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ANTIOXIDANT ACTIVITY AND SYNTHESIS OF SILVER NANOPARTICLES USING THE LEAF EXTRACT OF *LIMONIA ACIDISSIMA*

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ABSTRACT

A green synthesis of silver nanoparticles was done using a particular plant extract of *Limonia acidissima*. Silver nanoparticles were synthesised by the bioreduction of silver nitrate using different concentrations of plant extract, and UV–visible spectral analysis was carried out to confirm the formation of silver nanoparticles. The presence of various phytochemicals like flavonoids, steroids, polyphenols, and terpenoids was investigated by following standard biochemical methods. Later the antioxidant activity was performed by the phosphomolybdenum method, and the ethanolic leaf extract of *Limonia acidissima* was found to possess significant antioxidant activity. The biosynthesized silver nanoparticles were characterized by scanning electron microscopy (SEM).

KEYWORDS: **Silver nanoparticles, phytochemicals, *Limonia acidissima,* antioxidant activity**

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INTRODUCTION

Nanoparticle technology deals, with the production, characterization and manipulation of materials at the nanoscale. Synthesis of silver nanoparticles through a green method has attracted great interest in this area.[1] Among the various methods the biological and green methods are considerably preferred for the biosynthesis of silver nanoparticles using the plant extract that possesses phytochemicals with strong antioxidant properties.[2] The antioxidant effect of the plant extract is due to the presence of phenolic compounds such as flavonoids, phenolic acid, tannins and diterpenes. Antioxidants protect cells against cell damage causing molecules known as free radicals.[3] The development of nanoparticles used as therapeutic agents has introduced new opportunities for the improvement of medical treatment. Oxidation of biomolecules for the regulation of oxidative chain reaction and plant plays an effective role in nanoparticle synthesis as they are free from toxic chemicals as well as provide natural capping agents.[4] *Limonia acidissima* commonly known as Villam family of Rutaceae, well known in English as wood apple, used for the medicinal plants is cheaper, more associable to the most of the population in the world. This plant has various useful chemical constituents which are used in the treatment of diarrhea and diabetes as well as used as a cardio tonic.[5] *Limonia acidissima* is an important medicinal plant containing a number of phytoconstituents, and has a main role in the treatment of cancer, diabetes and wound healing. In the present study we carried out the biosynthesis of silver nanoparticles using a plant extract of *L. acidissima* evaluated the antioxidant activity and phytochemical screening.[6] A majority of antioxidants naturally present in food has the phenolic structure, especially flavonoids. Antioxidants can play a protective role in a number of diseases such as cardiovascular and neurodegenerative diseases in which oxidative stress and free radicals are the major contributors.[7] So far many studies have been conducted to evaluate the correlation between the phenolic structure and the antioxidant activity.[8] Phytoconstituents like flavonoids and phenolics have been reported as potent free radical scavengers and they frequently occur in medicinal and aromatic plants.[9]

MATERIALS AND METHODS

Collection of samples

Fresh leaves of *Limonia acidissima* (VUBT1006) were collected from Mayiladuthurai, Nagai District. The leaves were washed thoroughly with tap water and rinsed with distilled water then allowed to air dry at room temperature.

Preparation of the leaf extract

The shade-dried leaves of *L.acidissima* were blended and made into a fine powder. 10 g of fine leaf powder was immersed in 50 ml of ethanol solvent. It was incubated at room temperature for 24 hrs in an orbital shaker at 150 rpm. The suspension was filtered and the filtrate was stored in an airtight brown colored bottle and used for further processes.

Preliminary phytochemical screening

The ethanol extract of the leaves of *Limonia acidissima* was investigated for the presence of phytochemicals, like alkaloids, terpenoids, flavonoids, steroids, and polyphenols, by following standard biochemical methods.[10-11]

Synthesis of silver nanoparticles

1 mM $AgNO_3$ solution was prepared and stored in airtight bottles. 5 ml of the leaf extract was taken in a conical flask separately and 1 mM $AgNO_3$ solution was added dropwise with constant stirring at 50–60 °C; then the colour change of the leaf extract from yellow to dark brown was observed which confirmed the synthesis of silver nanoparticles.

UV–Vis spectral analysis

The initial characterization of synthesized silver nanoparticles was carried out using UV-Vis spectroscopy. The bioreduction of silver ions was indicated by their absorbance from 250 to 500 nm. The maximum peak was found to be at 420 nm for *L. acidissima*.

SEM analysis

The silver nanoparticles synthesized from the *L. acidissima* leaf extract were characterized using a scanning electron microscope. The image showed spherical shaped nanoparticles, observed at a magnification of x 9.99k. This confirms that the silver nanoparticles were obtained from the *L. acidissima* leaf extract.

Antioxidant activity

The antioxidant activity was evaluated by the phosphomolybdenum method[12]; the phosphomolybdate reagent solution was prepared by adding 1 ml of each 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate to 20 ml of distilled water and the volume was made up to 50 ml by adding distilled water. The hydroalcoholic extract of *L. acidissima* in different concentrations ranging from 100 µl to 500 µl was added to each test tube individually containing 3 ml of distilled water and 1 ml of molybdate reagent solution. These tubes were kept incubated at 95 °C for 90 min. After incubation these tubes were normalized to room temperature for 20–30 min and the absorbance of the reaction mixture was measured at 695 nm. Mean values of the *L. acidissima* leaf sample were calculated. Ascorbic acid was used as positive reference standard.

Statistical analysis

The experiments were carried out in triplicate and the results are given as mean ± standard deviation (SD). A sample analysis test was used for the comparison between the means of samples and standard. Correlation analysis was carried out for the antioxidant activity method (phosphomolybdenum) using Excel-2014.

RESULTS

Phytochemical, antioxidant and silver nanoparticle biosynthesis capacities were analysed in the medicinal

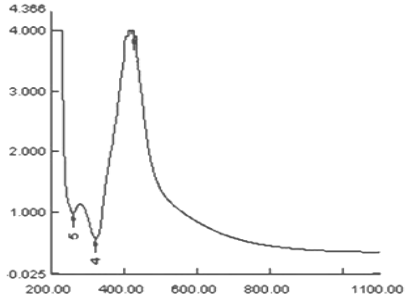
plant extract of *L. acidissima*. The results obtained by the qualitative screening of phytochemicals in *L.acidissima* are presented in Table 1; of the eight phytochemicals screened seven were found to be present in the ethanol extract. A significant amount of tannins, flavonoids, terpenoids, steroids, glycosides, reducing sugar and fatty acid is found; saponins were not present in the *L.acidissima* plant extract. The synthesis of silver nanoparticles was followed by the UV–visible spectral analysis with the absorption peak ranging from 400 nm to 450 nm. The UV–Vis spectra show a peak approximately at 450 nm (Fig 2) which clearly indicates the presence of nanoparticles in the sample. Scanning electron microscopy images (Fig 3) were acquired to analyse the shape and morphological structure of the silver nanoparticles that were synthesised using a biological method. The total antioxidant activity of the ethanol extract was evaluated (Table 2) by the phosphomolybdenum method according to the procedure described above.[12] The molybdenum assay is based on the reduction of Mo(VI) to Mo(V) by the extract and the subsequent formation of green phosphomolybdenum. It is a quantitative method to investigate the reduction reaction rate (Graph 1) of antioxidants.[13] It involves the thermal generation of an auto-oxidant during prolonged incubation at higher temperatures.[14] It shows the direct estimation of the reducing capacity of the antioxidant. According to the results obtained from antioxidant capacity assays, the ethanol extract of *L. acidissima* has shown a significant total antioxidant capacity. Biosynthesis of nanoparticles by a green method has more advantages over chemical and physical methods as well as it is cost effective and eco-friendly and does not require the use of pressure, energy, temperature, and toxic chemicals. The nanoparticles synthesis process has diverse applications in the field of healthcare, medicine, electronics, *etc*.[15]

Figure 1
Synthesised silver nanoparticles indicating the colour change.

Table 1
Phytochemical test carried out using the ethanol extract of L. acidissima.

S.No	Phytochemical constitutents	Chemical test	*L. acidissima* plant extract
1.	Tannins	Ferric chloride test	+
2.	Saponins	Foam test	-
3.	Flavonoids	Alkaline reagent test	+
4.	Steroids	Salkowski test	+
5.	Glycosides	Keller Killiani test	+
6.	Reducing sugars	Fehling's test	+
7.	Fatty acids	Detection of fatty acid	+
8.	Terpenoids	Salkowski test	+

Figure 2
Ultraviolet spectrum of Limonia acidissima plant extract



The UV–Vis spectrum shows an absorbance wavelength at 450 nm confirming the reduction of silver nanoparticles in the L.acidissima sample.

Figure 3
SEM image of silver nanoparticles from the extracts of L. acidissima showed Uniform size distribution at 2.21K x magnification

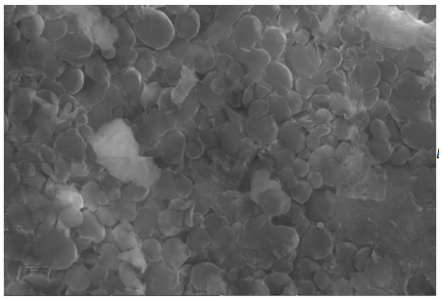
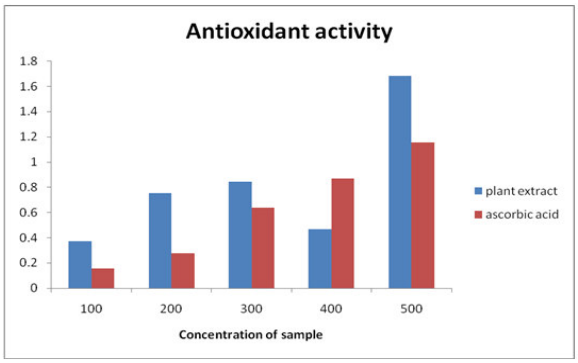


Table 2
Antioxidant activity of L.acidissima plant extract investigated using the phosphomolybdenum method.

Volume in µl	Absorbance at 695 nm *L. acidissima* plant extract	Ascorbic Acid
100	1.116	0.158 ± 0.01
200	2.265	0.278 ± 0.05
300	2.535	0.640 ± 0.02
400	1.404	0.871 ± 0.02
500	5.055	1.155 ± 0.01

Figure 1
Phosphomolybdenum method to determination of total antioxidant capacity in the L. Acidissima plant extract.



DISCUSSION

Qualitative screening for the presence of various phytochemical compounds was performed using the aqueous leaf extract. Biosynthesis of nanoparticles from an aqueous solution of $AgNO_3$ with the aqueous leaf extract of *L. acidissima* was confirmed by UV-Vis spectral analysis. The colour change of the reaction mixture into brown is due to the excitation of the surface plasmon resonance (SPR) vibration band of $AgNO_3$ (Figure1). In this study *in vitro* evaluation of antioxidant activity with different important antioxidant parameters was not done but involved the investigation in terms of total antioxidant capacity using novel methods such as phosphomolybdenum assay. Thus in the present study an effort was made to overview the total antioxidant capacity of the plant extract of *L. acidissima*. All values were expressed as mean ± S.D. and one-way ANOVA was done to analyse the significant difference using the statistical analysis by Excel-2014. Values with $p < 0.05$ were considered as significant. Biologically synthesised silver nanoparticles are most commonly used materials and are found to have antioxidant and phytoconstitute properties, in addition to having applications in textile, food, and paint industries and in other fields.[16] The total antioxidant capacity was determined for the *L.acidissima* plant extract by carried out *in vitro* antioxidant tests like phosphomolybdenum assay. Natural antioxidants are useful in protecting cells from oxidative damage.[17] Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxyl toluene (BHT) are rarely used in the food industry.[18] Natural phenolic antioxidants are mostly used as food ingredients and are added to various food products.The natural plants have antioxidant free radical scavenges which help in preventing pathologies like heart disease, cancer, arthritis, and liver disease.[19] The natural plant extract has clinical therapeutic potential due its antioxidant activity in reducing such free radical induced tissue injury and therefore has applications in the pharmaceutical industry.[20] The natural plant is found to possess a variety of antioxidants, so that medicinal plants can protect from free radicals originating from oxidative stress or exposure to UV radiation. In this study the total antioxidant capacity was determined for

the *L. acidissima* plant extract which indicated the highest free radical scavenging activity.

CONCLUSION

Synthesis of silver nanoparticles from the *L. acidissima* plant extract and their characterization by SEM and UV spectral analysis were successfully done. Phytochemical screening and evaluation of antioxidant activity of the *L.acidissima* plant extract were also performed. All phytochemical constitutents were present in the plant sample in significant amounts; saponins were not present in the *L.acidissima* plant extract. This plant extract shows the highest antioxidant capacity. According to the results the *L. acidissima* plant extract shows better *in vitro* antioxidant activity and phytochemical test.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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