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Original Research Article

Biosynthesis Of Silver Nanoparticles By Chrysanthemum Moriflorum Flower Extract: Characterization And Their Antimicrobial Activities

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ABSTRACT
The main objective of this study is to offer a rapid and efficient green production of silver nanoparticles with known antibacterial properties. In the current study, a biological approach was applied at room temperature to biosynthesize silver nanoparticles from Chrysanthemum moriflorum flower extract. Aqueous floral extract from Chrysanthemum moriflorum was used to make AgNPs from silver ions fast. This process became apparent when silver nitrate solution was added to the bright yellow flower extract solution, which caused it to turn brown. The biosynthesized AgNPs were examined using transmission electron microscopy (TEM) and ultraviolet-visible (UV-Vis) spectroscopy (TEM). The UV spectra revealed AgNPs' distinctive surface plasmon resonance band at 431 nm. The generated AgNPs, a powerful broad spectrum antibacterial agent, successfully inhibited Escherichia coli and Bacillus sp. To combat the rise in antibiotic resistance, AgNPs made from environmentally acceptable ingredients like flower extract offer a potential weapon.

Keywords: Chrysanthemum moriflorum, green synthesis, silver nanoparticles, TEM, antimicrobial property

INTRODUCTION
Nanotechnology is a contemporary field of study that focuses on the synthesis, design, and manipulation of particles with sizes ranging from 1 to 100 nm. The study of tiny objects, known as nanotechnology, has applications in all scientific disciplines, including chemistry, biology, physics, material science, and engineering. They are employed for coating surgical, cardiovascular, and urological instruments and materials [1]. The medical and pharmaceutical industries are about to undergo a revolution thanks to a plethora of new materials and methods that nanotechnology will bring to the table. It is expanding quickly thanks to the creation of nanoparticles and Nano products, both of which have unique physicochemical qualities that set them apart from larger matter. The AgNPs are the most well-known product in the arena of nanotechnology among all the noble metal nanoparticles because of their distinctive qualities, including chemical stability, high conductivity, antiviral, antifungal, and anti-inflammatory activity [2]. Due to their strong antibacterial efficiency against viruses, bacteria, and eukaryotic micro-organisms, silver nanoparticles have proven to be the most effective [1]. One of the fundamental substances that make up our world is silver. It is a rare, naturally occurring element that is much more malleable and flexible than gold and only slightly harder than gold.
Ag0, Ag2+, Ag3+ are four distinct oxidation states in which silver can exist. The latter are unstable in an aquatic environment, while the first two are the most common [2,3]. Metallic salts like AgNO3 and Silver chloride, however, are soluble in water [4]. Metallic silver itself is insoluble in

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water. The surgical prosthesis, splints, fungicides, and coins all include metallic silver. Infectious disorders including syphilis and gonorrhoea, as well as mental illness, gastroenteritis, nicotine addiction, and other conditions have all been treated with soluble silver compounds like silver slats [5].

Investigations have demonstrated that these quantities of Ag+ ions are too low to cause lead toxicity, even though the availability of free silver ions determines the acute toxicity of silver in the environment [6]. While soluble Ag compounds are more easily absorbed and have the potential to have negative impacts on health, metallic Ag appears to offer little risk to health [7]. Due to its many applications, silver can be exposed to the body through a multitude of entrance points. The main entrance point for Ag compounds and colloidal Ag proteins is through the digestive system. Estimated daily dietary consumption of silver is 70–90 gm. Silver is comparatively non-toxic [9] since it isn't thought to be hazardous to the immunological, cardiovascular, neurological, or reproductive systems and isn't thought to be carcinogenic [8]. Silver nanoparticles have been created using a variety of techniques, including physical, chemical, and biological ones. The earlier processes for creating silver nanoparticles were poisonous, and dangerous substances were employed.

Thus, "green synthesis" refers to the employment of environmentally benign technologies for the synthesis of AgNPs. Because it is more affordable, eco-friendly, and only requires one stage, green synthesis is favoured to conventional synthesis [10]. It also requires less energy, harmful chemicals, high pressure, and temperature. Numerous studies have documented the creation of silver nanoparticles using substances such plant leaf extract, root, stem, bark, leaf, fruit, bud, and latex [11]. Plant extracts are simply combined with a solution of the metal salt at room temperature to create NPs. Within minutes, the response is finished. This method has been used to create nanoparticles of silver, gold, and numerous other metals [12]. It is known that the type of plant extract, its content, the concentration of the metal salt, temperature, die pH, and contact duration all have an impact on how quickly nanoparticles are produced, how many there are, and other factors [13]. AgNP sizes and morphologies are strongly influenced by the solution's concentration. For instance, their distribution and size are dependent on the concentration of the precursor metal salts as well as the polysaccharides. It is well known that AgNPs' morphology has a significant impact on how they behave.

Complex combinations of molecular, surface, and crystalline characteristics lead to the morphological change [14]. The stimulation of localised surface plasmon resonance, which is a phenomenon, is what causes AgNPs to absorb electromagnetic radiation in the visible range between 380 and 450 nm, according to various studies [15]. A light wave confined inside conductive nanoparticles (NPs) that are slighter than the wavelength of light causes the optical phenomenon known as (LSPR). The interaction of electrons in a conduction band with incoming light causes the phenomenon [16].

MATERIALS AND METHODS

Preparation of the extract

Fresh *C. moriflorum* flowers were chopped into small pieces after being properly cleaned with tap water, then double distilled water. 100ml of double-distilled water was used to boil about 5g of cut flowers before they were filtered through Whatmann No. 1 filter paper. The filtrate was utilized as a bioreductant for the production of AgNPs after being cooled to room temperature.

ISSN 2515-8260 Volume 10, Issue 01, 2023

Bio synthesis of silver nanoparticles

With a ratio of 1:9 ratio-optimized concentrations (10 ml of extract to 90 ml of aqueous solution of 1 mM AgNO3), the solution of 1 mM AgNO3 was added dropwise in the reaction mixture of *C.moriflorum* flower extract in separate 250 ml Erlenmeyer flasks. Both were then dark incubated at 35 °C for approximately 48 h. By changing the colour from light yellow to dark brown and then spectrophotometric measurement, silver ions (Ag+) were reduced to AgNPs (Ag0) (Netala et al., 2015)

CHARACTERIZATION OF THE SYNTHESIZED SILVER NANOPARTICLES

UV–Visible spectrum for synthesized nanoparticles

The spectrum 50 ANALYTIKJENA Spectrophotometer, which was operated in the wavelength range of 400-800 nm, was used to capture the UV-Vis spectrum of this solution. The silver nanoparticles' unique UV-Vis absorption, known as the localized surface Plasmon resonance (LSPR), was observed.

FT-IR analysis

The mid-IR range of 400–4000 cm-1 is where the FT-IR spectrum was recorded. Utilizing the attenuated total reflectance approach, the spectrum was captured. The sample was directly inserted into the KBr crystal, and the transmittance mode was used to record the spectrum.

XRD analysis

AgNPs were synthesised, and the composition and structure were determined by XRD analysis. An X'Pert Pro X-ray diffractometer (PAN analytical BV) operating at a voltage of 40kV and a current of 30mA was used to measure the generation of AgNPs using Cu Ka radiation in h-2h configurations. The crystallite domain size was calculated using the Scherrer formula and the presumption that non-uniform stresses are absent from the XRD peaks. D1 = 40.94 k/b cos h, where k is the X-ray wavelength and h is the diffraction angle, and b is the full width at half maximum. The average crystallite domain size parallel to the reflecting planes is denoted by the D.

TEM analysis

The diameter of the biologically produced AgNPs was measured and seen via TEM analysis. The sample was dissolved in water that had twice been refined. On a "staining pad," a drop of thin dispersion was applied. The coated side of a carbon-coated copper grid was put into the drop. The grid was removed and air dried after roughly 10 minutes. JEOL JEM 2100 Transmission Electron Microscope was then used to screen the results (JOEL Corp, Tokyo, Japan).

Antibacterial activity of synthesized AgNPs

The classic Kirby-Bauer disc diffusion method was used to test the antibacterial effectiveness against both Gram-positive and Gram-negative bacteria. On the Mueller Hinton Agar plates, the bacterial suspension (108 colony-forming units/mL) was swabbed using sterile cotton swabs. The 6 mm-diameter sterile disc from Hi-Media Laboratories Pvt Ltd was impregnated with the following four substances: an aqueous *C. moriflorum* extract, AgNO3, and manufactured AgNPs at quantities of 25 g/disc. As a control, chloramphenicol was utilized. After being gently squeezed, the discs were incubated for 24 hours at 37°C.

A zone scale developed by Hi-Media Laboratories Pvt Ltd was used to measure the zone of inhibition in millimeters inside the diameter of each disc. The conventional broth microdilution method was utilized to determine the MIC. The tests were carried out in triplicate.

RESULT

UV-Visible Spectroscopy

ISSN 2515-8260 Volume 10, Issue 01, 2023

An indirect method to investigate the bioreduction of AgNPs from aqueous AgNO3 solution is UV-vis spectroscopy. Figure 1 displays the produced AgNPs' UV-vis absorption spectrum over three different time intervals (30min, 3 hour and 24 hour). Due to the simultaneous vibration of the AgNPs electrons in resonance with a light wave, the AgNPs exhibits the SPR absorption band with free electrons. The stimulation of longitudinal Plasmon vibrations of AgNPs in the solution led to the observation of a large absorption peak at 431 nm, a band characteristic of the Ag [17].

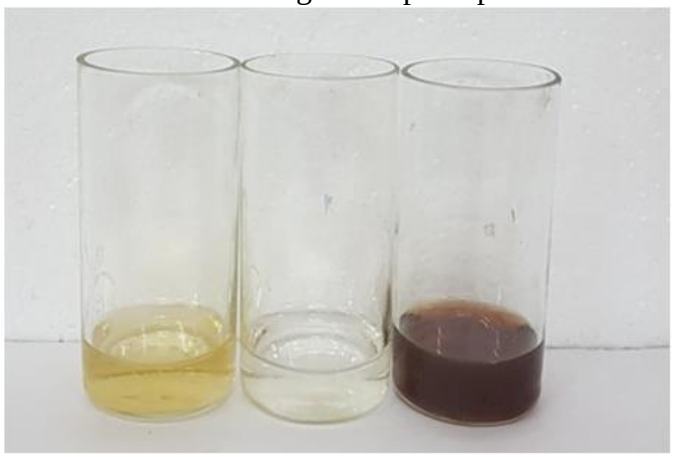

Fig 1: Visual observation (A) *C. moriflorum* flower extract (B) $AgNo_3$ solution(C) Synthesized AgNPs

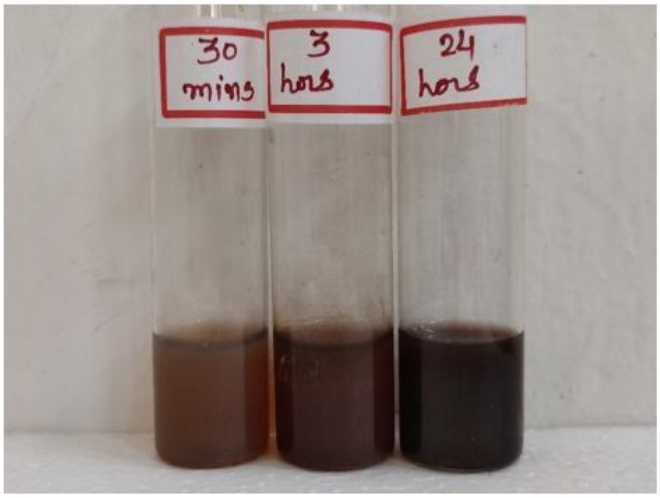

Fig 2: Showing different time interval color intensity of *C. moriflorum* flower extract with AgNPs

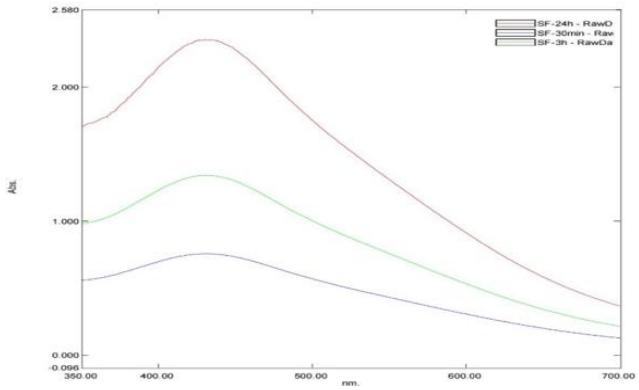

Fig 3: UV-visible spectra of *C. moriflorum* AgNPs obtained using flower extract of *C. moriflorum* at different time interval

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FTIR Analysis

The potential biomolecules that could serve as a capping and reducing agent for the AgNPs produced by extract were investigated using the Fourier transform infrared spectroscopy (FTIR) measurement (Figure 1B). The N-H stretching mode is responsible for the intense broad band at 3458.51 cm-1, while the aldehydic C-H stretching mode is responsible for the one at 2081.01 cm-1. 1632.40 cm-1 C=C, 1384.43 cm-1 -C-N, 1102.23 cm-1 alkyl amine, 829.63 cm-1 C-Cl alkyl halides, and 612.31 cm-1 C-Br are the highest values. The occurrence of the aromatic group is confirmed by the exciting band at 1592 cm-1, which results from C=C stretching in the aromatic ring [18]. Another study [19] supports the hypothesis that water-soluble flavonoids are involved in the reduction of metal ions utilising plant extracts.

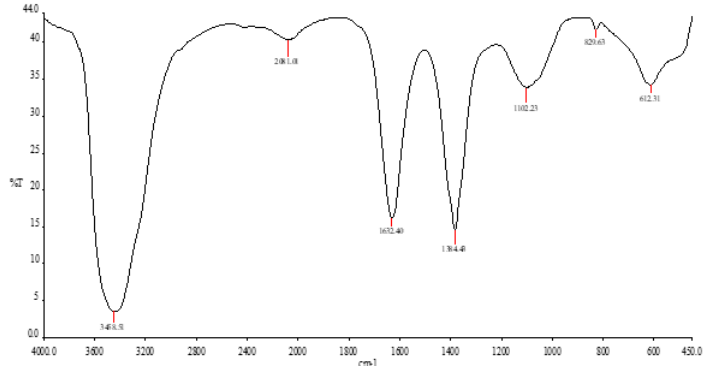

Fig 4: FTIR spectra of synthesized AgNPs of *C. moriflorum* flower extract

XRD Analysis

The XRD patterns for AgNP produced by extracts are shown in Figure 2A. At 2 = 38.18, 44.33, 64.51, and 77.46, which correspond to the (111), (200), (220), and (311) based on the band for face-centered cubic structures of silver, respectively, four key characteristic diffraction peaks for silver were found (JCPDS Card Number 87-0717). The absence of peaks from other phases indicates that single-phase Ag with cubic shape nanoparticles was directly produced. In general, crystallite size is correlated with the breadth of XRD peaks. From the half width of the diffraction peaks, the average crystallite diameter was calculated using the Debye-Scherrer equation: $D = (k\lambda)/ (\beta \cos \theta)$ (1)

Where D is the average size of the powder's crystals, k is a constant, is the wavelength of CuK, is the full width at half-maximum, and is the angle of Bragg diffraction. To determine the size of the crystal, the (111) plane was used. The Debye-Scherrers equation indicates that the produced AgNPs has an average crystallite size of 38.08 nm. The traditional design of bio-created silver nanoparticles is discovered to have an FCC structure when compared to JCPDS-file no: 89-3722. Using the Debye-equation, Scherrer's the average crystalline size of the silver nanoparticles was calculated [20].

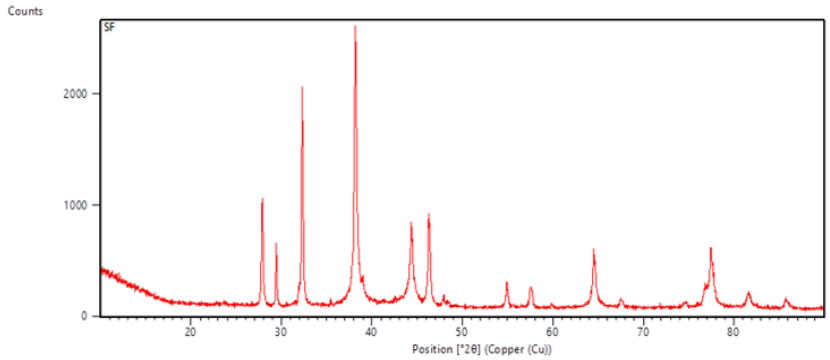

Fig 5: XRD pattern of AgNPs synthesized using the leaf extract of *C. moriflorum*

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TEM Analysis

In Figure, the TEM picture of AgNPs is displayed. According to the picture, the AgNPs had a spherical shape and range of 17 nm, which is in decent arrangement with the estimated particle size from XRD analysis. TEM was employed to evaluate the morphology, size, and shape of nanoparticles. It was discovered that the AgNPs were evenly distributed and primarily spherical in shape, although some NPs had irregular shapes and were between 5 and 17 nm in size. The silver nanoparticles created from the leaf extract of *Terminalia arjuna* appeared round and uneven in the TEM pictures [21].This demonstrated that the plants actively participated in the synthesis of silver nanoparticles and controlled their development.

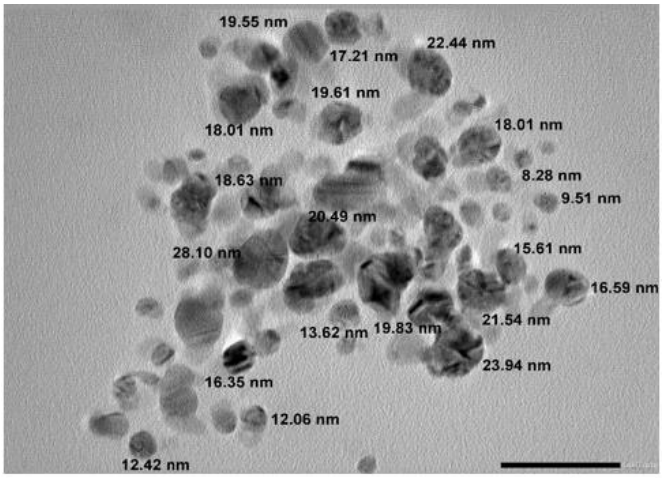


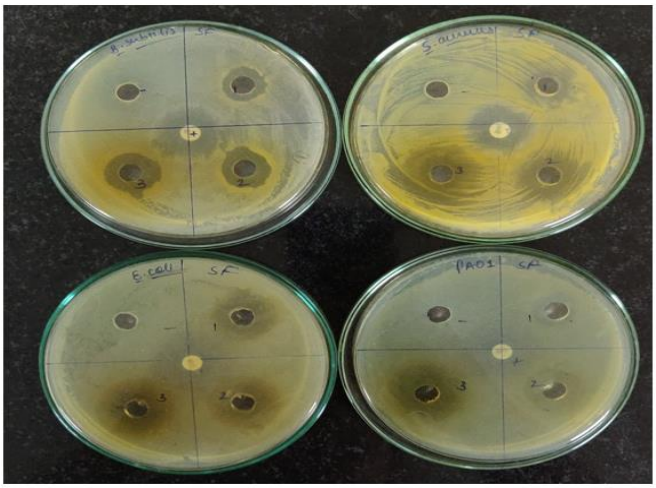
Fig 6: Transmission electron microscopy (TEM) images of AgNPs by *C. moriflorum at* 50 nm

Antibacterial Activity

AgNPs and plant bark extract were tested for antibacterial activity, and the findings are displayed in Figure. The antibacterial activity was evaluated using the well diffusion method. The pure floral extract is less effective than the nanoparticles created utilizing the plant extract, as shown by a comparison of the activities of the plant extract and NPs capped with the plant extract in Table 1. The results also suggest that, in comparison to floral extract, which exhibits less inhibition zone, nanoparticles exhibit a strong inhibition zone against several bacteria, including *staphylococcus aureus, pseudomonas aeruginosa, E. coli, and Bacillus subtilis*.

According to Table 1, the ZOI increased as AgNP concentrations rose. Gram-positive *S. aureus* (26.01±41 mm) by AgNPs made from *C. moriflorum* flower extracts, followed by Gram-negative *P. aeruginosa* (20.44 ±41 mm) and *E.coli* (25.15±0.15) and Gram-positive *B.subtilis* (19.32±0.19mm) at 70 µl concentration as shown in the table 1.

ISSN 2515-8260 Volume 10, Issue 01, 2023



Note: 1= Negative Control, 2= Plant extract, 3=$AgNO_3$, 4= AgNPs

Fig 7: Antibacterial activity of *C. moriflorum* -AgNPs prepared by *C. moriflorum* flower extract against (A) *Bacillus subtilis* (B) *Staphylococcus aureus* (C) *Escherichia coli* (D) *Pseudomonas aeruginosa*

(NOTE: Values are the mean of three replicates ± standard error.)

Table 1: Antibacterial activity of AgNPs synthesized by using *C. moriflorum* flower extract

Bacteria Class	Name of the organism	Concentrations (µl)	Zone of Inhibition(mm)		
			Plant extract	**Silver nitrate ($AgNO_3$)**	**Silver nanoparticles**
Gram negative	***Escherichia coli***	50 µl	17.12±0.12	18.44±0.12	21.71±0.12
		60 µl	19.41±0.21	20.24±0.21	23.13±0.21
		70 µl	20.21±0.15	22.14±0.15	25.15±0.15
	Pseudomonas aeruginosa	50 µl	10.21±0.70	14.14±0.70	17.11±0.70
		60 µl	11.21±0.51	16.71±0.51	19.37±0.51
		70 µl	13.44±0.41	17.77±0.41	20.44±0.41
Gram positive	***Bacillus subtilis***	50 µl	10.52±0.31	15.27±0.31	16.14±0.31
		60 µl	12.42±0.25	16.65±0.25	18.41±0.25
		70 µl	14.47±0.19	17.81±0.19	19.32±0.19
	Staphylococcus aureus	50 µl	11.15±0.15	18.78±0.15	23.24±0.15
		60 µl	12.46±0.56	20.45±0.56	25.71±0.56
		70 µl	14.43±0.41	22.41±0.41	26.11±0.41

CONCLUSION

Ag NPs were created using the green synthesis method and various quantities of *C.moriflorum* flower extract. Spectroscopic study of UV-visible absorption supported the production of Ag NPs. Ag NPs' XRD pattern reveals that the produced particles have a face-centered cubic crystalline structure and average in size 18 nm. The produced Ag NPs are spherical, according to TEM studies. In addition, a TEM research determined that the Ag NPs size for a 5 ml extract concentration is 5 to 17 nm range. The antibacterial activity of the produced Ag NPs against both gram-positive and gram-negative bacteria is the best.

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ISSN 2515-8260 Volume 10, Issue 01, 2023

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