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Research Paper

Phyto-synthesis of silver nanoparticles using *Mentha sylvestris*: An eco-friendly and cost effective approach

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Abstract

Nanoparticle synthesis is gaining tremendous attention of researcher now a days. Due to high energy requirements and environmental risks associated with physical and chemical techniques, the utilization of plant sources is an attractive and effective choice for synthesis of nanoparticles. Current study has revealed the silver nanoparticles (AgNPs) fabricating potential of aqueous leaf extract of *Mentha sylvestris* without any additional capping or stabilizing agent in a very cost effective as well as environmental friendly way. The bio-reduction of AgNPs was indicated by the change of colour in the $AgNO_3$ solution. The characterization of the prepared AgNPs was carried out by employing sophisticated techniques including Energy dispersive x-ray spectroscopy (EDX), UV-visible spectrometry, X-ray diffraction (XRD) and scanning electron microscopy (SEM) analyses. AgNPs exhibited an absorption peak value at 453 nm as revealed by the UV-visible spectral analysis whereas the reduction rate of the silver ions in was quantified by using Flame Atomic Absorption Spectroscopy (FAAS). The particles were found to exhibit a crystalline zine averaging as 33.3 nm raging from a minimum of 16.2 to a maximum of 56.4 as confirmed by the X-ray diffraction (XRD) analysis confirmed the average crystalline size. The synthesized AgNPs were characterized by a variety of morphological shapes including spherical and rectangular shaped shown by SEM analysis. Presence of Elemental Ag validated the green synthesis of AgNPs confirmed by Energy dispersive X-ray analysis. Present study concludes that *M. sylvestris* species have great potential to bio-fabricate AgNPs in rapid and facetious way.

Introduction

The field of nanoscience and technology is escalating day by day creating an impact on all aspects of human life due to tremendous applications of nanoparticles (Ahmed et al., 2022). It has been widely reported in the literature that the attributes of the nano particles regarding their symmetry and size can exhibit significant variations and differ from those of the macro materials, indicating their potential for a vast array of industrial applications. The properties of nanoparticles related to size may differ significantly from the properties of bulk materials (Nair and Pradeep, 2002). A variety of methods including biological, physical as well as chemical approached have been used across the globe for synthesis of Nanoparticles (NPs). Although the physical and chemical methods are widely practiced due to their effective results, reliability, and high quality NPs production, they pose serious environmental threats to the biological organisms as well as physical environment (Bachheti et al., 2022). Synthesis of NPs by employing metallic substrates has gained enormous popularity owing to the excellent catalytic potential, better magnetic as well as optic characters that eventually lead to better quality NPs production (Suganthy et al. 2018).

Notably gold and silver nanoparticles stand out among the metal nanoparticles due to their exceptional attributes including controllable size, uniform dispersity, robustness, biocompatibility, and effective absorption capacity making them highly promising for various biological application (Wang et al. 2021). Silver metal is renowned since ages attributed to its potential to be used in medicinal practices, especially its antimicrobial potential (Fouda et al. 2021). It has also been used extensively in the industrial sector in a wide array of products and commodities, where the projections have revealed that consumption is expected to touch an annual 800 tons by the year 2025 (Pulit-Prociak and Banach, 2016). Researchers have been experimenting with several fabrication options for AgNPs involving biological organisms mostly Fungi, bacteria, and algae. However, utilizing plants has emerged as a very sustainable and affective fabrication option for AgNPs.

Utilizing plant based materials for AgNP synthesis has many benefits that include their ecologically friendly and facile nature, easy availability, and cost effectiveness. Plant metabolites have proven themselves as excellent reducing as well as stabilizing agents for AgNPs synthesis. These plant metabolites mainly include sugars, phenolic compounds, terpenoid sand flavonoids, along with alcohol (Rani et al.,2023). Different plant parts including leaves, stem, roots, buds along with fruits, seeds, and secretions like latex and other exudates can yield NPs (Rajoriya et al., 2021). Literature review shows that a large number of plants have been utilized for synthesis of AgNPs that include *Mentha arvensis* (Sharma et al., 2018), *Curcuma longa* (Maghimaa and Alharbi, 2020), Fenugreek Rizwana et al., 2021), *Phyllanthus emblica* (Dhar et al., 2021), *Rhizophora stylosa* (Willian et al., 2022) *Azadirachta indica* (Ansari et al., 2023), *Parthenium hysterophorus* (Leyu et al., 2023), *Eupatorium adenophorum* (Dua et al., 2023) and *Vernonia amygdalina* (Tesfaye et al., 2023). Recently, AgNPs have been synthesized by using leaf extracts of *Ricinus communis*, *Musa balbisiana*, *Cardiospermum halicacabum* and *Tridax procumbens* (Okafor et al., 2023).

Mentha sylvestris L. (Lamiaceae) is a perennial medicinal herb widely recognized for its numerous pharmacological and toxicological properties. However, limited information is available regarding its allelopathic characteristic (Islam and Kato-Noguchi 2013). The major chemicals include menthone, menthol, 1, 8-cineole, terpineol-4, pulegone and piperidone. Specific objectives of the study include to evaluate the plant based synthesis of AgNPs using *Mentha sylvestris* and the characterization of final product using advanced analytical characterization techniques. These techniques include X-ray diffraction (XRD) analysis, scanning electron microscopy (SEM), UV-visible spectrometry, Energy dispersive x-ray spectroscopy (EDX).

Materials and Methods

Plant extract Preparation.

The selected plants of *Mentha arvensis* and *Mentha sylvestris* (Fig.1) were collected from the local area and fresh leaves were removed and washed thoroughly in distilled water. For preparation of boiled extracts, finely chopped leaves were boiled in distilled water for 10 minutes. The boiling was carried out in an electric oven followed by the cooling of the sample to room temperature. The cooled solution was than filtered by using filter paper (Whatman No.1).

Silver Nano particles Synthesis

The prepared filtrate was used for NPs synthesis. The leaf extracts up to a volume of 100 ml was poured into an Erlenmeyer flask in which we added 50 ml each of deionized water and silver nitrate aqueous solution of 20mM. The mixing was carried out at ambient temperature followed by thorough shaking. The resultant solution was centrifuged after 24 hrs. for a time of 4 minutes at a speed of 14000 rpm in centrifuge apparatus in order to separate the synthesized AgNPs from the rest of the substrate, mainly raw biomass. The AgNPs were thoroughly washed by using acetone as a solvent for purification.

The prepared AgNPs were than characterized by using analytical techniques to reveal their morphological properties as well as crystalline nature. UV-vis spectroscopy analysis was carried out by using Perkin Elmer Spectrophotometer (Lambda 950) whereas XRD analysis was carried out by using Bruker D-8 diffractometer. Formula and protocols given by the Debye-Scherrer were followed for the quantification and measurement of crystalline size of NPs inferred from the XRD peaks width. Scanning Electron Microscope (Model JEOL JSM-6510 LV) was used for the EDX analysis as well as Scanning electron microscopy of the AgNPs.

Results

Silver Nanoparticles Synthesis

The synthesis of AgNPs was determined by using visual indicators i.e., the colour change stage of the solution during the synthesis reaction, that indicated successful synthesis of NPs. The colour of the stalk solution used for NPs synthesis changes to Dark Brown from the initial Pale yellow tone in a very short time (seconds), indicating NPs synthesis (Fig.1).

Figure 1 *Mentha sylvestris* L. (Huds) and Leaf extract (a) AgNPs in solution (b)

UV-Visible spectroscopy analysis

The plant based green synthesis of Nano particles was confirmed by the results of the UV-visible spectroscopy analysis that were acquired after a time of 24 hrs. of the synthesis reaction. The mixture used for the reaction was contained in the cuvettes made up of quartz material and were exposed to spectral analysis. The UV-vis spectral range of the analysis was characterized with a wide wavelength range starting from a minimum of 300 nm to the highest value of 700 nm. The analysis results indicated the presence of a sharply defined prominent absorption peak that was recorded at a value of 453 nm. The recording of this peak is attributed to the phenomenon of Surface Plasmon Resonance that is a characteristic identifying feature of synthesized silver nanoparticles (Fig. 2).

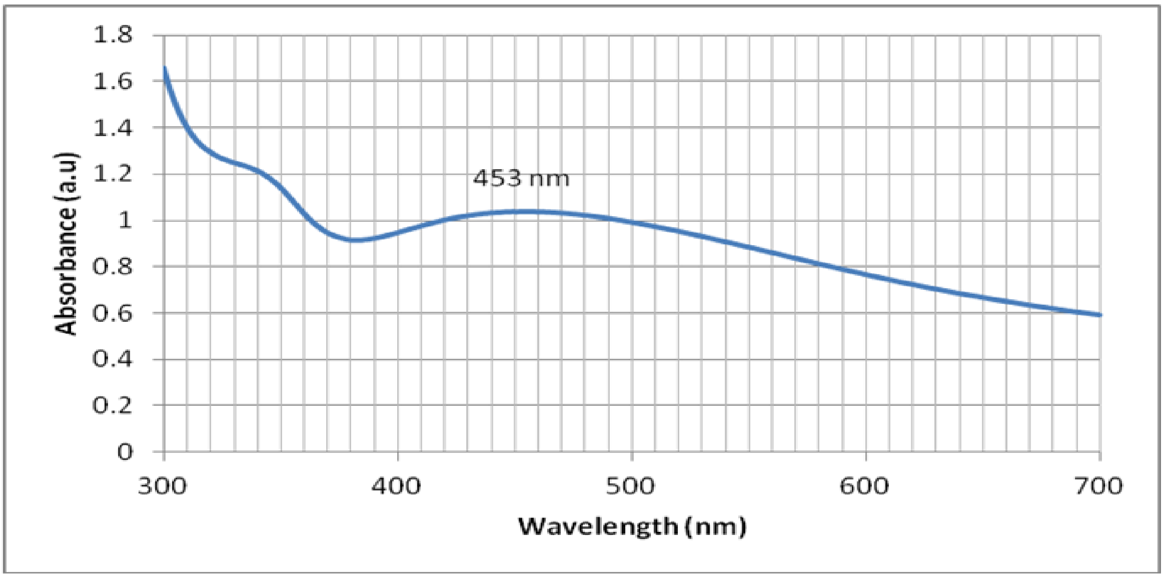

Figure 2 UV-Visible spectrum of AgNPs

Flame Atomic Absorption Spectroscopy (FAAS)

The concentration and quantity of the metal atoms in the analyzed samples was determined by employing Flame Atomic Absorption Spectroscopy (FAAS) (Fig. 3). The sample was extracted after 0, 1 and 6 hours of reaction. It was revealed that the silver ions were present in the solution having a concentration of 216 ppm/ml when leaf extract was added into $AgNO_3$ solution at initial stage. A rapid decline in the Ag ions concentrations was recorded as the value dropped to 49 ppm/ml after 60 minutes whereas it further dropped to a mere 6 ppm/ml value after 6 hours.

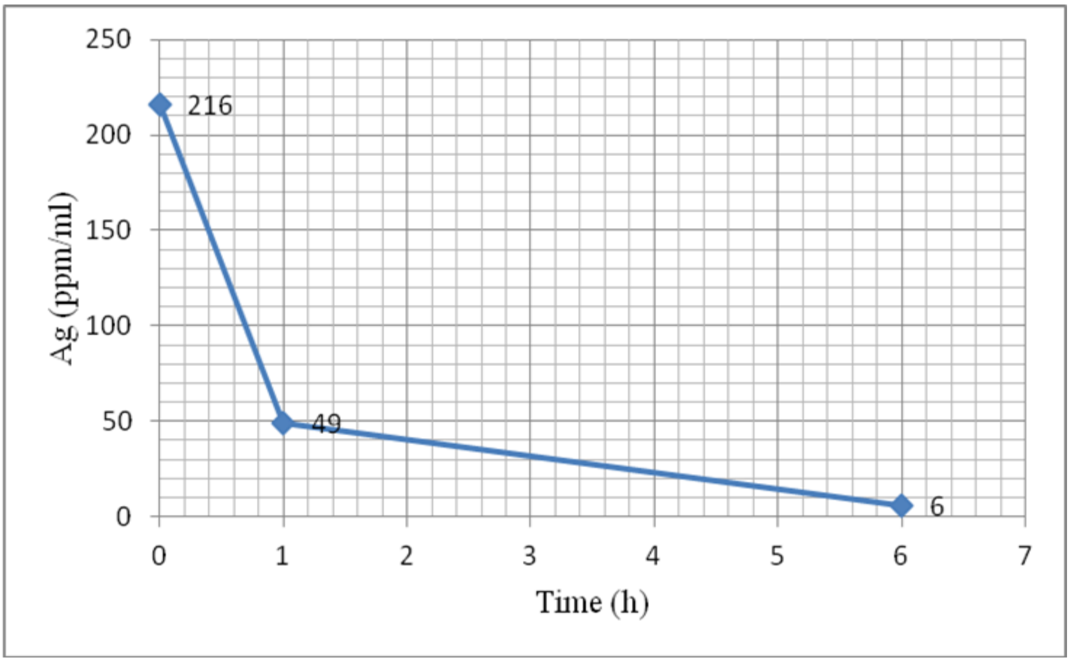

Figure 3 FAAS analysis of AgNPs solution

Scanning electron microscopy (SEM) analysis

The Morphological characterization of the synthesized AgNPs was carried out by SEM analysis. The results of the morphological characterization of the synthesized silver nanoparticles (AgNPs), as revealed by scanning electron microscopy (SEM) analysis, present insights into their structural diversity and distribution. The SEM micrograph depicts a high density of silver nanoparticles, indicative of a successful synthesis process. SEM Analysis revealed that AgNPs exhibited a diverse Morphology. The NPs were characterized with multiple appearances and shapes including spherical, polyhedral, floral as well as irregular (Fig. 4). Unlike conventional nanoparticles that often display a uniform shape, the AgNPs in this study showcased a variety of appearances and shapes. The presence of spherical, polyhedral, floral, and irregular-shaped nanoparticles suggests a complex interplay of factors during the synthesis process, such as precursor concentrations, reaction kinetics, and stabilizing agents.

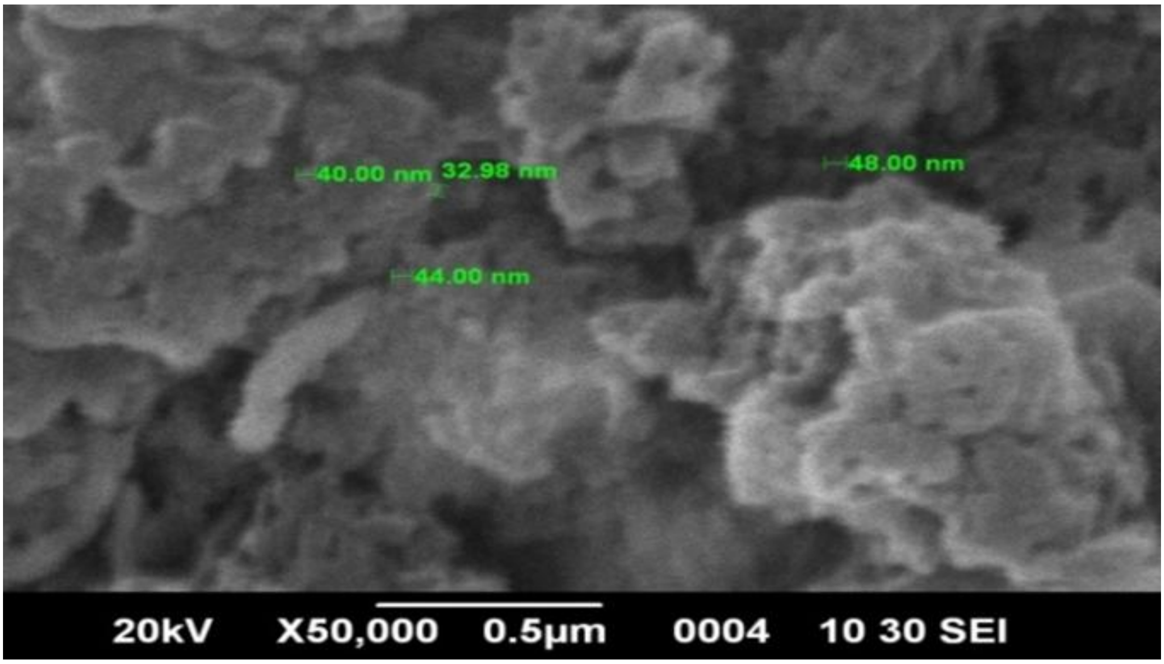

Figure 4 SEM images of silver nanoparticles

EDX Analysis

The results obtained by the spectrum of energy dispersive X-ray validated and confirmed the green synthesis of AgNPs using plant material. A sharp peak indicating significant optical resonance was recorded at 3 KeV attributed to the surface plasmon resonance phenomenon which further confirmed the reduction of AgNPs by the plant based substrate, i.e., Phyto-reduction. The results reveal the crystalline nature of the synthesized AgNPs as the recorded absorbance peak at 3 KeV corresponds with that of metallic nanocrystals of Silver.

Table 1 XRD analysis of the silver nanoparticles

Peak	2-Theta	d-spacing (Å)	FWHM	Relative peak intensity (%)	Particle size (nm)
111	38.2720	2.35177	0.3149	100.00	56.42
200	44.4996	2.03604	0.3542	25.62	27.05
220	64.6452	1.44185	0.2558	24.16	16.20
311	77.6003	1.22932	0.4320	22.03	35.45

X-Ray Diffraction Analysis (XRD)

The size of the synthesized AgNPs by using *Mentha sylvestris* leaf extracts was evaluated and measured by employing X-ray Diffraction (XRD) technique. Multiple diffraction peaks were revealed by the XRD analysis which is attributed to the Bragg's reflection phenomenon.

The sharp diffraction peaks in the 2θ spectrum were observed at 38.27, 44.49, 64.64, 77.60. The recorded values ranged from a minimum of 20 to a maximum of 80 synchronized and correlated with the 111, 200, 220 and 311 planes respectively (Fig. 5). The protocol and indices given by Debye Scherrer were followed to deduce the information about the size of synthesized Crystalline AgNPs. The width of peaks generated by the XRD analysis were quantified for size determination which revealed an average size of 33.38 nm for the Synthesized NPs. A wide range of variation was observed in the NPs size ranging from a minimum of 16.2 nm to a maximum of 56.4 nm (Table 2).

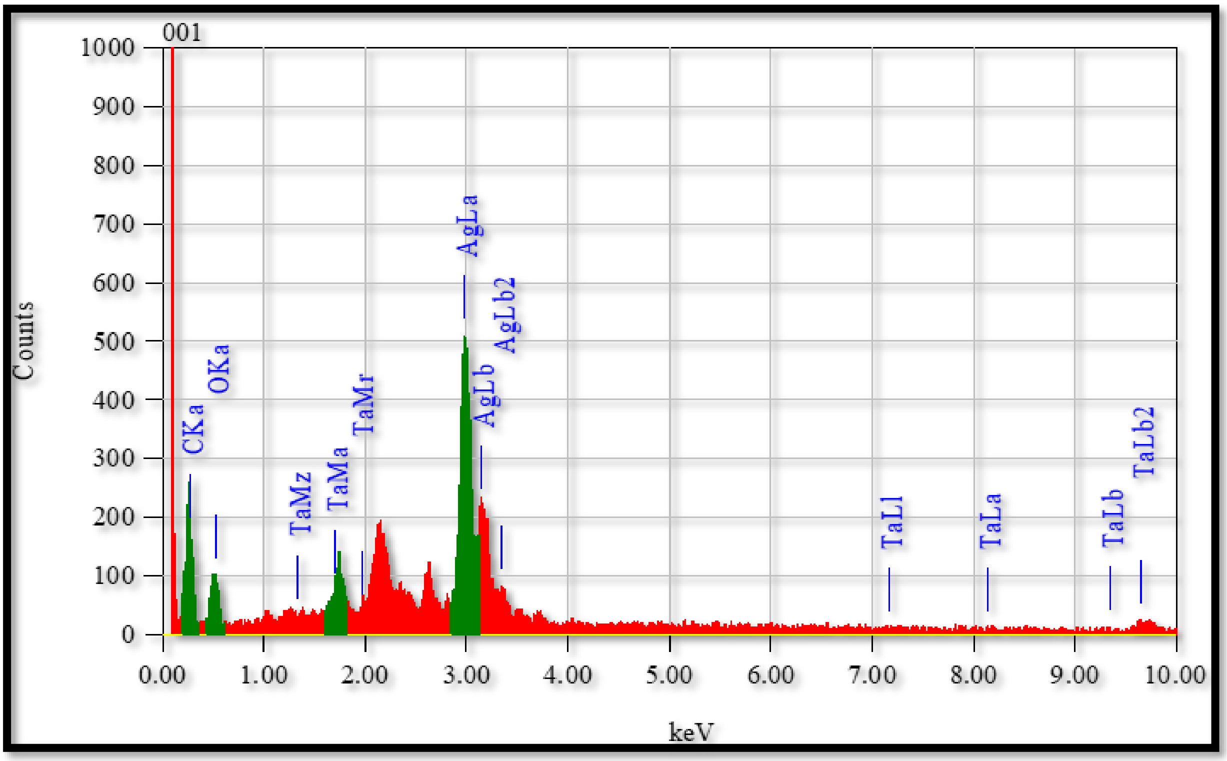

Figure 5 EDX spectrum of AgNPs

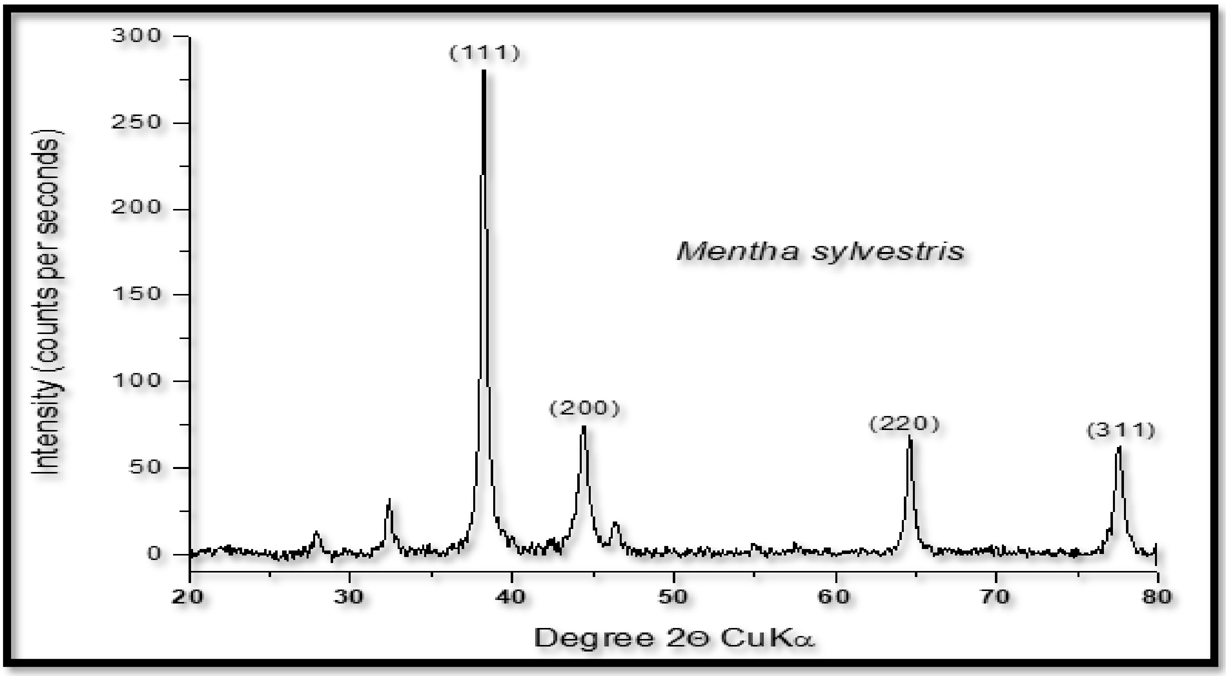

Figure 6 XRD spectrum of AgNPs

Table 2 EDX Analysis of AgNPs

Element	(keV)	Mass%	Error%	Atom%	K
C K	**0.277**	**15.71**	**0.39**	**38.96**	**12.3801**
O K	**0.525**	**24.32**	**3.72**	**45.30**	**11.6834**
Ag L	**2.983**	**52.51**	**2.12**	**14.51**	**68.6078**
Ta L	**3.132**	**7.46**	**2.98**	**1.23**	**7.3287**
Total				**100.00**	

Discussion

The Phytosynthesis of AgNPs is a very integrated and complex process involving inputs from both the $AgNO_3$ salt as well as the plant biomass. The Ag ions are obtained from the salt whereas the plant based green substrate acts as a stabilizing as well as reducing agent for the synthesis of NPs (Ahn et al., 2019). Visual colour change indicator was used to detect the formation of the NPs in our scheme where the rapid change of pale yellow substrate into a final dark brown tone indicated the NPs presence. This state was recorded immediately after addition of the plant extract into the silver salt solution. The dark brown color is indicative and representative colour of the AgNPs which is attributed to the plasmon resonance phenomenon (Elumalai et al., 2010).

Phyto-reduction of Ag+ to Ag0 was further monitored by analyzing reaction mixture in UV-Vis spectrometer. A broad peak at 453 nm wavelength showed the polydisperse AgNPs. Similar absorption band has been reported in previous investigation due to surface plasmon resonance of AgNPs in solution (Pirtarighat et al., 2019). FAAS analysis determined the amount of AgNPs in sample. Our results show that the reaction mixture exhibited an Ag ion concentration of 216 ppm/ml at the initial starting state i.e., Zero time of the reaction. The rate of phyto-reduction was initially faster as after one hour's amount of silver ion left in the mixture was 49 ppm/ml. Then, the amount of ionic silver has further decreased to 6 ppm/ml after 6 hours of reaction.

These results showed that phyto-reduction of ionic silver into AgNPs using leaf extract of *M. sylvestris* is quick and rapid. (Singhal et al., 2011). SEM analysis showed the morphology of AgNPs. They were agglomerated with irregular, polyhedral, floral, and spherical shapes. The diverse morphology observed in the synthesized AgNPs holds significant implications for their properties and potential applications. Different shapes and surface structures can influence the optical, catalytic, and antimicrobial properties of nanoparticles, making them highly versatile for various biomedical, environmental, and industrial applications. Understanding the morphology–property relationships of AgNPs is therefore essential for tailoring their functionality to specific application requirements (Jain et al., 2021). The irregular-shaped nanoparticles further add to the complexity of the morphological landscape, suggesting potential nucleation and growth mechanisms that deviate from classical models. These irregular shapes could arise from variations in nucleation sites, surface energy effects, or aggregation phenomena occurring during the synthesis process. EDX spectrum showed that silver is the chief component of synthesized AgNPs. EDX spectrum revealed presence of C, O and Cd. Presence of these ions suggests that the plant extract used for the NP preparation may have acted as a source of these ions which finally have adsorbed to the surface of synthesized NPs and were detected by the analysis (Erdogan et al., 2019). Sharply defined prominent diffraction peaks have been revealed by the XRD analysis XRD analysis in the 2θ spectrum. These peaks were recorded at values of 38.27, 44.49, 64.64, 77.60 respectively exhibiting wide range of variations. The detected peaks ranged from a minimum value of 20 to a highest value of 60 in this reaction which corresponds to the planar values of 111, 200, 220 and 311. This validates the crystalline cubic symmetry of the Siver NPs due to Bragg's reflection (Dua et al., 2023). XRD analysis presented contributes significantly to the characterization of AgNPs synthesized using *Mentha sylvestris* leaf extracts. By providing detailed information about the crystalline structure and size distribution of the nanoparticles, this study lays a solid foundation for further investigation and utilization of these AgNPs in various biomedical, environmental, and industrial applications This variation could be attributed to various factors such as the concentration of leaf extracts used in the synthesis, reaction conditions, and inherent properties of the leaf extract-mediated synthesis process. Understanding this size variation is essential for tailoring the synthesis process to achieve desired nanoparticle characteristics for specific applications. Our analysis has shown an average NP size of 33.38 nm for the synthesized particles. These findings are in line the results of several NP synthesis projects where the Synthesized NPs were found characterized with a size range of 33.5-30.5. These NPs have been synthesized using leaf extracts *Parthenium hysterophorus* (Leyu et al., 2023). Current study discovered that leaf extracts of *M. sylvestris* species are useful for simple, quick, cheap and environment friendly fabrication of AgNPs.

Conclusion

We have utilized the extracts obtained from the leaves of *Mentha sylvestris* for the green synthesis of AgNPs. The Plant extract has acted as a significant reducing agent for Ag^+ ions obtained from $AgNO_3$ aqueous solution of silver nitrate ($AgNO_3$), reducing it successfully to Ag^0. Primary indication of AgNPs synthesis as confirmed by visual indicators of color change stage observed in the mixture of NPs preparation reaction. Plant based synthesis of the AgNPs was validated by the recorded spectral band at the wavelength of 453 nm as shown by UV-Vis spectroscopy analysis. SEM analysis revealed that the NPs were crystalline in nature characterized by an average size recorded as 33.38 nm successfully prepared using an aqueous leaf extract of *M. sylvestris* at an ambient temperature without any addition capping or stabilizing agent. SEM analysis provides valuable insights into the morphological diversity of the synthesized AgNPs, highlighting their complex and multifaceted nature. Further investigations into the underlying synthesis mechanisms and structure–property relationships are warranted to fully harness the potential of these silver nanoparticles for practical applications. It is concluded that using *M. sylvestris* for the synthesis of nanoparticles can provide quick, cheap, and eco-friendly alternative over the conventional synthesis methods. Also, it can be easily scaled up commercially.

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