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Research Article

Green synthesis of silver nanoparticles by leaf extract of *Leea macrophylla* Roxb. Ex Hornem. and their antioxidant activity

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Abstract

The present investigation focuses on synthesizing silver nanoparticles through a green approach using leaf extract of the medicinal plant *Leea macrophylla*. Phytosynthesised nanoparticles were characterized by UV-Vis spectrophotometer, AFM, FTIR, HR-TEM, and Zeta potential. The formation of AgNP was indicated by visual observation. The color changes from colorless to brown color and the formation of AgNPs were further confirmed by UV-Vis of reaction mixture spectrum showing characteristic absorbance resonance peak at 418 nm. FTIR data revealed the phytochemicals involved in reducing Ag (I) ions to Ag (0) and capping of AgNPs. The transmission electron microscope images showed the AgNP as anisotropic and spherical in shape with a size range between 10 nm. The XRD data depicted nanoparticles' crystalline nature, and the minimum size of the particles is 10 nm. HR-TEM and Zeta Potential analysis images confirm the size, shape, and dispersion. This investigation aimed at the antioxidant activity of silver nanoparticles by using a DPPH assay. Silver nanoparticles showed higher antioxidant activity than the plant extract. It has found to be that the results showed that the antioxidant activity of silver nanoparticles was directly proportional to the concentration.

INTRODUCTION

Green synthesis of AgNP has offered some advantages in the fabrication process, such as low cost, quick and hassle-free synthesis, and the end products are non-toxic. Several methods have been employed to synthesize AgNPs however, the use of plants allows for the inexpensive production of AgNP on a large scale (Mathura *et al.*, 2018). Various sources of reducing agents have been used in the green synthesis of AgNPs, Such as microbes (Hulakoti and Taranath, 2014), algae and enzymes (Mukharjee *et al.*, 2001), and plant extracts of *Samanea saman* (Daphedar and Taranath 2017), Among these biological methods, Plant-mediated synthesis of nanoparticles is an efficacious approach which finds immense application in the medical field (Inbathamizh *etal.*, 2013).

An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule (Anand *et al.*, 2017). The main characteristics of an antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanism that lead to degenerate diseases (Bansode *et al*., 2022). Reactive oxygen species (ROS) are generated in the body as by-products of several cellular metabolic reactions; they consist of radical and non-radical oxygen species formed by partial oxygen reduction (Wang *et al.*, 2018). Low levels of ROS are necessary for cellular processes such as intracellular signalling, cell progression and cell defence. Conversely, high ROS levels or the antioxidant system's inability to regulate ROS levels efficiently results in oxidative stress. Oxidative stress directly or indirectly ROS-mediated damage of nucleic acids, proteins, and lipids. Consuming dietary antioxidant supplements to fight diseases, especially cancer, has become popular among the general public (Abambagade *et al.,* 2017). Herbal plants considered as natural good antioxidants since ancient times.

One species of the genus *Leea* of the family *Vitaceae*, namely *Leea macrophylla* (LM) Roxb. Ex Hornem. has been ascribed with abundant therapeutic claims for its ethnomedicinal and economical uses. In India, LM is distributed in the sub-Himalayan tract and the Western Ghats, mounting up to 2250 m in the Himalaya. Uttar Pradesh, Bihar, West Bengal, Sikkim, Assam, Meghalaya, Odisha, Madhya Pradesh, Maharashtra, Andhra Pradesh, Karnataka, Tamil Nadu, Kerala, and Andaman. It is distributed in other countries such as Nepal, Bhutan, Bangladesh, Myanmar, Laos, Cambodia, and Thailand. Due to its therapeutical potential, traditional healers have used it since the pre-historic period for various ailments. Many ethnobotanical claims have been conveyed during survey studies in the tribal regions of various states of India and other parts of the world (Sarvade *et al.,*2016)). It is commonly called in Mandal gida in kannada, Hathikarna in Hindi. Shrubs to 2 m high; young branches, rachises, petioles, petiolules and inflorescences hairy to mealy-pubescent. Leaves alternate, 1 or 3-foliolate or 1-3 pinnate; if 1 or 3-foliolate; petioles to 20 cm long; rachises 10-15 cm long; stipules obovate, 2-6 x 1-4 cm; leaflets oblique, broadly ovate, oblong-ovate, elliptic or rhomboid, 15-60 x 10-50 cm, cordate at base, acute or short acuminate at apex; if 1-3 pinnate leaflets 7-21 or more, oblong, ovate-lanceolate or elliptic, 9-30 x 4-9 cm, rounded at base, serrate at margin, acuminate to caudate at apex, chartaceous to subcoriaceous, glabrous to sparsely hairy above, sparsely to densely hairy sometimes mealy-pubescent beneath; lateral nerves to 14 pairs, pubescent to hairy; petiolules to 25 mm long. Inflorescences 12-45 cm long, much branched; peduncles to 25 cm long; bracts deltoid to narrowly 3-angled, to 6 mm long; pedicels 1-2 mm long, pubescent. Flowers are greenish white. Calyx 1.5-3 x 2.5-4 mm, 5-lobed, mealy-pubescent; lobes 3-angled, 0.75-1 x 0.8-1 mm. Corolla tube with staminal lobes 3-4 mm long; corolla lobes 5, linear-ovate, thick, 2-4 x 0.8-1 mm, greyish-pubescent to papillose. Staminal lobes slightly retuse or shallowly cleft; stamens free, in between staminal lobes; staminal column 2-3 mm long; filaments ca 1 mm long; anthers oblong, ca 1 mm long, medifixed. Ovary globose, 1-1.5 mm, 6-loculed; style to 2 mm long. Fruits subglobose or globose-depressed, 10-15 mm across, green; seeds usually 6, 3-gonous, ca 4 x 3 mm (Sarvade 2019). In this context, this study aims to be green synthesis of silver nanoparticles by aqueous leaf extract of *L.macrophylla* and investigates its antioxidant activity.

MATERIALS AND METHOD:

Collection of plant material:

Leea macrophylla Roxb. ex Hornem. plant were collected from the Botanical Garden, Karnatak University, Dharwad. Karnataka, India.

Preparation of Plant Extract:

Healthy fresh leaves were collected and washed with tap water to remove the dust particles, continued with distilled water, and leaves were shade dried to remove excess water present on the surface of leaves. Dried leaves were cut into small pieces. 5gm of incised leaves were boiled in 50 ml distilled water at 800 C for 45mins. After 45 mins, cooled to the room temperature and filtered through the whatman no 1 filter paper and stored at 4^0 C in the refrigerator until use for further analysis.

Preparation of 1mM Silver nitrate solution:

0.016 gram of Silver nitrate (AR-Grade Himedia) were dissolved in 1000 ml milipore water and stored in dark bottle.

Biosynthesis of Silver nanoparticles and their characterization:

5ml of leaf extract was mixed with 95 ml of 1mM AgNO3 solution and incubated at room temperature. In this period reaction mixture changes from colourless to dark brown colour accurately at 30 mins. The dark brown colour of the reaction mixture indicates the formation of silver nanoparticles. Silver nanoparticles were investigated using a UV-Vis spectrophotometer (Jasco V-670UV-Vis NIR spectrophotometer. Further, the dry powder was analysed using Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Diffractometer to confirm the silver nanoparticle's crystalline nature and phase purity. The shape and size of the nanoparticles were determined by atomic force microscopy (AFM) and high-resolution electron microscopy (HR-TEM).

Antioxidant activity:

Preparation of DPPH solution and test samples:

4 mg of DPPH (Himedia) were dissolved in 100 ml of ethanol and stored in dark bottle. 1mg/ml of plant extract and synthesized silver nanopartilces were dissolved separately in ethanol. **DPPH radical-scavenging activity:**

Hydrogen-donating activity was measured by direct hydrogen donation to the DPPH radical explained by (Zhang *et al*., 2011 ; Azam Chahardoli et al., 2017) with minor modification. Different concentrations (12.5-400 µg/ml) of the test samples were taken into the test tubes and 3ml of DPPH added to each test tube. Reaction mixture was incubated in dark for 30 mins. At the end of reaction absorbance was measured at 517 nm against blank. DPPH in ethanol was used as control. Ascorbic acid considered as a standard solution. The percentages of inhibition of free radicals were determined by the following equation. Experiments were carried out in triplicate (Azam *et al.,* 2018; Zhang *et al*., 2001).

Percentage of inhibition= (Ab_C - Ab_S / Ab_C) X 100

Result and Discussion:

Phytogenic silver nanoparticles were synthesized from silver nitrate solution and were determined using aqueous leaf extract of *L.macrophylla.* The color of the reaction mixture changes from pale yellow to a dark brown color indicating the formation of silver nanoparticles. Its excitation of surface Plasmon resonance effect shows the reduction of Ag (I) to Ag (0). UV-Visible spectrometry shows optical absorption spectra of AgNPs ranging from 300-700nm. Biogenic AgNPs of *the L. macrophylla* plant shows an intensive peak at 419 nm at pH 9, confirming the surface plasmon resonance of AgNPs (Horwat *et al.,* 2011).

Fig.1. Visual observation of formation of silver nanoparticles

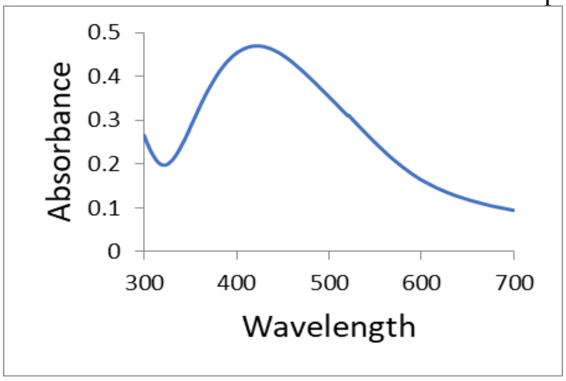


Fig.2. UV-Vis absorption spectra of silver nanoparticles synthesized by *Leea macrophylla* Roxb.ex Hornem.

The crystalline nature of the silver nanoparticles was confirmed using an X-ray diffractometer. The obtained XRD patterns show some Bragg reflection peaks were identified as (111), (200), (220), and (311) sets of lattice planes detected at the angle of 2θ. The average size of silver nanoparticles was calculated by using the Debye-Scherer equation = $K\lambda/\beta.\cos\theta$. The average crystalline size of the silver nanoparticles was found to be 10 nm (Hoag *et al.*, 2009).

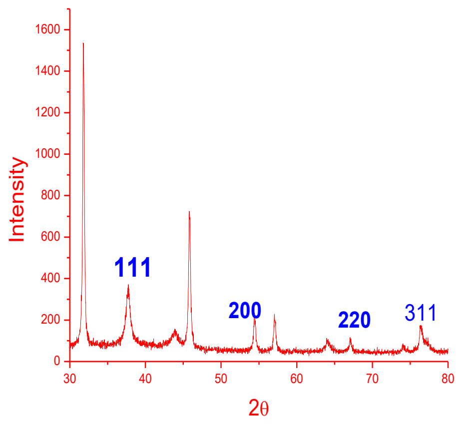


Fig.3. XRD of silver nanoparticles synthesized by leaf extract of *Leea macrophylla* Roxb.ex Hornem.

An atomic Force Microscopy result reveals the size, shape and distribution of the nanoparticles Fig 3. In Fig 3 A and B shows the two and three dimensional image of the silver nanoparticles and it reveals that the size of the nanoparticles ranges between 50 to 80 nm and are polydispersed. Fig3 C The topograph image of the silver nanoparticles shows the distributions of nanoparticles are poly dispersion (Subramaniyam *et al.*, 2015).

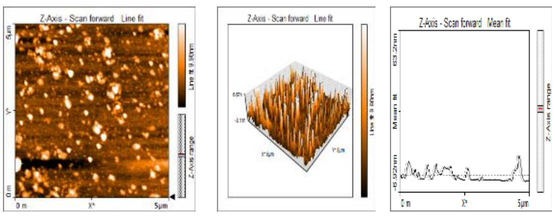


Fig.4. Atomic Force Microscopy (AFM) images silver nanoparticles A) 2D image B) 3D image C) Distribution of nanoparticles.

High resolution electron microscopy analysis images showed that the particles size ranges between 50 & 20 nm scale. The image of 20 nm scale shows the spherical shape of nanoparticles and formation of nanofringes on the surface morphology. Dark bands of nanofringes are proved that these AgNPs have strong stability in nature. The 5 nm scale of nanoparticle is clear showed that capping of the AgNP (Hussain *et al.*,2016).

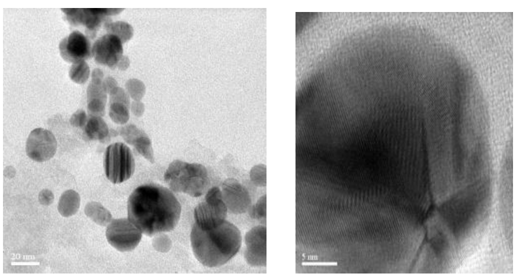
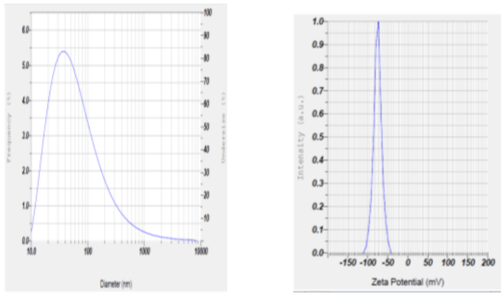


Fig.5. HR-TEM Images of the silver nanoparticles synthesized by *Leea macrophylla*

Zeta potential value indicates the size of the nanoparticles 50.5nm with polydispersity index of 0.854. zeta potential values shows -49.2mV and it indicates that the molecules involved in capping on the surface of silver nanoparticles as well as moderate stability in nature (Ibrahim *et al.*,2015).



A B

Fig.6. Zeta potential images of silver nanoparticles A) Size distribution of AgNP B) Zeta potential of AgNP.

FTIR spectra of the nanoparticles were recorded to identify the possible biomolecules involved in the reduction and capping/stabilization of the silver nanoparticles. Polyphenolic compounds could be adsorbed on the surfaces of silver nanoparticles, possibly through π-electron interactions in the absence of other strong ligating agents. Table.1 shows the FTIR spectra of Silver nanoparticles. 3431.73 cm^{-1} O-H stretching of alcohols and N-H stretching of amines, 2922.65 cm^{-1} Asymmetric stretching of aldehydes, 2852.00 cm^{-1} C-H stretching of alkenes, 2426.36 cm^{-1} O-H stretching of carboxylic acid, 1628.09 cm^{-1} N-H bending of primary amines, 1384.02 cm^{-1} C-H bend of alkyls, 1077.99 cm^{-1} C-N stretching of benzene, 841.54 cm^{-1} C-Cl stretch of alkyl halides, 542.36 cm^{-1} C-Br stretch of alkyl halides. The functional group of 1384.02 cm^{-1} mainly involved the bioreduction of AgNPs and involving in the capping of the AgNPs (Majumdar *et al.*, 2015; Martinez *et al.*, 2016; Mohanraj *et al.*, 2014).

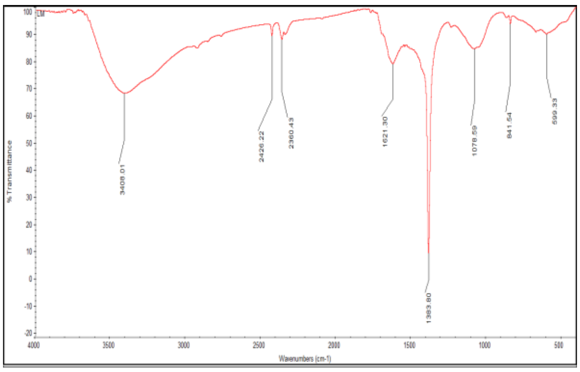


Fig.7. FTIR spectra of Silver nanoparticles synthesized by leaf extract of *L.macrophylla* Roxb.ex Hornem.

Absorption peak in Cm^{-1}	**Functional groups**
3431.73	O-H Stretching of alcohols and N-H amines
2922.65	Asymmetric stretching of aldehydes
2852.00	C-H stretching of alkenes
2426.36	O-H stretching of carboxylic acid
1628.09	N-H bending primary amines
1384.02	C-H bend of alkyls
1077.99	C-N stretching of benzene
841.54	C-Cl stretch of alkyl halides
542.36	C-Br stretch of alkyl halides

Table.1. FTIR functional group of silver nanoparticles synthesized by *L. macrophylla* Roxb.ex Hornem.

Con in µg/ml	Ascorobic acid	Aqueous leaf extract	Silver nanoparticles
12.5	0.306±0.176	0.212±0.122	0.577±0.033
25	0.206±0.166	0.405±0.234	0.404±0.023
50	0.200±0.115	0.811±0.104	0.351±0.020
100	0.265±0.153	0.428±0.247	0.342±0.019
200	0.450±0.260	0.307±0.177	0.404±0.023
400	0.578±0.136	0.535±0.203	0.410±0.036

The antioxidant efficacy of silver nanoparticles was illustrated the antioxidant potentiality compare with the standard ascorbic acid. The antioxidant activity of silver nanoparticles synthesized by leaf extract of *L. macrophylla* was examined at different concentration (12.5, 25, 50,100,200 and 400 µg/ml) depicted in the fig 22. The IC_{50} value of silver nanoparticles, leaf extract and ascorbic acid is, 32 µg/ml, 80 µg/ml, 60 µg/ml. The experimental results of DPPH activity of AgNPs were presented in the Standard deviation and standard error by using SPSS 2.0 software in the Table 2. The mean result of standard deviation of ascorbic acid is 0.306±0.176, 0.206±0.166, 0.200±0.115, 0.265±0.153, 0.450±0.260, 0.578±0.136, leaf extract is, 0.212±0.122, 0.405±0.234, 0.181±0.104, 0.428±0.274, 0.307±0.177, 0.353±0.203, AgNPs of *L. macrophylla* is, 0.577±0.083, 0.404±0.023, 0.351±0.020, 0.342±0.019, 0.404±0.023 and 0.410±0.036 respectively. *In-vitro* expression antioxidant activity of silver nanoparticle is reliably higher than plant extract when compared with the ascorbic acid (Islam *et al.*,2019).

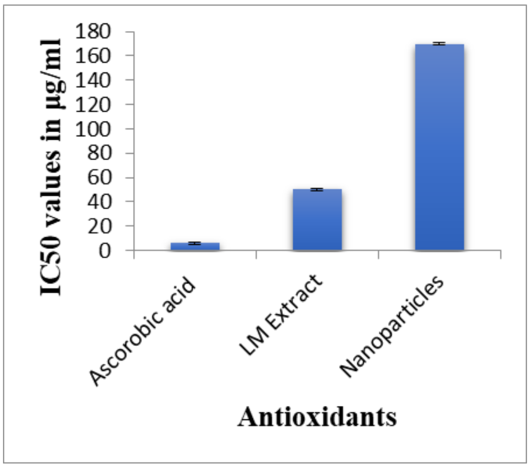


Fig.8. Antioxidant activity of silver nanoparticles synthesized by *L.macrophylla*

Table.2: Percentage of radical scavenging activity represented in the SD±SE.

Conclusion: This study shows that using an aqueous extract of *Leea macrophylla* leaf to synthesise silver nanoparticles is a green, environmentally friendly method. These nanoparticles had a spherical form and were formed of nano crystals. When compared to leaf extract, silver nanoparticles have the strongest antioxidant activity at lower doses. Its antioxidant qualities may potentially increase its usefulness in biomedicine.

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