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Biogenic synthesis of silver nanoparticles from aqueous flower extract of *Bougainvillea spectabilis* and their antibacterial activity

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Abstract
In this study, silver nanoparticles were biologically synthesized by using aqueous flower extract of *Bougainvillea spectabilis* wild. Synthesis and formation of silver nanoparticles were confirmed by colour change from pink colour to brown colour and it was further characterized by ultraviolet (UV) visible spectroscopy at the range of 300 to 800 nm. The peak showed at 431 nm. Further morphology, shape and size of the synthesized nanoparticles were characterized by field emission electron microscopy and the presence of metal silver was analysed by energy dispersive x-ray spectroscopy. Reducing and capping agents for synthesized silver nanoparticles were studied by Fourier transform infrared spectroscopy. The *in vitro* antibacterial activity of silver nanoparticles was tested against both Gram-positive and Gram-negative bacterial strains. Result showed that synthesized nanoparticles have potential and high antibacterial activity against Gram-positive bacterial strains compared to Gram negative bacterial strains.

Keywords: *Bougainvillea spectabilis*, silver nanoparticles, antibacterial activity FESEM, FTIR

Introduction
Nanoparticles are fundamental building blocks of nanotechnology. Nowadays, metal nanoparticles have been subject of focused research due to their magnetic, electronic, optical, mechanical and chemical properties that are notably different from those mass resources [1, 2]. Effectively studied nanoparticles today are those made from metals, especially gold, silver, copper, and zinc nanoparticles [3, 4]. Among these four, silver nanoparticles has attracted recognition due to their different properties and application like pharmaceutical industries [5], DNA sequencing [6], antimicrobial activities [7], textile industries [8] etc.,
Different methods were used for synthesis of silver nanoparticles such as evaporation, condensation, phase transfer process, microwave treatment, laser ablation, electrochemical synthesis, plant-mediated and microorganisms [9-11]. Among these methods, plant-mediated synthesis provides a natural capping and reducing agents for conversion of silver ions to silver nanoparticles as well as free from toxic chemicals and also reduce the cost and time of nanoparticles synthesis [12].
The objective of this study was to synthesize silver nanoparticles from aqueous flower extract of *Bougainvillea spectabilis* wild and which was tested against eleven bacterial strains such as *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Enterococcus faecalis*, *Corynebacterium diphtheriae*, *Streptococcus pneumoniae*, *Klebsiella pneumonia*, *Escherichia coli*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa* to study its antibacterial activity.

Materials and Methods
Preparation of aqueous flower extract
Flowers from *B. spectabilis* were collected from Maruthamalai hills, Coimbatore, Tamilnadu, India. Freshly collected flowers were cleaned up with tap water. Ten grams (10 g) of the flower were mixed with 200 ml double distilled water in a 500 ml conical flask and boiled for 20 minutes to facilitate the formation of aqueous flower extract. Obtained aqueous extract was filtered with Whatman No.1 filter paper and stored for further use.

Synthesis of silver nanoparticles
Biogenic synthesis of silver nanoparticles was carried out in a 500 ml conical flask containing

180 ml of 1 mM $AgNO_3$ and 20 ml of flower extract (9:1) and then it was kept at dark room. After some period of incubation, the colour of the mixture solution changed from pink colour to brown colour. This colour change indicates the formation of silver nanoparticle.

Characterization of NPs
An ultraviolet - Vis spectroscopy (JASCO UV Vis NIR V-67) was used to conduct optical measurement. UV-Vis spectroscopy was operated in the range from 300 to 800 nm. The size and shape of the synthesized nanoparticles were analysed by field emission scanning electron microscopy (ICON Quanta) operated at an accelerating 10 Kv. Field emission scanning electron microscope (FESEM) samples were prepared on carbon coated copper grid by just dropping a very small amount of nanoparticle into the grid. The presence of metals were analysed by energy dispersive x-ray spectroscopy.
The presence of functional and composition of silver nanoparticles were characterized by Fourier transform infrared spectroscopy (FTIR, Brucker) in the range of 500 to 3500 cm^{-1}.

***In vitro* antibacterial activity**
The *in vitro* antibacterial activity was assayed by standard Kirby- Bauer well diffusion method [13]. against 11 bacterial strains such as *Salmonella typhi, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Enterococcus faecalis, Corynebacterium diphtheria, Streptococcus pneumonia, Klebsiella pneumonia, Escherichia coli, Enterobacter aerogenes, a*nd Pseudomonas aeruginosa. Both Gram-positive and Gram negative bacterial strains were swabbed on Muller Hinton agar (MHA) plates using cotton swabs. Four wells were made on 5 mm in diameter in MHA agar plates with the help of gel puncture, which was impregnated with different concentration (10, 20, 30 and 40 µl) of synthesized silver nanoparticles and then bacterial strains swabbed plates were incubated at 37 °C for 24 h.

Results and Discussion
Synthesis of silver nanoparticles
After the exploration of plant extract into the 1 mM silver nitrate, the colour change was observed from pink colour to brown colour (Figure 1). The appearance of brown colour in the reaction flask suggested the synthesis of silver nanoparticles [14].

Characterization of NPs
Concentration of the presence of metal nanoparticles were analysed by using UV-Vis spectral analysis. UV visible absorption spectrum was noted at 431 nm (Figure 2) and a broadening of the peak indicated that the particles were polydispersed. Similar phenomenon was reported by Nisha *et al*., 2012 [15].
Field emission scanning electron microscope has been used to analyze the size, shape and morphology of synthesized silver nanoparticles from aqueous flower extract of *Bougainvillea spectabilis*. FESEM images showed the presence of nanoparticles which are like spherical with size range from approximately 16 to 83 nm and it showed poor dispersion (Figure 3). The presence of metal silver was confirmed by energy dispersive x-ray spectroscopy. Silver nanocrystallites exhibit an optical absorption band peak at 3 KeV (Figure 4). It also recorded the presence of other elements such as sulfur, oxygen, and sodium. This indicated the presence of reducing and capping agents.
Capping and reducing agents for biosynthesis of silver nanoparticles form aqueous flower extract of B. spectabilis were analysed by using a FTIR. Obviously, infrared bands are observed at 554.77, 596.28, 639.48, 1637.27, 2113.48 and 3336.28 cm^{-1} (Figure 5). This bands represent the presence of alkyl halide, alkynes function groups, C-H stretches, C-O stretching aromatics, and C=C-H stretching compounds [16]. Analysis of these spectra strongly suggested the presence of flavonoids and phenols, which were mainly responsible for the formation of silver nanoparticles by reducing silver nitrate.

***In vitro* antibacterial activity**
Antibacterial activity of silver nanoparticles was examined against bacterial strains by using standard zone of inhibition. The synthesized silver nanoparticles showed inhibition zone against, *B. cereus*, *C. diphtheria*, *S. pneumonia*, *E. coli*, *Salmonella typhi*, *B. subtilis*, *E. aerogenes*, *K. pneumonia*, *S. aureus*, *P. aeruginosa*, and *E. faecalis* (Figure 6). Different concentrations (10, 20, 30 and 40 µl) of synthesized silver nanoparticle were used for the study of *in vitro* antibacterial activity. The highest antibacterial zone of inhibition was recorded in *B. subtilis* followed by *B. cereus*, *S. pneumonia*, *S. aureus*, *E. faecalis*, *C. diphtheria*, *E. coli*, *S. typhi*, *E. aerogenes*, *K. pneumonia*, and *P. aeruginosa*. Measurement of the inhibitory concentration is as shown in Figure 7. It was reported that ethanol, methanol, chloroform and ethylacetate extract of *Bougainvillea spectabilis* leaves showed highest antibacterial activity against gram negative bacterial strains compared to positive bacterial strains (Umamaheswari *et al.*, 2008) [17]. Ethanol and methanol flower extract of *Bougainvillea spectabilis* showed excellent antibacterial activity against *salmonella typhi* compared to other (acetone and aqueous) extract (Sharif *et al.*, 2013) [18].

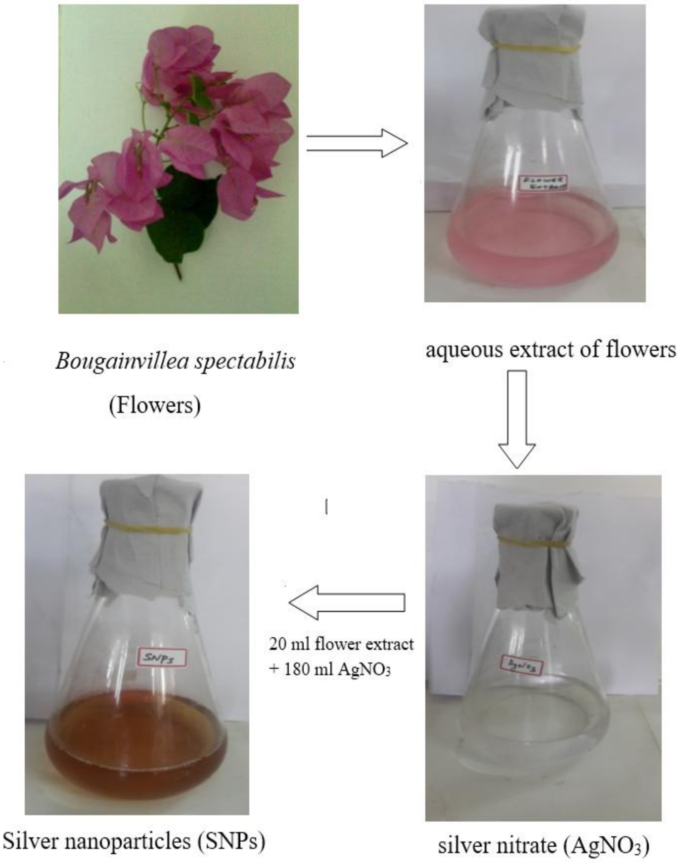

Fig 1: Biogenic synthesis of silver nanoparticles from aqueous flower extract *Bougainvillea spectabilis*.

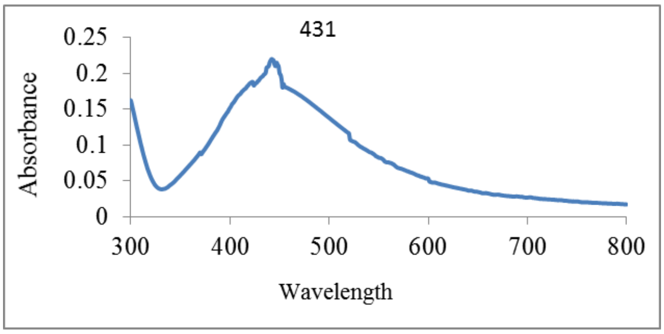

Fig 2: UV-Vis spectroscopic recording of synthesized silver nanoparticles and the peak noted at 431nm.

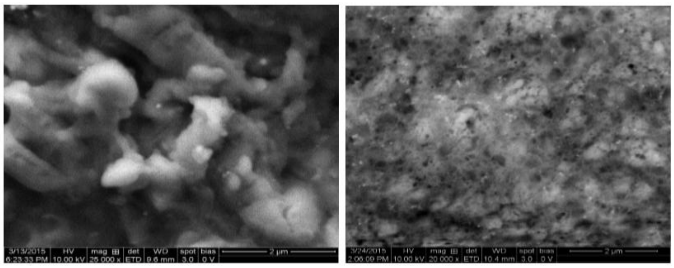

Fig 3: FESEM images of synthesized silver nanoparticles

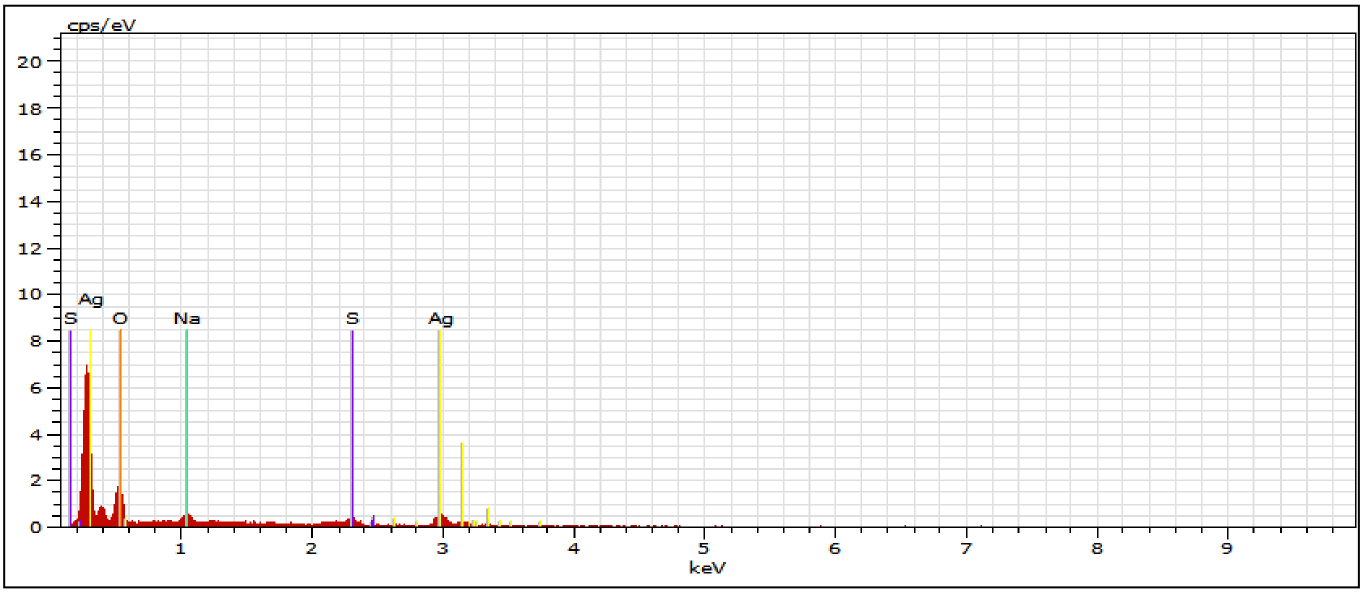

Fig 4: Elemental analysis of synthesized silver nanoparticles by energy dispersive x-ray spectroscopy

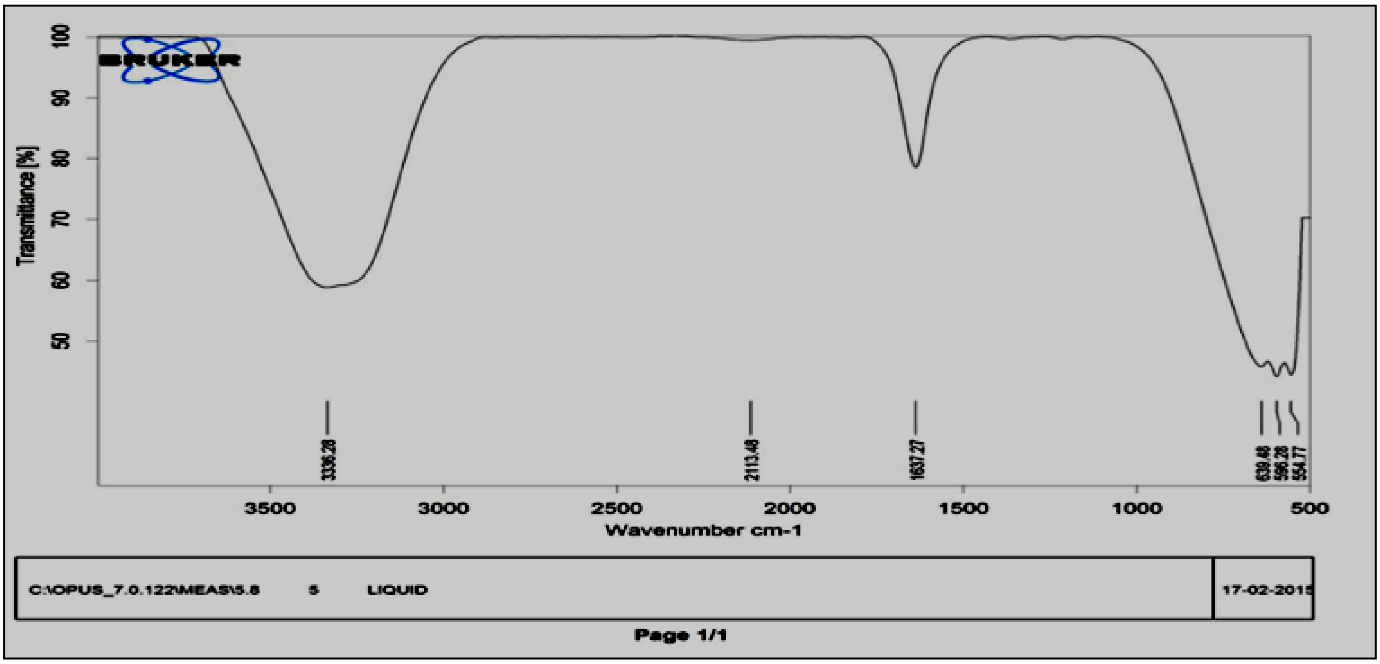

Fig 5: Fourier transform infrared spectral analysis of synthesized silver nanoparticles.

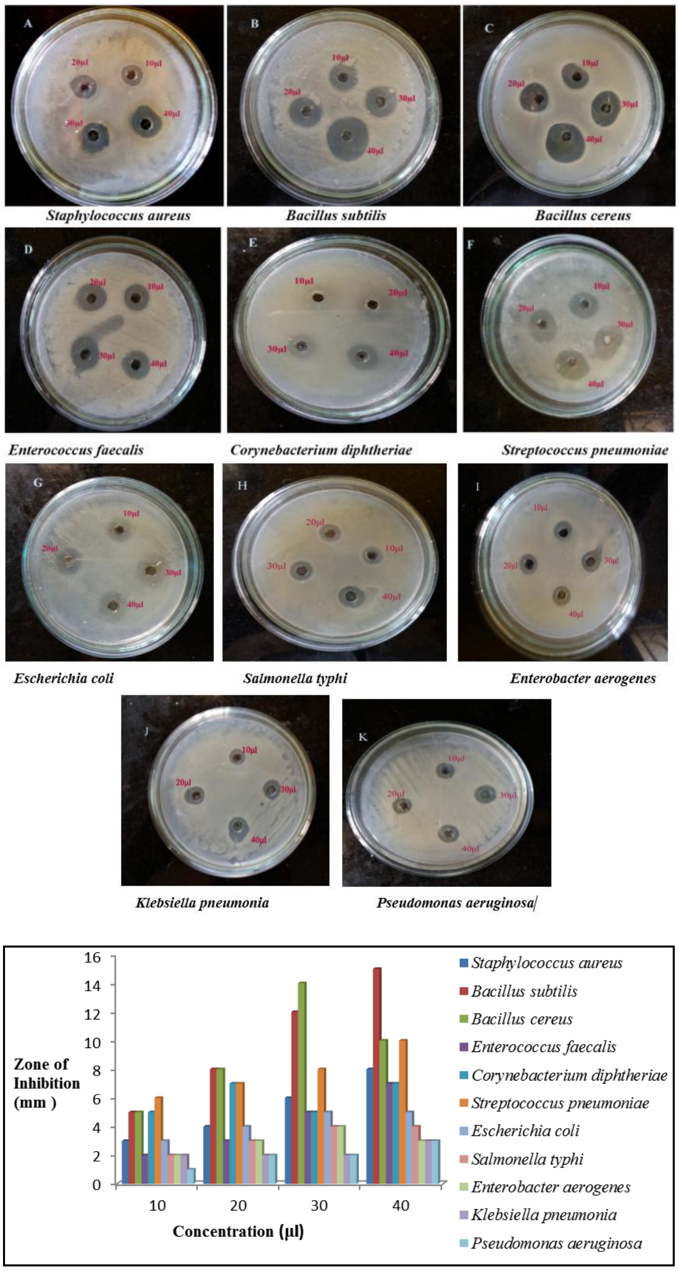

Fig 6: *In vitro*-antibacterial activity of silver nanoparticles from flower extracts of *Bougainvillea spectabilis*

Conclusion

In this study, a simple, green and efficient route to synthesize silver nanoparticles was developed by treating Ag ions with aqueous flower extract of *Bougainvillea spectabilis* at room temperature without using any harmful agents. Flower extract act as a reducing, capping and stabilizing agent for converting silver ions to silver nanoparticles. The synthesized silver nanoparticles are spherical with sizes in the ranges from 16 to 83 nm. Additionally, the antibacterial activity of the silver nanoparticles was measured by Kirby-Bauer method. The result of this study clearly demonstrates that the synthesized AgNPs has high antibacterial activity against Gram-positive bacterial strains compared to Gram-negative bacterial strains.

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References

1. Sengottaiyan A, Aravinthan A, Sudhakar C, Selvam K, Srinivasan P, Govarthanan M *et al*. Synthesis and characterization of *Solanum nigrum*- mediated silver nanoparticles and its protective effect on alloxan-induced diabetic rats. J Nanostruct Chem. 2016; 6:41-48.
2. Anarkali J, Vijayaraj D, Rajathi K, Sridhar S. Biological synthesis of silver nanoparticles by using *Mollugo nudicaulis* extract and their antibacterial activity. Arch. Appl. Sci. Res. 2012; 4(3):1436-1441.
3. Awwad A, Albiss B, Ahmad AL. Green synthesis, characterization and optical properties of zinc nanoparticles using *Olea europaea* leaf extract. Adv. Mater. Lett. 2014; 5(9):520-524.
4. Sonradu, Yuet YL, Boung WC, Mitsuaki N. Synthesis of silver nanoparticles by using tea leaf extract from *Camellia sinensis*. Int. J Nanomed. 2012; 7:4263-4267.
5. Rajathi K, Sridhar S. Green synthesized silver nanoparticles from the medicinal plant *Wrightia tinctoria* and its antimicrobial potential. Int. J. Chem Tech Res. 2013; 5(4):170-1806.
6. Wu J, Tan LH, Hwang K, Xing H, Wu P, Li W *et al*. DNA sequencing dependent morphological evolution of silver nanoparticles and their optical and hybridization properties. J Am. Chem. Soc. 2014; 136(43):195-202.
7. Balu AC, Richa singh, priyanka wagh, sweety wadhwni, Sharvari G, Avinash K *et al*. Synthesis, optimization and characterization of silver nanoparticles from *Acinetobacter calcoaceticus* and their enhanced antibacterial activity when combined with antibiotics. Int J Nanomed. 2013, 8:4277-4290.
8. Mohanapriya C, Srividhya M, Akilan AK, Kathirvelan K, Subitha AP, Sundaramahalingam MA. Bioreduction based synthesis of silver nanocoats and their application in development of nanoembedded medicalfabrics. Int J Pharm Rev Res. 2014; 27(2):210-215.
9. Kalishwaralal K. Extracellular biosynthesis of silver nanoparticles by the culture suspension of *Bacillus licheniforms*. Mater. Lett. 2008; 62(29):4411-4413.
10. Rout Y, Behera S, Ojha AK, Nayak PL. Green synthesis of silver nanoparticles using *Ocimum sanctum* and study of their antibacterial and antifungal activities. J Microbiol Antimicro. 2012; 4(6):103-109.
11. Nishibuchi, Mitsuaki, chieng, Mitsuaki N, Yuetying L. Synthesis of silver nanoparticles by using tea leaf extract from *Camellia sinensis*. Int J Nanomed. 2012; 7:4263-4267.
12. Anitha kp, ashely berry, labethani may, Paul BT. Genotoxicity of silver nanoparticles in vicia faba: A pilot study on the environmental monitoring of nanoparticles. Int j environ Res Public Health. 2012; 9:1649-1662.
13. Kyoung KH, Arokiyaraj S, Arasu MV, Vincent S, Prakash NU, Choi SH *et al*. Rapid green synthesis of silver nanoparticles from *Chrysanthemum indicum L* and its antibacterial and cytotoxic effects: an *in vitro* study. Int J Nanomed. 2014; 9(1):379-388.
14. Savithramma N, Rao ML, Rukmini K, Devi PS. Antimicrobial activity of silver nanoparticles synthesized by using medicinal plants. Int J Chem Tech Res. 2011. 3(3):1394-1402.
15. Nisha P, Abhijeet SD, malik CP. Biogenic synthesis of silver nanoparticles, using *Bougainvillea spectabilis* Wild bract extract. National academy science letters. 2012; 35(5):383-388.
16. Rafiq HS, Mujeeb K, Merajuddlin K, Syed FA, Muhammad NT, Wolfgang T *et al*. Green synthesis of silver nanoparticles mediated by *Pulicaria glutinosa*

extract. Int J Nanomed. 2008; 8:1507-1516.
17. Umamaheswari A, Shreevidya R, Aparna nuni. *In vitro* antibacterial activity of *Bougainvillea spectabilis* leaves extract. Adv. Bio. Res. 2008; 2(1-2):01-05.
18. Sharif N, Farza na R, Ijaz A, saima S, Fakhar UN, shagufta N. phytochemical analysis and inhibitory activity of ornamental plants. A. J Plant sci Res. 2013; 3(2):1-5.