250 ml conical flask. 100 ml distilled water added into the conical flask and placed it on a magnetic stirrer for 1 hour at 60 oC. After boiling, the mixture cooled and filtered using Whatman no. 1 filter paper. The extract of brownish green colour was obtained which was covered with aluminium foil and stored at 4oC for further use [10].

**Preparation of Aloe vera extract**

Twenty g fresh leaves of Aloe vera collected from botanical garden of LCWU. The leaves washed thoroughly with distilled water. By using mortar and pestle, the Aloe vera leaves chopped into fine particles and 100 ml of distilled water added in it. Then the mixture was heated at 80°C for 20 minutes on a magnetic stirrer at 500 rpm. Finally, the extract of yellowish green colour was obtained which was filtered by using Whatman No. 1 filter paper and stored at 4°C for further study [11].

**Preparation of Ficus extract**

Twenty-five grams fresh Ficus leaves collected from botanical garden of LCWU and washed three times with distilled water. The leaves were sun-dried for 4 days followed by the cutting of dried leaves into minute pieces and mixed with 100 ml distilled water in a 250 ml conical flask. The mixture was boiled at 80°C for 5 minutes on a magnetic stirrer at 500 rpm. After that the extract of brownish green colour was obtained which was filtered by Whatman No 1 filter paper and stored at 4°C for further study [12].

**Preparation of Papaya extract**

Twenty-five grams fresh Papaya leaves collected from botanical garden of LCWU and washed thoroughly with distilled water three times. The leaves chopped into tiny pieces and added into 250 ml conical flask. Then 200 ml of distilled water added to the flask. The conical flask placed in the oven at 60°C for 10 minutes followed by the removal of half of the leaves from the flask and crushed into a fine paste by using mortar and pestle. Then this paste shifted into a 250 ml conical flask. 200 ml of distilled water added into a paste containing conical flask. The mixture-containig flask then heated at 60°C for 10 minutes. Then the flask sealed with aluminium foil and placed in the incubator at 37 oC for 30 minutes. In the end, an extract of green colour was filtered by using Whatman No. 1 filter paper and stored at 4°C for further study [13].

**Preparation of seed solution**

One millimolar solution of AgNO₃ prepared by adding 0.0084 g of AgNO₃ in 50 ml of distilled water. A magnetic stirrer used to boil the solution at 80°C for 40 minutes by adjusting it at 500 rpm. Ten ml of already prepared different leaves extract added dropwise into the AgNO₃ solution. The colour of the solution started to change from colourless to different colours depending upon the extract. The colour change showed the synthesis of silver nanoparticles. After the change of colour, the solution removed from magnetic stirrer. Hence the four seed solutions were prepared namely So, Sa, Sf and Sp corresponding to the extract of Ocimum, Aloe vera, Ficus and Papaya respectively [3].

**Preparation of growth solution**

A five-millimolar solution of AgNO₃ prepared by adding 0.0425g of AgNO₃ in 50 ml distilled water. Twelve millilitre of already prepared extracts added into the 0.15 ml of AgNO₃ solution. Then this mixture placed on the magnetic stirrer for heating at 50 oC for 25 minutes along with mixing at 500 rpm. After that, 6 ml of already prepared seed solution added. The light colour changed to dark in the growth solution and four growth solutions were prepared namely Go, Ga, Gf and Gp corresponding to So, Sa, Sf, Sp respectively [3].

**Characterization**

The prepared silver nanoparticles characterized by UV-Visible Spectroscopy and Fourier Transformation Infrared Spectroscopy to investigate the optical and structural properties.

**UV-Visible spectroscopy**

The optical property of silver nanoparticles was determined
by UV-Vis spectroscopy using UV-1800 Shimatsu Double Beam Spectrophotometer. It refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and ultraviolet ranges. The absorption or reflectance in the visible range directly affects the perceived colour of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. The instrument designed so that it can make a comparison of the intensities of the two beams as it scans over the desired region of the wavelengths. In UV-Vis spectrophotometer, a beam of light split into two beams before it reaches the sample. One beam used as a reference and other beam passes through the sample. If the compound absorbs light at a particular wavelength, the intensity of the sample beam (IS) will be less than that of the reference beam.

From the Beer-Lambert’s law, the absorbance (A) of nanoparticles can be calculated as:

$$A = \log\left(\frac{I_0}{I}\right) = \varepsilon l c$$

where $I$ is sample light intensity, $I_0$ is reference light intensity, $\varepsilon$ is material dielectric constant, $l$ is length of optical light path and $c$ is precursor concentration [14].

Fourier Transform Infrared (FTIR) Spectroscopy

The Fourier transform infrared spectroscopy is one of the powerful tools for identification of compounds by matching spectrum of an unknown compound with reference spectrum like fingerprinting. It is used for the identification of functional groups in unknown substances. There are three basic components: a radiation source, interferometer and detector. The IR radiations from a broadband source are first directed to the interferometer, where it is divided and then recombined to generate constructive and destructive interference. The resulting beam passes through the sample compartment and reaches the detector. When infrared light is passed through a sample of the compound, some frequencies are absorbed, while other frequencies are transmitted without being absorbed. The transitions involved in the infrared absorption are associated with vibrational changes in the molecule. Different bonds groups have different vibrational frequencies and hence the presence of these bonds in a molecule can be detected by identifying this characteristic frequency as an absorption band in the infrared spectrum [15].

Antibacterial activity assessment by agar well method

Agar well diffusion method used to assess the antibacterial activity of silver nanoparticles. Twenty-five milliliters of nutrient agar poured on the petri plates and allowed to solidify. Then petri plates containing agar medium was streaked by a sterile cotton swab having Staphylococcus aureus. After that, agar material punched up to 6mm size to pour 100µL sample with a micropipette to check inhibition zone. These plates incubated for 24 h at 37 oC. After 24 hours the inhibition zone appeared which measured with a scale [16].

Statistical analysis

Recorded data subjected to ANOVA (analysis of variance) by employing SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) followed by Tukey’s HSD test was used to determine significant differences between treatments at P<0.05.

Results and discussion

UV-visible spectroscopy

The absorbance of seed solutions of AgNPs is placed along with that of its respective growth solution in each figure of UV-visible spectroscopy for the comparison. The studied UV-visible spectra and their results are discussed below.

Silver nanoparticles by Ocimum

The addition of Ocimum leaf extract to the silver nitrate solution resulted in a color change of solution from transparent to the brownish-green colour due to the production of silver nanoparticles. The colour changes were due to the surface plasmon resonance phenomena. Previous results also cleared that Silver and other metal nanoparticles have surplus (free) electrons that cause surface plasmon resonance (SPR) when treated with light. The SPR is because of the collective vibration of nanoparticles’ electron with lightwave [10]. The absorbance spectra of silver nanoparticles synthesized by Ocimum leaf extract along with a comparison of So and Go is given in fig. 1. The highest absorbance was recorded at 352 cm and 427 cm for seed and growth solution respectively. These results were also supported by the previous study carried out by Medda et al. (2015) [11]. Shifting of peak to the higher wavelength showed the formation of more and higher silver nanoparticles. The expansion of the absorbance peak represented that the particles are polydispersed in nature. The similar type of results was also documented by the Atta, Al-Lo-hedan, and Ezzat (2014) [17]. Expansion of the absorbance peak indicated the synthesis of polydispersed AgNPs [12].
Silver nanoparticles by Aloe vera
The silver nanoparticles showed the yellowish-green colour in the aqueous solution due to the surface plasmon resonance phenomena. Medda et al. (2015) [11] reported that silver nanoparticles synthesized from Aloe vera were of reddish-brown colour. The change of colour of newly synthesized AgNPs was because of SPR phenomena [18]. The different absorbance spectra of Sa and Ga is illustrated in fig. 2. The silver nanoparticles prepared by using Aloe vera showed a maximum absorbance spectrum at 221 cm and 229 cm for seed solution and growth solution respectively. This showed that the silver nanoparticles are small sized. The synthesis of AgNPs is illustrated by the absorbance of light in UV-visible spectrum [11]. The size of nanoparticles is very important to inhibit bacterial activity and the size also play role in the determination of inhibition path [10].

Silver nanoparticles by Ficus
The UV-visible spectra of both seed and growth solutions of AgNPs prepared using green Ficus leaf extract as a reducing agent are shown in fig. 3. The silver nanoparticles by Ficus showed the maximum absorbance peak at 360 cm and 389 cm for seed solution and growth solution respectively. The peak is shifted to a higher wavelength implying the formation of more and larger silver nanoparticles. Baia and Simon (2007) [19] reported a similar type of results. Shankar et al. (2004) [20] described that the more synthesis of AgNPs when leaf extracts of different plants used. The maximum wavelength during the reaction did not shift. It is feasible to quantitatively observe the concentration of AgNPs.

Silver nanoparticles by Papaya
The nanoparticles’ characterization was performed by an efficient technique called UV-visible technique [21]. The silver nanoparticles are formed when papaya leaf extract was added to the silver nitrate solution. The colour changed from transparent to brownish-red colour due to the surface plasmon resonance (SPR) phenomena. Praba et al. (2015) reported that the colour of silver nanoparticles was changed from yellowish to brown in an aqueous medium. The research work of Banala, Nagati, and Karnati (2015) narrated that the absorbance spectra during UV-visible spectrometric analysis showed the formation of AgNPs. The UV-visible absorbance spectrum of silver nanoparticles synthesized from Papaya leaf extract along with the comparison of Sp and Gp is given in fig. 4. The peaks were observed at 421 cm and 415 cm wavelength. This indicated that the peak shifted towards the shorter wavelength.
wavelength due to the formation of further small sized silver nanoparticles. Banala, Nagati, and Karnati (2015) also mentioned the variation in the size of silver nanoparticles synthesized from Papaya.

**Fourier transform-Infrared (FTIR) Spectroscopy**

**Ocimum leaf extract**

FTIR measurement was performed to check the biomolecules for efficient stabilization and capping of the silver nanoparticles produced by the leaf extract of Ocimum. The observed FTIR spectrum of AgNPs is mentioned in Fig. 5. Spectra of AgNPs synthesized from Ocimum showed 668, 1635 and 3354 cm⁻¹ transmission peaks. The sharp transmission peak at 3354 cm⁻¹ is assigned to phenolic compounds and OH-containing alcohols. The transmission peak at 1635 cm⁻¹ is assigned to the C-N bond of the amine group. The sharp transmission peak at 668 cm⁻¹ is showing the C-N stretching of esters and alcohols. This IR spectroscopic study has proved that the carbonyl group of protein and amino acid have high ability to bind metal which leads to stabilization of medium and these results are also supported by the research work of Mallikarjuna et al. (2011) [10]. These results also confirm the presence of possible proteins which act as stabilizing and reducing agents and Sathyavathi et al. (2010) also reported the somehow similar findings [22].

**Aloe vera leaf extract**

The outcomes of FTIR analysis performed to check the stabilization potential of AgNPs synthesized from leaves extract of Aloe vera is presented in Fig. 6. It illustrates the IR transmission spectrum of green synthesized AgNPs and performed to check the possible link between AgNPs and protein. Transmission Spectrum of AgNPs showed peaks at 668, 1635 and 3363 cm⁻¹. The transmission peak at 1635 cm⁻¹ shows the presence of primary amines. The transmission peak at 3363 cm⁻¹ and 668 cm⁻¹ indicates alcoholic, phenolic and nitriles group in AgNPs. The above results are also supported by the study of Medda et al. (2015) [11]. The graph of FTIR analysis demonstrates a sharp transmission peak at 3363 cm⁻¹. The above results also explain the fact that metals are strongly adsorbed to C=O of proteins showing that the proteins have the ability to develop a layer with the organic molecules, protecting interaction with green synthesized AgNPs [23]. The FTIR spectroscopic analysis proved that C=O of amino acid has a strong ability to bind different metals indicating the development of a layer which covers the metal’s nanoparticles. These results prove the occurrence of different proteins acting as stabilizing and reducing agents [24].
The FTIR spectroscopic analysis of silver nanoparticles synthesized from leaf extract of Ficus is shown in the fig. 7. FTIR Spectrum of AgNPs demonstrated the transmission peaks at 659, 1643, and 3354 cm⁻¹. The transmission peak at 3354 cm⁻¹ indicates the carbonyl (C=O) group as well as OH group of alcohols and phenolics. The sharp transmission peak at 1643 cm⁻¹ corresponds to the N-H bond present in protein and Praba et al. (2015) also demonstrated the somehow similar results [12]. The transmission peak at 659 cm⁻¹ could be due to the presence of ethers and esters [22]. FTIR spectroscopic analysis proved the reduction of silver cations to AgNPs is because of the reducing agents present in the plant extract [12].

### Papaya leaves extract
The FTIR spectroscopic analysis of AgNPs synthesized from Carica papaya leaf extract is shown in the fig. 8. FTIR spectrum of silver nanoparticles showed the transmission peaks at 668, 1635 and 3329 cm⁻¹. The FTIR analysis of AgNPs synthesized from Papaya leaves extract showed two sharp transmission peaks at 668 cm⁻¹ and 3329 cm⁻¹ representing the interaction between silver nanoparticles and proteins [25]. The transmission peak at 3329 cm⁻¹ is because of the presence of C=O or N-H groups. The transmission peak at 1635 cm⁻¹ is due to the amide (C=N) or carboxyl group [26]. The transmission peak at 677 cm⁻¹ could be because of the ether and ester. The components present in the Papaya leaf extract react with metal salts through the above listed functional groups to start to reduce these salts into nanoparticles [27, 28].

### Antibacterial activity
The antibacterial activity of the prepared silver nanoparticles checked for both of its seed and growth solution against gram-positive *Staphylococcus aureus* by Agar Well method. The resulted inhibition zones observed, measured and compared for all seed and growth solutions of AgNPs prepared from four different leaf extracts. The inhibition zone is the area on agar medium where the growth of the bacteria ceased. The areas are obvious in fig. 9, 10 and 11.
It was observed that AgNPs synthesized from different plant leaf extracts had different antibacterial potential. The inhibition zones of all the samples are given in table 1. Among the seed solutions, papaya seed solution (Sp) had the highest potential to inhibit the bacterial growth, so it showed the biggest inhibition zone and Ocimum had the smallest inhibition zone because of its least tendency to stop bacterial growth. But when these samples of AgNPs were grown through the Seed-Mediated Growth approach by adding a seed solution to respective growth solution, the antibacterial activity of each sample changed. The antibacterial activity of the growth solutions of AgNPs synthesized from the aloe vera (Ga) and Ocimum (Go) was enhanced than their respective seed solution giving inhibition zones of 25mm and 21mm respectively. Whereas in the case of papaya, the growth solution Gp gave the reduced size of the zone i.e. 13mm as compared to 19mm in seed solution. The reason of enhanced antibacterial effect in Go was due to the increase in the concentration of components [29] and in case of Ga, the reason is that the aloe vera itself has the antibacterial property and it showed the large inhibition zone [30]. Whereas reduced antibacterial effect in Gp is probably due to the decrease in the size of silver nanoparticles [31]. On the other hand, in the case of ficus, both seed and growth solutions of AgNPs showed the same inhibition zone. This also shows that the experimental conditions have not affected the size of nanoparticles in growth solution in case of ficus.

Table 1

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<th>Samples</th>
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Antibacterial potential of synthesized silver nanoparticles from four different leaves extracts against gram-positive *S. aureus*

Conclusion

In this research work, we first focused on the biosynthesis of silver nanoparticles by using aloe vera, ficus, Ocimum and papaya leaves extract through a seed-mediated growth approach. We used this method very first time for the green synthesis of AgNPs because it is a simple, cheap and environment-friendly. The synthesized AgNPs were characterized by UV-visible spectroscopy and Fourier Transformation Infrared (FTIR) spectroscopy to study their optical as well as structural properties. The antibacterial potential of the green synthesized silver nanoparticles were tested for both of its seed and growth solutions against the gram-positive *Staphylococcus aureus* by Agar Well method. The results deduced from our research contributed to the virgin and unique area of nanotechnology as a substitute for bactericidal.

Conflict of interest

The authors of this manuscript declare that they have no conflict of interest.

References


