RESEARCH ARTICLE

HPTLC Finger Print Development and Green Synthesis of Silver Nanoparticles Using *Alstonia scholaris* Linn. Root Extract

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ABSTRACT

Aim: The present research is grounded in a comprehensive approach encompassing pharmacognostic study, high-performance thin layer chromatography (HPTLC) profiling, and the preparation and characterization of silver nanoparticles derived from the aqueous root extract of *Alstonia scholaris* Linn. R. Br.

Methods: Successive extraction of root parts was conducted, and preliminary phytochemical screening was employed to identify the presence of primary and secondary metabolites, including alkaloids, glycosides, and tannins. HPTLC densitometric analysis of the aqueous root extract was performed using the CAMAG HPTLC system, yielding chromatograms scanned at wavelengths of 254 and 366 nm. The study further delves into the green synthesis of silver nanoparticles, utilizing the aqueous root extract. The reduction of silver ions and the subsequent formation of silver nanoparticles were monitored using a UV-vis spectrometer.

Results: Phytochemical screening confirmed the diverse array of primary and secondary metabolites present in the aqueous root extract. HPTLC densitometric analysis provided chromatographic results, offering insights into the chemical composition at specific wavelengths. The green synthesis of silver nanoparticles using the plant extract demonstrated successful reduction of silver ions, with further investigation of particle size distribution through transmission electron microscope (TEM) and field emission-scanning electron microscope (FE-SEM) revealing morphological characteristics of the prepared silver nanoparticles.

Conclusion: The findings from this multidisciplinary study shed light on the pharmacognostic attributes, chemical composition, and green synthesis potential of *A. scholaris* Linn R. Br. The documented presence of bioactive compounds and the successful synthesis of silver nanoparticles underscore the therapeutic potential of this plant. These results contribute to understanding the plant's medicinal properties and hold promise for future applications in healthcare and nanotechnology.

Keywords: *Alstonia scholaris*, Phytochemicals screening, HPTLC profiling, Green synthesis, Silver nanoparticles.

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INTRODUCTION

Medicinal plants are a reliable source of medication discovery and development; scientists have been focusing more on this topic in the last decade. Herbal medicines are non-hazardous, and with fewer side effects, phytoconstituent-based formulations are widely accepted and continuously used all over the world. *Alstonia scholaris* Linn Family: Apocynaceae is commonly referred to as the "Devil's tree" or Saptaparni. It is extensively found in the Southern region of the Western Ghats, the Western Himalayas, and the dry forests of India. It is a well-known medication used in India's Ayurvedic, Homoeopathic, and Folklore medical systems for a variety of ailments.[1,2] *A. scholaris* plant parts contain mainly a high concentration of alkaloids and other important phytoconstituents that can be extracted and used as a natural source of fungicide and bactericidal properties. Plant parts are used in the treatment of different types of diseases like respiratory infections, parasitic diseases, and other types of infectious diseases. Moreover, it has traditionally been used as a tonic, antimicrobial agent, antiperiodic, and anti-anthelmintic.[1] *A. scholaris* a tall evergreen tree that can be found growing in the western peninsula and north India. It is also found in various other Eastern Asian countries. Its leaves are arranged in whorls of five to ten (not always seven, which is the most common pattern), hence the name Saptaparna (sapta: seven, parna: leaves) in Sanskrit. It is also known as Chhatim in Bengali and devil tree or dita bark in English.[3] Recent years have seen significant scientific advancements in technology and research because of nanotechnology. The term "nanotechnology" refers to the process of creating, illustrating,

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modifying, and applying things by manipulating size and form at the nanoscale.[4] The more recent field of nanotechnology uses solid colloidal particles of materials that are small (10–100 nm), shaped, and naturally highly different from one another. Because of its many beneficial properties and uses in various sectors, it is widely used in many fields. Alfalfa sprouts were used in the first method of employing plants to create metallic nanoparticles. This was the first instance of silver nanoparticle production carried out in a living plant system. Alfalfa roots can take up silver from agar media and transfer it to the plant's shoots in the same oxidation state. These silver atoms organized themselves to form silver nanoparticles in shoots.[5] Researchers have discovered the exact green paths to avoid these issues. The traditional processes used to produce nanoparticles are costly, hazardous, and unfriendly to the environment. The substances that are found naturally and the byproducts that can be used to create nanoparticles. The use of microorganisms such as bacteria, actinomycetes (prokaryotes), yeasts (eukaryotes), and fungi is one way that green synthesis is classified. Another way is through the use of plants and plant extracts are used to prepare metallic nanoparticles. Using models such as membranes, DNA from viruses, and diatoms to prepare nanoparticles.[6–9] In the present work, we performed a Pharmacognostic study and the high-performance thin layer chromatography (HPTLC) profile of the roots of *A. scholaris* Linn. R. Br. (Saptaparṇa). The different root extracts showed different results. Consequently, this work aims to compare the phytochemical analysis, HPTLC profile, and preparation of synthesis-based silver nanoparticles using root extract and their characterization.

MATERIALS AND METHODS

Reagents and Chemicals

All the reagents and chemicals used in the present work were procured from M/s Merck India, Ltd. Bombay.

Collection of Plant Part Root

A completely mature and fresh *A. scholaris* tree was chosen from the campus of Vikram University, Ujjain. A botanist from the botanical department of Vikram University, Ujjain verified and authenticated the plant.

Processing of Plant Materials

Roots were collected from the healthy and mature tree in the dry weather and the soil and dust were washed with water, dried, and carefully stripped the root wood core. These were ground separately with a grinder to obtain 60 mesh size root powder. The rest of the roots were chopped into small pieces and ground to a coarse powder.

Macroscopic Studies

In macroscopic evaluation, we performed the organoleptic evaluation and measured the properties of crude drugs. We determined the properties (e.g. shape, size, color, odor, taste, base, and texture of root) observed.[10]

Microscopic Studies

Microscopic examinations are used to identification of organized drugs. *Alstonia* root powder histology studies were performed and photographs from different magnifications were taken. Microscopic studies were carried out by preparing the slides of powder drug (Table 2). [11]

Physicochemical Parameters

The following physicochemical properties were measured using well-established methods described in the Ayurvedic Pharmacopoeia of India and WHO recommendations standards (Table 3).[10]

Successive Solvent Extraction

The air-dried root powder is successfully extracted in soxhlet assembly with petroleum ether, chloroform, methanol, and distilled water. Finally, the drug is macerated with chloroform water. Each time, before extraction with the next solvent, the powdered material is dried in a hot-air oven below 50°C. Each extract is concentrated by distilling off the solvent and then evaporating to dryness in a water bath. The extract obtained with each solvent is weighed. Its percentage is calculated in terms of the air-dried weight of plant material. The color and consistency of the extract are noted.[12]

Qualitative Phytochemical Investigation of Root Extract

The plant serves as a biosynthetic laboratory for a wide range of chemicals, including glycosides, alkaloids, volatile oils, tannins, carbohydrates, proteins, and lipids that humans use as sustenance. Secondary metabolites are typically the molecules that give a medication its therapeutic properties. An in-depth analysis of plant metabolism's primary and secondary metabolites is part of a comprehensive study of crude medicine. The current study explores and identifies the greatest number of phytoconstituents that can be found in the various extracts.[13]

HPTLC Fingerprinting Profile of *A. scholaris* Roots Extracts

Dried and powdered roots aqueous extract were subjected to HPTLC analysis.

Sample preparation

1g of the dried and powdered plant part of *A. scholaris* was taken in a 50-round bottom flask and refluxed with distilled water (25 mL) for 1-hour. The extract was filtered through fluted filter paper (Whatman No. 40). The filtrate was concentrated to 10 mL, and taken for HPTLC profiling.[14,15]

Stationary phase

Precoated (support on Aluminium Sheets) silica gel plate. specification: TLC Silica gel 60, Merck.

Mobile phase

Acetone: Water (7:3.) v/v [GR grade solvents, Merck, India]

Plate layout

Stationary phase: Merck, TLC Silica gel 60, Plate format:100 x 100 mm, Application type: Band, Application: Position Y: 8.0 mm, length: 8.0 mm, width: 0 mm, Track: First position X: 15.0 mm, distance: 11.4 mm, Solvent front position: 70 mm.

Application

1 - Linomat 5 (S/N: 280010), Sample solvent type: Methanol,Dosage speed: 150 nL/s**,** Pre dosage volume: 0.20 µL**,** Instrument diagnostics:V alid diagnostics.

Development: Application

Tank: TTC 10x10, Saturation time: 20 minutes, Use saturation pad: true, Use smart ALERT: false, Volume front through 5 mL, Volume rear through 10 mL, Drying time: 5 minutes, Drying temperature: Room temperature

Observation

The chromatograms were visualized in CAMAG TLC visualizer, and scanned using a CAMAG TLC Scanner 4. Software version 3.2.22297.5, Linomat 5 S/N: 280010, TLC Visualizer 2 S/N: 280227. The HPTLC chromatograms are taken at 366nm.

Green Synthesis of Silver Nanoparticles using *A. scholaris* Root Aqueous Extract

For the green synthesis of silver nanoparticles, a 0.01M aqueous solution of silver nitrate (9.99%) was used in the first step. About 100 mL of 0.01M silver nitrate aqueous solution was mixed with 10 mL of aqueous root extract, and the resultant mixture was left to react at an ambient temperature. At regular time intervals, the reaction mixture's color shift from transparent yellow to dark brown observed shows the development of silver nanoparticles. After collecting the silver nanoparticle solution, the reduction process of the silver ions into nanoparticles in the solution was examined using UV-vis spectral analysis. After centrifuging the silver nanoparticle solution, the remaining solvent was evaporated in a dryer, producing a blackish-brown silver nanoparticle powder.[16,17]

Characterization of Silver Nanoparticles

Spectroscopical analysis

The aqueous root extract of *A. scholaris*-based silver nanoparticles was analyzed by UV-visible spectroscopy with sufficient dilution to determine absorbance and λ_{max}. UV spectrum was obtained using Shimadzu 1800 (Shimadzu Corporation, Kyoto, Japan).[5,18]

Particle size analyser by Malvern

The variations in laser light intensity scattered by the particles determine the translational diffusion coefficient of particles due to Brownian motion which dynamic light scattering (DLS) detects. The particle hydrodynamic size is determined using the Mie and Rayleigh scattering theories and modeling the particles as perfect solid spheres.[4]

FE scanning electron microscopy

In a vacuum, silver nanoparticles were left to dry. Morphological evaluation was carried out using Carl Zeiss, Model Supra 55 (Made in UK).[19]

Transmission electron microscopy

The size of the nanoparticles was determined using transmission electron microscopy (TEM) (Tecnai T20, 200 Kev FEI instrument) from Sprint Testing Solutions Mumbai. TEM grids loaded with nanoparticles are kept in a desiccator before transferring to the specimen holder. Image J 1.45 s software (NIH, USA) was used to analyze the particle size of nanoparticles.[20]

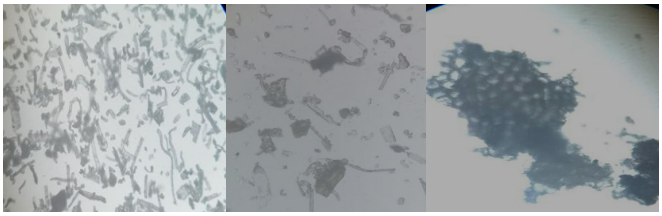
RESULTS AND DISCUSSION

Macroscopic Studies

The macroscopic characters of the root are summarized as follows in Table 1. The outer surface of the root is light yellowish to brown in color. Characteristic odour root and surface were rough. The fracture is short and smooth. Taste bitter.

Powder microscopy of the root part

Powder microscopic description: wood color powder showing abundant groups of stone cells with various sizes, shapes, and thicknesses with distinct radiating pits and striations. Narrow fiber and xylem cell walls are thick. Sclereids with highly thickened and striated walls of various shapes and sizes, different shapes like pentagonal beaded cork cells. Vessel elements are common in the powder and calcium oxalate crystal (Figure 1).



(I) (II) (III)

Figure 1: Powder microscopy of root *A. scholaris*

Table 1: Macroscopic study of *A. scholaris* root

S. No.	*Parameters*	*Plant part (Root)*
1	Color	Light yellowish to brown
2	Odor	Characteristic
3	Size	Short pieces
4	Shape	Taproot is thick and lateral roots developed
5	Taste	Bitter

Table 2: Physicochemical evaluation of *A. scholaris* root

S. No.	*Parameters*	*Part (Root) results in (w/w %)*
1	Loss on drying	4.40
2	Total ash	5.52
3	Acid insoluble ash	1.12
4	90% Alcohol soluble extractive	7.90
5	Water soluble extractive	10.23

Table 3: Estimation of %yield of various extracts of the *A. scholaris* root of the plant

S. No.	*Plant part*	*Parameters*			
		Solvent	*Nature*	*Color*	*%Yield in gm*
1	Root	Petroleum ether	Semi-solid	Brownish	1.2
		Chloroform	Solid	Light brownish	1.1
		Methanol	Semi-solid	Brownish to dark	2.3
		Distill water	Solid	Brownish to dark brown	5.42

Table 4: Phytochemicals analysis of root extracts of *A. scholaris*

S. No.	*Phytoconstituents*	*Petroleum ether*	*Chloroform extract*	*Methanol extract*	*Aqueous extract*
1	Flavonoids	-	+	+	+
2	Alkaloids	-	-	+++	+++
3	Tannin	-	+	+	++
4	Protein	-	+	-	++
5	Saponin	-	+	++	++
6	Glycosides	-	+	+	+
7	Phenols	-	+	++	+++
8	Thiols	-	-	++	-
9	Steroids	+	+	+	++
10	Carbohydrate	-	+	++	+++
11	Volatile oil	+	+	-	_-

Results of the tests indicate very intense (+++), intense (++), Weak (+), or negative (-) reactions against, the particular crude drug sample in the respected solvent.

Qualitative Phytochemicals Screening

The extracts obtained as above are then subjected to qualitative tests for the identification of different phytoconstituents present in it, as mentioned in Table 4.

General comments of HPTLC fingerprinting

In each case, a large number of bands were obtained in Figure 2 at 366 nm. Some of the listed bands could be composite bands for present phytoconstituents. The chromatograms showed that the different alkaloidal components present in the aqueous root extract were very similar to each other.

UV–vis analysis

UV visible spectroscopy spectra of the aqueous root extract of *A. scholaris*-based silver nanoparticles with suitable dilutions. The intensity of the absorption peak at 272 nm is recorded. (Figure 3) shows spectra of aqueous root extract of *A. scholaris*-based silver nanoparticles. UV range from 200 to 700 nm and Millipore water was used as a blank to adjust the baseline.

Particle size

Dynamic light scattering size measurements were performed on a Zetasizer Nano (Malvern Panalytical Ltd., UK) equipped with a maximum 4 mW He−Ne laser emitting at 633 nm and Zetasizer Software, v. 7.13. Samples were contained in ZEN0040 (Malvern Panalytical) low-volume disposable cuvettes. Each measurement was performed at a noninvasive back-scattering angle of 173° (in the dispersant) after thermally equilibrating the sample at 25°C for 3 minutes (Figure 4).

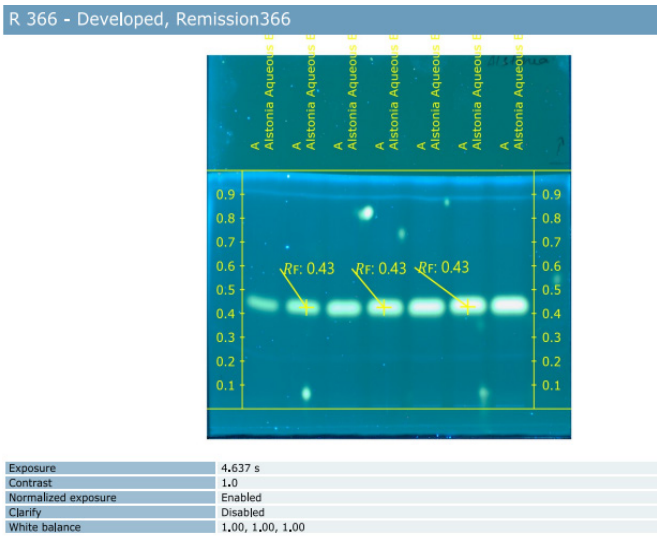


Figure 2: Photograph of HPTLC plate -visualization at 366 nm

FE-SEM

The reflected secondary electrons are captured by detectors and converted into image. Among all the microscopy techniques, SEM is the most efficient method for the measurement of nano-level and identified easily different particle size and shapes of

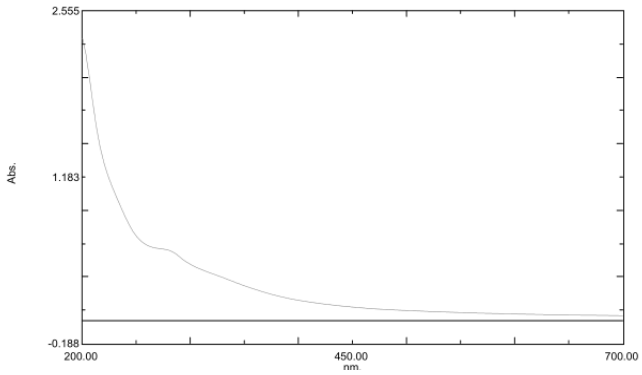


Figure 3: UV-visible spectra of silver nanoparticles prepared by *A. scholaris* aqueous root extract

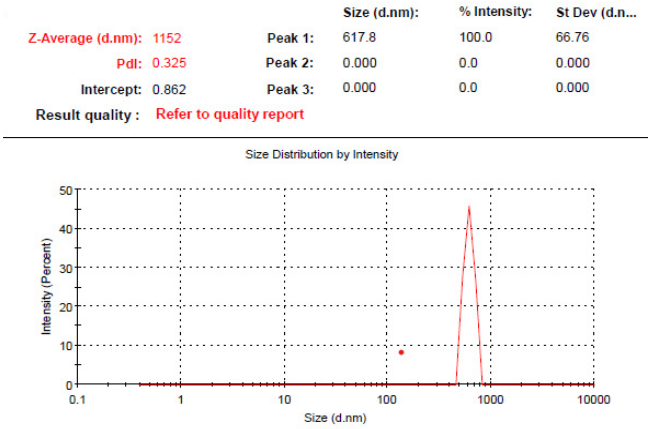


Figure 4: Particle size distribution report graph of silver nanoparticles

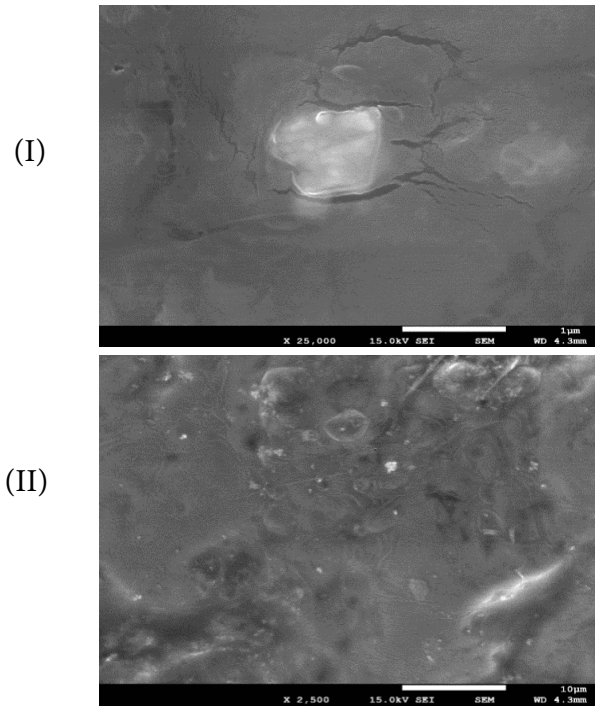


Figure 5: FE-Scanning electron microscopy images of silver nanoparticles

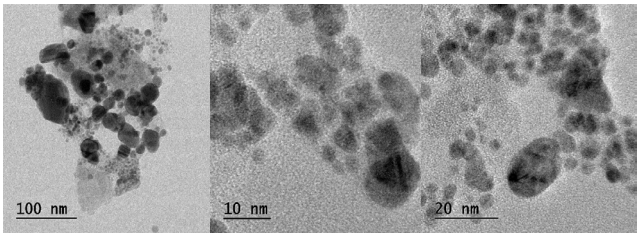


Figure 6: Transmission electron microscopy images of silver nanoparticles

nanoparticles. It can also determine the basic information about surface morphology of nanoparticles in the nanoscale. Figure 5 (I–II) illustrates the FESEM studies of silver nanoparticles.

TEM

Transmission electron microscopy (TEM) is an important method of characterizing nanoparticlemorphology (size and shape).[21-23] TEM images of nanoparticles are typically acquired in brightfield mode, based on the contrast generated by electron scattering from heavy atoms. Different image analysis software are used to obtain statistical distributions in particle size and shape. Individual silver nanoparticles can also be assessed for uniformity in shape or size. TEM study shows measured minimum particle size for silver nanoparticles of 10 nm Figure 6 (I-III).

CONCLUSION

The present study pharmacognostic, HPTLC profiling, and preparation and characterization of silver nanoparticles of aqueous root extract of the plant *A. scholaris* Linn. R. Br. Were performed. These data can be utilised to identify and authenticate the roots of this significant medicinal plant. Important diagnostic and microscopic characteristics include phloem fibers, stone cells, and calcium oxalate crystals. Performed the successive extraction of the plant's root parts, e.g., preliminary phytochemical screening. Phytochemical screening results confirm the presence of different primary and secondary metabolites like alkaloids, glycosides, tannins, etc. HPTLC densitometric analysis of the aqueous root extract was carried out using the CAMAG HPTLC system, and the results were obtained in the form of chromatograms (scanned at the wavelength of 254 and 366 nm). The green synthesis-based method of silver nanoparticles using aqueous root extract of *A. scholaris* was shown to be fast, eco-friendly, and cheaply produced nanoparticles that are nanosized and nano-shaped. So, it can be concluded that green synthesis is an effective and eco-friendly method of producing metal nanoparticles.

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