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Synthesis and Characterization of Silver Nanoparticles using Aqueous Extract of *Citrus maxima* Leaf

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Plant-based synthesis of silver nanoparticles is an environmental friendly method which is gaining increasing attention among the researchers as plants are readily available, non-toxic, safe to handle, cost-effective and possess a wide variability of secondary metabolites which act as both natural reducing and capping agents in silver nanoparticles synthesis. In present study, silver nanoparticles have been synthesized successfully using aqueous extract of leaves of *Citrus maxima* Merr. Silver nanoparticles formed were characterized by ultraviolet-visible spectrophotometer, scanning electron microscopy, transmission electron microscopy, selected area electron diffraction pattern, EDX and FT-IR spectrometer. The synthesized nanoparticles are spherical in shape, polycrystalline nature and ranged from 2 to 25 nm in size. The leaf of *Citrus maxima* has a strong potential for environmental friendly synthesis of silver nanoparticles.

Keywords: Silver nanoparticles, *Citrus maxima*, Aqueous extract, Environment friendly.

INTRODUCTION

In recent times, research and development in the field of nanotechnology is one of the most important and potential area for synthesis and applications of nanomaterials with size ranging from 1 to 100 nm. Interest on nanoparticles is increasing rapidly because of their extremely small size and large surface to volume ratio, and they show unique and different properties compared to the bulk materials [1]. Several metal nanoparticles are extensively used in consumer goods such as soaps, detergents, shampoos, shoes, toothpaste and cosmetic products in addition to their applications in pharmaceutical products [1], medicine [2] and drug delivery and disease diagnostic systems [3,4]. Silver nanoparticles (AgNPs) have recently been studied due to their several applications in the field of medicine, sensors, optics, optoelectronics and catalysis [5,6]. Antimicrobial activity of AgNPs is one of the most important properties. Other properties of AgNPs include acaricidal [7], antiparasitic [8], antiproliferative [9], larvicidal [10], antibiofouling [11], anticancer [9,12] and antiviral activities [13]. Various methods such as chemical, photochemical, radiation, electrochemical and biological methods have been employed to synthesize AgNPs [6,9]. Silver nanoparticles syntheses using physical and chemical methods have some disadvantages as these are capital and energy intensive processes and in the synthesis procedure, non-polar solvents and toxic chemicals are employed, and then capping agents or other synthetic additives are also utilized for maintaining the morphology, size, stability and longevity of synthesized nanoparticles [1,14]. The biosynthesis of silver nanoparticles through biological method has benefits over chemical and physical methods as no toxic chemicals are used in biological processes. Moreover, this method is environment friendly, cost effective and the biomolecules present themselves act as reducing as well as capping agents [6,10,15]. Synthesis of AgNPs through biological methods includes synthesis utilizing plants [1,16], microorganisms [17-19], egg shells [20], fish scales [21] and marine crabs [22]. Plant-based synthesis of AgNPs is better compared to other biological resources such as microorganisms due to faster rate of reaction and availability of the plant resources in abundance. Moreover, plants are non-toxic, environment friendly, safe to handle, cost-effective and possess a wide variability of secondary metabolites which act as both natural reducing and capping agents in AgNPs synthesis [1,16,17]. However, microorganism-mediated synthesis of AgNPs possesses multi-step procedures like microbial isolation, culture preparation, maintenance, *etc.* which is tedious and time-consuming compared to plants [1,18,23]. Several studies have reported that biomolecules from plant extracts such as polyphenols, flavonoids, alkaloids, essential oils, proteins, enzymes, polysaccharides, *etc.* have the excellent power to reduce silver ions and effectively cape the produced AgNPs inhibiting agglomeration and thus providing good stability of the particle [5,16,17,23]. In this study, silver nanoparticles have been synthesized successfully using aqueous extract of leaves of *Citrus maxima* Merr. and the silver nanoparticles formed were characterized using various instrumental techniques.

EXPERIMENTAL

Plant materials: Fresh leaves of *Citrus maxima* Merr. were collected from the village of Debargaon near Bodoland University, Kokrajhar, India in the month of February, 2017.

Preparation of aqueous extract from leaves: The fresh leaves were thoroughly washed with water for several times to remove the dust and then dried in oven at 55 ºC for 3 days to remove the moisture. The dried leaves were grinded to fine powder with the help of mortar and pestle. The aqueous extract was prepared by mixing 20 g of powdered material with 100 mL of distilled water. The mixture was stirred vigorously at 60 ºC for 1 h. Then the extract was filtered with Whatmann No. 1 filter paper and the filtrate was stored at 4 ºC for further use.

Synthesis of silver nanoparticles: In 100 mL $AgNO_3$ (1 mmol) solution, 2% of aqueous extract was added. The mixture was taken in a 250 mL conical flask and stirred in a magnetic stirrer at an ambient temperature (27-28 ºC). The formation of silver nanoparticles was monitored by UV-visible spectrophotometer at regular intervals (0 min, 1 h, 6 h, 12 h and 24 h). The initial light yellow colour of the mixture gradually changed to dark brown. After the synthesis, it was dried in a hot air oven at 75 ºC.

Characterization of synthesized silver nanoparticles: The optical property of AgNPs was determined by UV-visible spectrophotometer. After the addition of aqueous extract to $AgNO_3$ solution, the spectra were taken at different intervals upto 24 h between 300 nm to 700 nm. Scanning electron microscopy (SEM) analysis was performed at IASST (SEM, ZEISS, SIGMA VP, No. 01-94), Paschim Boragaon, Guwahati (India). Transmission electron microscopy (TEM), selected area electron diffraction (SAED) pattern and EDX analyses were performed at Sophisticated Analytical Instrumentation Centre, Tezpur University (TEM, TECNAI G2 20 S-TWIN-200KV, Resolution: 2.4A0, FEI COMPANY, USA), India. The synthesized AgNPs was also analyzed using FT-IR spectrometer (4000-400 cm^{-1}).

RESULTS AND DISCUSSION

UV-visible analysis of synthesized silver nanoparticles: In this study, 2% of aqueous extract from *C. maxima* leaf was used for the biosynthesis of AgNPs and the reaction was performed at an ambient temperature (27-28 ºC). Silver formation was monitored by UV-visible spectrophotometer at regular intervals *viz*. 0 min, 1 h, 6 h, 12 h and 24 h. In this study, the reaction progress leading to conversion of Ag^+ to Ag^o was monitored by observing the colour change of the solution and UV-visible absorbance peak in the range of 300-700 nm (Fig. 1). It was initially seen that the colour of the solution was pale yellow which turned to yellowish brown after few hours and then eventually turned to dark brown indicating the formation of AgNPs. This colour change is due to the surface plasmon resonance (SPR) of AgNPs in the visible region. The silver nanoparticles have free electrons which give the SPR absorption band due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. It was reported that AgNPs show SPR band in the range of 400-450 nm [5,20]. In this study, SPR band was not observed at 0 min, but was seen at 421 nm in 1 h of reaction at 27-28 ºC, which confirms the bioreduction of Ag ions to AgNPs (Fig. 1). It was also observed that the intensity of the peak gradually increased with time. Silver nanoparticles were found to be stable after 24 h as no characteristic change in the SPR band was observed after 24 h along with no further colour change in the solution. Hence, from UV-visible study, it was confirmed that silver ions were reduced to silver nanoparticles using 2 % aqueous leaf extract of *C. maxima*.

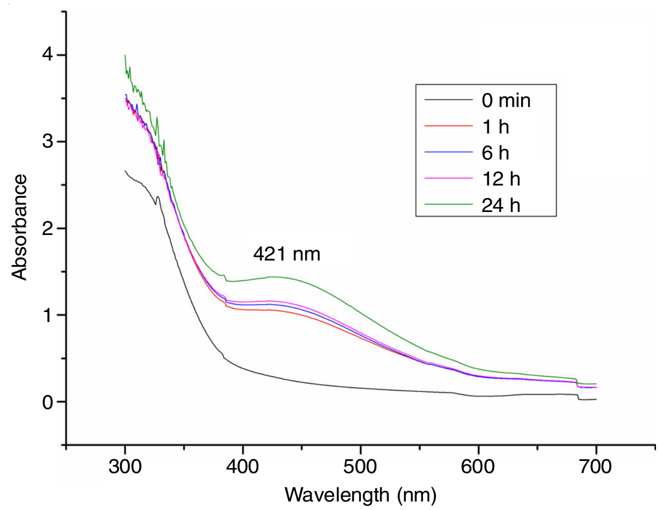

Fig. 1. UV-visible spectra of colloidal solution of synthesized AgNPs at different intervals

SEM, TEM, SAED and EDX analyses: Fig. 2 depicted the SEM image obtained from films of AgNPs synthesized from aqueous leaf extract of *C. maxima*. SEM image provides the idea that the produced AgNPs are spherical in shape. The size and morphology of AgNPs have been investigated using TEM and SAED pattern. TEM images of synthesized AgNPs were depicted in Fig. 3. The TEM images show that most of the nanoparticles are spherical and some are elliptical in shape. The sizes of the synthesized AgNPs ranged from 2 nm to 25 nm. Sinha and Ahmaruzzaman [20] also reported in their study that AgNPs formed were mainly spherical in shape with the particle size ranging from 6 nm to 26 nm. Selected area electron diffraction (SAED) pattern depicted in Fig. 4 with bright spots and concentric diffraction rings indicated the polycrystalline

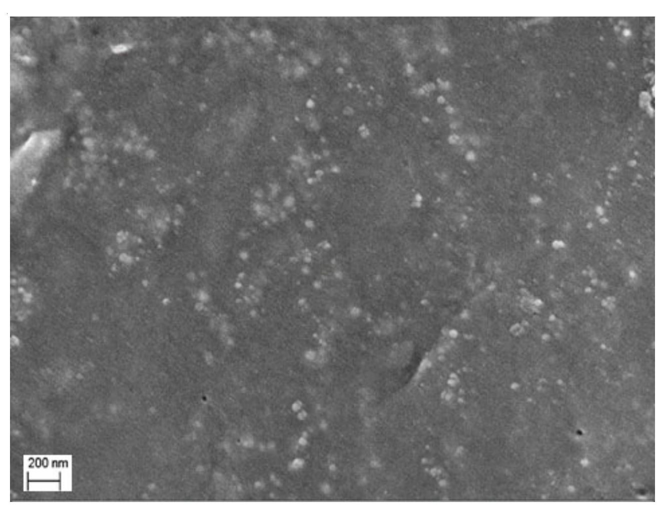

Fig. 2. SEM image of AgNPs

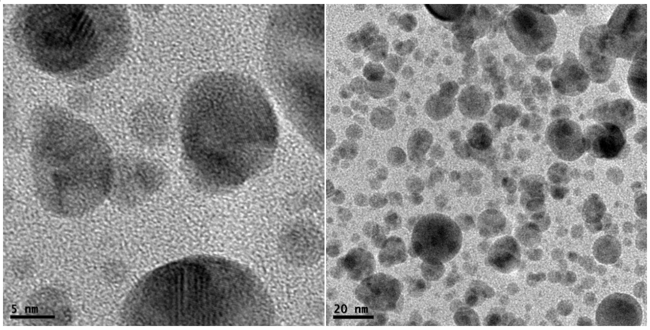

Fig. 3. TEM images of synthesized AgNPs

Fig. 4. SAED diffraction pattern

nature of AgNPs. From the particle size distribution of nanoparticle as shown in Fig. 5, the average particle size of AgNPs was found to be 4-8 nm, which suggests *C. maxima* leaf extract as a very good reducing agent. EDX analysis provides idea about qualitative as well as quantitative status of the elements that may be involved in fabrication of nanoparticles. Fig. 6 showed the presence of elemental silver of AgNPs with a sharp signal at around 3 keV. Silver nanoparticles generally show typical optical absorption band in the range of 3-4 keV due to surface plasmon resonance [24,25]. The signal of Cu shown

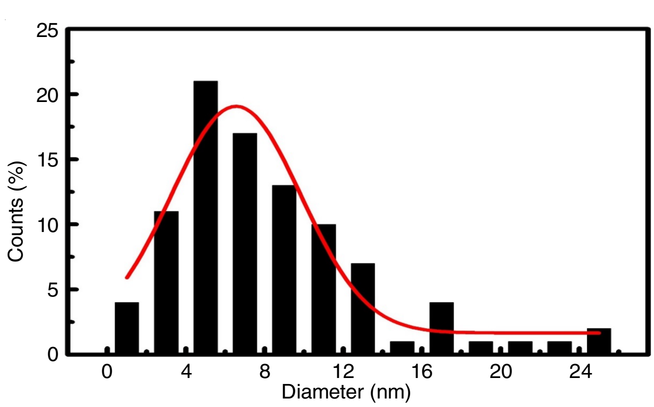

Fig. 5. Particle size distribution of AgNPs

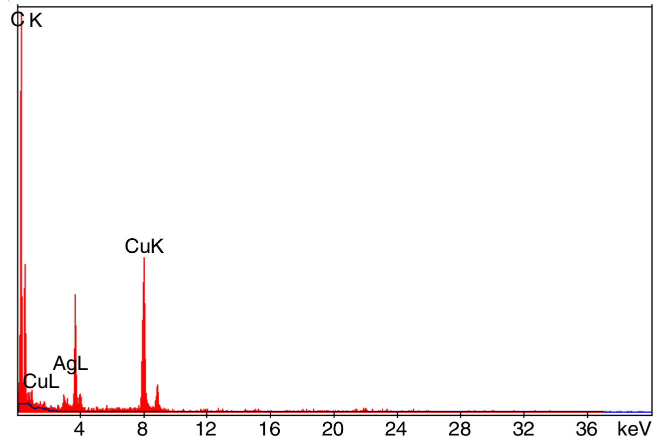

Fig. 6. EDX spectrum of synthesized AgNPs

in EDX spectrum (Fig. 6) is due to TEM grid of Cu and other peaks (like carbon), which may be attributed the presence of biomolecules in the plant extract.

FT-IR analysis: A FT-IR study is used to carry out to ascertain the biomolecules present in the plant extracts which act as capping agents and efficiently stabilizes the synthesized nanoparticles [5]. The FT-IR spectrum (Fig. 7) of silver nanoparticles showed various peaks at 3422.4, 2793.1, 1598.6, 1423.8, 1339.8, 1073.4, 906, 834.8, 671.4 and 551 cm^{-1}. The broad band at 3422.4 cm^{-1} may be the characteristic peak of O-H stretching vibration and N-H stretching vibration. The characteristic absorption band at 2793.1 cm^{-1} is attributed due to C-H stretching vibration. The peak at 1598.6 cm^{-1} may be due to C=O stretching vibration or N-H bending vibration. The peak at 1423.8 cm^{-1} is due to C=C stretching frequency of aromatic compounds. The peak at 1339.8 cm^{-1} may be due to the existence of -N-O group. The peak at 1073.4 cm^{-1} is due to -C-O-C- linkage or C-O bonds. Therefore, it can be inferred that the synthesized nanoparticles might be surrounded by proteins and other secondary metabolites having functional groups like carbonyl, amide, ester, phenolic -OH, ether, *etc*. From infrared study, it can be confirmed that the carbonyl groups from the amino acid residues has the ability of capping with silver (metal) nanoparticles to stop agglomeration. This suggests that the biological molecules could possibly perform dual roles of formation and stabilization of silver nanoparticles.

In present study, the synthesized silver nanoparticles are found in the range of 2 to 25 nm in size. Several plants used

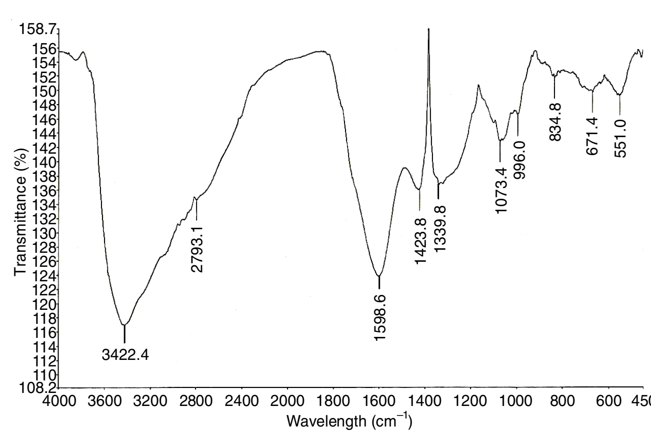

Fig. 7. FT-IR spectrum of silver nanoparticles

TABLE-1
BIOSYNTHESIS OF SILVER NANOPARTICLES USING LEAF EXTRACTS OF VARIOUS PLANTS

Name of plant	Plant part	Shape	Size (nm)	Ref.
Lonicera japonica	Leaf	Spherical	~7.8	[26]
Cycas	Leaf	Spherical	2-6	[27]
Paederia foetida	Leaf	Spherical	4-15	[28]
Azadirachta indica	Leaf	Spherical	~34	[29]
Ceratonia silique	Leaf	Spherical	5-40	[30]
Manilkara zapota	Leaf	Spherical	70-140	[7]
Euphorbia prostrata	Leaf	Rod shaped	25-80	[8]
Acalypha indica	Leaf		20-30	[31]
Lippia citriodora	Leaf	Spherical crystalline	15-30	[32]
Ocimum sanctum	Leaf	Circular	4-30	[33]
Murraya koenigii	Leaf	Spherical	40-80	[34]
Mangifera indica	Leaf	Triangular, hexagonal	~20	[35]
Hibiscus rosa sinensis	Leaf	Spherical	~13	[36]
Chenopodium album	Leaf	Spherical, triangular	10-30	[37]
Coleus amboinicus	Leaf	Triangle, spherical, decahedral, hexagonal	~17.6, ~30.6, ~35.8	[38]
Hibiscus cannabinus	Leaf	Spherical	~9	[39]
Prosopis juliflora	Leaf	Triangles, tetragons, pentagons, hexagons	35-60	[40]
Malva parviflora	Leaf	Face centered cubic (fcc)	19-25	[41]
Annona squamosal	Leaf	Spherical	20-100	[42]
Vitex negundo	Leaf	fcc	~20	[9]
Mukia scabrella	Leaf	Spherical	18-21	[43]
Ocimum tenuiflorum	Leaf	fcc	25-40	[44]
Albizia adianthifolia	Leaf	Spherical	4-35	[12]
Citrus maxima	Leaf	Spherical	2-25	This work

for the synthesis of silver nanoparticles reported in the literatures are presented in Table-1 and results of present study are found to be comparable to these reports (Table-1). Hence, the leaves of *Citrus maxima* plant can also be used an effective and potential plant material for biosynthesis of silver nanoparticles.

Conclusion

The present study showed that aqueous leaf extract of *Citrus maxima* Merr. can be used efficiently for the synthesis of silver nanoparticles at ambient temperature (27-28 ºC). The synthesized silver nanoparticles ranged from 2 to 25 nm in size and the average particle size was found to be 4-8 nm. The nanoparticles are spherical in shape and polycrystalline nature. The leaf of this plant has a strong potential for synthesis of silver nanoparticles and the method is simple, cost-effective and ecofriendly.

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