



Synthesis of silver nanoparticles using aqueous extract of *Impatiens balsamina* leaves and analysis of antimicrobial activity

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Abstract

Green synthesis of metallic nanoparticles has gained significant attention in the field of nanomedicine as an environment-friendly and cost-effective alternative in comparison with other physical and chemical methods. Several metals such as silver, gold, iron, titanium, zinc, magnesium and copper have been subjected to nanoformulation for a wide range of useful applications. Silver nanoparticles (AgNPs) are playing a major role in the field of nanomedicine and nanotechnology. They are widely used in diagnostics, therapeutic and pharmaceutical industries. AgNPs have been synthesized using silver nitrate and leaf extract of *Impatiens balsamina* that belongs to the Balsaminaceae family. Synthesized nanoparticles were characterized by UV-Vis spectroscopy, FT-IR, TEM and SAED. Furthermore, the antimicrobial effect of the plant extract and the nanoparticles were evaluated against *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pyogenes* which was assessed by well diffusion method. The average size of AgNPs synthesized, was 20 nm with the spherical shape. *Rhazya stricta* based nanoparticles exhibited improved antimicrobial activity against bacterial and fungal strains.

Keywords: *Impatiens balsamina*, silver nanoparticle synthesis, characterization, antimicrobial activity

Introduction

The broad spectrum of nanotechnology is important in the major fields of biology, chemistry, physics, and material sciences. Nanotechnology deals with the study of materials at the nanometers (Elumalai *et al.*, 2010; Singh *et al.*, 2009) [9, 45]. The day to day development of nanotechnology creates a major interest in the development and fabrications of different dimensioned nanoparticles (Marambio-Jones and Hoek, 2010) [25]. The nanomaterials can be synthesized by different methods including chemical, physical, irradiation, and biological methods. The development of new chemical or physical methods has resulted in environmental contaminations, since the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous byproducts (Zhang *et al.*, 2008) [50]. Thus, there is a need for "green nanotechnology" that includes a clean, safe, ecofriendly, and environmentally nontoxic method of nanoparticle synthesis, and in this method there is no need to use high pressure, energy, temperature, and toxic chemicals (Jeong *et al.*, 2005; Savithramma *et al.*, 2011) [18, 37]. The biological methods include synthesis of nanomaterials from the extracts of plant, bacterial, fungal species, and so forth. The synthesis of nanoparticles from the plant extracts is considered to be a process (Saxena *et al.*, 2010) [30]. The preparation and maintenance of fungal and bacterial cultures are time consuming and require aseptic conditions and large manual skills to maintain the cultures (Schultz *et al.*, 2000) [39].

Plant extracts include bark, root, leaves, fruit, flowers, rhizoids, and latex and are used to synthesize the nanoparticles. These nanoparticles show different dimensions including the size, shape, and dispersion which have more efficacy than those synthesized from the

chemical and physical procedures. Therefore, the use of green plants for similar nanoparticle biosynthesis methodologies is an exciting possibility which has compatibility for pharmaceutical and other biomedical applications, as they do not use toxic chemicals for the synthesis of nanoparticles (Vijayaraghavan and Nalini, 2010; Crooks *et al.*, 2001) [47, 7].

Nanoparticles had a wide variety of application in the major fields of medicine, electronics, therapeutics, and diagnostic agents. Silver nanoparticles have wide application in biomedical science like treatment of burned patients, antimicrobial activity and used the targeted drug delivery, and so forth (Singh *et al.*, 2010) [44]. Nowadays the nanoparticles are coated on the medical appliances, food covering sheets, and cans for storing the beverages and food (Sharma *et al.*, 2009; Prasad *et al.*, 2011; Ram *et al.*, 2012) [42, 31, 33]. However, there are many problems and toxicity of using metal oxide nanoparticles on the human health. Use of plants for the synthesis of nanoparticles does not require high energy, temperatures, and it is easily scaled up for large scale synthesis, and it is cost effective too (Ghosh *et al.*, 2012; Vankar and Shukla, 2012; Mukunthan *et al.*, 2011) [10, 46, 26].

Impatiens balsamina is belonging to the family, Balsaminaceae and commonly known as Balsam plant in English, Pokok Keembung in Malay, Kasi Thumbai in Tamil and Tou Gu Cao in Chinese. It has cathartic, diuretic, emetic, anti-haemorrhoidal and antibacterial properties (Basanti, 2011) [5]. Moreover, the leaves are crushed and used as a poultice on wounds and skin inflammations. They are combined with salt and castor oil to make a poultice that is packed around a finger affected by whitlow: the whitlow disappears in a short time. The previous study revealed that the significant analgesic and anti-inflammatory effects of

water extract of *Impatiens balsamina* leaves (Debashree *et al.*, 2013) [8]. The extracts of *I. balsamina* also showed a long lasting skin moisturizing effect and prevent dryness, rough skin chap, dandruff and splitting hair ends, hence are used to prepare lotions, creams, hair tonics, cosmetics, bath preparations and detergents (Jin *et al.*, 2011) [19]. Different parts of the plant are used to treat disease and skin afflictions: the leaves, seeds and stems are also edible if cooked. *Impatiens balsamina* has been used as indigenous medicine in Asia for the treatment of rheumatism, fractures and fingernail inflammation (Anonymous, 1959) [2]. The *I. balsamina* has traditionally been used for the treatment of thorn or glass-puncture wounds, abscesses, in grown nails and chronic ulcers caused by allergic reaction of detergents. The groups of compounds commonly found in this plant are naphthoquinones, coumarins, phenolic acids, flavonoids, anthocyanidins and steroids (Park *et al.*, 2003) [28]. Modern chemical and pharmacological studies have identified flavonols and naphthoquinone derivatives, some of which have strong antimicrobial, anti-anaphylaxis, anti-inflammatory as well as itch alleviating and anti-dermatitis activities, as the main chemical components of this plant (Archana and Bratati, 2014) [4]. The seed extract in various solvents have been found to possess promising antibacterial (Abhijeet *et al.*, 2014) [1] and antifungal activities (Binorkar and Jain 2012) [44]. Reported pharmacological activities of *Impatiens balsamina* such as antibacterial and antifungal activities (John *et al.*, 2013; Kang *et al.*, 2013; Sakunphueak *et al.*, 2012; Rajendra *et al.*, 2014; Yang *et al.*, 2001) [20, 22, 35, 32, 49], anti-androgen effect (Ishiguro *et al.*, 2000), antioxidant activity (Archana and Bratati, 2014) [4], anthelmintic activity (Wang *et al.*, 2009) [48], anti-inflammatory, analgesic and antidiabetic activity (Imam *et al.*, 2012; Shivaji *et al.*, 2013) [12, 42], anti-anaphylactic activity (Ishiguro *et al.*, 2002; Oku and Ishiguro, 1999; Ishiguro and Fukumoto, 1997) [16, 27, 13], antipruritic (Ishiguro *et al.*, 1998) [14], anti dermatitic activity (Jalalpure *et al.*, 2007) [17], anti tumor activity (Herrera *et al.*, 2013; Pei *et al.*, 2012) [11, 29]. The present investigation was carried out to synthesis, characterize the silver nanoparticles and to test the antimicrobial efficacy of the leaf extract of *Impatiens balsamina* against some clinically isolated pathogenic bacterial strains.

Materials and Methods

Source of chemicals and reagents

Silver nitrate were purchased from Sigma Aldrich (USA). All the reagents utilized for the analytical grade (AR Grade).

Collection of plant materials

The leaves of *Impatiens balsamina* were collected from Thanjavur, Tamil Nadu, India. The collected leaves were washed several times with distilled water to remove the traces of impurities from the leaves. Healthy flowers were spread out in a plain paper and shade dried at room temperature for about 10 days and makes a fine powder using grinder mixture. The powder materials were used for further studies.

Preparation of extract

Fresh leaves of *Impatiens balsamina* were thoroughly washed using tap water and rinsed with distilled water. The leaves were dried for 5 min in an oven at 28°C for one week

and then pulverized to a fine powder with the aid of a Starlite blender (Model SL-999). The aqueous extract was prepared by weighing out (20 gm) of the milled powdered leaves of *Impatiens balsamina* were soaked in 100 ml of cold distilled water in a conical flask and stirring vigorously with a glass rod for proper extraction. The combination was allowed to settle for 24 hours at room temperature. The extracts were then filtered using Whatman no.1 filter paper (125 mm × 100 circles) and used for further experiments.

Synthesis of silver nanoparticles

For synthesis of silver nanoparticles (AgNPs), the Erlenmeyer flask containing 100ml of AgNO₃ (1mM) was reacted with 12 ml of the aqueous extract of *Impatiens balsamina* leaves. This setup was incubated in dark (to minimize the photo activation of silver nitrate), at 37°C under static condition. A control setup was also maintained without *Impatiens balsamina* leaves extract. The colour changes from pale yellow colour to dark brown colour.

Characterization of silver nanoparticles

Ultraviolet–Visible (UV-Vis) spectroscopy, FT-IR analysis, Transmission electron microscope (TEM) and selected area electron diffraction pattern (SAED) analysis.

Antimicrobial assay by disc diffusion method

All things considered, dispersion technique was utilized to watch the measurements subordinate antibacterial action of AgNPs on *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, and *S.pyogene* species. Bacterial inoculums were set up by growing a solitary province overnight in supplement stock and modifying the turbidity to 0.5 McFarland norms. Organism species were developed in Potato dextrose soup for 72 h and utilized for the investigation. Mueller–Hinton agar (MHA) plates were vaccinated with this bacterial and parasitic suspension and different volumes of AgNPs in 5, 10, 15, 20 and 25 g/mL were added to a middle well with a width of 8 mm. These plates were brooded at 37 °C for 24 h and room temperature for the contagious plates. The zone of inhibition (ZOI) was measured by subtracting the good diameter from the total inhibition zone diameter.

Results and Discussion

Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ion when exposed to herbal extracts was reduced in solution, there by leading to the formation of silver hydrosol. Silver nanoparticles with their unique chemical and physical properties are proving to be an alternative for the development of new antimicrobial agents. Silver nanoparticles have also found diverse application in the form of wound dressings, coating for medical devices and silver nanoparticle impregnated textile fabrics, etc. (Begum *et al.*, 2009) [6]. Leaf extract with silver nitrate solution is brown colour and after one hour, the colour changes (Figure 1). This conform that silver nitrate was reduced and transformed into silver nanoparticles.



Fig 1: Visual observation of flowers extract and silver nitrate solution and Biosynthesized silver nanoparticles

UV-Visible spectroscopy Analysis

The reduction of Ag^+ ions in solution was monitored by aqueous component and measuring the UV-spectra of the solution. Figure 2 shows that the absorption spectra of silver nanoparticles formed in the action media has an absorbance peak at 448.35 nm. UV-Vis spectroscopy was used to determine the formation and the stability of the synthesized silver nanoparticles in aqueous colloidal solution. The bio matrix present in the stem bark extract of *Impatiens balsamina* may leads to the change in the absorbance and transmittance of UV- Vis spectra. The bio-reduction of silver nanoparticles is extracellular and promises the vast development of green synthesis of metabolic nanoparticles using the plant bark sources.

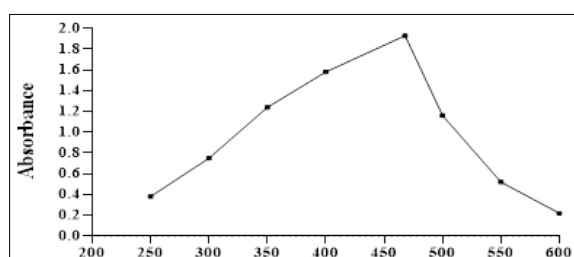


Fig 2: UV peak values of silver nanoparticles synthesized from aqueous extract *Impatiens balsamina*

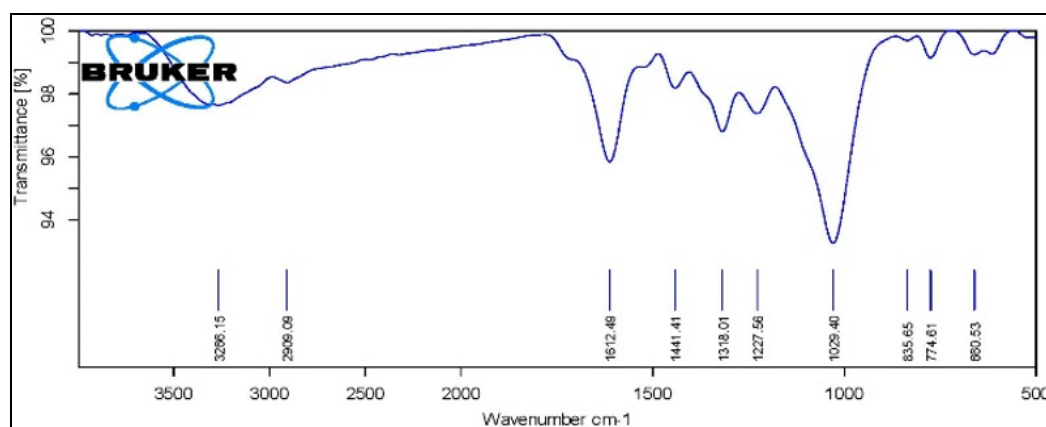


Fig 3: FTIR peak values of silver nanoparticles synthesized from aqueous extract of *Impatiens balsamina*

Peak present at 1224.19, indicate C-O-C Vibration of dialkyl. The peak at 112.40, indicate C-Hstretching vibration of alcohol. The peak present at 773.60, indicate vibration of C=C1 alkayhalides. From the FTIR spectra of *Impatiens balsamina* bark extract, change in wave number if the functional groups was observed due to the reduction and stabilization of metal group Prasad and Elumalai, (2011)^[26]. When the spectrum of *Impatiens balsamina* bark extract was compared with the spectrum of samples, the functional groups of alkanes and carboxyli acids and were observed

Fourier transforms infrared spectroscopy (FI-TR) Analysis

FTIR analysis was used for the characterization of silver nanoparticles. FTIR absorption spectra of synthesis of silver nanoparticles are shown in Table 1 and Figure 3. Possible functional group involved in the synthesis and stabilization of silver NPs was studied by FTIR spectroscopy. The FTIR was recorded in the range of 500-400 cm^{-1}

The functional group – alcohols, phenols, alkenes, alkanes, carbonyls, aromatics, nitro compounds, alkyl halides and aliphatic amine were identified from FTIR spectrum recorded from the bark extract of *Impatiens balsamina*. The spectrum showed the bands for the functionl group located at 3266.15, 2909.09, 1612.49, 1441.41, 1318.01, 1227.56, 1029.40, 835.65, 774.61 and 660.53 cm^{-1} . The peak present at 1612.49, indicate C=C stretching alkene. The peak present at 1029.40, indicate C=O vibration of ketone. The peak present at 1384.48, indicate CH3 bend stretching vibration of alkene.

Table 1: FTIR peak value of silver nanoparticles synthesized from aqueous extract of *Impatiens balsamina*

S.No.	Group of frequency cm^{-1} of the sample	Functional group
1	3266.66 cm^{-1}	Unknown
2	2909.09 cm^{-1}	Unknown
3	1612.49 cm^{-1}	OH –Amide
4	1441.41 cm^{-1}	C-N
5	1318.01 cm^{-1}	Diketones
6	1227.56 cm^{-1}	CH-CH2
7	1029.40 cm^{-1}	Alkyketone,
8	835.65 cm^{-1}	Alkyl amide
9	774.61 cm^{-1}	Aromatic
10	660.65 cm^{-1}	C-Br strong
11	555.35 cm^{-1}	C-ClHalogen

from the spectrum of sample due to the stretching of alkynes and alkanes (H-C=O), and carboxlic acids (-C=O-) bands.

Transmission Electron Microscope (TEM) Analysis

The TEM image showing AgNPs synthesis using plant extract confirmed the development of silver nanostructure by *impatiens balsamina*. The silver nanoparticles used in this study were having the mean diameter of 20 nm as show by TEM micrographs.

Figure 4 shows the absence of surface protecting ligands and it is spherical in shape. The particles were crystalline in

nature as revealed by the high magnification TEM image of nanoparticles the lattice of silver was clearly seen.

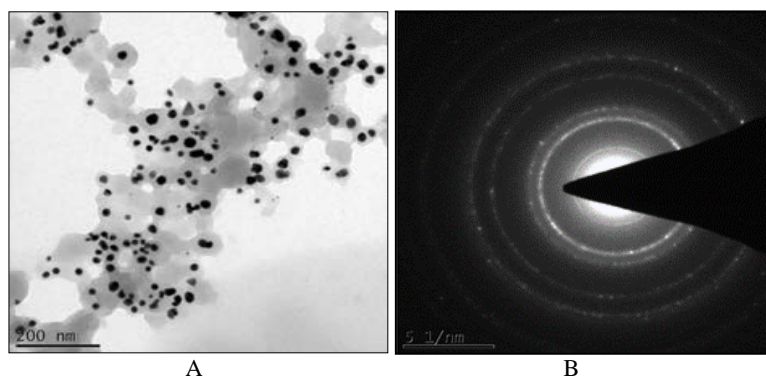


Fig 4 A: TEM analysis of AgNPs, B: SAED pattern images of AgNPs

Antimicrobial activity by Disc Diffusion Method

The effect of different concentration silver nanoparticles (100µg/ml, against different bacterial and fungus species were reported in case of *Impatiens balsamina* (Table 2 and Figure 5). The zone of inhibition was found to be in the bacteria $13.1 \pm$ to 11.6 ± 0.5 and fungi 9.5 ± 1.3 mm.

Maximum antibacterial activity was shown by AgNPs extract of *Impatiens balsamina* with inhibition zone minimum of 13.1 ± 0.2 mm (at AgNPs concentration of 100 µg/ml) against *K. pneumoniae*. The lowest activity shown in *S. aureus* 11.6 ± 0.5 Moreover, AgNPs have shown more antimicrobial activity as well as standard drug.

Table 2: Antimicrobial activity of synthesized AgNPs against different bacterial and fungal strain

Sample	pathogens	Nanoparticles (100 µg)	Ampicillin (10µg)	Miconazole (30 µg)
Zone of inhibition (mm)				
<i>Impatiens balsamina</i>	<i>K.pneumoniae</i>	13.1+0.2	25.1+1	-
	<i>E.coli</i>	09.5+1.3	-	7.8+07
	<i>S.aureus</i>	11.6+0.5	13.5+0.5	-
	<i>S.pyogenes</i>	-	-	7.5=0.8

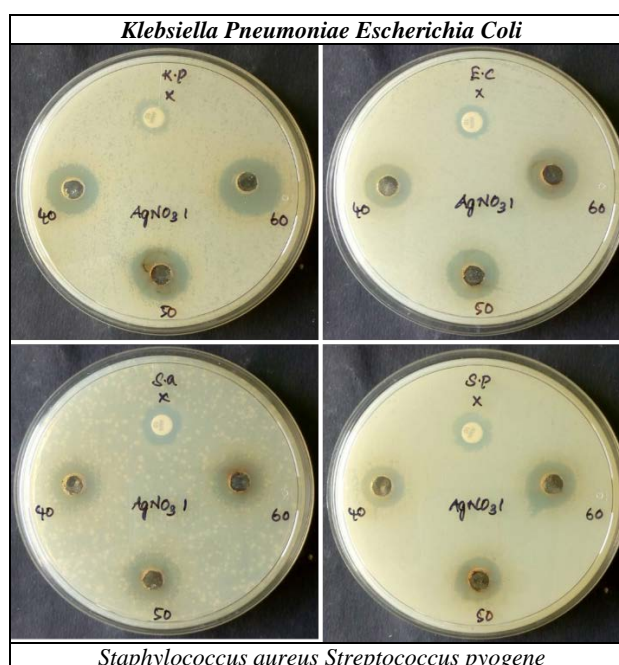


Fig 5: Antimicrobial activity of synthesized AgNPS against different bacterial and fungal strain

The different species of bacteria show zone of inhibition in the well diffusion method of antimicrobial activity. The different patterns of the zone of inhibitions are observed in Table 2 and Figure 5. Synthesized silver nanoparticles showed antibacterial activity against both Gram positive and negative bacteria. The highest zone of inhibition was

observed for *K.pneumoniae* even at lower concentration. The exact mechanism of the inhibition of the bacteria is still unknown, but some hypothetical mechanisms show that the inhibition is due to ionic binding of the silver nanoparticles on the surface of the bacteria which creates a great intensity of the proton motive force, and the one hypothesis from the

research states that the silver nanoparticles invade the bacterial cell and bind to the vital enzymes containing thiol groups (Sharma *et al.*, 2009; Ramteke *et al.*, 2013; Kavita *et al.*, 2011) [42, 34, 23]. Also, the findings of Sereemasun *et al.* (2008) [40] and Linga and Savithramma, (2012) [24] suggested the inhibition of oxidation-based biological process by penetration of metallic nanosized particles across the microsomal membrane. The molecular basis for the biosynthesis of these silver crystals speculated that the organic matrix contains silver binding properties that provide amino acid moieties that serve as the nucleation sites (Prabhu *et al.*, 2010; Savithramma *et al.*, 2011) [37].

Conclusion

The biological synthesis of the silver nanoparticles is rapid, ecofriendly, cost-effective, and simple method of synthesis. In the present study-silver nanoparticles are synthesized at room temperature within a less span of time. The synthesized silver nanoparticles were characterized by UV-visible spectrometer, FT-IR, TEM and SAED analysis. The size of the nanoparticles ranges from 15-20 nm with spherical shape. AFM and SEM reveal that the synthesized silver nanoparticles are well dispersed showing no agglomeration. These nanoparticles showed a broad spectrum antimicrobial activity against both Gram positive and Gram negative bacteria. Investigation on the antimicrobial activity of synthesized silver nanoparticles using *Impatiens balsamina* extract against *K.pneumoniae*, *E.coli*, *S.aureus* and *S.pyogene* reveals high potential as antimicrobial agent in pharmaceutical, food, and cosmetic industries.

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