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Phytochemical screening and antimicrobial efficacy of green synthesized silver nanoparticles from *Neolitsea sericea* (Blume) Koidz

[1] Srividhya Pattabiraman, [2] Sinthia Ganesan, [3] Thilagam Muthunathan, [*4] Palanichamy Mehalingam
[1, 2, 3] Research Department of Botany (DST-FIST Sponsored) V. H. N. Senthikumara Nadar College, Virudhunagar, Tamil Nadu, India
[4] PG Department of Zoology and Research Centre, Sri Parasakthi College for Women, Courtallam, Tamil Nadu, India

Abstract
The present study we reported for the synthesis of AgNPs using *Neolitsea sericea* (Blume) Koidz. Leaf extracts were used as reducing and capping agent from silver nitrate solution. The green synthesis of silver nanoparticles was done by the bio reduction of silver nitrate using the plant extracts made from the leaves of *Neolitsea sericea.* Hence, the present study was focused to control the Gram positive strains such as *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis* and Gram negative strains such as *Klebsiella pneumoniae Escherichia coli*, *Pseudomonas aeruginosa* and a fungus *Candida albicans* by screening their antimicrobial activity and synthesizing the silver nanoparticles. Subsequently this plant was screened to evaluate the phytochemical activities due to their high medicinal value. The dried leaves were extracted with ethanol. The extracts were subjected to various phytochemical tests. The test confirmed the presence of various phytochemicals in plant extracts. The synthesized leaf extract based silver nanoparticles were confirmed by the colour change from pale yellow to dark brown appearance. UV – visible spectroscopy studies were carried out to assess the formation of silver nanoparticles. Further, efficient antimicrobial activity of the synthesized silver nanoparticles proves the potential application of green synthesis in the area of nano-medicine.

Keywords: *Neolitsea sericea,* green synthesis, silver nanoparticles, phytochemical screening and antimicrobial activity

1. Introduction
Nanotechnology deals with the production and stabilization of various types of nanoparticles (Feymen, 1991) [7]. In order to obtain nanoparticles in large quantities within a short period, physical and chemical procedures are used (Bigall and Eychmuller, 2010) [3]. Biologically synthesized silver nanoparticles (Ag-NPs) have wide range of applications because of their remarkable physical and chemical properties (Balantrapu and Goia, 2009; Tripathi *et al.,* 2010) [1, 20]. The use of nanoparticles is gaining impetus in the present century as they posses defined chemical, optical and mechanical properties (Gong *et al.,* 2007; Rai *et al.,* 2009). The field of nanotechnology is one of the most active areas of research in modern material sciences. Nanotechnology is a field that is developing day by day, making an impact in all spheres of human life (Singh *et al.,* 2010) [18, 20] and creating a growing sense of excitement in the life sciences especially biomedical devices and biotechnology (Prabhu *et al.,* 2010) [16]. In nanotechnology, silver nanoparticles are the most prominent one.

Silver nanoparticles of range between 1 nm and 100 nm in size and have attracted intensive research interest. It is generally recognized that silver nanoparticles may attach to the cell wall, thus rupturing cell-wall permeability and cellular respiration. The nanoparticles may also penetrate inside the cell causing damage by interacting with phosphorus and sulfur containing compounds such as DNA and protein. Generally, silver does not adversely affect viable cells and does not easily provoke microbial resistance.

Biological methods have paved way for the "greener synthesis" of nanoparticles and these have proven to be better methods. Due to slower kinetics, they offer better manipulation and control over crystal growth and their stabilization. This has motivated an upsurge in research on the synthesis routes that allow better control of shape and size for various nanotechnological applications. The use of environmentally benign materials like plant extracts (Jain *et al.,* 2009), bacteria (Saifuddin *et al.,* 2009), fungi (Verma *et al.,* 2010) [18, 22] and enzymes (Willner *et al.,* 2007) for the synthesis of silver nanoparticles offer numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need for special circumstances such as use of high pressure, energy, temperature and toxic chemicals (Singh *et al.,* 2010) [18, 20]. Silver-containing materials were also employed in textile fabrics, as food additives, and in package and plastics to eliminate microorganisms. Because of such a wide range of applications, numerous methods concerning the fabrication of silver nanoparticles, as well as various silver based compounds containing ionic silver (Ag+) or metallic silver have been developed (David *et al.,* 2010) [6].

In the present study, the leaf extract of *Neolitsea sericea* reduces Ag^{+} ions to Ag^{o} (nano silver) using Surface Plasmon Resonance in a first approach. The leaf extract used in

synthesizing Ag nanoparticles acts both as stabilizing as well as reducing agent and the reaction is carried out in a simple eco–friendly technique to produce nanoparticles with smaller crystalline size. The efficiency of the Ag nanoparticles has been evaluated against different kinds of microbial strains.

1.1 Taxonomical Classification

Kingdom : Plantae
Order : Laurales
Family : Lauraceae
Genus : *Neolitsea*
Species : *N.sericea* (Blume) Koidz.

Neolitsea sericea (Blume) Koidz.is a medium-sized evergreen tree which grows up to 10 m tall and belongs to the Lauraceae family. It is well known for its young leaves densely covered with silky, yellow-brown hairs which appear golden in the sunshine. (Figure 1)The species has historically been associated with Buddhism and is locally honoured as the 'Buddhism tree'. Because of its graceful shape and religious connotation, *N. sericea* is commonly used as an ornamental tree in public gardens. The rapid degradation and destruction of *N. sericea* original habitats have resulted in a continuous decline of natural populations.

Figure 1

Fig 1: *Neolitsea sericea* leaves used for the synthesis of AgNPs.

2. Materials and Methods

2.1 Chemicals

All the reagents purchased were of analytical grade and used without any further purification. Silver nitrate (AgNO3) was received from Sigma-Aldrich from India. Nutrient agar for bacterial culture and Mueller–Hinton broth and agar for antimicrobial activity were purchased from Hi-Media, Mumbai, India. Double distilled water was used throughout the experiments.

2.2 Preparation of plant extract

The *Neolitsea sericea* leaves were collected from Sirumalai hills, Dindigul District, Tamil Nadu (India), were washed thoroughly with distilled water and were shade–dried for about 10 days. The dried leaves were powdered by using kitchen blender. Exactly 30 g of this leaf powder was taken and mixed with 200 mL of methanol and boiling at 65° C with the help of heating mantle for 20 minutes. The extracts were filtered with Whatman No.1 filter paper. The filtered extract was collected and used as such for further study.

2.3 Synthesis of silver nanoparticles

For synthesis of silver nanoparticles, 20ml of methanol leaf extracts of *Neolitsea sericea* was added to the 250 mL Erlenmeyer flask containing 100 mL of $AgNO_3$ (1mM) and maintained at room temperature for 48 hours. The synthesis of silver nanoparticles in the solution was monitored by using UV–visible spectrophotometer (UV-1800v, Shimadzu, Japan). The final products obtained were centrifuged at 10000 rpm for 30 minutes and washed several times with deionized water and ethanol. Finally, the particle was dried at 60^0C and was used for further characterization study.

2.4 Preliminary Phytochemical Screening

The collected plant leaf extracts used for the synthesis of silver nanoparticles and synthesized particles were used for phytochemical analysis. (Table: 1).

2.5 Test for Alkaloids (Mayer's Test)

To 1 ml of synthesized AgNP solution, 6 drops of Mayer's reagent was added. The formation of yellowish creamish precipitate indicated the presence of alkaloids (Rajkumar *et al.*, 2011; Aslam *et al.*, 2012).

2.6 Test for Tannins (Braymer's Test)

1ml of the AgNP solution was mixed with 2ml of water. To this 2 drops of 5% ferric chloride solution was added. Appearance of dirty green precipitate indicated the presence of tannins (Rajkumar *et al.*, 2011; Aslam *et al.*, 2012).

2.7 Test for Steroids (Salkowski Test)

To 2ml of the solution, 2ml of chloroform was added and followed by concentrated sulphuric acid. Formation of reddish brown ring at the junction showed the presence of steroids (Abe *et al.*, 1998).

2.8 Test for Terpenoids

2ml of the sample was treated with 2ml acetic acid. Then concentrated sulphuric acid was added. Deep red color development showed the presence of terpenoids (Abe *et al.*, 1998).

2.9 Test for Coumarins

2ml of the solution was taken and 3ml of 10% sodium hydroxide was added. Formation of yellow coloration indicated the presence of coumarins (Abe *et al.*, 1998).

2.10 Test for Catechins

2ml of solution was treated with few drops of Echrilich reagent and few drops of concentrated HCL. The pink color formation indicated the presence of catcehins (Abe *et al.*, 1998).

2.11 Test for Phenols

1ml of the solution was treated with 3% ferric chloride. The appearance of deep blue color, shows the presence of phenol (Benzie and Strain, 1996; Hodges *et al.*, 1999) [2, 9].

2.12 Test for Flavonoids

1ml of the solution was treated with 1ml of sulphuric acid. Orange color formation confirmed the presence of flavonoids (Benzie and Strain, 1996; Hodges *et al.*, 1999) [2, 9].

2.13 Test for Quinones
1ml of the extract was treated with 5ml of HCL. Formation of yellow color precipitate indicated the presence of quinine (Benzie and Strain, 1996; Hodges *et al.,* 1999) [2, 9].

2.14 Test for Saponins (Foam test)
1ml of the leaf solution was mixed with 5ml of distilled water. The contents were heated in a boiling water bath. Frothing indicated the presence of saponins (Edeoga *et al.,* 2005; Harborne, 1973) [5, 8].

3. Antimicrobial Assay
The synthesized AgNPs from the leaf extract of *Neolitsea sericea* was analyzed for antimicrobial activity against three gram positive bacterial strains – *Bacillus subtilis, Staphylococcus aureus, Streptococcus faecalis*, three gram negative bacterial strains – *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and one fungal strain – *Candida albicans*. Well diffusion assay method was followed by using standard protocol. For this, plant extract 10µl, $AgNO_3$ 10µl and synthesized AgNPs of three different concentrations (100μg/ml, 150μg/ml, and 200μg/ml) were poured on a well. The streptomycin (10 µg/ml) was also poured on the plate and incubated at 37º C for 24 h. Diameter of the zones was measured in (mm) with the help of scale and the results were tabulated.

4. Results and Discussion
4.1. Visual identification
In the present study, the formation of silver nanoparticles in the solution was visually identified by the appearance of pale brown colour in the solution. Figure 2 shows the reduction of silver metals ions into silver nanoparticles. This color change was observed 48 hours after addition of $AgNO_3$ to the extract when kept under incubation at Room temperature. At the end of the reduction process a dark brown color is produced with silver nanoparticles settling at the bottom of the conical flask.

Figure 2

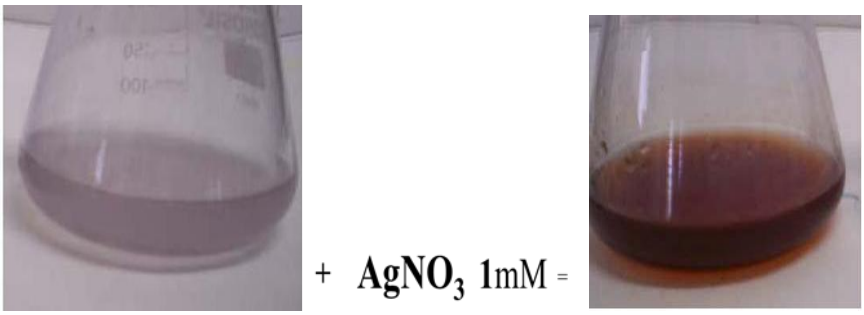

Fig 2: Color change produced in the plant extract on addition of silver nitrate solution.

4.2 UV absorbance Analysis
The fixed ratio of *Neolitsea sericea* leaf extract to metal ion solution (1:9) led to color change of the solution from pale yellow to dark brown colour due to the formation of silver nanoparticles. The change of color is due to excitation of surface plasmon vibrations with the nanoparticles. Figure 3 shows the Surface Plasmon Resonance of silver nanoparticles producing a peak in the range of 435 nm. The reduction of silver ions started immediately and completion of the reaction took place after 24 hours at room temperature, indicating the rapid biosynthesis of silver nanoparticles.

Figure 3

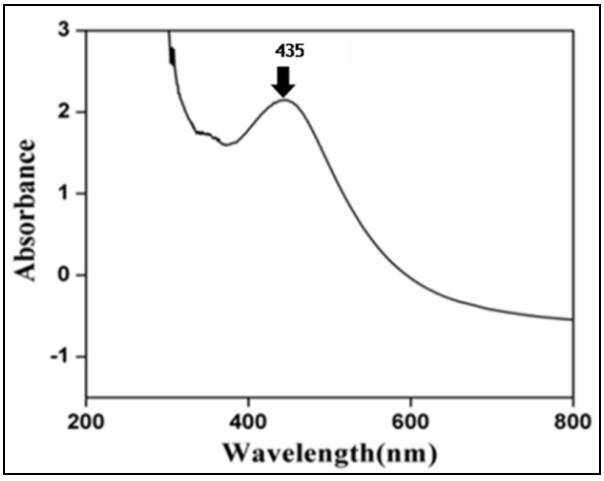

Fig 3: Absorbance of Ag nanoparticles synthesized at 1 mM concentration

4.3 Phytochemical screening

The present study was carried out with the aqueous extract of a *Neolitsea sericea* leaf, a plant that possesses a number of bioactive compounds. The qualitative phytochemical screening of methanol plant extract was analyzed and revealed the presence of alkaloids, flavonoids, phenols, steroids, tannins, saponins, quinines, terpenoids except coumarines and catachins. The results are shown in Table.1.

Table 1: The results of phytochemical screening

	Synthesized AgNPs in room temperature	**Synthesized AgNPs kept in Shaker**
Tanins	-	-
Flavonoids	+	+
Terpenoids	-	-
Saponins	-	-
Steroids	-	-
Coumarin	-	+
Alkaloids	-	-
Phenol	-	-
Quinone	-	-
Catachine	-	-

4.4 Antimicrobial activity for silver nanoparticles

The silver nanoparticles were tested for their antimicrobial activity against bacterial and fungal strains and zone of inhibition was measured (Table 2). In this study, the antimicrobial property of AgNPs was investigated by growing gram positive *(Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis)* bacterial colonies, gram negative (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *E.coli*) bacterial colonies and fungus (*Candida albicans*) on Mueller Hinton agar plates supplemented with AgNPs. Streptomycin was used as standard control and distilled water was used as control for the extract. The inhibition zones obtained indicates maximum antibacterial activity of the prepared test sample. Figure 4 shows the zone of bacterial inhibition by AgNPs prepared from *Neolitsea sericea* leaf extract and shows maximum inhibition for *Bacillus subtilis* followed by *Candida albicans.* The fungal strain also exhibited significant zone of inhibition. Hence, it may be concluded that these silver nanoparticles exhibited good antimicrobial property. Also, when compared with $AgNO_3$ and AgNPs, there is no such prominent antimicrobial activity in case of the plant extracts when used in crude form. The present study corroborates with the observation of several workers (Krishnaraj *et al.*, 2010; Yamini and Lakshmi, 2011; Sharma *et al.*, 2013) [11, 23].

Table 2: The Antimicrobial activity of AgNps synthesized using leaves of *Neolitsea sericea*

Name of the culture	Plant extract 10 µl	$AgNO_3$ 10 µl	Zone of inhibition (mm)			Streptomycin 10 µl
			100µl	150 µl	200 µl	
Bacillus subtilis	15	12	11	12	12	29
Staphylococcus aureus	11	10	11	12	11	28
Streptococcus faecalis	13	11	10	11	13	28
Klebsiella pneumoniae	11	10	11	12	11	30
Pseudomonas aeruginosa	14	11	11	12	13	28
E.coli	14	11	10	12	12	30
Candida albicans	15	10	10	11	12	28

Figure 4

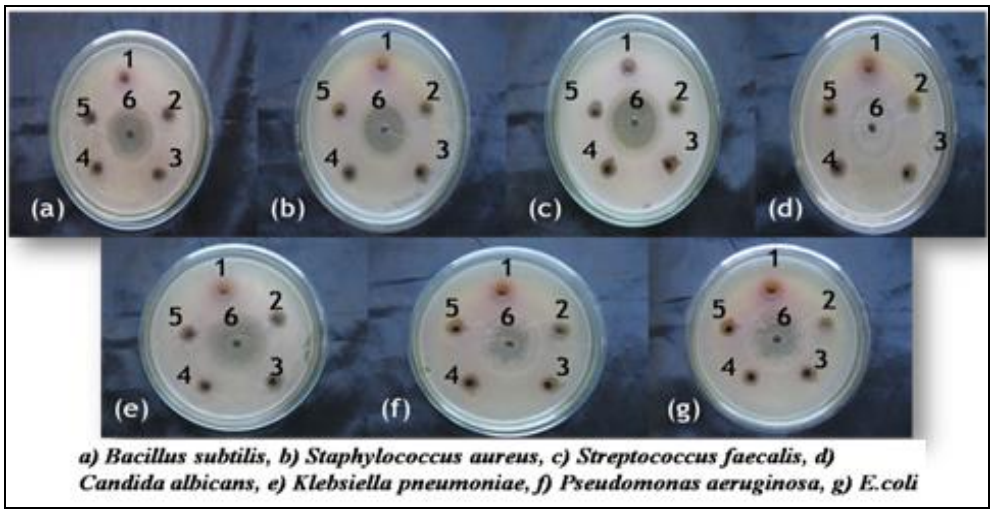

Fig 4: Antimicrobial activity of various concentrations of Silver nanoparticles against Selective bacterial pathogens:

1. Plant extract (10µl), 2. silver nitrate (10µl), 3. AgNps (100 µg/ml), 4. AgNps (150 µg/ml), 5. AgNps (200 µg/ml), 6. streptomycin (10 µg/ml.).

Figure 5

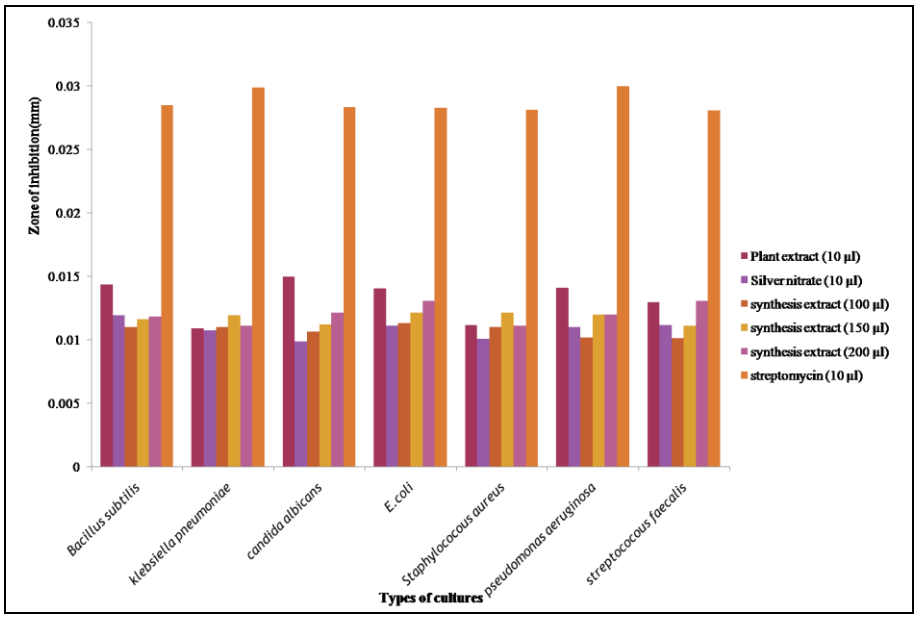

Fig 5: Antimicrobial activity of silver nanoparticles synthesized from *Neolitsea sericea*

5. Conclusion

In this green synthesis technique, no hazardous solvents were engaged. The synthesis of leaf extract based silver nanoparticles was confirmed by the colour change from pale yellow to dark brown appearance. UV – visible spectroscopy studies were carried out to assess the formation of silver nanoparticles. Subsequently this plant was screened to evaluate the phytochemical constituents. The dried leaves were extracted with ethanol. The extracts were subjected to various phytochemical tests. The tests confirmed the presence of various phytochemicals. The silver nanoparticles produced from *Neolitsea sericea* plant extract have a great potential to be used as antimicrobial agents against various pathogens. The present protocol is an eco-friendly and cost- effective way of synthesize silver nanoparticles in the laboratory as well as in room conditions.

6. Acknowledgements

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