J. Surface Sci. Technol., Vol 29, No. 1-2, pp. 47-66, 2013

Green Synthesis of Silver Nanoparticles and their Antibacterial Activity

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Abstract — Silver nanoparticles have been prepared by the reduction of silver ions using respectively castor oil (*ricinus communis*), khat (*catha edulis*) and sun flower (*helianthus annuus*) leaf extracts as reducing and stabilizing agents. The as-synthesized material was characterized by using spectroscopic, XRD and TEM techniques. As-synthesized silver nanoparticles are found to have face centered cubic structure with average crystallite size 28 nm. and showed antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*.

Keywords : *Ag-nanoparticles, synthesis, green reducing agents, antimicrobial activity*.

INTRODUCTION

There is huge interest in metal nanoparticles because of their unusual physical and chemical properties at their nanoscale. In metal nanoparticles, because of the quantized motion of the collectively excited conduction band electrons and the size dependent surface plasmons, there is extraordinary large electromagnetic field enhancements upon interaction with an electromagnetic field [1]. The plasmon resonance is strongly dependent on the size, shape, dielectric properties of the particle and its surroundings. By altering the synthesis routes, nanoparticles with different shape and size can be synthesized.

Noble metal nanoparticles exhibit unique electronic, catalytic, optical, magnetic, mechanical and chemical properties which significantly differ from those of their bulk materials. These special properties of nanoparticles can be attributed to their small size and large specific surface area. Metal nanoparticles find several applications in

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electronics, catalysis, photonics, and diagnostic biological probes [2].

The synthesis of metallic nanoparticles is an active area of academic research in the field of nanotechnology. A variety of chemical and physical procedures could be used for synthesis of metallic nanoparticles. However, these methods are fraught with many problems including use of toxic solvents, generation of hazardous by-products, and high energy consumption. Accordingly, there is an essential need to develop environmentally benign methods for synthesis of metallic nanoparticles. A promising approach to achieve this objective is to exploit the array of biological resources in nature. Indeed, over the past several years, plants, algae, fungi, bacteria, and viruses have been used for the production of low-cost, energy-efficient, and nontoxic metallic nanoparticles [3].

To date, metallic nanoparticles are mostly prepared from noble metals (i. e., Ag, Pt, Au and Pd) [4]. The use of metallic nanoparticles in the field of catalysis, optoelectronics, diagnostic biological problems and display devices uncovered many significant findings. Amongst the noble metals, silver (Ag) is of choice for use in the field of biological systems, living organisms and medicine [5, 6].

The dispersions of metal nanoparticles display intense colors due to the plasmon resonance absorption. Surface Plasmon resonance is a collective excitation of the electrons in the conduction band at the surface of the nanoparticles. Metallic nanoparticles have characteristic optical absorption spectra in the UV-Vis region [7].

The investigation on silver nanoparticles has also gained importance due to their use in the field of opto-electronics and their anti-microbial activity. Antibacterial activity of silver-containing material has proved useful to reduce infections as well as to prevent bacteria colonization on vascular grafts [8], textile fabrics [9] human skin [10], dental material, stainless steel materials [11] and prosthesis [12].

A number of different synthetic processes for metal nanoparticles are known, these include ultrasonic irradiation [13] electrochemical synthesis [9], reversed micelle processes [10,14,15], sonochemical synthesis [16], thermal decomposition [17], solvothermal synthesis [18] and bioreduction [19,20]. Chemical methods for metal nanoparticle fabrication are not cost-effective and also involve the use of toxic chemicals as reductants and stabilizers, which can be dangerous to our environment.

Many reports have been published in the literature on the biogenesis of silver nanoparticles using several plant extracts, particularly Neem leaf broth (*Azadirachta indica*) and Geranium leaves (*P. graveolens*) [21] *Capsicum annuum* leaf extract [22], *Aloe vera* plant extract (Maensiri *et al*., 2008); Papaya fruit extract (Jain *et al*., 2009), *Mentha piperita* leaves [23]; and *Eucalyptus hybrida* (Safeda) leaf [24] Citrus limon

(lemon) aqueous extract [25]. The reducing property of different plant constituents such as geraniol may play a critical role in the reduction of Ag^+ to silver nanoparticles [26].

Castor Oil (*Ricinus communis*), Khat (*Catha Edulis*) and Sun Flower (*Helianthus Annuus*) plant leaf were coosen in the present study for the reduction of silver ions. The leaves of Castor Oil (*Ricinus communis*) are reported to contain three monoterpenoids : 1, 8-cineole, camphor and α-pinene, and a sesquiterpenoid : β-caryophyllene, as the main constituents [27]; flavonoids [28]; proteins and aminoacids [29]. Besides, Khat (*Catha Edulis*) leaf extract contains alkaloids, cathine and the dimer of cathinone, triterpenes, sterols, and fatty alcohols, hydrocarbons, fatty acids and saponins [30]. In addition, the leave extracts are chosen, since they are environmentally available in the study area and contain chemical constituents that could act as reducing and capping agents.

Studies have indicated that biomolecules like proteins, phenols and flavonoids, terpinoids play a role in reducing and capping the nanoparticles [31]. Currently, there is a growing need to develop environmentally benign nanoparticle synthetic processes that are free from toxic chemicals in the synthesis protocol. As a result, researchers in the field of nanoparticles synthesis and assembly have turned to biological system [32].

Silver is known for its antimicrobial properties and has been used for years in the medical field for antimicrobial applications and even has shown to prevent HIV binding to host cells [33]. The Ag-NPs are also reported to be nontoxic to human and effective against bacteria, viruses, and other eukaryotic micro-organisms at very low concentration and without any side effects [34]. Silver nanoparticles, because of their large specific surface area, are highly active and can play a crucial role in inhibiting bacterial growth in aqueous and solid media. The antimicrobial activity of colloidal silver is influenced by the size of the particles. Smaller the particle size more is its antimicrobial effect [35].

Biosynthesis of metal nanoparticles, using plant leaf material as reductants as well as capping agent, is currently under exploitation. It is an eco-friendly, cost effective and more efficient alternative method for large scale synthesis of metal nanoparticles. Under the proposed research, kinetic study of silver ions reduction has been carried out using different plant leaf extracts as reductants and capping material. The antibacterial activities of as-synthesized silver nanoparticles against some pathogenic bacteria have been investigated.

EXPERIMENTAL

Chemicals and Reagents :

The chemicals used were as follows : Silver nitrate ($AgNO_3$) (Blulx laboratories; 99.9 %, MW = 169.87 g/mol); agar agar; potassium bromide (KBr) (99.5%, BDH) plant leaf of Castor Oil (*Ricinus Communis*), Khat (*Catha Edulis*) and Sun Flower (*Helianthus Annuus*). Clinical isolates of gram negative bacteria *Escherichia coli* and gram positive bacteria *Staphylococcus aureus* were received from Ethiopian Health and Nutrition Research Institute (Addis Ababa).

Methods :

Preparation of Plant Leaves Extract —

A 20 gm each of fresh leaves of castor oil *(Ricinus Communis*), khat (*Catha Edulis*) and sun flower (Helianthus Annuus) were, separately, washed with distilled water and dried. The air-dried leaves were mashed using mortar and pestle and mixed with 100 ml double distilled water (DDW) in a 250 ml Erlenmeyer flask, stirred for 5 minutes and filtered. The extract was stored at 4^oC.

Preparation of Silver Nanoparticles —

1.5 mL leaves extract was added to 30 mL 10^{-3} M $AgNO_3$ aqueous solution and kept at room temperature [36]. After 24 hours, the solution was centrifuged at 2500 rpm for 30 minutes. The supernatant liquid was decanted off and the residue was repeatedly washed each time with 10 ml of de-ionized water. The residue was dried in an oven at 40^0C for 12 hrs. A portion of it was kept for FTIR study and the remaining sample was calcined at 400^0C in a ceramic crucible for 4 hours, cooled and then used for XRD analysis.

UV-Visible absorbance Study —

Absorbance spectra of reaction mixture (10^{-3} M $AgNO_3$ + plant leaf extract) as a function of time was recorded over 300–800 nm, spectrophotometrically. The progress of reaction was monitored by the observed increasing intensity of the absorbance peak.

X-Ray Diffraction (XRD) Analysis —

XRD pattern of as-synthesized nanomaterial was recorded using Cu targeted K_α radiation of wavelength 1.5405 Å. The scanning was carried out over 2θ range : 4^o to 64^o at current : 30 mA and voltage : 40 kV.

Fourier Transform Infrared (FTIR) Spectroscopic Analysis —

The uncalcined Ag nanoparticles sample was mixed thoroughly with KBr and cast into pellet and analyzed on FTIR spectrophotometer (SHIMADZU 1730 model,

JAPAN) in the diffuse reflectance mode operating at a resolution of 4 cm^{-1}.

Study of Antibacterial Activity —

Antibacterial activity of as-synthesized silver nanoparticles against *Escherichia coli* and *Staphylococcus aureus* was studied using paper disc diffusion technique. The test bacterial strains were streaked on nutrient agar in sterile Petri dishes and incubated for 24 hours. Using a micropipette, 10, 20 and 30 µl of 5 mg/ml of as-synthesized silver nanoparticle dispersion in water were impregnated, separately, on paper discs of 6 mm diameter. Zones of inhibition were measured after 24 hours of incubation. The magnitude of antimicrobial effect against, *Escherichia coli* and *Staphylococcus aureus* was determined based on the inhibition zone measured.

Data Analysis —

Origin version six and SAS version eight software's were used to analyze the data collected from UV-Visible spectrophotometer and zones of inhibition of silver nanoparticles against test organisms, respectively. Level of significance among means was compared at $\alpha = 0.05$ and data was investigated using one way ANOVA analysis.

RESULTS AND DISCUSSION

UV-visible Spectroscopic Study :

The progress of the bio-reduction of Ag^+ ions using leaves extract of Castor Oil (*Ricimus communis),* Khat (*Catha Edulis*) *and* Sun Flower (*Helianthus annuus),* as reducing and stabilizing agent, was monitored from the increasing intensity of surface plasmon resonance/absorption peak of silver nanoparticles around 450 nm as shown in Figs. 1 to 3.

Surface plasmon resonance (SPR) abserption patterns, of metal nanoparticles, depends on particle size and the dielectric constant of the medium. SPR bands observed, with increase in the reaction time indicates the formation of anisotropic molecules that are stabilized in the medium [6]. Similar observation have also been reported by [1,7,37] on surface plasmon resonance. Absorption of visible radiation is due to the induced polarization in conduction electrons of metal nanoparticles with respect to their immobile nucleus. When a particular wavelength is matched to the size of a nanoparticle, dipole oscillation is generated in the compensated form of the induce polarization and then the electrons in the nanoparticle resonate, resulting in absorption of radiation [38].

Metal nanoparticles exhibit weak absorbance around 300–400 nm. The

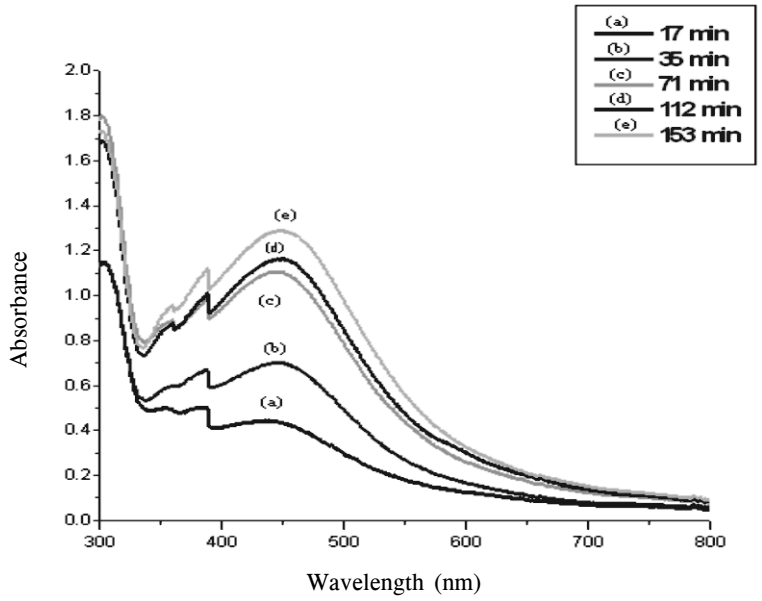

Fig. 1. UV-vi sible absorption spectra of aqueous silver nitrate solution mixed with Castor oil (*Ricinus communis*) leaf extract as a function of reaction time (in minutes).

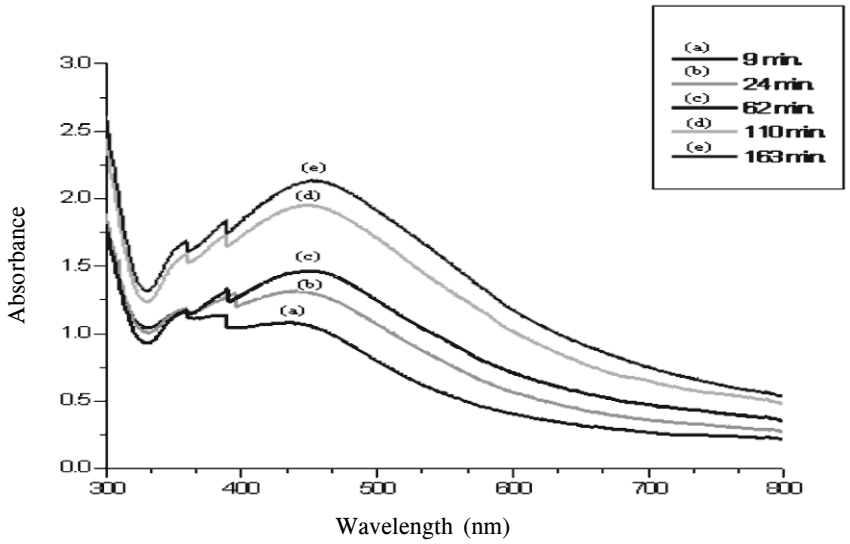

Fig. 2. UV-visible absorption spectra of aqueous silver nitrate solution mixed with Khat (*Catha edulis*) leaf extract as a function of reaction time (in minutes).

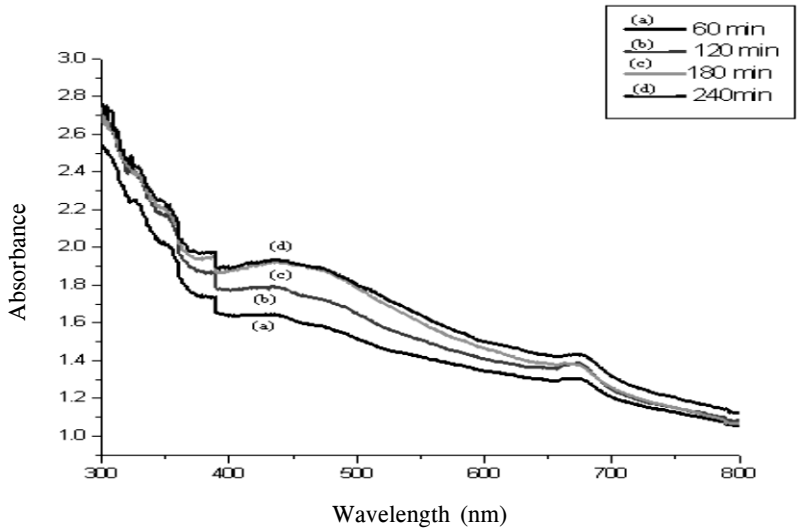

Fig. 3 . UV-visible absorption spectra of aqueous silver nitrate solution mixed with Sun Flower (*HelianthusAnnuus*) leaf extract as a function of reaction time (in minutes).

absorption peak at 370 nm corresponds to the transverse plasmon vibration in the Ag nanoparticles, whereas the peak at 440 nm is due to the excitation of longitudinal plasmon vibrations [26, 39]. An absorption band at 270 nm is attributed to the aromatic amino acid of proteins present in the leaf extract. It is well known that the absorption band at 270 nm arises due to electronic excitations in tryptophan and tyrosine residues in the proteins. A red shift in the absorption peak with increasing reaction time indicates an increase in nanoparticle size. The increase in the intensity of absorption peak with time is attributed the increasing amount of the absorbing metal nanoparticles [25],

Fourier Transform Infrared (FTIR) Spectroscopic Study :

FTIR spectroscopic studies were carried out to identify the chemical nature of biomolecules in the leaves of Castor Oil (*Ricinus communis*), Khat (*Catha edulis*) and Sun Flower (*Helianthus annuus*) which may be responsible for capping of Ag nanoparticles for the stabilization of the silver nanoparticles. FTIR spectra of synthesized silver nanoparticles using the above leaf extracts as reductants are presented in Figs. 4 to 6, respectively and the absorption frequencies are listed in Table 1.

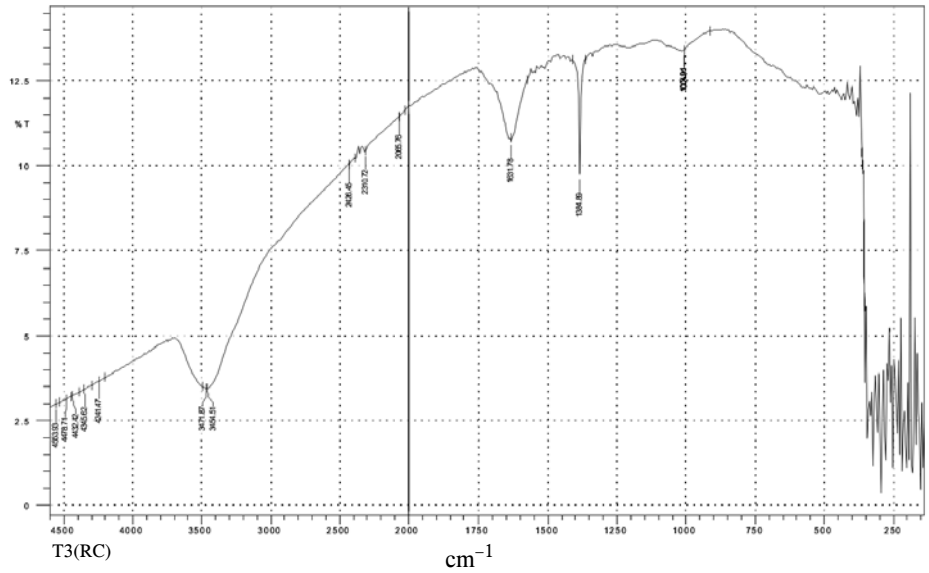

Fig. 4. FTIR spectra of Castor Oil (*Ricinus communis*) plant leaf extract mediated silver Nanoparticles.

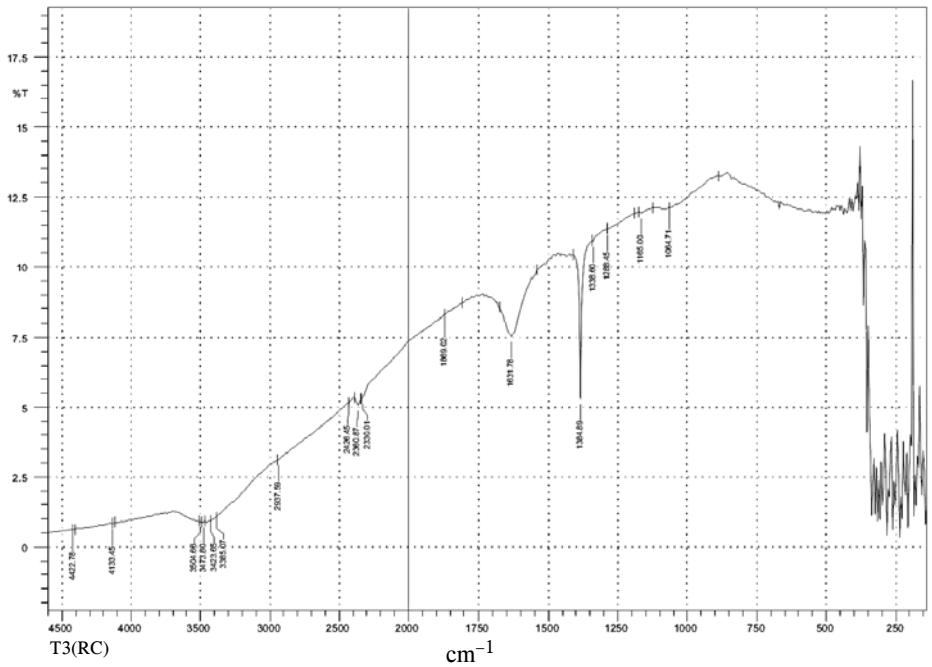

Fig. 5. FTIR spectra of Khat (*Catha Edulis*) plant leaf extract mediated silver Nanoparticles.

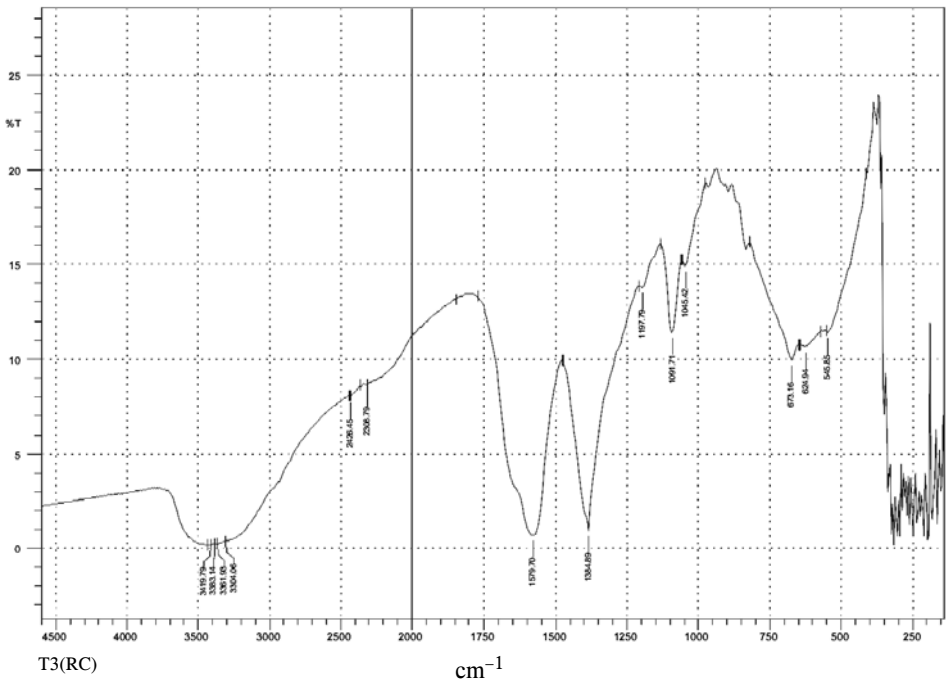

Fig. 6. FTIR spectra of Sun Flower (*Helianthus Annuus*) mediated silver nanoparticles leaf extract.

TABLE 1.

FTIR absorption frequencies of Castor Oil (*Ricinus communis*), Khat (*Catha edulis*) and Sun Flower (*Helianthus annuus*) leaf extract mediated silver nanoparticles

Sample*	Wave number (cm^{-1})
CO	3471(3454), 2426, 2310, 1631, 1384, 1004
KH	3404 (3473), 3304, 2937, 2426,2360,1631,1384
SF	3418(3330), 2426, 2308, 1579,1384,1091,1045

*CO = Castor Oil (*Ricinus Communis*); KH = Khat (*Catha Edulis*) and SF = Sun Flower (*Helianthus Annuus)*

The peaks near 3430 cm^{-1} and near 2920 cm^{-1} were assigned to O–H stretching and aldehydic C–H stretching, respectively. The band at 1635 cm^{-1} is due to carbonyl stretching in proteins. The peak at 1579 cm^{-1} is for N-H bending, 1038–1389 cm^{-1}

corresponds to C–N stretching vibrations of the amine. These suggest that the free carbonyl and NH_2 group in amino acid residues of proteins present in plant leaf extract may bind metal nanoparticles while encapsulating/capping the later to prevent their agglomeration. There biological molecules such as proteins perform dual functions : (a) reduction of Ag^+ ions to Ag^o and (b)stabilization of silver nanoparticles in the aqueous medium [36]. Similar conclusion regarding the encapsulation of metal nanopartiles by proteins during their biosynthesis have also been reported, earlier [40].

X-ray Diffraction (XRD) Analysis :

XRD spectra of as-synthesized Ag nanoparticles obtained by bioreduction of Ag^+ ions using Castor oil (*Ricinus communis*), Khat (*Catha Edulis*) and Sun Flower (*Helianthus Annuus*) leaf extract are presented in Fig. 7.

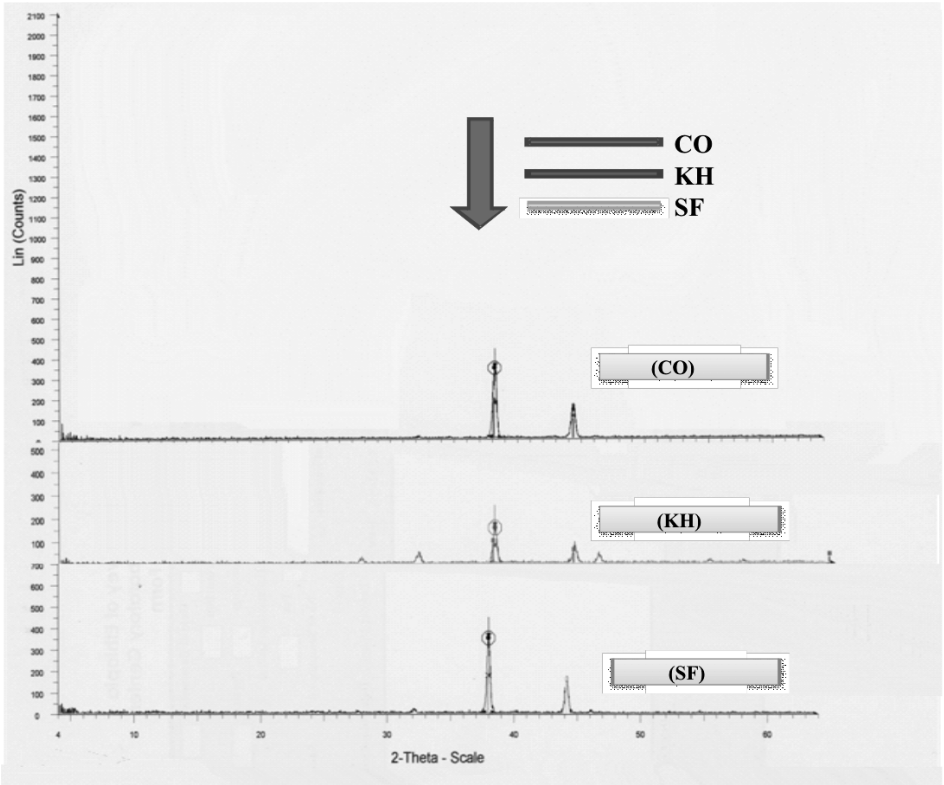

Fig. 7. XRD pattern of silver nanoparticles synthesized using different plant leaf extracts as reductants : CO = Castor oil (*Ricinus Communis*); KH = Khat (*Catha Edulis*) and SF = Sun Flower (*Helianthus Annuus*).

The observed diffraction peaks at $2\theta = 38^0$, 44.5^0 and 64^0 corresponding to (111), (200) and (220) crystal planes, suggest face centered cubic (fcc) structure of as-synthesized Ag nanoparticles crystallites, in agreement with the previous reports [4, 6, 29, 39]. The observed XRD pattern with broadening of the Bragg diffraction peaks indicates the formation of nano-size silver particles.

The crystallite size of silver nanoparticles was calculated using Scherer formula–

$$D = 0.94.\ \lambda/(\beta.\cos\theta) \quad (1)$$

Where, D = crystallite size in nm; β = full width at half maximum (FWHM) in radians; λ = wave length of the X-ray (0.15406 nm) for Cu target Kα radiation and θ = Bragg's angle in degrees. The calculated averge size of silver nanoparticles obtained using Castor oil (*Ricinus communis),* Khat *(Catha Edulis)* and Sun Flower *(Helianthus Annuus)* leaf extracts as reductants are : 26 nm, 27 nm and 32 nm, respectively (Table 2).

TABLE 2.

Average crystallite size of silver nanoparticles obtained from bioreduction of silver ions using some plant leaf extracts as reductants

Reductant*	FWHM (indegree)	crystallite size (nm)
CO	0.322	26
KH	0.317	27
SF	0.268	32

*: CO = Castor Oil (*Ricinus Communis*), KH = Khat (*Catha Edulis*) and SF = Sun Flower (*Helianthus Annuus*)

TEM Analysis :

Transmission electron microscopic (TEM) images of silver nanoparticles synthesized from Ag^+ ions using Castor oil (*Ricinus Communis*), Khat (*Catha Edulis*) and Sun Flower (*Helianthus Annuus*) extracts as reductants and observed at 80 KV and 30000 x magnification, are presented in Figs. 8 to 10, respectively. Average size of the silver nanoparticles prepared using the above plant leaf extracts as reductants are 30 35 and 40 nm in fair agreement with the values obtained from XRD analysis.

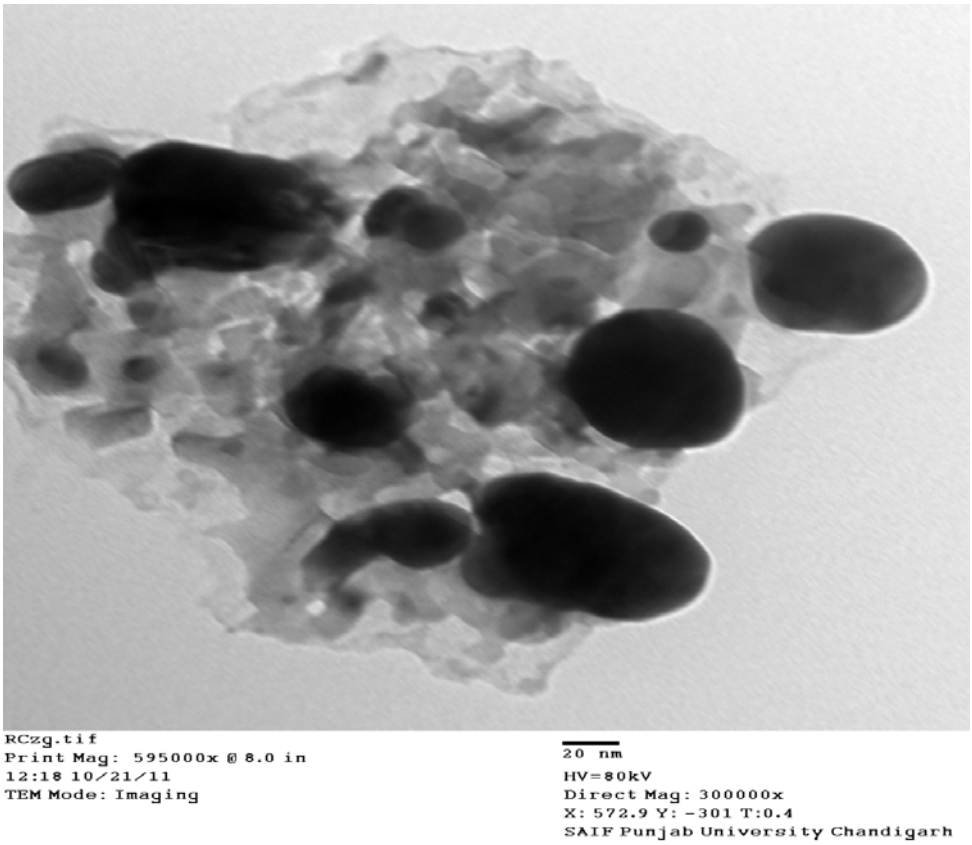

Fig. 8. TEM image of silver nanoparticles synthesized using Castor oil (*Ricinus Communis*); extract as reductants and observed at 80KV and 30000x magnification.

Antibacterial Activity of Silver Nanoparticles :

Silver nanoparticles prepared by the reduction of silver ions using castor oil (*ricinus communis*), khat (*catha edulis*) and sun flower (*helianthus annus*) leaf extracts as reductants as well as stabilizing agent were tested for their antibacterial activity against

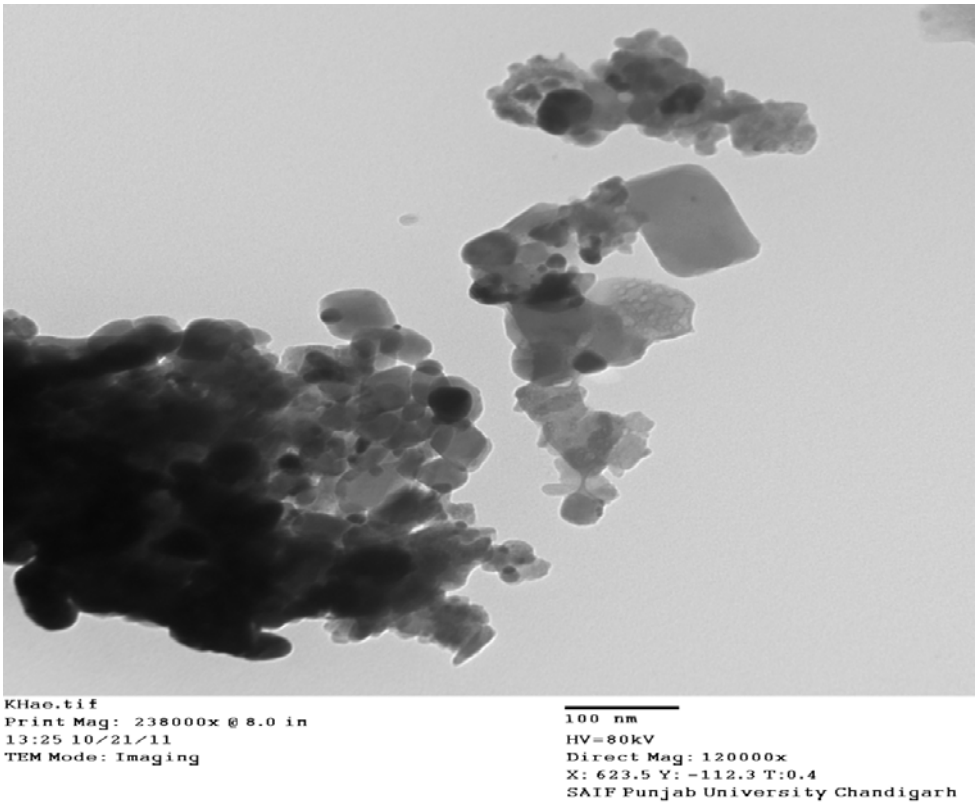

Fig. 9. TEM image of silver nanoparticles synthesized using Khat (*Catha Edulis*) extract as reductants and observed at 80KV and 30000x magnification.

two pathogenic bacteria : gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* using paper disk diffusion method. Different volumes (10, 20 and 30 μL) of 5 mg/ml silver nanoparticle dispersion in water were applied on

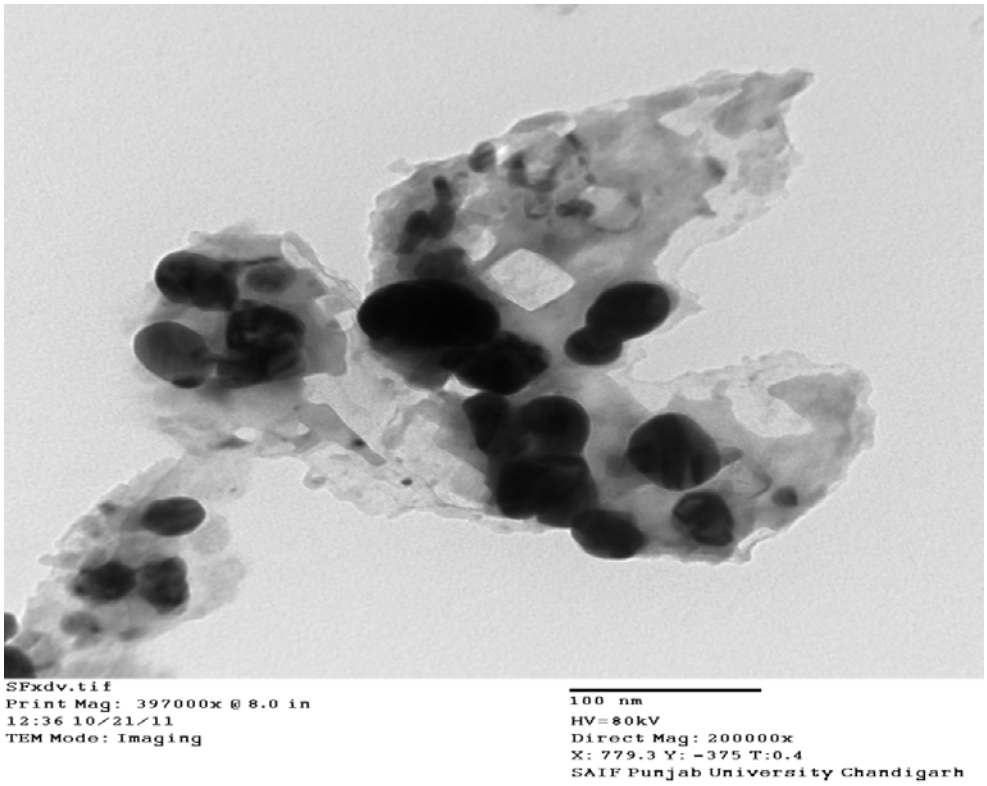

Fig. 10. TEM image of silver nanoparticles synthesized using Sun Flower (*Helianthus Annuus*) extract asreductants and observed at 80KV and 30000x magnification.

nutrient agar taken in sterile Petri dishes and incubated for 24 hours. Some of the observed results exhibiting zone of inhibition (in mm) are displayed in Figs. 11 to 13. The mean of three replicates of zone of inhibition (mm) around well of silver nanoparticles are presented in the Table 3.

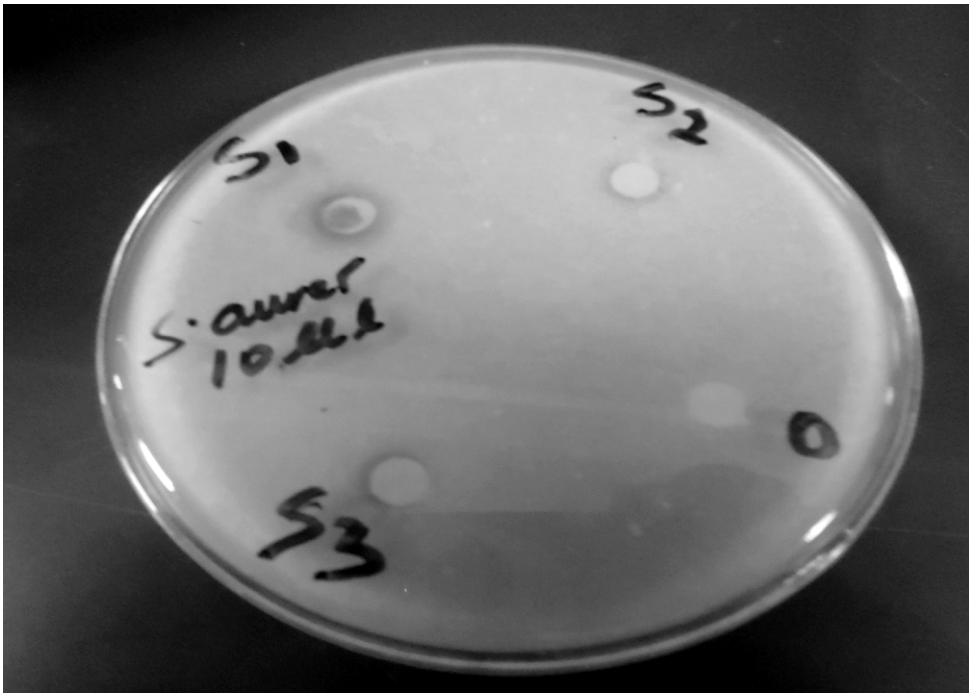

Fig. 11. Zones of inhibition by 10 µl of 5 mg/ml suspension of silver nanoparticles synthesized using different plant leaf extracts as reductant and stabilizers against *Staphylococcus aureus;* S_1 = sun flower (*helianthus annuus*) mediated Ag nanoparticles; S_2 = Castore Oil (*Ricimus Communes*) mediated Ag nanoparticles; S_3 = Khat (*Catha Edulis*) mediated Ag nanoparticles; O = Control (Distilled Water).

As synthesized silver nanoparticles of the present study exhibited good antibacterial activity against gram negative bacteria *Escharichia coli* as well as gram positive bacteria *Staphylococcus aureus*. The inhibition of bacterial growth at various applied concentrations of the test solutions are significant indicating that Ag nanoparticles exhibit good biocidal activity against gram-positive and gram-negative bacteria.

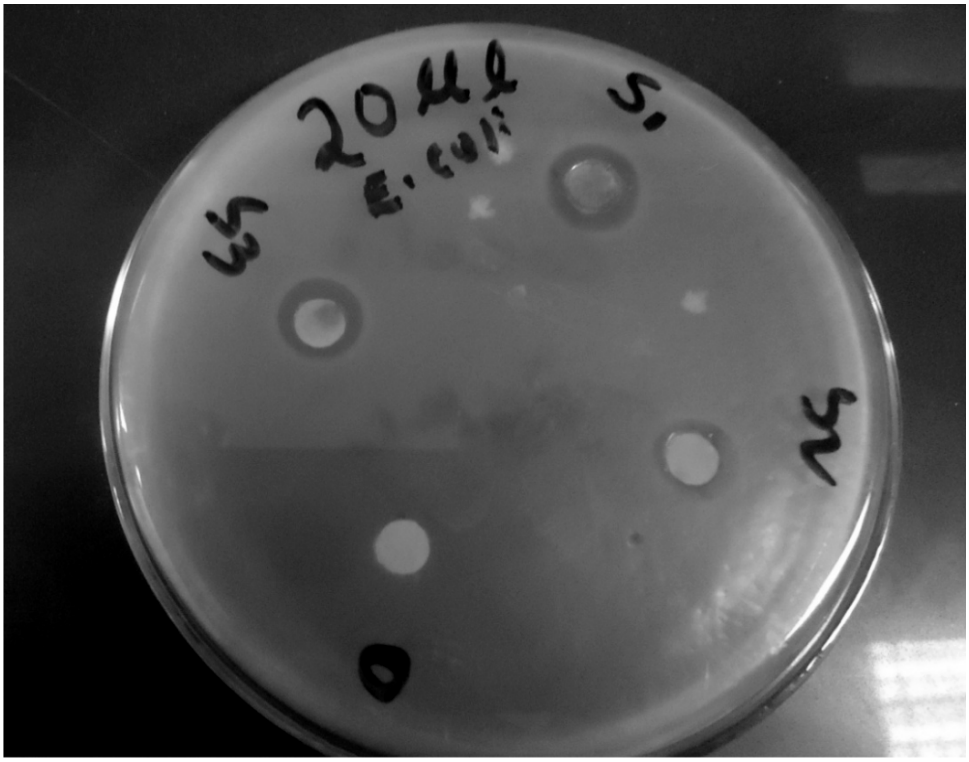

Fig. 12. Zones of inhibition by 20 µl of 5 mg/ml suspension of silver nanoparticles synthesized using different plant leaf extracts as reductant and stabilizers against *Staphylococcus aureus;* S_1 = sun flower (*helianthus annuus*) mediated Ag nanoparticles; S_2 = Castore Oil (*Ricimus Communes*) mediated Ag nanoparticles; S_3 = Khat (*Catha Edulis*) mediated Ag nanoparticles; O = Control (Distilled Water).

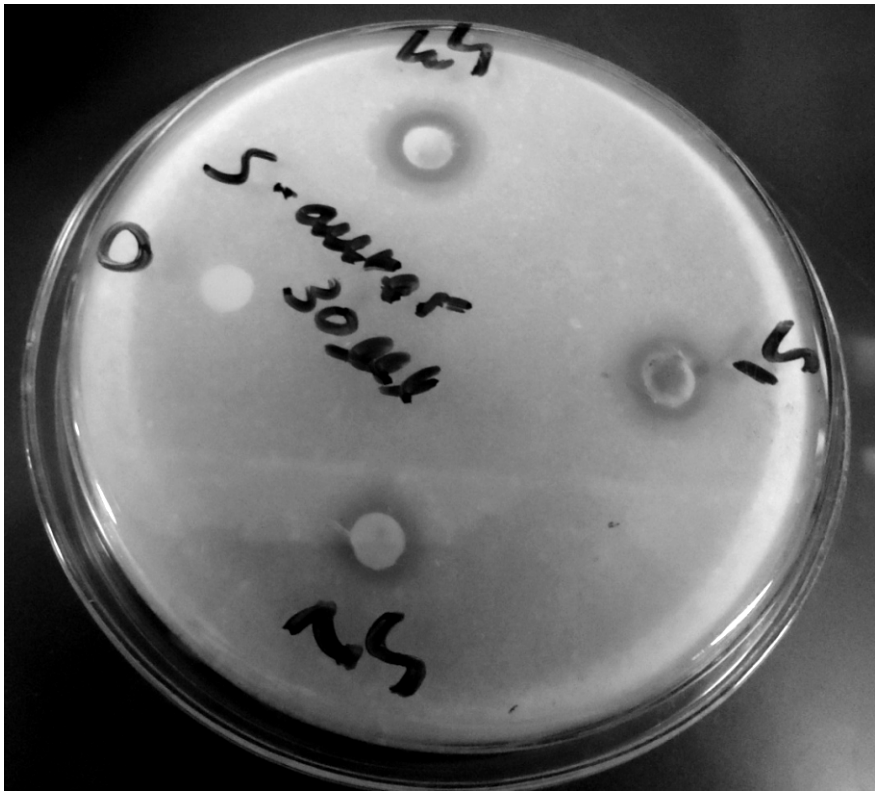

Fig. 13. Zones of inhibition by 30 µl of 5 mg/ml suspension of silver nanoparticles synthesized using different plant leaf extracts as reductant and stabilizers against *Staphylococcus aureus;* S_1 = sun flower (*helianthus annuus*) mediated Ag nanoparticles; S_2 = Castore Oil (*Ricimus Communes*) mediated Ag nanoparticles; S_3 = Khat (*Catha Edulis*) mediated Ag nanoparticles; O = Control (Distilled Water).

TABLE 3.

Zone of inhibition (mm) of sun-flower (*helianthus annuus*), castor oil (*ricinus communes*), khat (*catha edulis*) -mediated Ag nanoparticles

Sample	Test Organism	Concentration of Silver Nanoparticles				P-value
		0 μL	10 μL	20 μL	30 μL	
SF	*Escherichia coli*	0	7.5 $\pm$ 0.5	10.8 $\pm$ 0.3	12.5 $\pm$ 0.5	0.0012
	Staphylococcus aureus	0	10 $\pm$ 0.3	13.7 $\pm$ 0.6	15.5 $\pm$ 0.5	0.0005
CO	*Escherichia coli*	0	8.8 $\pm$ 0.3	10.7 $\pm$ 0.3	11.8 $\pm$ 0.3	0.0008
	Staphylococcus aureus	0	7.8 $\pm$ 0.3	11.8 $\pm$ 0.3	13.0 $\pm$ 0.9	0.0002
KH	*Escherichia coli*	0	7.8 $\pm$ 0.3	10.5 $\pm$ 0.5	13.7 $\pm$ 0.3	0.0001
	Staphylococcus aureus	0	7.7 $\pm$ 0.3	12.8 $\pm$ 0.3	13.7 $\pm$ 0.6	0.0001

Note : Values are mean of three replicates $\pm$ standard deviation.. Values are statistically significant ($p < 0.05$) at 95% confidence interval. SF = sun-flower (*helianthus annuus*); CO = castor oil (*ricinus communes*); KH = khat (*catha edulis*) mediated Ag nanoparticles, respectively.

It shows that silver nanoparticles synthesized via green route are promising antimicrobial agent against pathogens and may have a great potential in biomedical applications [42].

CONCLUSION

The bioreduction of aqueous Ag^+ ions to silver nanoparticles using leaf extracts of castor oil (*racinus communis*), khat (*catha edulis*) and sun flower (*helianthus annuus*) as bioreductants and stabilizing agents have been carried out. The progress of Ag^+ ions bioreduction was monitored, spectrophotometrically.

The FTIR spectra revealed that the reduction of silver ions and stabilization of the resultant silver nanoparticles occur through the participation of plant leaf proteins. As-synthesized silver nanoparticles exhibited antimicrobial activity against two pathogenic bacteria : gram-negative *escherichia coli* and gram-positive *staphylococcus aureus*. Therefore, Silver nanoparticles may be a prominent product with potential application in medicine and hygiene.

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