Journal of Optoelectronic and Biomedical Materials Vol. 15, No. 2, April - June 2023, p. 65 - 79

Anti–oxidant and anti-inflammation activities of nanostructured assemblies of silver sulfide nanoparticles using an extract of Cinnamomum tamala leaves

C. Upadhyay, R. Kothari*
Department of Chemistry, School of Sciences, ITM University, Gwalior (M.P.), 474005, India

Silver sulfide nanoparticles have gained applications in industrial areas due to their tunable physical, chemical, and biological properties. Among various nanoparticles silver sulfide nanoparticles have gained worldwide attention due to their assessment as an anti-microbial agent. The microstructure of nanostructured silver sulfide nanoparticles determine its electronic, structural, optical and electrical properties, and possible applications of silver sulfide nanoparticles in modern electronics, biology and medicine. The appearance of non-stoichiometry in silver sub-lattices of monoclinic silver sulfide at decreasing size particles to the nanometer scale is considered. The interdependent changes in non-stoichiometry and crystal structure at the transformation of a non- conducting nano crystalline silver sulfide in super-ionic conductors are also very important aspects in biosynthesis of Silver sulfide nanoparticles. The effect of nano crystalline state on the peculiarities of crystal structure, non-stoichiometry, optical and thermal properties of bio synthesized Silver sulfide nanoparticles are explained. Silver sulfide nanoparticle have provide a significant role in the biomedical field for various application- oriented products such as IR cameras, solar panels, optical fiber and filters. Among modern biomedical potential of silver sulfide nanoparticles tremendous interest is oriented towards the therapeutically enhanced personalized health care practices. The biosynthesized silver sulfide nanoparticle using aqueous extract of Cinnamomum leaves has been documented in our present research work. The presence of secondary metabolites like flavonoid, tannins, steroid, cardiac glycosides, and alkaloids was confirmed by phytochemical analyses of the aqueous extract of Cinnamomum tamala leaves and these secondary metabolites can be used as reducing stabilizing and capping agent. After three months, the biosynthesized Silver sulfide nanoparticles were found to be stable without the evidence of agglomeration at room temperature. Structural and morphological properties of Silver sulfide nanoparticles were analyzed by UV-VIS, FT-IR, XRD, EDX, TEM, and SEM spectroscopic techniques. The surface Plasmon resonance for Silver sulfide nanoparticles was obtained around 290nm. Biosynthesized Silver sulfide nanoparticles was spherical in shape with effective diameter size of 50nm.Our novel approach provides a promising and effective method to large scale synthesis of eco-friendly, and cost effective pharmacologically active silver sulfide nano particles.

(Received December 15, 2022; Accepted May 2, 2023)

Keywords: Silver sulfide nanoparticle, Cinnamomum tamala leave, Structural, Morphological, Pharmacological activities

1. Introduction

In nano biotechnology, using green synthesis of metallic nanoparticle from plant parts has gained very much important due to inclusion of ecofriendly protocols for preparing the wide range of nanoparticles. Biosynthesis of metallic nanoparticles from various plant parts is regarded as an important and efficient tool for reducing toxic effects of chemicals which are used in classical chemical methods. In the green approach nontoxic, aqueous environment and ecofriendly chemicals have been used for the synthesis of metallic nanoparticles. Nanoparticles (NPs) have been increasingly used in medicine due to their unique physicochemical properties, stability as

* Corresponding author: richakothari@itmuniversity.ac.in
https://doi.org/10.15251/JOBM.2023.152.65

well as thermal and photochemical features [1-3]. Among the various noble metal nanoparticles, silver nano particle have received particular interest due to their ability to overcome the multidrug resistance (MDR) problems [4-6]. The pharmacological activities of nanoparticles are generally derived from their ability to reach the molecular target within which specific biochemical reactions are initiated. Thus, smartly designed metallic nanoparticles with special physicochemical properties like small size (<100nm), large surface –to- volume ratio, and effective pharmacological reactivity are considered as some excellent drug candidates for anticancer, antioxidant, anti-inflammatory and antimicrobial agents. [7-11].

Since ancient times, silver salts have been widely used for various medical purposes e.g. as an antiseptic, antimicrobial, anticancer and wound healing agents [12-13]. Recently, silver nitrate and silver nanoparticles are FDA – approved for the use in antibacterial wound dressing, food supplements and medical devices (i.e. antibacterial surface impregnation) [14-15]. Overall in medical application, silver nanoparticles are less toxic than other forms of silver, exhibit negligible toxicity (depending upon the particle size of nanoparticles) and possess a diverse range of biomedical applications. The increasing use of silver nanoparticles in biomedical science raises hopes for their use as an alternative anticancer agent that may be involved in interfering with the mitochondrial respiratory chain, leading to increased reactive oxygen species (ROS) formation and ATP synthesis arrest, and consequently DNA damage [16-19]. The significant biological activity of Ag NP's against breast (MCF-7, SKBR 3, and 8701-BC), leukemia and color (HT-29, HCT-116 and Caco-2) cancer cells was reported. Silver nanoparticles have been well explored as vesicle-like nano systems. For example, silver nanoparticles based hydrogels embedded in polymer cross linked network, which were conjugated with doxorubicin have been tested as an antitumor agent against malignant melanoma. Moreover, similar hybrid nano composites exhibited antimicrobial activity against gram positive and gram negative bacteria [20-22]. It was also reported that hydrogen containing Ag/Ag @ Agcl/Zno promotes wound healing and shows high antimicrobial activity against E. coli and S. aureus after exposure to visible light [23]. Additionally, Au/Ag hybrid nanoparticles have been found to be effective theramostic agents for photoacoustic imaging and therapeutics of bacterial infections. Gold and Silver nanoparticles have been used to visualize bacteria through fluorescence change analysis, making the antibacterial treatment procedure more clear and allowing accurate control of anti-bacterial agent doses, thereby avoiding possible drug resistance [24]. The cytotoxicity of silver nanoparticles may be tunable by their change and size – alterable properties. Zwitter ionic modified silver nanoparticles have been shown to be highly effective antibacterial materials capable of eradicating bacterial biofilms without damaging healthy cells, indicating their potential further use in humans [25]. Recently, silver nanoparticles have shown excellent agents as novel virucides. Silver nanoparticle conjugated oseltamivir (Tamiflu R, neuraminidase inhibitor) carrier inhibited H1N1 influenza virus activity via ROS mediated signaling pathways [26]. Moreover, it has also been shown that the silver nanoparticles can conjugate with coronaviruses and bind to the host cells [37,38, 43]. Now- a -day the increasing number of virological studies have given some hope for the therapeutic use AgNp^' Sagainst SARS – COV-2 [27-28].

In the present study, an attempt has been made to examine the effect of nanoparticles size, charge and surface- volume ratio, importantly major factors that determine the properties of silver sulfide nanoparticles in pharmacological applications. To explore this issue, a seed mediated growth method was used to fabricate phytochemicals capped silver sulfide nanoparticles with three different size ranges optimal for biomedical applications (~5, ~10, ~20). Our main goal was to synthesize a range of silver sulfide nanoparticles with both smaller and much larger sizes (an equivalent approach for zeta potential) which was accomplished and explained that the biological properties of each nanoparticle fraction are fundamentally different.

2. Experimental

2.1. Chemicals

Thiosemicarbazide hydrochloride & Silver nitrate were purchased from Merck Pvt. Ltd India. Ethanol, dichloromethane and methanol were also purchased from Merck Pvt. Ltd India. used as solvents. Distilled water from a Millipore System (e=18.2MΩ) was used in all experiments and also in washing glassware's of the laboratory experiment.

2.2. Plant materials

The leaves of Cinnamomum tamala leaves were collected from local market of Gwalior (M.P.) in the month of March 2019 (figure 1). The authentication of the plant species was done by subject expert and a voucher specimen (ITM|4|CT|19) was deposited in the Department of Chemistry, School of Sciences, ITM University, Gwalior (M.P.).Cinnamomum tamala leaves have a clove like aroma with a hint of peppery and they are used for culinary and medicine purposes. It is thought to have been one of the major sources of the medicinal plant leaves known in classic and medieval time as malabathrum.

Table 1 . Scientific Classification of Cinnamomum tamala

Kingdome	Plantae
Clade	Tracheophytes
Clade	Angiosperm
Clade	Magnoliids
Order	Laurales
Family	*Lauraceae*
Genus	Cinnamomum
Species	C. Silver Sufide

Aroma attributes of Cinnamomum tamala leaves: The Cinnamomum tamala leaves have strong aroma attributes due to presence of Beta –caryophyllene, Linalool, Caryophyllene oxide and Eugenol. Cinnamomum tamala leaves also called India bay leaves. From ancient times, these are considered as Ayurvedic medicines which are used in the formulation of various topical gels and ointments as an analgesic, anti-inflammatory and antioxidant agents [29]. Hence biosynthesis of silver sulfide nanoparticles from Indian bay leaves is cost effective, ecofriendly, biodegradable green approach.

2.3 Preparation of the plant extract

An aqueous extract of the Cinnamomum tamala leaves was used for the green synthesis of silver sulfide nanoparticles. Leaves of Cinnamomum tamala have been used as an alternative anticancer agent that may be involved in interfering with the mitochondrial respiratory chain leading to increased reactive oxygen species formation and ATP synthesis arrest and consequent DNA damage. To remove debris and associated dust, fresh leaves were washed with double distilled water (DDW) and cut into very fine pieces before being dried in the presence in the sunlight. 20 g of the leaves was immersed in 100 ml distilled water and the leaves were incubated at 40□c for 100 min to prepare for extraction. After incubation, the extract of leaves was kept at room temperature for cooling, filtered using whatmann no.41 filter paper (ash less filter paper) and used for the synthesis of silver sulfide nanoparticles.

2.4 Phytochemical analysis

In order to elaborate the presence of important phytochemicals in the aqueous extract of Cinnamomum tamala leaves, the extract was subjected to qualitative screening. For this screening**,** some specific functional group test was performed to check the availability of biomolecules like flavonoid, cardiac glycosides, tannin, saponins , steroids, carbohydrates etc. The standard protocol for qualitative analysis was used to identify the presence of major phytochemicals in the aqueous extract of leaves, as shown in Table 2.

Table 2. Phytochemical analysis of aqueous extract of Cinnamomum tamala leaves.

S.NO.	Phytochemical constituents	Result
1.	Flavonoid	+
2.	Alkaloids	+
3.	Glycosides	+
4.	Steroid	+
5.	Phenols	+
6.	Terpenoids	+
7.	Saponins	-
8.	Resins	+
9.	Tannins	+
10.	Cardiac Glycosides	-
11.	Phytosterols	+
12.	Carbohydrates	+
13.	Fixed oils and fats	-

2.5. Visual characterization of Green synthesized silver sulfide nanoparticles

Green synthesis of Silver sulfideNP'S has been indicated by changing of colored solution of silver nitrate solution into black. (Fig.1) According to green technology, metallic nanoparticles were synthesized in three phases. The first phase is considered as an activation phase which indicates the metal ion reduction in the presence of reducing agents either in the form of chemical or phytochemicals after that nucleation process starts. The second phase is the growth phase which involves the synthesis of nano assemblies of metallic nanoparticles. In the third phase, which is known as termination phase which facilitates the formation of proper shape and geometry of metallic nanoparticles.

In our work, we use aqueous solution of AgNO3 for synthesis of silver sulfide NP's. Due to ionic nature of AgNO3, it immediate dissociates into Ag+ ions and No3- ions and when extract of Cinnamomum leaf extract was added along with Thiosemicarbazide hydrochloridesolution , silver ions present in the solution reduces into zero –valent state i.e. Ag0. In this work thiosemicarbazide hydrochloride used as a source of S2- ions for the formation of silver sulfide NP'S. This process leading to the formation silver sulfide NP'S followed by the growth phase, leaving behind the remaining component as by products. [30]

2.6. Green synthesis of silver sulfide nanoparticles

In an Erlenmeyer flask 100ml of silver nitrate solution (3mm) and 1mM of thiosemicarbazide hydrochloride solution was stirred for 20 minutes then add 100 ml of aqueous extract of Cinnamomum tamala leaves and continued to stirring for 24 hours at room temperature. After 24 hours the color of the solution turned from colorless to black color, indicating the formation of Silver sulfideNP'S and this was also confirmed by UV-visible spectroscopic measurement. After completion of reaction, the final reaction mixture was centrifuged at 10,000- 11,000 rpm and Silver sulfideNP'S were separated from the solution. Finally, the solution was filtered out via whatmann filter paper no. 41 and black colored Silver sulfideNP'S were collected.

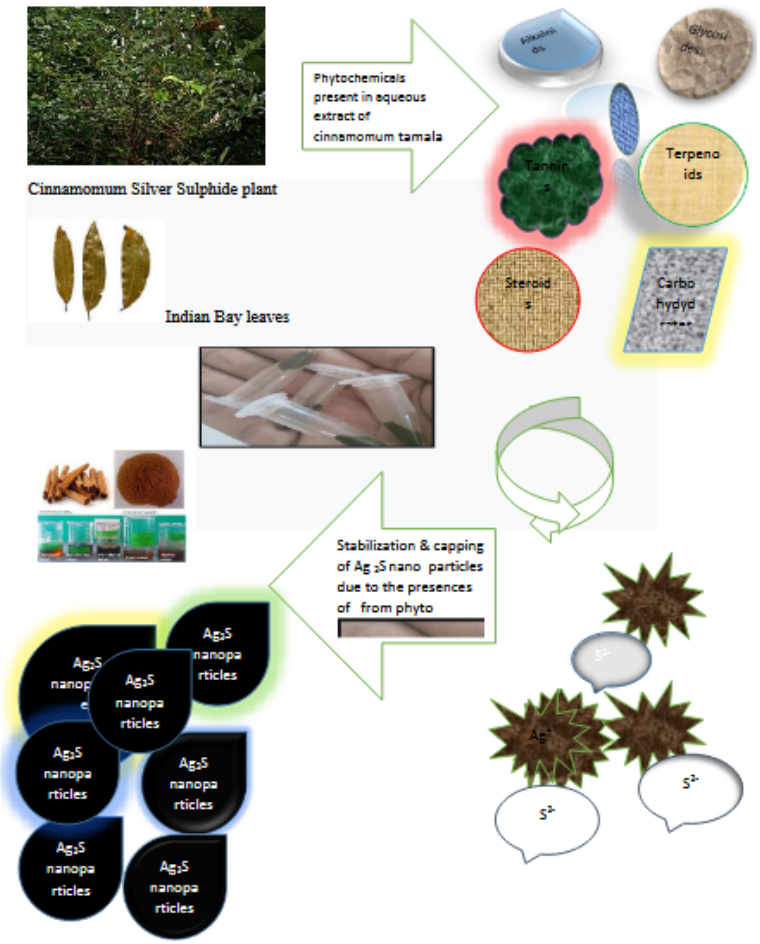

Fig. 1. Possible mechanism of Green synthesis of the silver sulfide nanoparticles from Cinnamomum tamala leaves.

2.7. Characterization of green synthesis silver sulfide nanoparticles

All the required chemicals were purchased from sigma- Aldrich and Merck Pvt. Ltd. All the chemicals were used without further purification. Green synthesized silver sulfide nanoparticles were collected and spectroscopically characterized by the structural, morphological and vibrational technique like UV-Vis, FT-IR, XRD, TEM, SEM & EXD. The structure and the particle size of silver sulfide nanoparticles were characterized by X-ray diffraction (XRD) at room temperature using a D_8 advanced BRVKER diffractometer equipped with CU K$\propto$ ($1.54A^0$) as the incident radiation. The Scherrer equation was used for the calculation of the crystal size. The Scherrer equation was:

$$D = \frac{k\lambda}{\beta \cos\theta}$$

where,

K= 0.9
D= Crystal size (A□)
λ= Wavelength of the Cu k ∝ radiations
β= Corrected half – width of the diffraction peak

The UV-Visible absorption spectra of silver sulfide nanoparticles were recorded on a Perkin Elmer UV – Vis Lambda 25 UV Visible spectrometer in PC Ray research Centre, ITM

University Gwalior (M.P) using 1cm path cuvette and used dichloromethane solvent for dissolution of $ilver\ SulphideNP'S$. The FT-IR spectra were recorded on a Perkin – Elmer FT-IR spectrophotometer (KBr pellets) in PC Ray research Centre, ITM University Gwalior, (M.P) with the wavelength range 400- 4000 cm^{-1}. The fine structure of the prepared sample of $Silver\ SulphideNP'S$ was analyzed by HR scanning electronic microscopy (HRSEM) by TECHAI G2F20 operated at 300 KV using a drop of suspension of sample in ethanol on carbon coated copper grid. Energy dispersive X-ray spectroscopy (EDX) of $Silver\ SulphideNP'S$was carried out on sigma ZEISS, Field Emission Scanning electron microscope. Anti-inflammatory and antioxidant activities of green synthesized silver sulfide nanoparticles were investigated using UV- Vis Spectrophotometer DPPH method. Protein denaturation, protease inhibition assay, heat induced heamolysis assay.

2.8. Antioxidant activities of green synthesized silver sulfide nanoparticles

The free radical scavenging activity of green synthesis **silver sulfide nanoparticles** at concentration 200,400,600,800 and 1000 **µ**g/ml was carried out in the presence of freshly prepared solution of stable radical DPPH (0.04%w/v) following Hataro's method using ascorbic acid as a standard substance for comparison of antioxidant activities of silver sulfide nanoparticles. All the test analysis was performed in triplicate manner and results are averaged. The results in percentage are expressed as the ratio of absorption decrease of DPPH free radical in the presence of $Silver\ SulphideNP'S$ and absorption of DPPH in the absence of silver sulfide nanoparticles at 517 nm by UV-Visible spectrophotometer. The percentage scavenging activity of DPPH free radicals was measured using following equation:

$$\% \text{ RSA} = \frac{AC-AS}{AC} \times 100$$

where,

A_c = Absorbance of control
A_s =Absorbance of Sample

2.9. Anti-inflammatory activities

(a) Inhibition of protein denaturation:

Inhibition of protein denaturation activities of sample was evaluated by standard protocol of Mizushima et al [31]. In this method, 100 ml of 1 % solution of BSA was added to sample, than heat the solution at 50□c for half an hour. After cooling the solution, absorbance was taken at 660nm using UV- visible spectrophotometer. In this procedure acetyl salicylic acid (Aspirin) was taken as a positive control. This experiment was performed in triplicates and percentage inhibition of protein denaturation activity of sample was calculated by the following formula:

$$\% \text{ Inhibition of protein denaturation} = 100\text{-}\left\{\frac{A-A}{A_o}\right\} \times 100$$

A_o = Absorbance of standard acetyl salicylic acid
A_1 = Absorbance of leaf extract
A_2 = Absorbance of $Silver\ SulphideNP'S$

b) Protease inhibition assay

This activity was evaluated by Saket et al [32]. In this method 100 ml of BSA solution and 100 ml $Silver\ SulphideNP'S.$ incubated at room temperature for 15 minutes then add 250 ml of trypsin solution for inhibition effect of protease enzyme and centrifuge the mixture for 5 minutes at 10,000- 12,000 rpm. After centrifugation, supernatant was collected and take the absorbance at 217nm with the help of UV- Vis spectrometer. Performed this experiment in the triplicates and the percentage protease inhibition activity was calculated by the following formula.

$$\% \text{ Protease Inhibition } = 100\text{-}\left\{\frac{A-A}{A_o}\right\} \times 100$$

A_o = Absorbance of positive control (Aspirin).
$A_{1=}$ Absorbance of leaf extract
A_2 = Absorbance of $Silver\ SulphideNP'S$

c) Heat induced hemolysis assay

This activity of sample was performed using a standard protocol of Cerda et al and Sasty M et al [33-34]. In this method, 5 ml of healthy human blood sample was centrifuged at 3000 rpm for 15 minutes. After centrifugation, cells were washed with saline solution (P^H -7.2), than add equal volume of leaf extract and silver sulfide nanoparticles solution. Both the solution was kept on water bath for 20 minutes for digestion. After digestion of samples, the absorbance of samples was monitored at 520 nm with the help of UV-Vi s spectrophotometer at the time interval of 10 minutes. In this method acetyl salicylic acid was taken as standard solution.

3. Results and discussion

This research work reports the green synthesis of silver sulfide nanoparticles from aqueous extract of Cinnamomum tamamla leaves which was free from any contamination of impurities and requires continuous stirring of reaction mixture which contains 100 ml of $AgNO_3$ solution, 100 ml thiosemicarbazide hydrochloride solution and 100 ml of aqueous extract of Cinnamomum tamala leaves in a ratio of 1:1 v/v with continuous stirring of reaction mixture. The color of solution changes form colorless to black indicates the formation of silver sulfide nanoparticles and this was verified by UV – visible spectroscopy.

The aqueous extract of Cinnamomum tamala leaves contains flavonoid, alkaloids, glycosides, steroids, phenolic compounds, terpenoids, resins, tannins ,phyto-sterols and carbohydrates phytochemicals that act as capping as well as stabilizing agents for green synthesis of silver sulfide nanoparticles and these phytochemicals may also be responsible for the reduction of silver ions into silver sulfide nanoparticles Due to the presence of phytochemicals in aqueous extract of Cinnamomum tamala leaves, aggregation of silver sulfide nanoparticles was not observed next three months after green synthesis of silver sulfide nanoparticles. The possible mechanism for the green synthesis of silver sulfide nanoparticles is shown in figure 1.

3.1. Optical properties

3.1.1. UV- Vis Spectroscopic Analysis

This is a very important and reliable technique for the primary identification of synthesis of metallic nanoparticles because the peak positions and shapes are very sensitive to particle size of nanoparticles. The sample of silver sulfide nanoparticles at a concentration of 1mg/ml was prepared in distilled water. The electronic absorption spectra were registered in quartz cuvette (L=1cm). The measurement was performed at room temperature and the range of 200- 800 nm, using a Perkin Elmer UV- Vis Lambda 25 UV- Vis spectrophotometer. Nano sized metallic nanoparticles exhibit unique optical properties having an exponential decay. The surface plasmon peak of silver sulfide nanoparticles has been reported to appear at 310 nm which confirm the green synthesis of silver sulfide nanoparticles A bathochromic shift i.e. blue shift from 285nm to 310 nm was observed with the increase in the amount of aqueous extract of cinnamomum tamala leaves. The observation of blue shift in UV spectra can be explained on the basis of increased nucleation rate due to greater amount of Ag_2S nano particles in the reaction mixture at the time of stirring of reaction mixture. Black colored silver sulfide nanoparticles were appeared when stoichiometric amount of silver nitrate solution, Thiosemicarbazide hydrochloride solution and aqueous extract of Cinnamomum tamala leaves are mixed. Due to the presence of the phytochemicals in leaves extract, a large number of silver sulfide nanoparticles were formed within a short time.

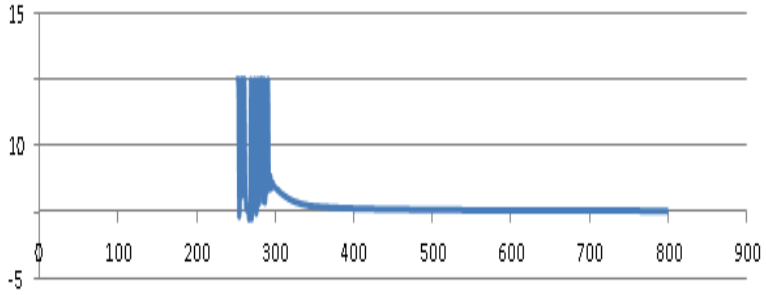

Fig. 2.UV spectra green synthesized silver sulfide NP'S

3.1.2. FT-IR Spectral analysis of green synthesized silver sulfide NP'S

FT-IR spectral analysis of aqueous extract of Cinnamomum tamala leaves and green synthesized silver sulfide NP'S were carried out to identify the presence of phytochemical which are responsible for the reduction, capping and stabilization of biosynthesized silver sulfide NP'S. Table-3 displays the major peaks, wave numbers, and the interpretation of the possible functional groups. The FT-IR spectra also shows that the phytochemicals of the leaves extract or their functional groups which are responsible for reducing and stabilizing the silver sulfide nanoparticles. The absorbance bands of leaves extract at 3370, 2410,1586, 1050,670 and 420 cm-1 are due to O-H, C-H, C=N, C-O, C-Cl functional groups, respectively. Simultaneously, absorbance bands for the silver sulfide nanoparticles at 3786, 2917, 2075,1605, 1515,1384,1075,830 and 361 cm-1 correspond to the functional groups of O-H, C-H, C=N, C-O and C-Cl and might be responsible for the bio reduction of Ag+ S2- ions into silver sulfide NP'S.

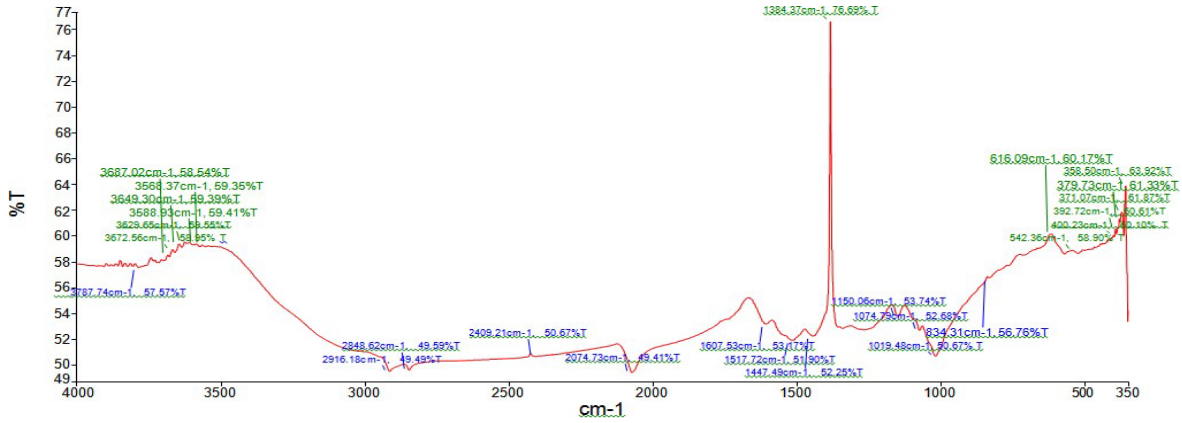

Fig. 3. FT-IR spectra of green synthesized silver sulfide NP'S.

3.1.3. Photoluminescence studies

Photoluminescence studies were carried for leaves extract and biosynthesized silver sulfide NP'S shown in figure 4. The optimized biosynthesized silver sulfide nano particle shows a strong emission peak at 290nm for an excitation at 350 nm. When silver sulfide nanoparticles excited at 300nm, it showed excitation at 350nm. The luminescence at 250 nm and 350nm may be due to the presence of phytochemicals in aqueous extract of leaves of Cinnamomum tamala.

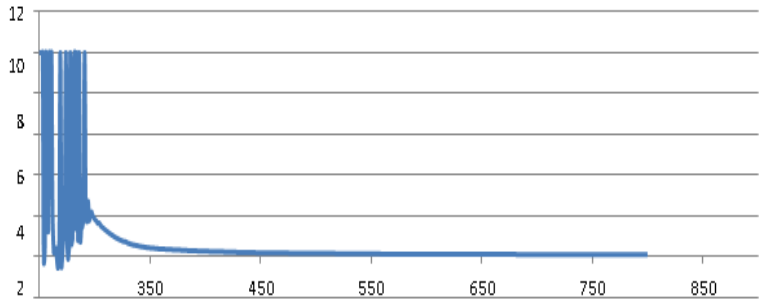

Fig. 4. Photoluminescence spectra of green synthesized Ag_2 SNP'S.

3.1.4. Powder X-ray Diffraction Studies

The crystalline phase of the silver sulfide nanoparticles was characterized by XRD analysis. It is an important technique which has been used for both molecular and crystal structures, qualitative identification of compound and quantitative study of functional groups which are responsible for the degree of crystallinity and small size of nanoparticles. The diffraction intensities were recorded from 5 to 80 at 2ɵ angles, suggesting a monoclinic configuration. Five characterization peaks were observed at 2ɵ angels of 38.1□, 44.4□,64.4□ 77.4□ and 81.4□ and these corresponds to the h, k, l values of the reflection from (110), (200), (220), (800) and (715), collaborating with the values of the JCPDS Card (card number – 893722). The Scherrer formula:

$$D = \left(\frac{0.9\lambda}{\beta cos\theta}\right)$$

where, $\lambda = wavelenth$ of X -rays

β= full width at half maximum at the ɵ angles was used to calculate the average crystalline was used to calculates the average crystalline size of the biosynthesized silver sulfide nanoparticles, showing 0.5732+ 1 nm on the (110) plane. The particle size was below 100 nm, the width of the peaks obtained in XRD pattern is related to the crystalline size of the particle. The small size of nanoparticles indicated the high surface area to volume ratio .[35]

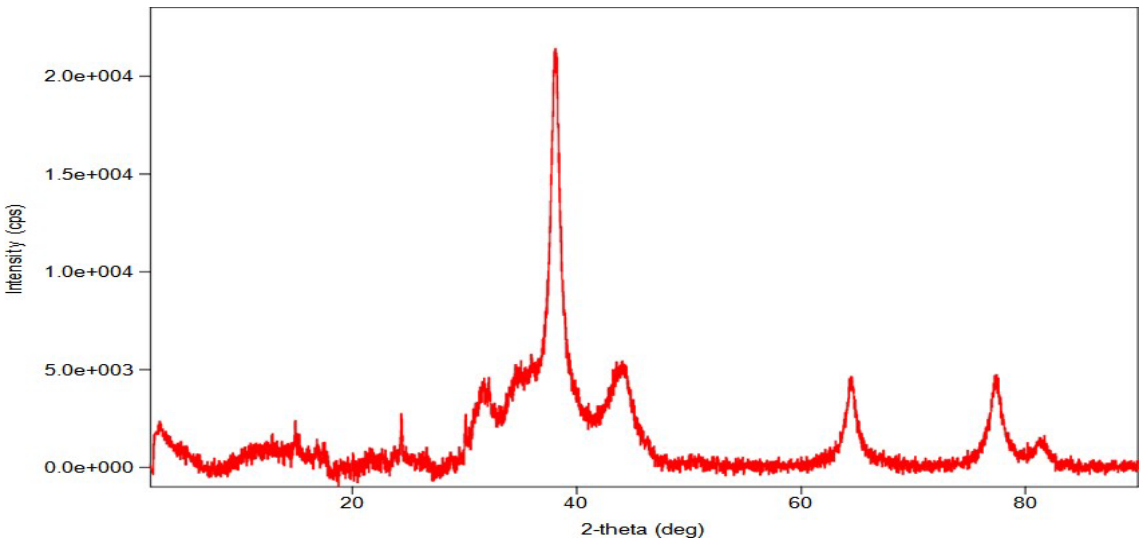

Fig. 5. XRD spectra of biosynthesized silver sulfide Nano particles.

3.1.5. Morphology (SEM and EDX) analysis of biosynthesized silver sulfide Nano particles

The morphology of the biosynthesized Ag_2 S nanoparticles was examined using Field Emission Scanning Electron Microscopy (FESEM).

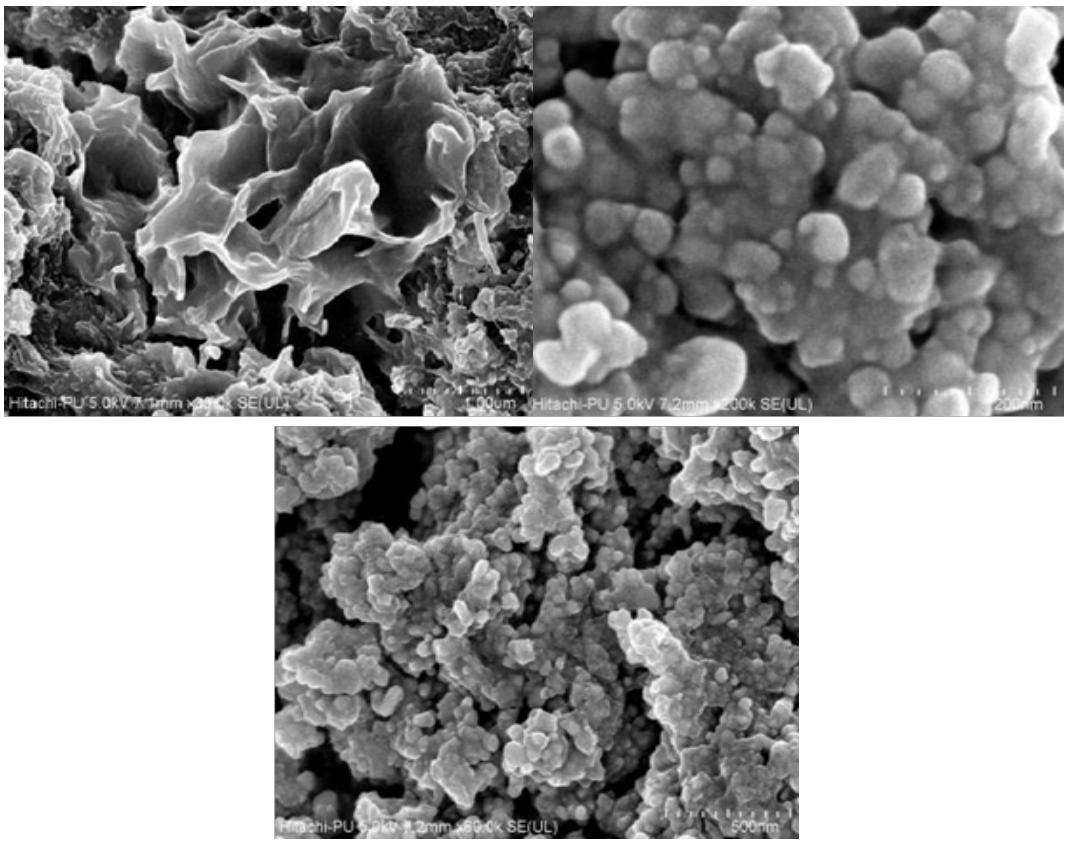

Fig. 6. SEM spectra of biosynthesized Ag_2 S Nano particles

Fig 6 represents the SEM images of the Ag_2 S nanoparticles at different resolutions. The elemental composition of the green synthesized silver sulfide nanoparticles was examined using EDX (Energy Dispersive) X- ray analysis. Figure 7 shows the EDX spectra displaying the % of composition and major elemental peak at 8Kev that is specific to the Ag metal and S nonmetal. Along with peaks of Ag and S in the EDX spectra other small peaks appeared for phytochemicals that were used for the capping of the Ag_2 S nanoparticles. The percentages of Ag $_2$ S and other photochemical are given in table elemental analysis and determination of weight percentage and atomic percentage of biosynthesized silver sulfide nanoparticles.

Table 3. Elemental composition of Elements present in EDX Spectra of biosynthesized silver sulfide nanoparticles.

Element	Weight %	Atomic %
Silver	95.10	98.02
Sulfur	4.90	1.98
Other	100	100

The signals appear in EDX spectra confirm the presence of elements. Some others impurities were also present in the sample such as C, O was identified become of the interaction of the phytochemicals present in leaf extract of Cinnamomum tamala with Silver sulfide during bioprocessing of silver sulfide nanoparticles. SEM monograph in figure- 7 explains the formation of well dispersed, versatile and spherical silver sulfide nanoparticles via green approaches. SEM monograph showed that silver sulfide nanoparticles were arranged in a very open and quasi linear structure. The presence of solvent does not change the shape of nanoparticles but presence of solvent in the reaction medium initiates the nucleation process in a very short time.

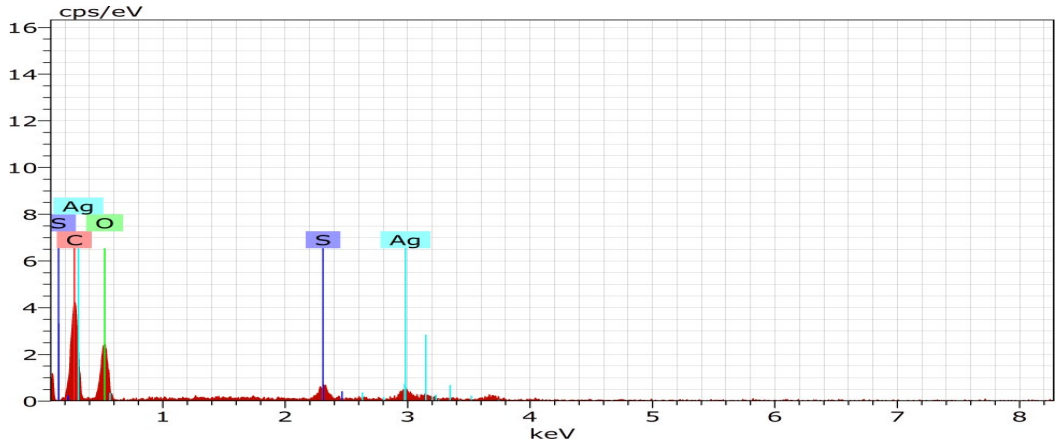

Fig. 7. EDX spectra of biosynthesized Ag_2 S Nano particles.

3.1.6. TEM Analysis of biosynthesized Ag_2 S nanoparticles

Transmission electron microscopy (TEM) in an important characterization tool for the direct image of nanomaterial's to obtain quantitative measure of particle size, size distribution, shape and lattice fringes. The particle size and lattice fringes are measured using HRTEM (SAIF Jiwaji University CIF Laboratory, Gwalior). The thickness and radius of silver sulfide nanoparticles were estimated using Gatan software. Reduced FFT shown in figure 8 indicates the highly crystalline behavior of biosynthesized silver sulfide nanoparticles and clear occurrence of lattice fringes in biosynthesized silver sulfide nanoparticles. The presence of dark and bright fringes confirms the formation of very small crystalline structure of nanoparticles. The particles have been separated by well- defined boundaries, and are visible and uniformly distributed, which was confirmed by microscopy visualizing under the high resolution. The TEM images also explain that the solver sulfide nanoparticles are capped and stabilized by the phytochemicals which are present in the aqueous extract of Cinnamomum tamala leaves.

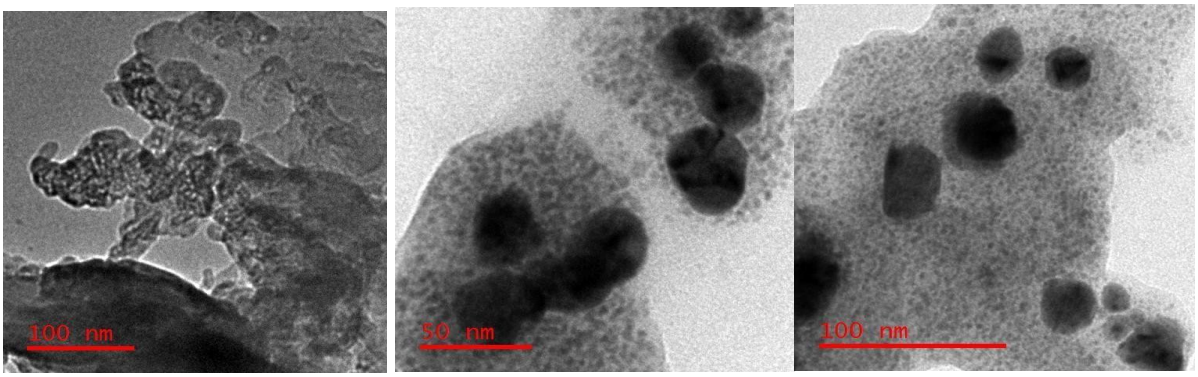

Fig. 8. TEM Images of Biosynthesized silver sulfide nanoparticles.

3.1.7. In-vitro antioxidant activity

Biosynthesized silver sulfide nanoparticles was screened for antioxidant activity via DPPH assay. DPPH is a stable free radical compound and has been widely used to test the free radical scavenging activity of numerous synthesized compounds as well as herbal products. In the in vitro antioxidant activity of sample the pairing of electrons takes place after reacting with reducing agent and it loses its pink color stoichiometrically depending upon the number of electron accepted by DPPH radical due to the presence of antioxidant agent in the reaction mixture. In this experiment ascorbic acid used as a standard substance (indicator) for comparison of antioxidant activity of synthesized sample. An unshared electron presents on the nitrogen atom of DPPH molecule is responsible for the absorbance of light at 517nm and also for the development of visible deep purple color. When DPPH radical accept an unpaired electron donated by an antioxidant compound, the color of DPPH solution fades, which can be quantitatively measured from the changes in the absorbance of light. The reverse reaction is evaluated by adding DPPH at the end of the reaction. If there is an increase in the percentage of remaining DPPH free radical at the plateau, the reaction is reversible; otherwise it is a complete reaction The DPPH assay evaluates the ability of the sample to donate H to the DPPH radical, resulting in de- colorization of DPPH solution. The greater de- colorization action of solution, the higher the antioxidant activity of compound and this was reflected in a lower IC_{50} value of silver sulfide nanoparticles.

Table 4. In-vitro antioxidant activities of biosynthesized silver sulfide nanoparticles.

S.no.	Compound	DPPH activity (IC_{50} μg/ml)
1.	Aqueous extract of Cinnamomum tamala leaves	2.59
2	Biosynthesized silver sulfide nanoparticles	3.50
3	Ascorbic acid (Standard)	3.76

3.1.8. Anti-inflammatory activity of slver sulpfde nanoparticles

Inflammation is an early immunological response against foreign particles by tissues which is confirmed by the increased production of pro –inflammatory cytokines, the activation of the immune system, and the release of prostaglandins and chemotactic substances such as complement factors interleukines-1 (IL-1), TNF- alpha (35) deep. Among several anti-inflammatory agents, silver sulfide nanoparticles have recently played an important role as an anti-inflammatory agent.

3.1.8.1. Inhibition of Protein Denaturation activity:

The aqueous extract of Cinnamomum tamala leaves and biosynthesized Ag_2 SNP'S showed excellent inhibition of (i) Protein denaturation activity (ii) Anti –proteinase activity (iii) Heat – induced hemolysis method. In this experiment, aspirin drug used as a standard anti-inflammatory drug.

Table 5. Inhibition of protein denaturation activity of compounds.

S.No.	Compound	Protection + SD (%)	P value	Error
1.	Aqueous extract of Cinnamomum tamamla Leaves	79%	0.01	0.079
2.	Biosynthesized silver sulfide nanoparticles.	82%	0.01	0.082
3.	Aspirin drug (standard)	89%	0.01	0.089

Table 6. Inhibition of anti-proteinase activity of compounds.

S.No	Compound	protection	P Value	Error value
1.	Aqueous extract of Cinnamomum tamala leaves	79%	0.01	0.079
2.	Biosynthesized Ag_2 S nanoparticles	84%	0.01	0.084
3.	Standard aspirin drug	89%	0.01	0.089

Table 7. Anti-inflammatory activities of compound by heat induced haemolysis method.

S. No	Compound	Protection + SD (%)	P value	Error Value
1.	Aqueous extract of Cinnamomum tamala leaves	79%	0.01	0.079
2.	Biosynthesized Ag_2 S nanoparticles	81%	0.01	0.081
3.	Standard aspirin drug	89%	0.01	0.089

3. Conclusion

In summary, novel biosynthesis of silver sulfide nanoparticle from aqueous extract of Cinnamomum tamala leaves is a simple, fast and ecofriendly green chemistry approach. During the interaction of phytochemicals of leaves with AgNO3 and thiosemicarbazide hydrochloride solutions, the color of the reaction mixture turned from colorless to black indicating the formation of silver sulfide nanoparticles and this was also confirmed from UV- visible spectroscopy. The absorbance peaks at 285 and 310nm in the UV visible spectroscopy spectral results confirmed the formation of stable silver sulfide nanoparticles via green approach. Also result of FT-IR spectroscopy also confirmed the green synthesis of Silver sulfide NP'S. From the various structural and morphological studies, it has been observed that the shape and size of silver sulfide nanoparticles depends upon the concentration of aqueous extract of Cinnamomum tamala leaves which was used as a precursor for the biosynthesis of silver sulfide nanoparticles.

The use of aqueous extract of Cinnamomum leaves helps to reduced silver nitrate and thiosemicarbazide hydrochloride very fast in the spherical shapes of Silver sulfide NP'S (silver sulfide nanoparticles) within a short period of time. This is a simple, very efficient, ecofriendly, fast and cost efficient method for the synthesis of silver sulfide nanoparticles. The use of plant extract plays a very important role in reducing reaction time, reducing minimum possibilities of side reaction and proper convention of silver nitrate and thiosemicarbazide hydrochloride solution into silver sulfide nano particles in a very short time. This ecofriendly method has very strong potential to be utilized for the large scale industrial production of silver sulfide nanoparticles. From the results of biological activities of green synthesized compound, it has been concluded that green synthesis of silver sulfide nanoparticles from Cinnamomum tamala leaves in a aqueous environment can be consider as a good drug candidate for various biomedical applications like antibacterial, antioxidant, and anti- inflammatory agents in future for human.

Acknowledgements

Richa Kothari and Chandani Upadhyay thanks to the management of ITM University, Gwalior for grating a mobility Grant in 2019. The authors are thankful to the SAIF Chandigarh,

PC Ray research centre ITM University, Gwalior for spectral analysis. Author also thankful to the multi-disciplinary laboratories of ITM University Gwalior for providing technical Support

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