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Green synthesis of Ag-Nps using *Glycyrrhiza glabra* L. *Sterculia foetida* L. and mixed extract and its antibacterial activity

Suganyaa Baskar [1], Sobana Alagumanian [2], Alagumanian Subramaniam[1*]
[1]PG and Research Department of Botany, H.H.The Rajah's College (Auto), Pudukkottai-622001 Tamil Nadu, South India
[2] Department of Physics, H.H.The Rajah's College (Auto), Pudukkottai-622001, Tamil Nadu, South India
1*Corresponding author : Alagumanian Subramaniam

ABSTRACT
Aqueous extract of *Glycyrrhiza glabra L.* (S_1),*Sterculia foetida* (S_2)and mixed samples (S_3) is utilized as reducing agent for the eco-friendly synthesis of Ag-Nps. The nanoparticles were synthesized and characterize utilizing UV–vis, X-ray diffraction (XRD) and FTIR analysis. Crystallinity of the Ag-Nps is proved from the XRD pattern and Scanning Electron Microscopy (SEM). Bi-molecules accountable for capping are a variety of Ag-Nps as established by the FTIR spectra. The temperament of Ag-Nps synthesized to all examined by UV- vis spectra. The Ag-Nps were by means of a regular size of 7–15 nm and typically spherical exact by XRD pattern. The antibacterial activity of synthesized Ag-Nps was assessed with that of aqueous *Glycyrrhiza glabra L.* (S_1),*Sterculia foetida* (S_2)and mixed samples (S_3) by diffusion technique. The Ag-Nps from S_3 sample prominently reserved bacterial development against multi drug resistant to the entire pathogens which is utilized in this investigation. Thus Ag-Nps displayed wide range antibacterial activity at lower attention and may be an excellent option therapeutic move toward in future.
KEYWORDS: *Glycyrrhiza glabra L, Ag-Nps,Sterculia foetida , XRD, SEM, FTIR*

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INTRODUCTION

Nanotechnology deals with the manufacturing and applications of materials with nanoscale diameter. Nanoparticles (NPs) have shown distinctive properties, which can be rightfully manipulated for several preferred applications due to their high surface area to volume ratio and vast electronic, optical, magnetic, chemical, and physical properties. Significant interest has arisen in the research of NPs during the last decade. Nanoparticles due to their controlled size and composition got fundamental and technological attention because they offer solutions to technological and environmental challenges, owe to their wide applications in various fields, such as in pharmaceutical and biomedical, biomedicine, catalysis, water treatment, energy conservation with tunable electrical conductivity, thermal conductivity, tensile strength, superior rigidity, hardness and erosion resistance, which are currently used for manufacturing of satellite components, aircraft spares, industry parts, and electronic microchips [1,2]. However, in most studies they are suggested to be nontoxic, but due to their small size and variable properties, they are suggested to be perilous to the environment to a significant extent [3,4]. The present chapter highlights the various synthetic schemes and diversified applications of silver nanoparticles (AgNPs).

Antibacterial effect against anaerobic and aerobic bacteria of AgNPs is widely studied by different scientist and research communities around the world. AgNPs show highly antibacterial effect due to their formation of free radicals on its surface to penetrate through the membrane of the cell to disturb the intracellular processes [5-10]. Furthermore, Furno research group has demonstrated the potential of silver nanoparticles as an antimicrobial agent. They showed that silicon discs impregnated with silver nanoparticles efficiently prevented bacterial adhesion and growth. Their potent antibacterial activity was reported against various strains of bacteria including highly pathogenic bacteria species including gram positive and gram negative bacteria. It was observed that a small amount of AgNPs is deadly for the majority of viruses and bacteria [11-13]. Sondi and Salopeck-Sondi reported the antibacterial activities of AgNPs against *E. coli* (representative species for gram negative bacteria) on Luria-Bertani agar plates [14-16]. In another study, Morones *et al*. reported experiments on the size-related properties of the AgNPs on

different species of gram negative bacterial strains. The results obtained from their study suggested that size of AgNPs is an important factor in preventing the bacterial cells from their normal functions [17]. Recently, Sonker *et al*. investigated an eco-friendly, green, cheap, and convenient biological method for the synthesis of AgNPs using the cell extract of the cyanobacterium *Nostoc sp*. strain HKAR-2. As prepared AgNPs showed a dose-dependent cytotoxic activity against human breast cancer MCF-7 cells with IC_{50} of 27.5 µg/ml and therefore exhibited excellent antibacterial and antifungal activities [18].

In recent time combination of AgNPs has concerned imperative due to their different properties such as catalysis [19], optical and magnetic [19], electrical property [12], and antimicrobial properties. The utilize of plants as the synthesis of Ag-Nps has drawn concentration, because of its fast, eco-friendly, non-pathogenic, economical protocol and suggests a single step method for the biosynthetic processes. The reduction and stabilization of silver ions by a mixture of metabolites which are previously introduced in the plant extracts containing therapeutic properties.

An analysis of previous literature provides that extracts from different plants such as *Gliricidia sepium,* [20], *Rosa rugosa* [21], *Chenopodium album* [22]*Cycas* [23]*Acalypha indica* [24], *Cassia fistula*[25], *Hibiscus rosa sinensis*, [26], *Ipomoea aquatica, Enhydra fluctuans, Ludwigia adscendens* [27], *Psidium guajava* [28], *Garcinia mangostana* [29], Krishna tulsi (*Ocimum sanclum*) [30], *Cocos nucifera coir*[31], *Origanum vulgare* [32]*Agrimoniae herba*[33], *Neolamarckia cadamba* [34], *Piper betle* [35] *Plumbago zeylanica* [36], Shikakai and Reetha[37], *Salvia spinosa* [38] etc. have been explored for the synthesis of Ag-Nps.

Glycyrrhiza glabra is one of the most popular medicinal plants belonging to the Fabaceae family (also known as Leguminosae), and its members are now commonly used as feed and food. The genus *Glycyrrhiza* is derived from the Greek words *glykos* (sweet) and *rhiza* (root). It is also called licorice, liquorice, glycyrrhiza, sweet wood, and *Liquiritiae radix* (in English); *süssholz* and *lakritzen wurzel* (in German); *reglisse* and *bios doux* (in French); *shirin bayan* and *mak* (in Persian); and *liquirizia* and *regaliz* (in Italian and Spanish, respectively). This species is a native of Mediterranean areas, but it is now also present in India, Russia, and China. The extracts are currently used in pharmaceutical and food industries, as well as in the manufacture of functional foods and food supplements [39].The use of liquorice predates the Greek and Roman empires, having a long history of traditional medicines and folk remedies. In fact, different geographical areas and periods are linked to different uses [40, 41]. In particular, it is still widely used to treat gastritis, peptic ulcers, respiratory infections, and tremors in folk medicine. Commonly, *G. glabra* root is employed to prepare a tea that is an excellent thirst quencher. The dried root has been described as a tooth cleanser [42]. Actually, the most important industrial use of *G. glabra* is the production of food additives, such as flavours and sweetening agents [43-45]. In particular, the root is used as a flavouring agent for American-type tobacco, chewing gum, candies, baked goods, ice cream, and soft drinks [46]. In beers and fire extinguishers, the root extracts are used as foaming agents, whereas the root fibbers are used in insulation, wallboard, and boxboard materials, after removal of the medicinal and flavouring constituents. In the cosmetic field, *G. glabra* is described as a skin depigmentation agent and is being incorporated in topical products for that purpose.

Sterculia foetida, first described in 1753 by Carolus Linnaeus, is a soft-wood tree grows up to 115 ft tall. It is commonly called Wild Almond, Hazel *Sterculia*, Poon tree, Java Olive, Jangli badam (Hindi), and Gorapu badam (Tamil). The name *Sterculia* genus originates from Sterculius, the Roman God of fertilizer or manure. *Sterculia foetida* seeds can be eaten raw or roasted and are not harmful to humans and animals. A variety of pharmacologically active compounds such as quercetin, apigenin and scopolin have been isolated from *Sterculia foetida* leaves [47]. Sterculinine-I, sterculinine-II, and soyacerebroside-I [48] were isolated from the *Sterculia lychnophora* seeds.

There is little suggestion on the use of natural resources like plants, bacteria, fungi, yeast and honey for synthesizing Ag-Nps. AgNPs synthesized by green route and alsoearlieranalysis recorded the Ag-Nps synthesis from single plants but, here we present a report on the facile, fast and single-pot aqueous biosynthesis of these nanoparticles using the combination of two extract (leaves extract of *Sterculia foetida*and root extracts of *Glycyrrhiza glabra).*

MATERIAL AND METHODS

Experimental

Materials

Silver nitrate 99.9 % was used in this research. Milli-Q water was used to prepare aqueous solution through the synthesis. All chemicals used in this analysis were high-quality analytical grade and it is used without purification.

Preparation of the plant extracts

The *Glycyrrhiza glabra* roots were systematically rinsed with a copious quantity of double distilled water at the beginning, followed by Milli-Q water and the root was cutted by small pieces. The extract was prepared by heating 100 g of roots and dispersed in 500 ml of Milli-Q water for 30 min in Erlenmeyer flask and it is used to water bath at ~100^{o}C. The extract was cooled at room temperature and filtered through a fourfold muslin cloth to find clear filtrate of the root extracts of *Glycyrrhiza glabra* (S_1).

At the first stage the *Sterculia foetida* leaves were thoroughly rinsed with a copious quantity of double distilled water and followed by Milli-Q water and the little pieces of leafs were cut. The extract was prepared by heating 100 g of aerial parts dispersed in 500 ml of Milli-Q water for 30 min in Erlenmeyer flask using water bath at ~100^{o}C. The extract was cooled to room temperature and filtered through a fourfold muslin cloth to obtained clear filtrate of the root extracts of *Sterculia foetida*(S_2). After that 100 ml from both leaves extract of *Sterculia foetida* and root extract of *Glycyrrhiza glabra* were mixed well and used for further analysis (S_3).

Synthesis of Ag-Nps from extract

Biosynthesis of Ag-Nps using aqueous plant extract of *Glycyrrhiza glabra* (S_1),*Sterculia foetida* (S_2)and mixed samples (S_3) wascarry out by addition of 12 ml of S_1, S_2 and S_3extract to a reaction mixture containing 88ml of 1mM $AgNO_3$. The Ag-Nps formed from the leaves and root extract and supernatant were divided at firstby centrifugation at 10000 rpm for 25 min and in also centrifugation of collected supernatant at 15000 rpm for 30 min and the pellets was washed by using Milli-Q water. The pellets were stored and utilized for more explanation and use as antibacterial activity against pathogens.

Characterization of Ag-Nps

X-ray diffraction (XRD) measurements were taken out on Phillips PW 1830 instrument operating at a current of 20 mA and radiation voltage of 40 kV with CuKα radiation (λ = 1.5406 Å). Fourier transforms infrared (FTIR) spectra were recorded at room temperature on a Nicolet 6700 FTIR spectrometer. For the FTIR measurements of capped Ag-Nps, a small number of Ag-Nps (0.01 g) dried at 60^{0}C for 4 h and it was mixed with KBr used to form a round disk appropriate for FTIR measurements. A suitable amount of the extract was combined with KBr to findthe FTIR spectrum of the extract. Morphological and chemical study of synthesized AgNPs was taken out utilizing JEOL 7001F FEG-SEM (REEQ/711/CTM/2005) equipped with EDX detector. UV-visible spectroscopic study was taken out by using UV-vis Spectrophotometer UV-1800 (Shimadzu).

Bacterial strains and cultivation

Bacterial strains having *Escherichia coli, S. haemolyticus, Aeromonas hydrophila, Cronobacter sakazakii, Aeromonas salmonicida, and Basillus subtilis* were utilized for experiment. 50ml of LB broth was prepared in the 250ml conical flask and the bacterial strains were improved in this medium at 370^{0}C on an orbital shaker. The culture flask was inoculated at 0.1 OD 600nm with presentlyprepared LB medium under same culture conditions. The mid-log phase bacterial cultures were utilized for the antibacterial analysis.

Disk diffusion method

0.1 OD of overnight different bacterial cultures was swabbed on the 25ml LB agar plates. Then the whatman disk was placed on the plates. About 30ul of S_1, S_2 and S_3 samples were contain on that whatman disc and incubate for overnight at 37^{o}C. Streptomycin was used as a standard.

Photocatalytic degradation

The photocatalytic activities of the samples were determined by degrading methylene blue dye with a starting concentration of 10 mg/L. A photocatalytic degradation test was carried out in the 200 ml capacity photo-reactor emitting in the range of 254 nm which works in a batch mode. The reactor was enclosed with a water system, made of quartz and this setting with a low pressure mercury vapor lamp (power of 6 W, UV lamp-Philips) for UV irradiation. The concentration of the photocatalyst utilizes the 0.1g of samples suspended into the dye solution and as the resulting suspension was ultrasonicated for 5 min to eliminate aggregate and the dye-catalyst suspension was reserved in the dark condition for 1 h to make sure an adsorption desorption equilibrium. Decolorization of dye solution was tested at disparate time intervals (30 min) by colorimeter and the removal efficiency was recorded.

RESULTS AND DISCUSSION

X-ray diffraction (XRD)

Fig. 1 display the X-ray diffraction (XRD) patterns of synthesized Ag-Npsutilizing S_1, S_2 and S_3 samples extract at room temperature. The XRD patterns of Ag-Nps extract represent that the structure of Ag-Nps is face-centered cubic (fcc) and in also the XRD peaks at 2θ of 38.103^{o}, 44.31^{o}, 64 ^{o} and 77 ^{o}could be ascribed to the 111, 200, 220, 311 crystallographic planes (JCPDS, file no.04-0783). The (2 0 0), (2 2 0), and (3 1 1) Bragg reflections are broadened and weak relative to the intense (1 1 1) reflection. This feature reveal [49] that the nanocrystals are (1 1 1)-oriented as proved by high-resolution SEM

measurements and following to the Bragg peaks representative of FCC silver nanocrystals, additional as yet unassigned peaks are also observed propose that the crystallization of bio-organic phase happen on the surface of the nanoparticles. The same outcome was recorded in Ag-Nps synthesized utilizing *geranium* leaf extract [50] and *mushroom* extract [51]. The sharpness of peaks displayed that the nanoparticles are in nanoregime. White *et al*. [52] has theoretically introduced that in the bio reduction of silver ions several other groups may be concerned *i.e*, amino acids, proteins as well as enzymes. This result display the maximum peak intensity in S_3related to S_1 and S_2, it is obvious that Ag-NPs formed using all extracts were basically crystalline from the XRD pattern. The average nanocrystalline size has been projected by using famous Debye–Scherrer formula,

$$D = (k\,\lambda)/(\beta\,\cos\theta)\ ,$$

Where D is particle diameter size, k is a constant equals 1, k is wavelength of X-ray source (0.1541 nm), β is the full width at half maximum (FWHM) and h is the diffraction angle corresponding to the lattice plane (111). The average crystallite size measured is obtained to be 15nm, 11nm and 7nm for S_1, S_2 and S_3 samples respectively according to Debye–Scherrer equation, the TEM image of Ag-NPs slightly higher compared to the particle size. This can be ascribed to the slight deviation of the spherical shape of the particles that is essentialfor the Debye–Scherrer formula[53,54].

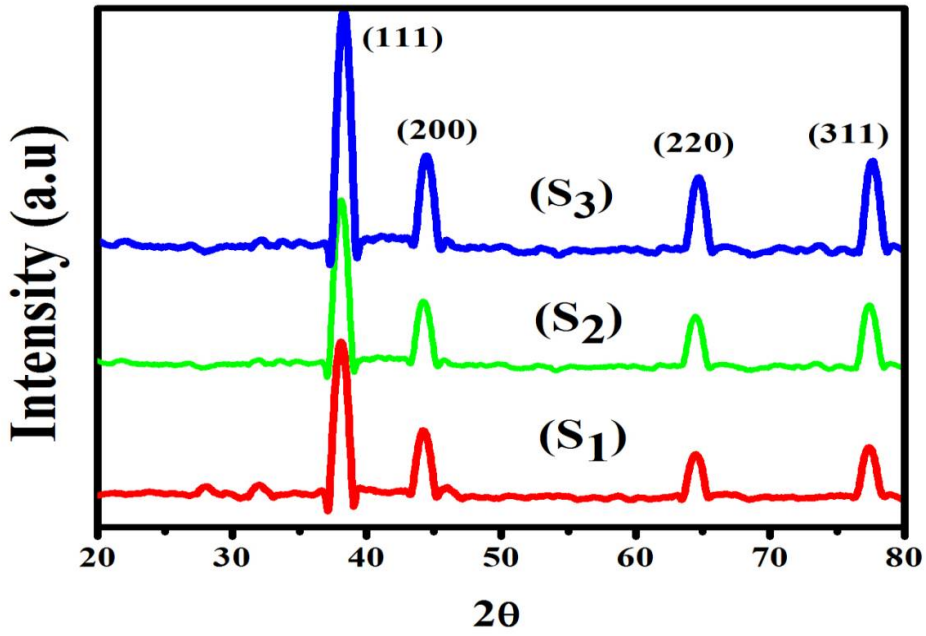


Fig-1.X-ray diffraction pattern of Ag nanoparticles prepared with aqueous extract of S_1, S_2 and S_3

FTIR Analysis

FTIR measurements were taken out to identify the possible biomolecules in S_1, S_2 and S_3 samples responsible for capping leading to efficient stabilization of the Ag-Nps. FTIR spectrum of the Ag nanoparticles synthesized utilizing S_1, S_2 and S_3 samples extracts was showed in (Fig-2), which displayed absorption peaks at 3777, 3395,3285, 2924, 2855, 1643, 1541, 1238, 1030, 780, 618, 566 and 466 cm^{-1}.The symmetric and asymmetric stretching of the O–H groups is demonstrated by bands at 3200–3600 cm^{-1}among them, the typical signal. The presence of C=O groups in extracts displayed by the absorption peak at 1643 $cm^{-1}$and the peak at 1541 cm^{-1} is attributed to C=C functional groups. Following that, the absorbance bands at 1541, 1238, 1030 and 780 cm^{-1} are compared with the in-plane flexing vibration of O–H, the asymmetric stretching of C–O–C, symmetric stretching of C–O–C and out-of-plane flexing vibration of O–H, respectively. polyphenolic compounds in the extracts, such as quercetin 3-rutinoside, delphinidin 3-sambubioside, cyanidin 3-sambubioside, etc are used to arise the absorption peaks. Compared with the spectrum of S3, the intensity of O–H stretching and flexing vibrations very much decreases following the reduction. The alteration could be caused by the fact that the polyhydroxy groups of polyphenols are major responsible for the reduced of Ag (I) and the occurrence of Ag–O groups on the surface of Ag-Nps [55-58]. The vibrational bands equivalent to the bonds such as –C=C–(ring), –C–O,–C–O–CandC=C (chain) are derived from water-soluble compounds such as flavonoids and terpenoids presence. Hence, it possibly inferred that these biomolecules are responsible for capping and efficient stabilization. These environmentally benign nanoparticles would obtain utilizing in cosmetics, food, and medicine.

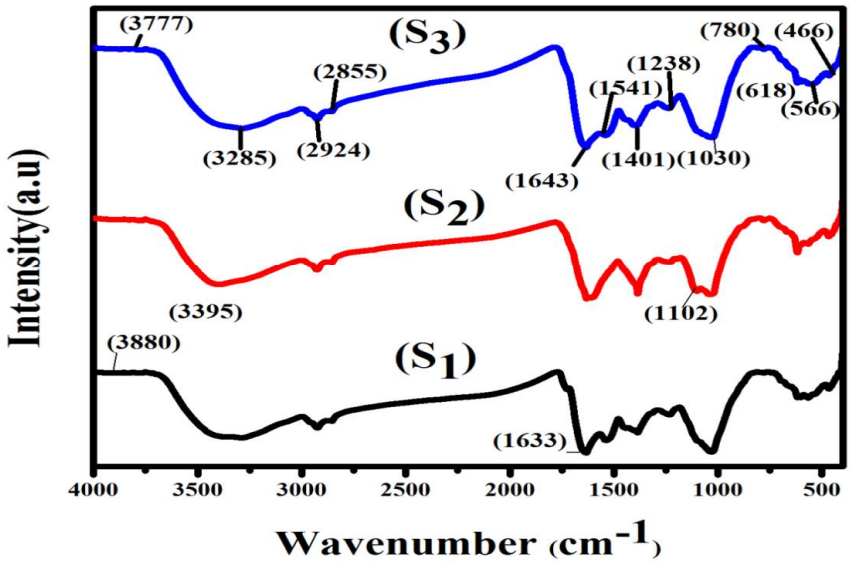
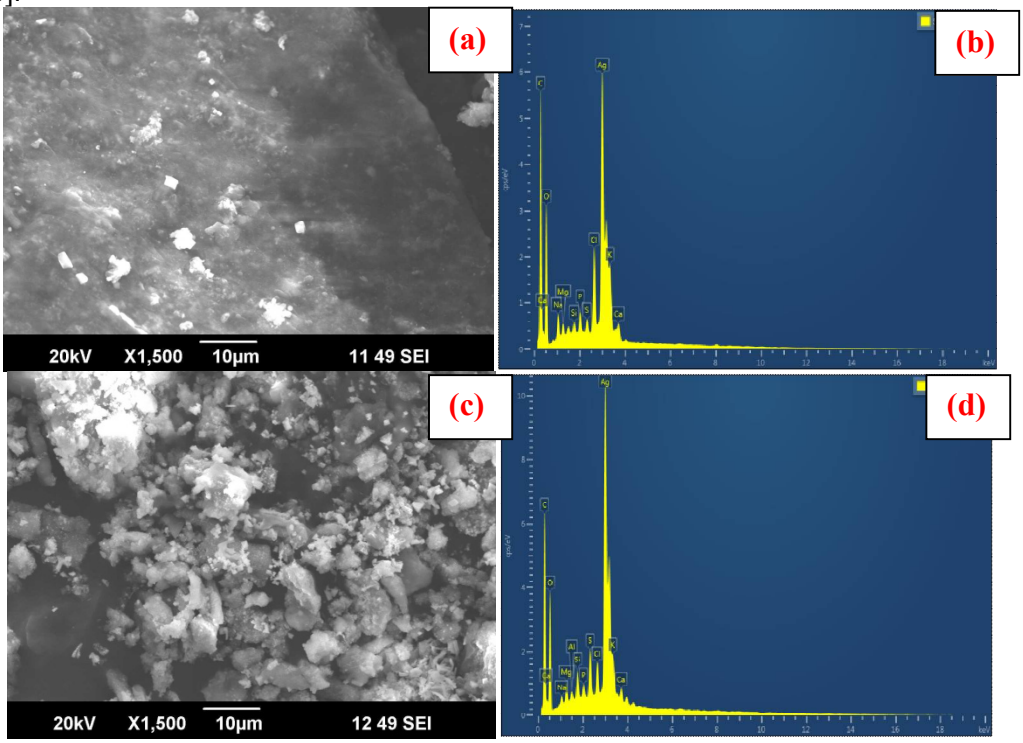


Fig-2.FTIR analysis of Ag nanoparticles prepared with aqueous extract of S_1, S_2 and S_3

SEM Analysis

The morphology S_1, S_2 and S_3extracts of the Ag-Nps were recognized through SEM images (Fig. 3a, c, and e). Ag-Nps were spherical in shape and there were a few oval. Green synthesized Ag-Nps had been increase thoroughly in the solution. The size of few chosensynthesized nanoparticles was 7-15 nm according to SEM images. Dynamic light scattering (DLS) investigation was used to calculate the average diameter of total particles. The majority of the Ag-Nps were 7.13 nm in diameter. In the SEM images the biomolecule coating of the biosynthesized Ag-Npswas showed and this layer introduced plant extract metabolites' role in the synthesis and stabilizing of the green synthesized Ag NPs. These outcomes are in agreement with Oves *et al.*'s [59] obtained. Deposited elemental analysis of synthesized Ag-NPs was taken out with EDX. EDX images of S_1, S_2 and S_3 sample (Fig. 3 b, d, and f) showstrong peaks for silver metal in each sample which proved the existence of Ag-NPs. Strong peaks of metallic nanoparticles appeared at 2.9 keV in all EDX images (Fig. 3b, d, f), as a maincomponent of nanoparticles used to verify the presence of silver [60].



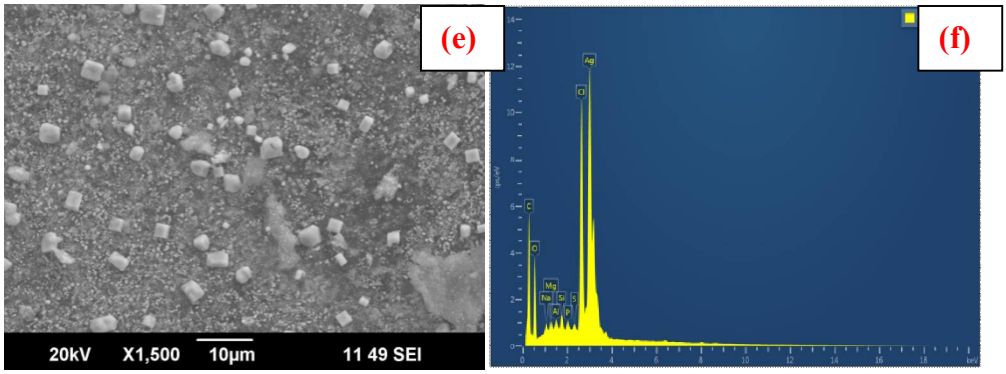


Fig-3.SEM and EDAX analysis of Ag nanoparticles prepared with aqueous extractof S_1, S_2 and S_3

UV–Vis absorption spectrum

UV–Vis absorption spectrum S_1, S_2 and S_3 samples of Ag-Npsareshowed in Fig.4. Broad bell-shaped spectrum curve was obtained from UV–Vis spectra. Different metabolites from plant extract introduced to solution make the plasmon band broad because they possibly read in this spectrophotometric range, too. Surface plasmon resonance (SPR) of silver happens at 450 nm and this peak increased with time up to 4 hours. According to Mie theory, spherical nanoparticles have displayed only a single SPR band. The number of peaks increases by increasing the diversity of particles shapes [61, 62]. Then, it can be completed that biosynthesized Ag-Npsare unanimously spherical in nature.

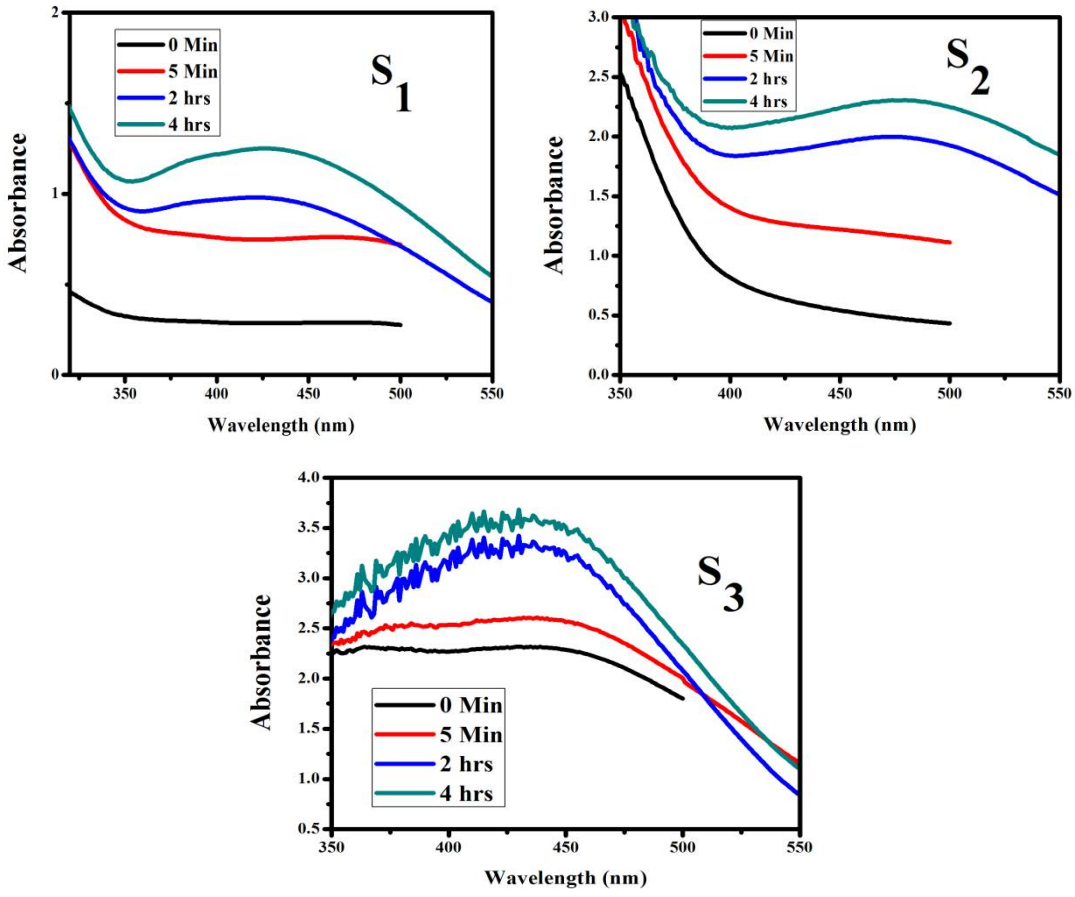


Fig-4.UV spectrum analysis of Ag nanoparticles prepared with aqueous extract of S_1, S_2 and S_3

Dynamic light scattering analysis

The dynamic light scattering technique is an efficient method to calculate particle diameters in the original grain size distribution after reaction [63]. Various factors such as pH value, temperature, and reaction time are the key factors that affect the size distribution of Ag-Nps. Fig. 5a had show the average

particle size of Ag-Nps green synthesized utilize silver nitrate solution at S_1. The size distribution pointed and the mean particle size reduced from 100 nm to 39 nm. When S_2 sample the average grain size of the Ag-Nps are available in Fig. 5b. It can be seen that the size of Ag-Nps prepared with S_2 as a reducing from 100 to 48nm. Fig. 5c demonstrated the average particle size of Ag-Nps synthesized using silver nitrate solution at S_3 as a lessening from 100 to 78nm.

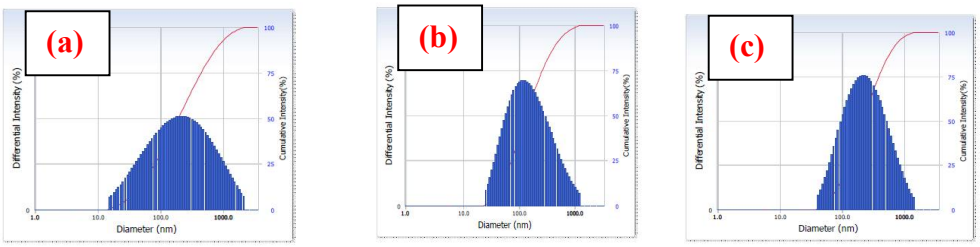


Fig-5. Dynamic Light Scattering analysis of Ag nanoparticles prepared with aqueous extract of S_1, S_2 and S_3

Antibacterial activity of plants based AgNPs

Infectious diseases caused by pathogenic bacteria constantly remain a severe threat to public health and communities. Plants and plant-derived compounds have been used to determine this global issue since prehistory [64]. By disk diffusion method Green synthesis of Ag-Nps was trialed for antibacterial activity against pathogenic bacteria. Silver ion and silver compounds are toxic to pathogens because they are highly effective towards microorganisms and contain large surface area. Silver ions, as well as Ag-Nps, were known to include strong antimicrobial activities. The antibacterial activity of S_1, S_2 and S_3 samples containing Ag-Nps demonstrated that bacteria were inhibited by various solutions with different extents. The outcome of the antibacterial analyze are depicted in Table-1. These results agreed with earlier work taken out by Bindhu and Umadevi, [65]. Highest UV absorption denoting formation of high amounts of Ag-Nps was displayed by the highest activity on all bacteria was detected with S_3.The difference in the sensitivity of bacteria to Ag-Nps was owing to the dissimilarity in thickness and constituents of their membrane structure [66].

Table 1. Antibacterial activity of Ag nanoparticles prepared with aqueous extract of S_1, S_2 and S_3

			Zone of inhibition(mm)		
	strains		S_1	S_2	S_3
E.coli (KF 918342)			9.3±0.6	10.3±0.3	14.6±0.9
S. Haemolyticus			10±0	11±0	14.3±0.7
Aeromonas hydrophila			9±1	10±1	13.3±1
Bacillus subtilis			12±1.1	10.6±1.1	15.2±0.8
Cronobacter sakazakii			11±0.6	12.3±0.6	14.7±0.9
Aeromonas salmonicida			13±1.5	12.6±1.5	18±0.7

Photocatalytic Study

The photocatalytic activity of the samples of S_1, S_2 and S_3 NPs for the degradation of methylene blue (MB) is illustrated in Figure 4.10. When S_1, S_2 and S_3catalyst were irradiated with visible light using mercury vapour lamp, electrons (e^-) in the valance band are excited to the conduction band with simultaneous generation of the same amount of holes (h^+) in the valance band. The figure4.11 shows the photocatalysts study of S_1, S_2 and $S_3$with methylene blue (MB) dye concentration (10 mg/L) and the photocatalyst dose of 0.1 g under UV light irradiation (λ=254 nm). The distinguishing peak of the methylene blue dye illustrates the absorption at 665 nm and depending upon the intensity peak of the methylene blue dye absorption decreases with the increase of the reaction time. The S_3 catalysts exhibit higher photocatalytic activities compared to S_2 and S_1. When the composition of metal ions has become more dominant to capture electrons examined in a greater photocatalytic activity. But if the amount of metal ions act as recombination centers of electrons and holes and hence the photocatalytic activity is found to increase [67]. The colour removal efficiency of the photocatalyst prepared in S_1, S_2 and S_3 is 87.0, 81.7 and 90.9 %

and these values were achieved within 120 min. S_3 has shown higher efficiency of 90.9 % compared to other photocatalysts. It has also been tested the reusability of S_3 for 3-4 cycles.

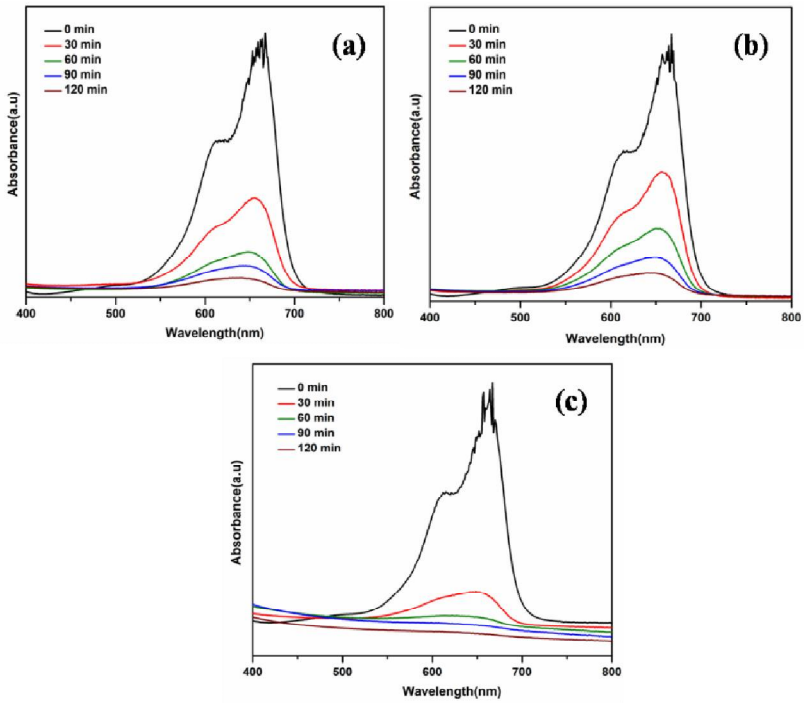


Figure 6 Variations in absorption spectra *vs*. wavelength for the photocatalytic degradation of MB as a function of irradiation time in the presence of photocatalyst S_1, S_2 and S_3

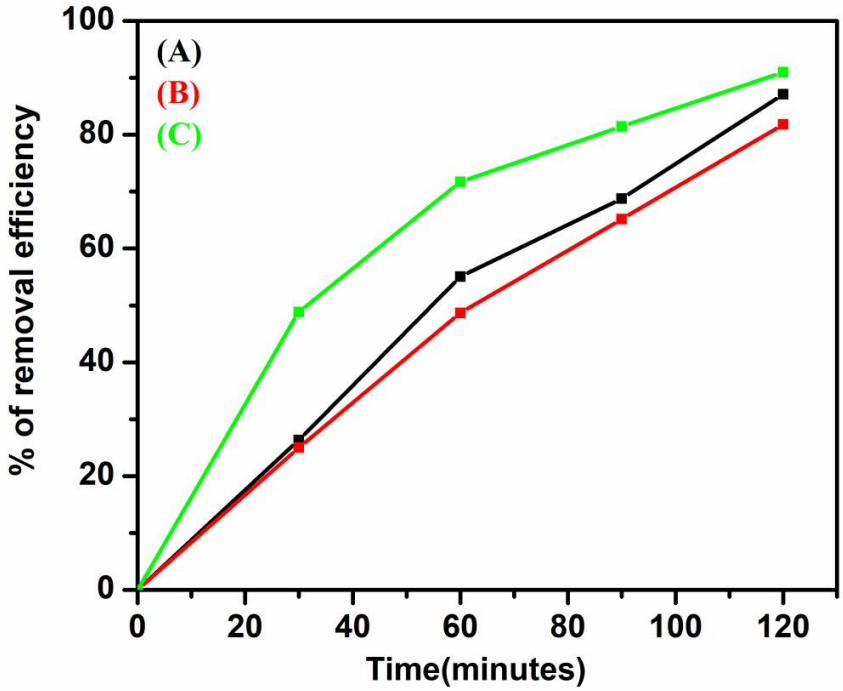


Figure 7 Photocatalytic degradation of MB in presence of (A) ZnO, (B) SnO_2 and (C) Zn_2SnO_4NPs

CONCLUSION

In our result, we have reportedfor the first time the green synthesis of highly constant spherical Ag-Nps of 7 to 15 nm diameter from the plant extract of *Chrysopogon zizanioide* (S_1), *Ocimum sanctum* (S_2) and mixed samples (S_3). The different equipment was measured by UV–vis, SEM, XRD and FTIR was described by the nanoparticles. In also, bactericidal activity assessment of the biosynthesized Ag-Nps displayed their inhibitory function against different bacterial pathogens. In this study, possible functional groups

and effective compounds responsible for the decrease of silver ions were assigned. Here we concluded that the S_3 sample appeared a promising candidate for Ag-Nps synthesis and antibacterial activity.

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