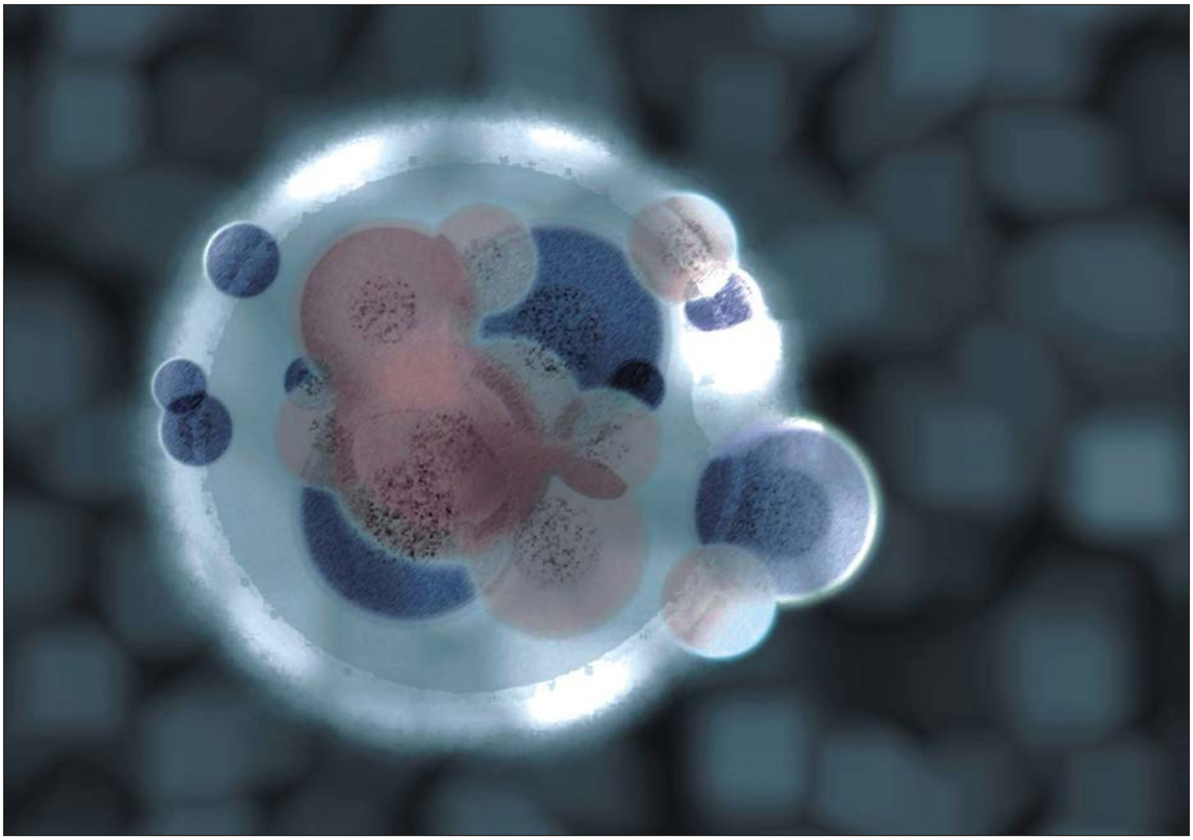

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Evaluation of Antibacterial Efficacy of Biologically Synthesized Silver Nanoparticles using Stem Barks of *Boswellia ovalifoliolata* Bal. and Henry and *Shorea tumbuggaia* Roxb.

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Abstract: The synthesis of metal nanoparticles using biological systems is an expanding research area due to the potential applications in nanomedicines. Nanoparticles synthesized by chemical method are not eco-friendly. The biological synthesis of silver nanoparticles is cost effective and eco-friendly to that of conventional method of nanoparticles synthesis. In the present study the silver nanoparticles synthesizes rapidly by using the stem barks of endemic medicinal plants *Boswellia ovalifoliolata* and *Shorea tumbuggaia.* After assessing the formation of silver nanoparticles with the help of UV-Visible spectroscopy and were characterized by using EDAX and SEM. Diversity has been observed in size and shape of the silver nanoparticles synthesized in two plants. These phytosynthesized silver nanoparticles were tested for their antibacterial activity. The test cultures of *Proteus, Pseudomonas, Klebsiella, Bacillus* and *E. coli* species were used. The bacterial property of silver nanoparticles was analyzed by measuring the inhibitory zone. These silver nanoparticles synthesized from bark extract of *Boswellia ovalifoliolata* showed moderately toxic to the *E. coli* and *Pseudomonas* species and highly toxic to *Proteus* species. Phytosynthesized silver nanoparticles of *Shorea tumbuggaia* bark extract were moderately toxic to *E. coli* and *Bacillus* species and highly toxic to *Klebsiella* species. The important outcome of the study will be the development of value added products from medicinal plants of India for biomedical and nanotechnology based industries.

Key words: Medicinal plants, silver nanoparticles, Scanning Electron Microscopy (SEM), inhibition zone, phytosynthesis

INTRODUCTION

One of the fields in which nanotechnology finds extensive applications is nanomedicine, an emerging new field which is an outcome of fusion of nanotechnology and medicine. Medicine is no more physician job exclusively; the materials and devices designed at the level of nanoscale are for diagnosis, treatment, preventing diseases and traumatic injury, relieving pain and also in the overall preservation and improvement of health (Naik and Selukar, 2009). Nanotechnology can improve our understanding of living cells and of molecular level interactions. A number of nanoparticles based therapeutics have been approved clinically for infections, vaccines and renal diseases (Malhotra, 2010). Oligodynamic silver having antimicrobial efficacy extends well beyond its virotoxicity and it have lethal effects spanned across all microbial domains (Prabhu *et al*., 2010). The application of silver nanoparticles in drug delivery, drug discovery and new drug therapies have declare war on many dead full diseases and they use the body natural transport pathway and natural mechanism of uptake of the drug by the diseased cells (Balaji, 2010).

Silver nanoparticles are widely used for its unique properties in catalysis, chemical sensing, biosensing, photonics, electronic and pharmaceuticals (Sarkar *et al*., 2010) and in biomedicine especially for antibacterial agent (Rai *et al*., 2009) and antiviral agent (Elechiguerra *et al.*, 2005). Silver nanoparticles have a great potential for use in biological including antimicrobial activity (Sap-Iam *et al*., 2010). Antimicrobial capability of silver nanoparticles allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances and in medical devices (Marambio-Jones and Hoek, 2010). Silver is an effective antimicrobial agent exhibits low toxicity (Farooqui *et al*., 2010). The antibacterial activity of silver species has been well known since ancient times (Shrivastava *et al*., 2007). The most important application of silver and silver nanoparticles is in medical industry

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such as tropical ointments to prevent infection against burn and open wounds (Ip *et al*., 2006). Biologic synthesis of nanoparticles by plant extracts is at present under exploitation as some researchers worked on it (Calvo *et al*., 2006; Bhyan *et al*., 2007) and testing for antimicrobial activities (Saxena *et al*., 2010; Khandelwal *et al*., 2010).

Boswellia ovalifoliolata Bal. and Henry is a narrow endemic, endangered and medicinal tree species. It is deciduous medium sized tree belongs to the family Burseraceae. This tree harbours on Tirumala hills of Seshachalam hill range of Eastern Ghats of India. Tribals like Nakkala, Sugali and Chenchu use the plant and indigenous community to treat number of aliments (Savithramma and Sulochana, 1998). *Shorea tumbuggaia* is an endemic endangered, red-listed and semi-evergreen tree species belongs to the family Dipterocarpaceae, restricted to the Southern Eastern Ghats in Andhra Pradesh and Tamil Nadu. The tree trunk used as flag poles for temples. The stem is a source of resin which is used as incense and as substitute in marine yards for pitch. The plant is used to cure different diseases by indigenous tribal people and local villages (Savithramma and Sudarshanamma, 2006). The present study was an attempt to test the antibacterial efficacy of silver nanoparticles produced by using the stem bark extracts of endemic medicinal plants which have been using in traditional medicine without validation.

MATERIALS AND METHODS

Biogenisis of silver nanoparticles: The stem barks of *Boswellia ovalifoliolata* and *Shorea tumbuggaia* plant species were collected in the year 2009 from the Tirumala hills of Andhra Pradesh, India and were air dried for 10 days then the barks were kept in the hot air oven at 60°C for 24 to 48 h. The dried barks were ground to a fine powder. One mM silver nitrate was added to the plant extracts separately to make up a final solution of 200 mL and centrifuged at 18,000 rpm for 25 min. The collected pellets were stored at 4°C. The supernatants were heated at 50 to 95°C. A change in the colour of the solutions was observed during heating of process. The reduction of pure Ag^{2+} ions were monitored by measuring the UV-Vis spectrum of the reaction media at 5 h after diluting a small aliquot of the sample in distilled water by using Systronic 118 UV-Visible Spectrophotometer. Thin films of the samples were prepared on a carbon coated cupper gird and SEM analysis of both the samples was carried out using Hitachi S-4500 SEM Machine. The EDAX measurements of the silver nanoparticles of the bark extracts were performed on Hitachi S-3400 NSEM instrument equipped with thermo EDAX attachments.

Antibacterial efficacy analysis: Pure cultures of *E. coli, Pseudomonas aeruginosa, Bacillus subtilis, Proteus* and *Klebsiella* were procured from the Department of Microbiology of Sri Venkateswara Institute of Medical Science (SVIMS). The experiments of antibacterial analysis were carried out in the Applied Microbiology of Sri Padmavathi Mahila Vishvavidyalyam (SPMV). The sensitivity testing of the plant extracts were determined by using disc diffusion method (Bauer *et al*., 1966). Eighteen hours old six bacterial broth cultures were used as inoculums after adjusting their population to cfu mL^{-1} using 0.9% (w/v) sterile saline by the method as described by Forbes *et al*. (1990). 0.5 mL of standard inoculums of bacterial species were pipeted separately into sterile petriplate contained 20 mL of melted agar medium in each plate and mixed well by gently swirling on the table top. The seeded plates were allowed to solidify. Sterile paper discs previously soaked in 10 μg mL^{-1} concentration of known plant extract with in silver nanoparticles were carefully placed on the labeled seeded plates. As where the control does not contain any exposure to silver got infected before with in 24 h at room temperature. Hence the disc for control was avoided in the petriplate. After 24 h inhibition of zone formation was noted. The experiments were repeated thrice and mean values of zone diameter were presented.

RESULTS AND DISCUSSION

The two plant species *Boswellia ovalifoliolata*-Bal and Henry and *Shorea tumbuggaia* Roxb. Critically endangered, globally threatened and listed in IUCN Red list. Both species are endemic to Tirupati-cuddapah-nallamalai hotspot of India. Indigenous tribal people and local villagers use the two plants to cure different diseases. 10 to 25 mL decoctions per day of the stem bark of *Boswellia ovalifoliolata* reduce rheumatic pains (Nagaraju and Rao, 1990). Shade dried gum is powdered, dissolved in water and mixed with curd and given orally to cure amoebic dysentery (Sudhakar, 1998). The equal mixture of gum and stem bark in one tea spoon full given daily with sour milk on empty stomach for a month to cure stomach ulcers (Savithramma, 2006). The gum of *Shorea tumbuggaia* also used in indigenous medicine as an external stimulant, duodenal ulcers and a substitute for Abrutis. The extract of the plant bark is used to cure ear-ache and leaf juice is used as ear drops (Patil *et al*., 2004).

Confirmation of metal-plant interaction: The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. The bark extracts were pale yellow in colour before addition of Ag $(NO_3)_2$ and these were changed to brownish colour suggested the rapid formation of silver nanoparticles. The time duration of change in colour varies from plant to plant. *Boswellia ovalifoliolata* synthesized silver nanoparticles within 10 min whereas *Shorea tumbuggaia* took 15 min to synthesize silver nanoparticles. The change of colour indicates the biosynthesis of silver nanoparticles exhibit brown colour in aqueous solution due to the surface plasmon resonance phenomenon. In this study the results obtained is very clear in terms of identification of potential medicinal plants for synthesizing the silver nanoparticles. This has been observed in several studies (Gilaki, 2010; Li *et al*., 2009). The synthesis of silver nanoparticles had been confirmed by measuring the UV-VIS spectrum of the reaction media. The UV-VIS spectrum of colloidal solutions of silver nanoparticles synthesized from *Boswellia ovalifoliolata* and *Shorea tumbuggaia* have absorbance peaks at 350 and 430 nm, respectively and the broadening of peak indicated that the particles are polydispersed (Fig. 1). Silver nanoparticles synthesized by using *Euphorbia hirta* showed absorbance peak at 430 nm (Elumalai *et al*., 2010). From EDAX spectrum it is clear that *Boswellia ovalifoliolata* and *Shorea tumbuggaia* have recorded weight percent 39.88 and 33.52% of silver nanoparticles respectively (Fig. 2). The energy dispersive analysis of X-rays showed the other elements synthesized from stem barks of *Boswellia ovalifoliolata* and *Shorea tumbuggaia* (Table 1). The SEM image (Fig. 3) showed relatively spherical shape silver nanoparticles formed with diameter ranging from 30 to 40 nm in *Boswellia* (Ankanna *et al*., 2010) and 40 nm in *Shorea* material. The same results were expressed in plant extracts of *Aloe vera* (Chandran *et al*., 2006), *Emblica officinalis* (Amkamwar *et al*., 2005), *Carica papaya* (Jain *et al*., 2009) where as the leaf extract of *Parthenium* showed irregular shapes silver nanoparticles of 30 to 80 nm with average size 50 nm (Parashar *et al*., 2009). Warisnoicharoen *et al*. (2011) were able to synthesized silver nanoparticles size diameter of 12 nm. Several proteins mainly cell wall bound enzymes with amino groups are responsible for the synthesis of nanoparticles and biological synthesis and characterization of silver and gold nanoparticles are different size ranging from 8 to 40 nm (Chandran *et al*., 2006). By altering the pH, strength of elements, plant sources and incubation temperature of the nanoparticle synthesis reaction mixture, the synthesis methods, it is possible to create a wide range of different nanoparticles. Nanoparticles of various sizes and properties may be obtained by further topping the plant bioresources of diverse type in wild environment (Gilaki, 2010).

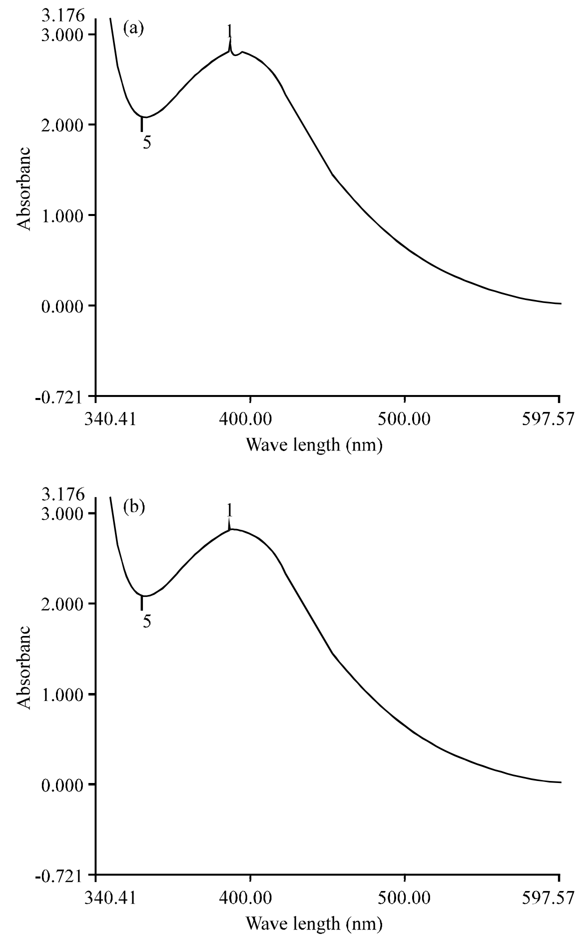

Fig. 1: UV-VIS absorption spectra of silver nanoparticle synthesized from bark extracts of (a) *Boswellia ovalifoliolata* and (b) *Shorea tumbuggaia*

Table 1: Energy dispersive analysis of X-rays (EDAX) of synthesized elements of bark of *Boswellia ovalifoliolata* and *Shorea tumbuggaia*

	Boswellia ovalifoliolata		*Shorea tumbuggaia*	
Element	Weight	Atomic (%)	Weight	Atomic (%)
C+K	24.19	44.33	31.16	51.67
O+K	33.17	45.63	32.39	40.32
Si+K	0.95	0.74	1.06	0.75
S+K	0.45	0.31	0.38	0.23
Cl+K	1.36	0.84	1.49	0.84
Ag+L	39.88	8.14	33.52	6.19
Total	100.00		100.00	

Antibacterial efficacy of SNPs: Toxicity studies on pathogen opens a door for nanotechnology applications in medicine. Biological synthesis of metal is a traditional method and the use of plant extracts has a new awareness for the control of disease, besides being safe and no phyto-toxic effects (Gardea-Torresdey *et al*., 2003). The biogenesis of silver nanoparticles using endemic

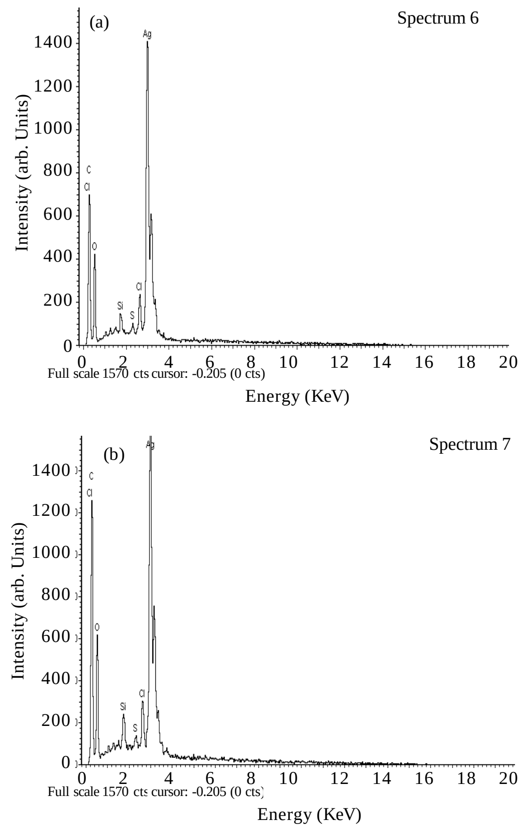

Fig. 2: Energy dispersive analysis of X-rays of synthesized silver nanoparticles of stem barks of (a) *Boswellia ovalifoliolata* and (b) *Shorea tumbuggaia*

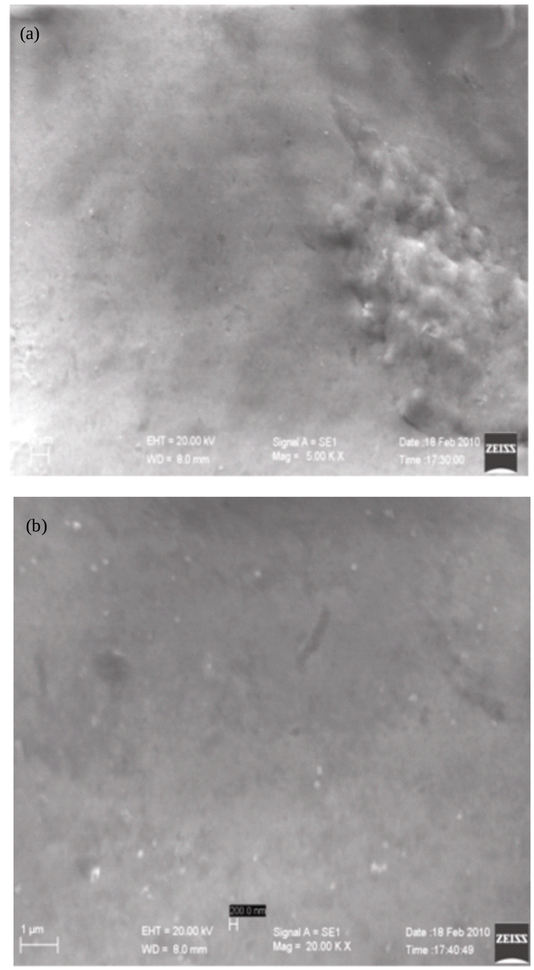

Fig. 3: SEM images of (a) *Boswellia ovalifoliolata* and (b) *Shorea tumbuggaia* (300 resolution power)

medicinal plants was found to be highly toxic against different pathogenic bacteria of selected species. The tested concentrations of plant extracts are 10 μg mL^{-1} in both the plants. The silver nanoparticles of *Boswellia ovalifoliolata* showed inhibition against *E. coli*, *Klebsiella* and *Bacillus*. Whereas the silver nanoparticles of *Shorea tumbuggaia* inhibited the growth of *E. coli*, *Pseudomonas* and *Proteus* species. The *Boswellia ovalifoliolata* showed maximum toxicity to *Proteus* and *Shorea tumbuggaia* to *Klebsiella* species (Fig. 4). Martinez-castanon *et al.* (2008) found that the antibacterial activity of the nanoparticles varies when their size diminishes. The use of plant extracts is effective against various microorganisms including plant pathogens (Mishra *et al.*, 2007). Silver nanoparticles interactions with bacteria are dependent on the size and shape of the nanoparticles (Pal *et al.*, 2007). The greatest surface area of silver nano particles the greatest the antibacterial activity (Thiel *et al.*, 2007). Oligodynamic silver antimicrobial efficacy extends well beyond its virotoxicity (Huang *et al.*, 2007). The silver nanoparticles synthesized from the extracts of the plant barks of two plants are toxic to multi-drug resistant bacteria. It shows that they have great potential in biomedical applications. Similar observations were found with the *Allium cepa* (Saxena *et al.*, 2010), *Argimone mexicana* (Khandelwal *et al.*, 2010). Warisnoicharoen *et al.* (2011) found that silver nanoparticles have an ability to interfere with metabolic pathways. The findings of Sereemaspun *et al.* (2008) suggested that the inhibition of oxidation based biological process by penetration of metallic nano-sized particles across the microsomal membrane. The use of silver ions as preventing agents in cosmetics was tested by a challenged list in a set of cosmetic dispersions with the addition of known

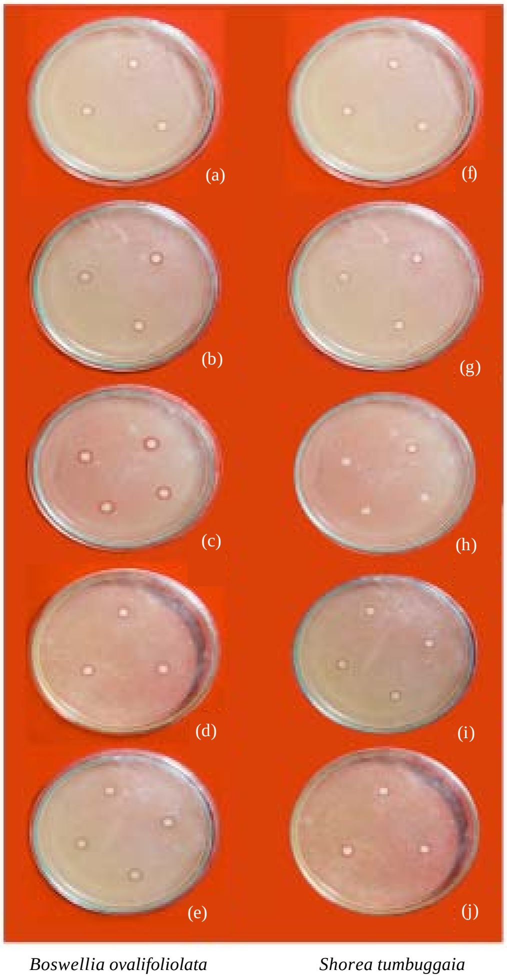

Fig. 4: Antibacterial activity shows (a, f) *Bacillus*; (b, g) *E. coli* (c, h) *Klebsiella*; (d, i) *Proteus* and (e-j) *Pseudomonas*

preservative inhibitors or micro orgamism's growth promoters. Silver has more microbial efficacy and more effective in the presence of proteinaceous material and inorgamic binding proteins that associated with inorganic structures *in vivo* using routine molecular biology techniques. The molecular basis for the biosynthesis of these silver crystals is speculated that the organic matrix contain silver binding proteins that provide amino acid moieties that serve as the nucleation sites (Prabhu *et al*., 2010). The efficiency of various silver based antimicrobial fillers in polyamide towards their silver ion release characteristics in an aqueous medium was also investigated and discussed in number of plants including algae, yeast and fungi (Arya, 2010). The selected two plant species have been using in traditional medicine, so far these plants have not been tested to antimicrobial activity The present study supports the medicinal values of these endemic plants and also revealed that a simple rapid and economical route to synthesis of silver nanoparticles and their capability of rendering the antibacterial efficacy. Moreover, the synthesized silver nanoparticles enhance the therapeutic efficacy of both the plants.

CONCLUSION

The present study included the bio-reduction of Ag^{++} ions by the endemic medicinal plant's stem bark extracts and their antibacterial activity. The study revealed that the two plant species are good source for synthesis of silver nanoparticles at fast rate. The aqueous silver ions exposed to the extracts, the synthesis of silver nanoparticles were confirmed by the brown colour formation within 10 to 15 min. the antibacterial efficacy against different species of bacteria confirmed that the silver nanoparticles are capable of rendering antibacterial efficacy and strengthen the medicinal values of the plants. The phyto-synthesis of silver nanoparticles is simple and convenient to handle and most advantage and also eco-friendly.

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