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GREEN SYNTHESIS AND CHARACTERIZATIONOF SILVER NANO PARTICLES USING LEAF EXTRACTOF *Cichorium intybus:* A SUSTAINABLEAPPROACH

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Abstract:

The utilization of eco-friendly and sustainable approaches in the synthesis of nanoparticles has gained significant attention due to their potential applications in various fields. In this study, we present a green synthesis method for the synthesis of silver nanoparticles (AgNPs) using the leaf extract of Cichorium intybus. Cichorium intybus commonly known as cichory, a widely available and easily cultivable plant, is rich in phytochemicals with inherent reducing and stabilizing properties. The leaves of Cichorium intybus were collected and authenticated. The aqueous leaf extract of Cichorium intybuswas prepared using cold maceration method and the extract was packedtightly. The Physicochemical parameters and phytochemical screening of extract were assessed. The green synthesis process was conducted by mixing aqueous silver nitrate (AgNO3) with Cichorium intybus leaf extract under controlled reaction conditions. The reduction of Ag+ ions to AgNPs was monitored by changes in the solution's color, confirming the formation of nanoparticles. The resulting AgNPs were characterized using various analytical techniques such as UV-Visible spectroscopy, zeta sizer, zeta potential, scanning electron microscopy(SEM), transmission electron microscopy (TEM). The green synthesis of AgNPs using Cichorium intybus leaf extract showcases a sustainable and environmentally friendly approach to nanoparticle production. This study contributes to the growing the knowledge on green nanotechnology and highlights the potential of Cichorium intybus as a valuable source for nanoparticle synthesis.

Keywords:
Green synthesis,Cichorium intybus,Silver nanoparticles, Zeta sizer, Zeta potential

INTRODUCTION

Nanotechnology has created an important change in the pharmaceutical industry, bringing significant technologies in delivery of drugs, diagnostics and personalised treatments. The ability to design materials on the nanoscale has enabled precise and regulated medication administration, better imaging tools and personalised therapy strategies(Shi et al., 2010).A nanoparticle, defined as an extremely small particle with at least one dimension less than one hundred nanometers, is critical to these achievements(Asif et al., 2022). Materials functioning at the nanoscale, typically encompassing from 1 to 100 nanometers, have vastly different characteristics than their bulk counterparts. The green synthesis of silver nanoparticles (AgNPs) is a novel and environmentally friendly method of producing nanoparticles. Green synthesis, contrary to traditional approaches, which frequently involve the use of hazardous chemicals and high-energy procedures, utilises the reducing and stabilising characteristics of natural substances such as plant extracts, microorganisms and biocompatible polymers. This environmentally friendly strategy not only reduces the environmental impact but also provides a safer and more cost-effective method of producing AgNPs(SK et al., 2016). This study aims to explore green synthesis of silver nanoparticles using *Cichorium intybus* leaf extract, seeking to offer an eco-friendly method for silver nanoparticle production with applications in medicine, nanotechnologyand environmental science.

MATERIALS AND METHODS

Collectionof plantmaterials

The leaves of *Cichorium intybus* werecollected by Mr. V. Chelladurai (Retried Research Officer Botanist, Central Council for Research in Ayurveda and Siddha, Govt. of India) from Sengottai,Tirunelveli, Tamilnadu in the month of January 2023. The plantmaterials were identified and authenticated by Dr. S. Mutheeswaran Scientist,Xavier research foundation, Palayamkottai. The collected plant materials were free from diseases andalsocontamination ofotherplants. The registered authentication number of *Cichorium intybus* is XCH-40507.

Plant profile

Cichorium intybus

Fig 1: ***Cichorium intybus***

Synonym: Chicory , blue sailor's succory
Biological source: *Cichorium intybus*
Family: Asteraceae
Geographical source: In India Chicory is found in the north western regions like Kashmir and Punjab and in areas of south India(Shailja Choudhary et al., 2021).

Vernacular names

- Hindi: Kasni, Hinduba
- Tamil: Kasni
- Marathi: Kachani
- Telugu: Kasini, kasini-vittulu
- Kannada: Chikory
- Urdu: Kasni, tukme-e-kasni, barg-e-kasni
- Sanskrit: Kasni

Chemical constituents:

Alpha-amyrin, Taraxerone, Baurenyl acetate, Beta-sitosterol,Sonchuside A, Cichoriolide, Chlorogenic acid, 3,5- Dicaffeolyquinic acid, Cichoralexin, Malic acid, Caffeic acid

Dicaffeoyltartaric acid, chicoric acid , Cyanidin, Glucoside , sesquiterpene lactones and caffeic acid derivatives

Uses:
*Cichorium intybus*possesses various medicinal properties such as hepatoprotective, anti-diabetic, anti-cancer, anti-inflammatory, analgesic, cardiovascular, anti-oxidant, anti-microbial, anthelmintic, anti-malarial, anti-allergic and gastroprotective activities(R & SN, 2013)

Preparation of extracts
The leaves of *Cichorium intybus* was air-dried and coarsely powdered. 500g of each coarse powder was macerated by cold maceration with deionized water with occasional shaking for 72 hr , 48 hrs, 24 hrs. Extract was decanted, filtered, concentrated and kept in a desiccator for complete removal of solvent. Residue was then packed in air tight container.

Physico-chemicalparameters

Ashvalue
The ash component of the crude drug was taken to be the residue left after incineration. It often represents inorganic salts naturally occurring in the drug and perched to it, although it may also include inorganic materials inserted for adulteration purposes. Individual drugs ash values vary within limited bounds, whereas different drugs' ash values vary significantly(Shailja Choudhary et al., 2021)

Totalash

The official analytical methods established by the Pharmacopoeia (I.P.1998) were used, total ash was calculated. One gramme of the sample was weighed into a silica crucible that had been burnt and cooled before being used to measure the total amount of ash. Repeated ignitions were used until the weight remained constant.

Watersolubleash

The ash was filtered through an ash-free filter paper after being heated in 25^0 C of water. The next step was hot water washing. In the silica crucible, the filter paper was fired, cooled and the water-insoluble ash was weighed. By deducting the water insoluble ash from the total ash, the water soluble ash was computed.

Acidinsolubleash

Acid insoluble ash found by five minutes of boiling with 25^0 Cof diluted hydrochloric acid and filtering through ash-free filter paper. In the silica crucible, the filter paper was fired, cooled and weighed.

Extractivevalues

Successive extraction with organic solvents was carried out in order of increasing polarity by maceration using guidelines prescribed by Indian pharmacopoeia.

Watersolubleextractive

In closed flasks, 5g of the whole plant's air-dried coarse powder was macerated for 24 hours with 100ml of chloroform water (99.5ml water + 0.5ml of chloroform), shaking frequently after which it was left to stand for an additional 18 hours. It was quickly filtered after that. In three flat-bottomed shallow dishes with tarred bottoms, 25ml of the filtrate was evaporated to dryness, dried at 105° Celsiusand weighed(Sriwastava et al., 2010).

Ethanol soluble extractive

100ml of 90% ethanol was macerated with 5g of dried, coarse plant powder for a total of 24 hours, with the first 6 hours involving regular shaking and the final 18 hours involving standing. It was quickly filtered after that and 25 ml of the filtrate was evaporated to dryness in three shallow dishes with flat bottoms that had been covered in tar, dried at 105 °Cand weighed.

Lossondrying

Each drug's weight was precisely measured at 1gand it was dried for 5 hours at 105 °C in a china dish coated with tar. Calculating the percentage of weight to weight using the starting weight.

Phytochemicalevaluation

The Phytochemicals including alkaloids, coumarins, tannins, phenols, saponins, flavonoids, carbohydratesand terpenoidswere individually assessed for the aqueous leaf extracts of *Cichorium intybus* using specific tests**(Kennedy & Wightman, 2011)**.

Green synthesis of silver nano particles

- The 10ml aqueous extract of *Cichorium intybus* was taken for the green synthesis of silver nanoparticles.
- To this 90ml of silver nitrate was added to each separately.
- The nano particles were synthesized in (1:9) ratio. The resulting solution was then heated for 30 mins at 50^{o} C in order to increase the synthesis of the silver nanoparticle.
- The colour change was observed as dark brown, Finally, the *Cichorium intybus* silver nanoparticles were separated by centrifugation (8000 rpm) and it was washed with distilled water and allowed to dry at room temperature(Alharbi et al., 2022).

Characterization of Silver Nano Particles

Visual Inspection

The reduction of metal ions in the reaction medium of AgNPs suspension was visually evaluated for colour change.

UV-Vis spectroscopy

A spectrophotometer (UV 4000) was used to scan UV-Vis spectra of nano suspensionfrom 400 to 800 nm for the wavelength of maximum absorbance (max)(Liaqat et al., 2022).

Zeta Sizer and Zeta potential

Zeta potential and size of silver nanoparticles in suspension were detected with Zeta sizer Nano series (Malvern instrument).The size of the nano particles were determined and the potential were reported.

Scanning electron microscopy

A scanning electron microscope (Quanta 250 FEG) operated in high-vacuum mode with a 15 kV acceleration voltage was used to examine the morphology and size of AgNPs.

Transmission electron microscopy

TEM was performed with a JEOL microscope (JEM-1011) at an accelerating voltage of 80 keV. The specimens were dried under vacuum after drying an aqueous Ag NPs drop on the carbon-coated copper TEM grids and they were then loaded into a sample hold. The purpose of this analysis was to assess the shapes and sizes of Ag NPs.

RESULTS

Table: 1 Physiochemical parameters of aqueous leaf extract of *Cichorium intybus*

S.no	Parameters	LECI %
1	Total ash value	4.8
2	Water soluble ash	1.45
3	Acid insoluble ash	2.25
4	Loss on drying	6.24
5	Water soluble extractive	5.81
6	Alcohol soluble extractive	4.43

The table 1 illustrated that the analysis of physicochemical parameters in the powders of *Cichorium intybus* revealed significant differences. The recent study on physicochemical screening provides useful information which may help in authenticating the genuine plant along with nature of phytoconstituents present in it.

Table: 2 Preliminary phytochemical analysis of aqueous leaf extract of *Cichorium intybus*

S.No	Particulars	LECI
1	Phenols	+
2	Flavonoids	+
3	Quinones	-
4	Amino acid	-
5	Terpenoids	+
6	Coumarins	+
7	Alkaloids	+
8	Tannins	+
9	Saponins	+
10	Proteins	-
11	Carbohydrates	+

The aqueous leaves extract of *Cichorium intybus showed* the presence of flavonoids, terpenoids, alkaloids, saponins etc. The result of different phytochemical tests for leaves extracts were given in table. (+)indicates the presence of compounds (-) indicates absence of *compounds*.

Characterizationof Silver Nano Particles

The Characterization of the silver nano particles synthesized using aqueous extract of *Cichorium intybus* demonstrated their stability and desirable characteristics.

Visual inspection

The visual inspection of silver nanoparticles of *Cichorium intybus* revealed a noticeable color change. Initially, the samples exhibited a slight yellow color. However, after the addition of silver nitrate using the green synthesis method, the color transformed into a dark brown color. This alteration in color was attributed to the reduction of silver ions in the presence of plant extracts, leading to the successful formation of silver nanoparticles.

UV –Vis spectroscopy

The UV-visible spectra analysis of silver nano particles revealed characteristic absorption peaks in the wavelength range of 400nm-500nm. The silver nano particles of *Cichorium intybus* showed a strong and sharp peak at 444nm, corresponding to the surface plasmon resonance of the nano particles.These results confirm the successful synthesis of silver nano particles with appropriate size and shape using the respective plant extracts. The intensity of the peaks indicates a high degree of homogeneity and stability in both samples.

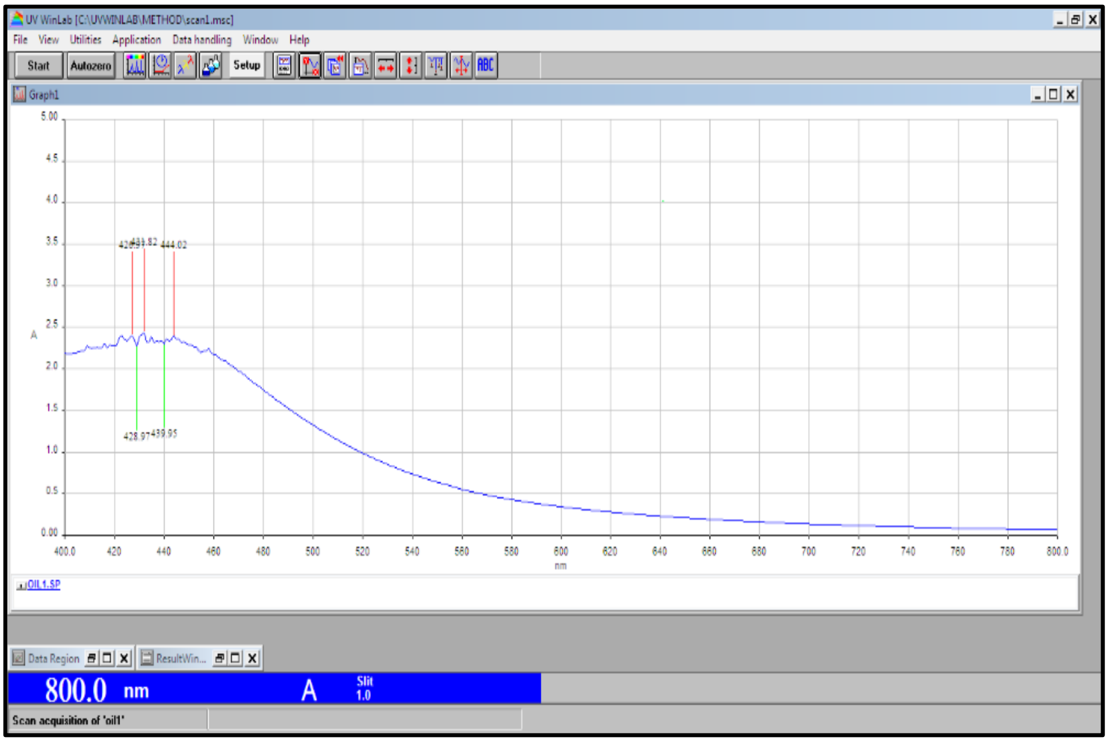

Fig 2: UV-vis spectra of silver nano particles of*Cichorium intybus*

Zeta Sizer and Zeta potential

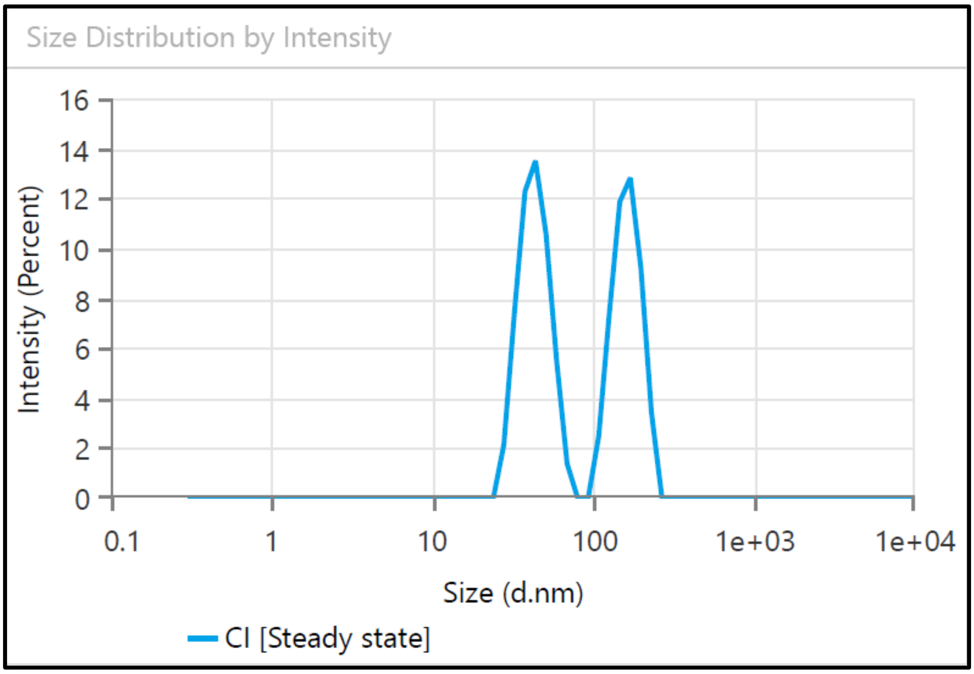

Fig3: Particle Size analysis of *Cichorium intybus* silver nanoparticles

Table 3: Particle Size analysis of *Cichorium intybus* silver nanoparticles

Statistics Table

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Z-Average (nm)	237.9	-	-	237.9	237.9
Polydispersity Index (PI)	0.3917	-	-	0.3917	0.3917
Mean Count Rate (kcps)	271.1	-	-	271.1	271.1
Peak 1 Mean by Intensity ordered by area (nm)	43.69	-	-	43.69	43.69
Peak 2 Mean by Intensity ordered by area (nm)	163.5	-	-	163.5	163.5
Intercept	1.034	-	-	1.034	1.034
Fit Error	0.04711	-	-	0.04711	0.04711

Fig 3& table 3 illustrated thatzeta sizer analysis of silver nano particles prepared using aqueous extracts of *Cichorium intybus* revealed a mean particle size of (237.9 nm) with a narrow size distribution. The recorded data indicates that the majority of the particles fall within this size range, suggesting a uniform and well-dispersed sample.

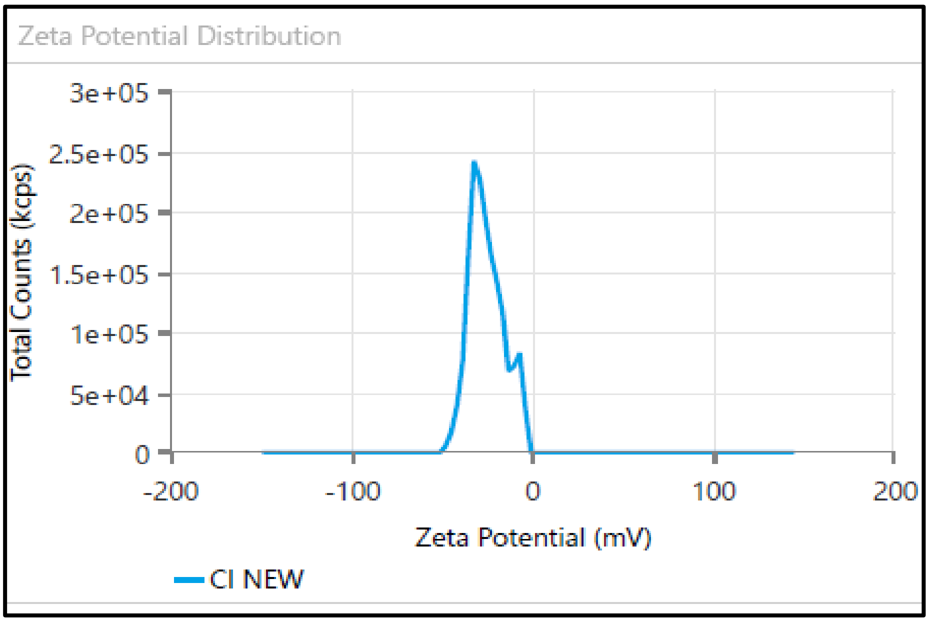

Fig 4: Zeta potential analysis of*Cichorium intybus* silver nanoparticles

Table 4: Zeta potential analysis of *Cichorium intybus* silver nano particles

Statistics Table

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-25.01	-	-	-25.01	-25.01
Zeta Deviation (mV)	9.571	-	-	9.571	9.571
Conductivity (mS/cm)	0.3295	-	-	0.3295	0.3295
Zeta Peak 1 Area (%)	84.87	-	-	84.87	84.87
Zeta Peak 1 Mean (mV)	-27.34	-	-	-27.34	-27.34
Zeta Peak 1 Width (mV)	7.501	-	-	7.501	7.501
Mean Count Rate (kcps)	494.8	-	-	494.8	494.8
Derived Mean Count Rate (kcps)	4123	-	-	4123	4123

Fig 4& Table 4 shows zeta potential measurement demonstrated a (-25.01mV) charge on the surface of the silver nano particles of *Cichorium intybus* .The obtained zeta potential value indicates a stable colloidal suspension, preventing particle agglomeration. These results confirm the successful synthesis of *Cichorium intybus* silver nano particles with desired size and charge characteristics.

Scanning electron microscopy

The SEM study of the synthesised silver nanoparticles of*Cichorium intybus* yielded detailed images of their surface appearance and structure.

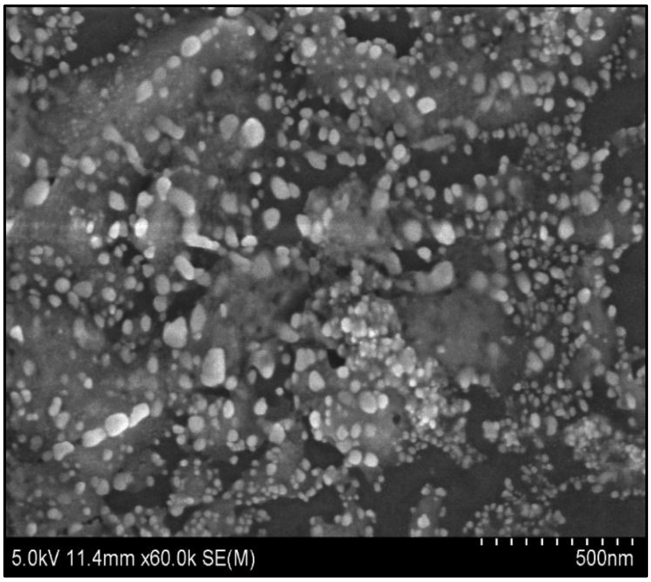

Fig5: SEM images of synthesized silvernanoparticles of *Cichorium intybus*

Fig 5 shows that SEM images revealed well-defined and spherical particles, indicating a successful green synthesis process.These results confirmed the production of silver nanoparticles of *Cichorium intybus*with the desired properties, making them ideal for prospective applications in a variety of disciplines.

Transmission electron microscopy

The TEM study of the synthesised silver nanoparticles of*Cichorium intybus* andrevealed the presence of well defined and spherical nano particles.

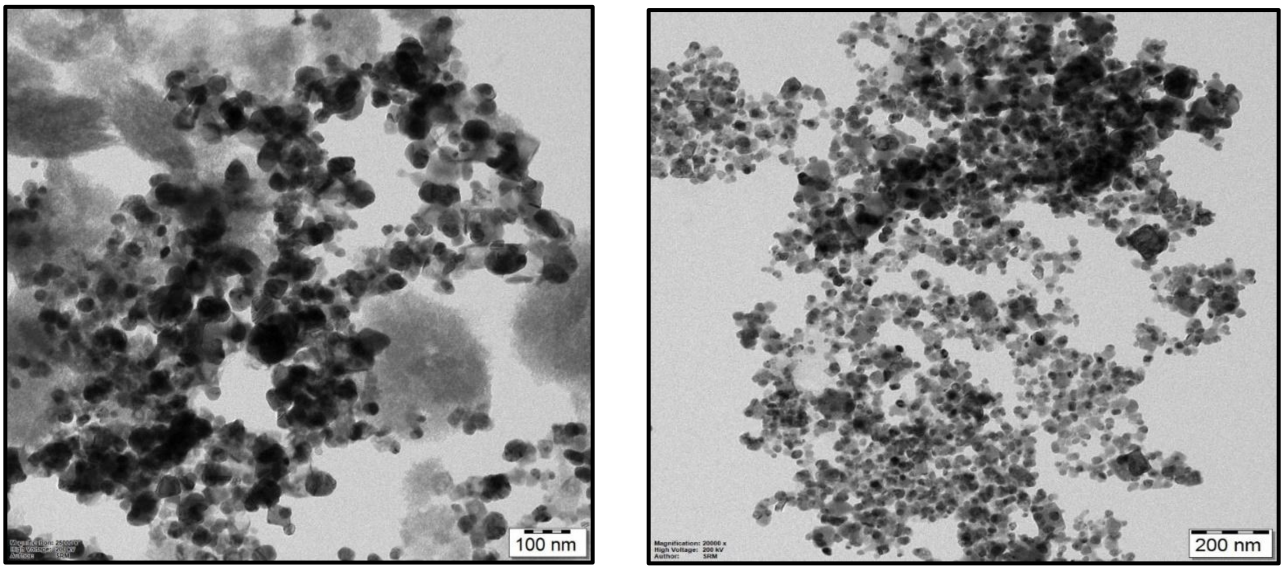

Fig6: TEM images of synthesized silver nanoparticles of *Cichorium intybus*

Fig 6shows TEM images that reveal well-defined and spherical particles, indicating a successful green synthesis process of silver nanoparticles.The silver nanoparticles of *Cichorium intybus* with a uniform size distribution and smooth surface. These desirable properties make silver nano particles promising candidates for various potential applications.

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Authentication Certificate

Based on the Taxonomic features and personal observations the plant material/s submitted by V. Anantharaj is botanically identified and confirmed as here under with the Register No:

Botanical Name: Cichorium intybus L.

Syn. Name:

Family: Compositae - Asteraceae

Part:

Reference: Flora of Tamil Nadu Vol. I, Page 35, 1987 Botanical Survey of India, Coimbatore

Place: Palayamkottai

Signature
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Fig 7: Authenticate certificate Fig8: Prepared silver nano particles

DISCUSSION

In this study, *Cichorium intybus* leaf extract was utilized for eco-friendly silver nanoparticle (AgNP) synthesis. The leaves of *Cichorium intybus* wascollected and authenticated. The aqueous extract of *Cichorium intybus*was preparedand the physicochemical and phytochemical parameters were assessed.The green synthesis involved mixing $AgNO_3$ with *Cichorium intybus* leaf extract, resulting in a color change indicating AgNPs formation. The Silver nanoparticles were characterized using UV-Visible spectroscopy, zeta sizer, zeta potential, SEM and TEM analysis.Our findings showedthat*Cichorium intybus* leaf extract as an eco-friendly and sustainable method for AgNPs production, contributing to the field of green nanotechnology and showcasing its potential for nanoparticle synthesis.

CONCLUSION

The study convincingly demonstrated the successful and eco-friendly synthesis of silver nanoparticles, achieved by utilizing *Cichorium intybus* leaf extract. These silver nanoparticlesderived from *Cichorium intybus* hold a significant promise for developing novel formulations to target various diseases. This research finding emphasizes the potential of plants as valuable sources in advancing nanoparticle synthesis, contributing to the expanding field of nanotechnology.

Ethical approval: Not applicable

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Conflicts of Interest - The authors report no conflicts of interest in this work.

Author contributions: Idea and planning of work was supervised by AV, SM, ER,SJ. Experimental work was carried out by AV under the guidance of SM, ER, SS, SJ.AV and SM wrote the manuscript. All authors read and approved the final manuscript.

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