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Pharmacological Applications of Silver Nanoparticles Synthesized using Aqueous Leaf Extract of *Ximenia americana*

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ABSTRACT

The *X. americana* leaf was extracted using water by cold extraction method. AgNPs were synthesized by phytoreducing method. The synthesized silver nanoparticles were characterized by using different techniques including UV–visible spectroscopy, FTIR analysis, scanning electron microscopy and energy dispersive X-ray spectroscopy. *In-vitro* antioxidant activity of synthesized AgNPs were evaluated by using FRAP, H_2O_2, and DPPH assays, anti-inflammatory activity was carried out by using protein denaturation *in-vitro* bioassay and anthelmintic activity by employing standard *in-vitro* method (*Pheretima posthuma* model). UV-Vis spectral analysis which revealed a higher absorbance peak (λ_{max}) at 413 nm. FTIR spectra showed shifts in some peaks of aqueous extract with functional groups. SEM studies show the size of AgNPs in the range of 30-150 nm with irregular shape. EDX shows sharp peak of silver at 3 kev with various elemental composition. *In-vitro* pharmacological study revealed that synthesized AgNPs exhibited potential antioxidant, anti-inflammatory and anthelmintic activity.

1. Introduction

Biotechnology has emerged up as integration between biotechnology and nanotechnology for developing biosynthetic and eco-friendly technology for synthesis of nanomaterials. Nanoparticles are cluster of atoms in the size range of 1-100 nm. The metallic nanoparticles are most promising in health care due to their large surface area to volume ratio [1]. Generally the nanoparticles synthesized from chemical methods but these methods of synthesis are medically non-applicable because of contamination from precursor chemicals [2]. Now a day's different types of nanoparticles of copper, zinc, titanium [3], manganese, magnesium, silver and gold are synthesized [4]. In the above mentioned metal nanoparticles, silver nanoparticles are biologically active and thus have immense application in the field of medicine [5]. Silver nanoparticles are gaining more attention due to their enormous applications, which includes biolabelling in optical receptors, catalyst in many chemical reactions and also possess different biological activities such as antibacterial, antifungal, antioxidant, antiviral and anti-inflammatory activities [6-9]. AgNPs can be produced by using physical and chemical methods but the nanoparticles synthesized from these methods have environmental defect and economically expensive [10]. Different biological sources can be used for synthesis of AgNPs such as bacteria, fungi, algae and plant material [11]. Green nanotechnology has received much attention due to its cost-effective and eco-friendly approach. Among various sources available, plants are considered as preferred choice due to their bio-reducing and stabilizing potential [12]. Plants serve as sources of many biochemical compounds which include alkaloids, flavonoids, tannins, phenols and saponins that could act as effective reducing agents for the bio-reduction of metal into nanoparticles which have a wide range of biological applications [13, 14]. The plant extracts mediated synthesis of nanoparticles is relatively fast as there is no need of maintaining specific media and cultural conditions, unlike microbial synthesis. With this background, the present study was undertaken using *Ximenia americana* plant belonging to Olacaceae family. In our previous studies, the aqueous leaf extract of *X. americana*are reported to have high phenolic content and antioxidant activity [15]. However there is no scientific reports on the use of *Ximenia americana* leaves for the synthesis of AgNPs, hence in the present study attempt was made to synthesize AgNPs using *X. americana* and also evaluate the antioxidant property of synthesized AgNPs.

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2. Experimental Methods

2.1 Plant Collection

Ximenia americana leaves was collected from Karnatak University Campus, Dharwad, India in the month of April 2018. The leaves were identified and authenticated by Dr. K. Kotresha, Dept of Botany, Karnatak Science College, Dharwad, Karnataka, India. A voucher specimen (001) was deposited in the Dept. of Botany, Karnatak Science College, Dharwad, Karnataka. Fresh leaves material was washed under running tap water, shade dried and then powdered using mechanical grinder. The powder leaves material was stored in airtight containers at -20 °C for further use.

2.2 Preparation of Plant Extract

About 25 g of powdered leaves material was extracted with 250 mL of distill water using soxhlet apparatus for 48 h. The aqueous extract was further concentrated using rotoevaporator, then dried in desiccator and was stored in air tight bottle at 4 °C until use. The aqueous extract was used as reducing and stabilizing agent for synthesis of AgNPs [16].

2.3 Solvents and Reagents

All the solvents and chemicals used were analytical grade and were obtained from Hi-media, India.

2.4 Preparation of Silver Nitrate Solution

Silver nitrate solution of 1 mM was prepared by dissolving 0.017 g amount of silver salt in 100 mL of distilled water and stored in amber color bottle.

2.5 Synthesis of Silver Nanoparticles

1 mL of leaves aqueous extract was added to 10 mL of 1 mM $AgNO_3$ aqueous solution. The overall reaction mixture process was carried out in a dark condition at room temperature to avoid unnecessary photochemical reactions. After the incubation and reaction time, the colorless reaction mixture changed to dark brown color that indicated the oxidation/reduction reaction [17]. After the desired reaction period aqueous mixture containing Ag-NP's was centrifuged at 10,000 rpm for 10 min, repeated centrifugation and re-dispersion in double distill water was carried out to remove the traces of aqueous extract in the newly synthesized AgNPs, which were allowed to dry in powder [18].

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2.6 Characterization of Newly Synthesized AgNPs

The synthesized AgNPs were characterized by using different techniques including UV–Visible spectroscopy, FTIR analysis, scanning electron microscopy and energy dispersive X-ray spectroscopy, TGA and DSC analysis.

2.6.1 UV–Visible Spectroscopy Analysis

The reduction of silver ions in the colloidal solution was confirmed and analyzed by UV spectrum of 1 mL of aliquot sample in quartz cuvette by using UV–visible spectroscopy and observed for wavelength scanning between 200-1100 nm with distilled water as a reference and 1 mM $AgNO_3$ as a blank [19].

2.6.2 FTIR Analysis

FTIR spectroscopy was used to recognize the functional groups (bio-groups) that bound on the silver surface and involved in the formation of AgNPs. After 72 hours of incubation the AgNPs were isolated by repeated centrifugation (3-4 times) of the reaction mixtures at 10,000 rpm for 15 min. The supernatant was replaced by de-ionized water and the pellet was stored as powder. The dried AgNPs were subjected to FTIR analysis by potassium bromide pelleting method in 1:100 ratio.

2.6.3 Scanning Electron Microscopy Analysis

Surface morphology of nanoparticles was determined by scanning electron microscopy. The sample was prepared by centrifuging colloidal solution after 4-6 h reaction at 10,000 rpm for 5 min. The pellet was redispersed in deionized water and re-centrifuged, this process was repeated for several times and then dried to get dry pellet. Then the purified AgNPs were sonicated for 5-10 min with one cycle making the suspension. Then the drop of the suspension was placed on carbon coated grid. The sample was kept under lamp until get complete dry. The prepared sample was subjected to SEM analysis using SEM Model - JSM-6360 at the University of Kohlapur, Kohlapur, India.

2.6.4 Energy Dispersive X-Ray Analysis

The reduced AgNPs were dried on carbon coated copper grid, which was then subjected to EDX and through this techniques the elemental composition was also detected.

2.7 Determination of In-vitro Antioxidant Activity

2.7.1 Ferric Ion Reducing Antioxidant Power Assay (FRAP)

Ferric ions reducing power was measured according to the method of Oyaizu with a slight modification [20]. Biosynthesized AgNPs in different concentrations ranging from 100 µL to 500 µL were mixed with 2.5 mL of 20 mM phosphate buffer and 2.5 mL 1%, w/v potassium ferricyanide, and then the mixture was incubated at 50 °C for 30 min. After incubation, 2.5 mL of 10%, w/v trichloro acetic acid and 0.5 mL 0.1%, w/v ferric chloride were added to the mixture, which was incubated for 10 min. Finally, the absorbance was measured at 700 nm using a UV-Vis spectrophotometer. Ascorbic acid was used as reference standard. All samples were assayed in triplicates.

2.7.2 Hydrogen Peroxide Scavenging Assay

The antioxidant activity of synthesized AgNPs with ascorbic acid as a standard was assessed based on their ability to scavenge the hydrogen peroxide [21]. 0.6 mL of 4 mM H_2O_2 solution in phosphate buffer (pH-7.4) was added to 0.5 mL of known concentration of standard ascorbic acid and to tubes containing different concentrations ranging from 100 µL to 500 µL of plant extracts in phosphate buffer (pH-7.4). Absorbance of the solution was measured at 230 nm after 10 min against the blank solution containing phosphate buffer without hydrogen peroxide. Control was prepared by replacing the sample or standard with phosphate buffer. All samples were assayed in triplicates. The percentage of inhibition was calculated by using formula method.

$$\text{Percentage of inhibition } \% = A_c\text{-}A_t/A_c \times 100$$

2.7.3 DPPH Free Radical-Scavenging Ability Assay

Radical scavenging activities of synthesized AgNPs were determined using the DPPH radical as a reagent, according to the methods of Rice-Evans *et al*. [22]. 100 µL of a DPPH radical solution in ethanol (60 µM) was mixed with 100 µL of sample solution in ethanol (different concentrations, w/v). The mixture was incubated for 30 min in the dark at room temperature and then absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Ascorbic acid was used as a reference standard. The DPPH scavenging activity of each sample was calculated using the following equation:

$$\% \text{ inhibition} = A_c\text{-}A_t/A_c \times 100$$

where, A_c is the absorbance of the control reaction (100 µL of ethanol with 100 µL of the DPPH solution), and A_t is the absorbance of the test sample. The experiment was done in triplicate. The IC50 value was calculated for all the samples used. Lower absorbance of the reaction mixture indicated higher free radical activity.

2.8 Evaluation of In-vitro Anti-Inflammatory Activity

Anti-inflammatory activity of synthesized AgNPs was evaluated by protein denaturation method as described by Padmanabhan *et al*. [23] with slight modifications. Diclofenac sodium, a powerful non-steroidal anti-inflammatory drug was used as a standard drug. The reaction mixture consisting of 2 mL of known concentration of synthesized AgNPs (100 µg/mL) with standard Diclofenac sodium (100 µg/mL) and 2.8 mL of phosphate buffered saline (pH 6.4) was mixed with 2 mL of egg albumin (from fresh hen's egg) and incubated at 27±1 °C for 15 min. Denaturation was induced by keeping the reaction mixture at 70 °C in a water bath for 10 min. After cooling, the absorbance was measured at 660 nm by using double distilled water as blank. Each experiment was done in triplicate. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = A_t\text{-}A_c/A_c \times 100$$

where, A_t=absorbance of test sample; A_c=absorbance of control.

2.9 In-vitro Anthelmintic Activity

The anthelmintic activity of synthesized AgNPs was evaluated by the following the method of Dash et al [24]. For each group of animals with three earthworms in each groups, each earthworm were separate released into 20 mL of desired formulation in normal saline, Group 1 earthworm were released in 20 mL normal saline in a clean petri plate. Groups 2-6 earthworms were released in 20 mL normal saline containing 50, 100, 150, 200 and 250 mg/mL of synthesized AgNPs respectively. Last group of earthworms were released in 20 mL normal saline containing standard drug piperazine citrate (100 mg/mL). Earthworms were observed; the time taken for paralysis and the time taken for death was monitored and documented in minutes. Paralysis time was analyzed based on the behavior of the earthworm with no revival body state in normal saline medium. Death was concluded based on total loss of motility with faded body color [25].

2.10 Statistical Analysis

All experiments were performed in triplicates (n=3) and the data are represented as the mean ± standard deviation. Differences between the means of the individual groups were analyzed using the analysis of variance procedure of SPSS software 20 Version (IBM). The significance of differences was defined at the $p < 0.05$ and $p< 0.01$ level.

3. Results and Discussion

3.1 Visual Observation and UV-Vis Characterization

Addition of aqueous extract of *X. americana* leaves to the aqueous solution of 1 mM $AgNO_3$, lead to formation of color change in the reaction mixture from yellowish to brownish color within 15 min, this indicates bioreduction reaction of $AgNO_3$ from silver metal ions (Ag^+) in solution state to silver nanoparticles (Ag^0). After 1 h incubation, complete reaction occurred with formation of reddish brown color (Fig. 1).

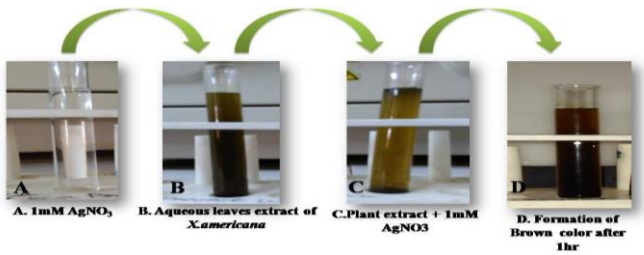

Fig. 1 Photograph showing formation of AgNPs with a color change after addition of aqueous leaf extract of *X. americana*

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Formation of silver nanoparticles was confirmed by the UV-Vis spectral analysis of colloidal solution for wavelength scanning between 200-1100 nm. UV-Vis spectral analysis reveals maximum absorption peak (λ_{max}) at 413 nm (Fig. 2).

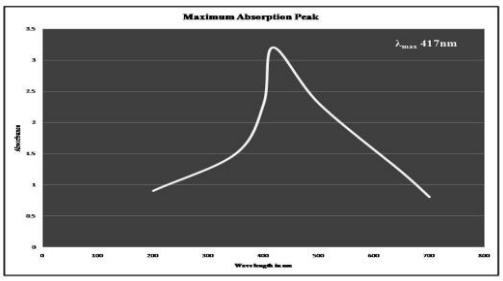

Fig. 2 UV-VIS absorption spectrum of Silver nanoparticles after 1 h of incubation

3.2 SEM and EDX Studies

Surface and morphology of the synthesized AgNPs was confirmed by the SEM studies. SEM images showed irregular shaped silver nanoparticles and size in diameter range of 30-150 nm. EDX studies helped to find out purity of silver nanoparticles and its elemental composition (Fig. 3). It reveals that strong signal of metallic silver region at 3 Kev which confirmed the formation of silver nanoparticles by the aqueous leaves extract of *X. americana*. Its elemental composition studies reveal that in addition to silver there was presence of different elements like carbon, nitrogen, silicon and oxygen in 47.04%, 1.83%, 0.64% and 3.20% respectively and silver as major element with percentage 47.29% (Table 1 and Fig. 4.).

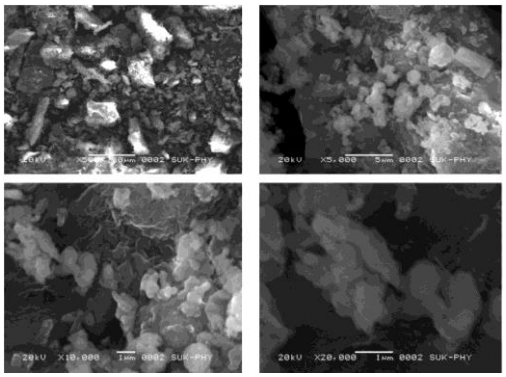

Fig. 3 SEM images of biosynthesized silver nanoparticles

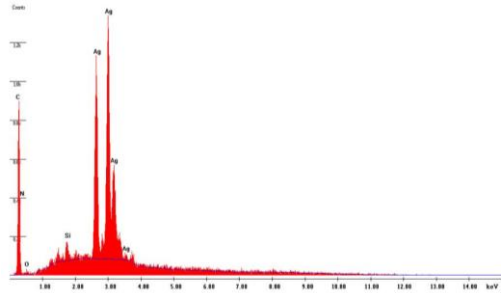

Fig. 4 EDX spectrum of biosynthesized silver nanoparticles

Table 1 EDX spectrum of biosynthesized silver nanoparticles

S.No.	Elements	Total Weight in %	Atomic Weight in %
1	Carbon (C)	47.04	83.18
2	Nitrogen (N)	1.83	2.78
3	Oxygen (O)	3.20	4.24
4	Silicon (Si)	0.64	0.48
5	Silver (Ag)	47.29	9.31

3.3 FTIR Analysis

FTIR spectroscopy was used to identify the functional groups responsible for bioreduction of Ag^+ into Ag^0 nanoparticles. FTIR spectra of plant extract and AgNPs are illustrated in Figs. 5 and 6 respectively. The FTIR spectra showed shifts in some peaks of aqueous extract of *X. americana* i.e 1520 cm^{-1}, 1446 cm^{-1}, 1215.17 cm^{-1}, 882.75 cm^{-1}, 826.82 cm^{-1} and 659.04 cm^{-1} with functional groups nitrocarbon compounds, alkanes, alkyl halides which were absent in FTIR spectra of AgNPs and responsible for synthesis of AgNPs. But in commonly some functional groups were present in both FTIR spectra of plant extract and AgNPs *viz.,* phenols, aromatics, alcohol and alkyl halides with peaks at 3405.89 cm^{-1}, 3410.30 cm^{-1}, 1611.02 cm^{-1}, 1626.02 cm^{-1}, 1321.34 cm^{-1}, 562.78 cm^{-1} and 563.04 cm^{-1} which may be acting as capping and reducing agents for synthesis of AgNPs (Fig. 5).

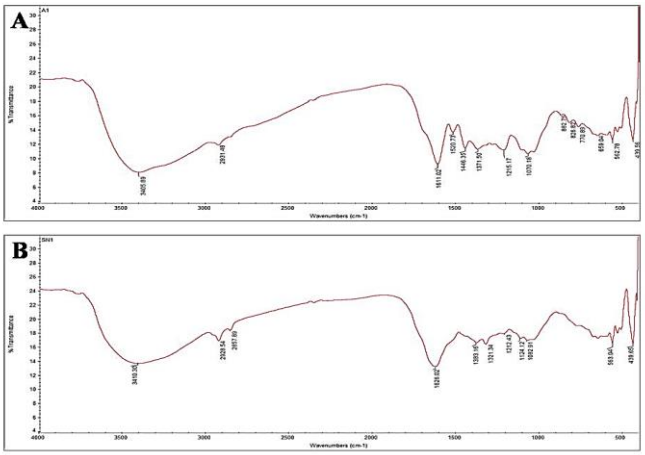

Fig. 5 FTIR spectrums of A) aqueous leaf extract of *X. americana* and B) biosynthesized silver nanoparticles

3.5 In-vitro Antioxidant Activity

In the present study, *in-vitro* antioxidant activity of synthesized AgNPs was evaluated by using FRAP, H_2O_2, and DPPH assays.

3.5.1 FRAP Assay

In the present study known concentrations of synthesized AgNPs were subjected to FRAP assay along with ascorbic acid as standard. Increase in the concentration of both standard and AgNPs lead to increase in antioxidant activity along with absorbance. The antioxidant activity of AgNPs was less on comparison with standard with absorbance of 1.1180±0.00300 but standard shows higher absorbance (1.3513±0.00351) (Table 2 and Fig. 6).

Table 2 FRAP Assay AgNPs

S.No.	Concentration	Std Ascorbic acid	AgNPs
1	100 µL	0.3863±0.00513	0.4843±0.00208
2	200 µL	0.5677±0.00351	0.5753±0.00306
3	300 µL	0.7947±0.00306	0.7313±0.00404
4	400 µL	1.0677±0.00252	0.9470±0.00458
5	500 µL	1.3513±0.00351	1.1180±0.00300

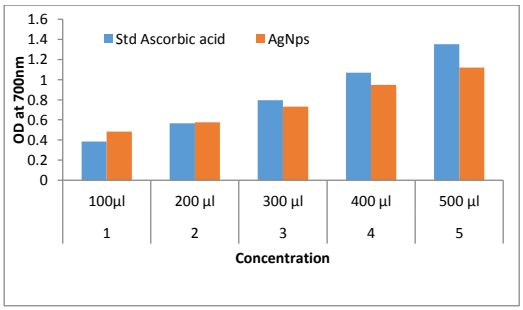

Fig. 6 H_2O_2 assay of silver nanoparticles

3.5.2 H_2O_2 Assay

Hydrogen peroxide radical scavenging assay revealed that AgNPs showed higher scavenging activity than the standard with 71.3967±0.11676% inhibition. The results are tabulated in Table 3 and Fig. 7.

Table 3 DPPH Assay AgNPs

S.No.	Concentration	Std Ascorbic acid	AgNPs
1	10 µg	71.5433±0.34152	69.7900±0.17349
2	20 µg	77.4267±0.44501	73.3667±0.39107
3	30 µg	79.2167±0.28919	78.1700±0.34044
4	40 µg	81.5567±0.33501	80.1800±0.34044
5	50 µg	85.4633±0.23007	82.2633±0.34152

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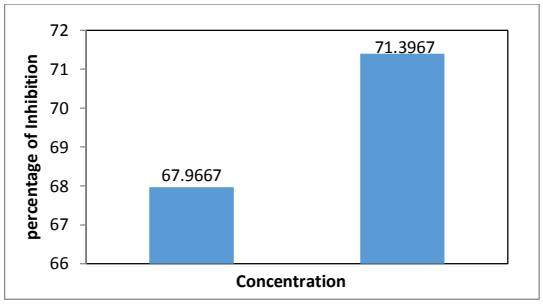

Fig. 7 H_2O_2Assay of silver nanoparticles

3.5.3 DPPH Assay

The antioxidant activity of AgNPs was compared with ascorbic acid as standard antioxidant. The results revealed that the antioxidant activity of synthesized AgNPs was comparable with antioxidant activity of standard i.e.82.2633±0.34152. The results are shown in Table 4 and Fig. 8.

Table 4 H_2O_2 Assay of AgNPs

S.No.	Concentration	Treatment	% Inhibition
1	500 µg	Standard	67.9667±0.20033
2	500 µg	AgNps	71.3967±0.11676

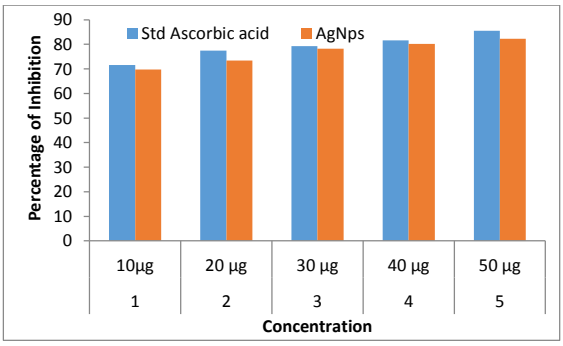

Fig. 8 DPPH assay of silver nanoparticles

3.6 In-vitro Anti-Inflammatory Assay

Anti-inflammatory study of known concentrations (100 µg) of synthesized AgNPs was subjected for anti-inflammatory activity through protein denaturation assay. The *in-vitro* anti-inflammatory activity of the extracts was comparable to the Diclofenac sodium, a reference drug. Significant difference was observed among within the group the denaturation of protein. The results revealed that synthesized AgNPs exhibited significant anti-inflammatory activity with percentage of inhibition 82.8464% than standard drug whereas standard drug Diclofenac sodium showed 74.4633% of inhibition of protein. The results are tabulated in Table 5.

Table 5 Anti-inflammatory Assay

S.No.	Concentration	Treatment	% Inhibition
1	100 µg	Standard	74.4633±0.13051
2	100 µg	Synthesized AgNPs	82.8464±0.32648

Table 6 *In vitro* anthelmintic activity of silver nanoparticles synthesized from aqueous extract of *Ximenia americana* against *Pheretima posthuma*

Test Samples	Concentration in mg/20 mL	Paralysis Time in minutes	Death time in minutes
Control (Normal Saline)	-	112.66±4.48454	204.33±3.17984
Silver nanoparticles of *Ximenia americana*	50	38.66±1.76383**	52.33±2.02759**
	100	33.00±2.64575**	49.33±2.40370**
	150	28.00±1.15470**	43.33±2.40370**
	200	24.00±2.40370**	36.00±2.30940**
	250	17.33±1.76383**	34.66±1.76383**
Standard Drug Piperazine citrate	100	31.33±1.76383**	36.00±1.15470**

Results are expressed as Mean±SE (n=3); * significant at the $p < 0.01$
Correlation is significant at the 0.01 level (2-tailed)**
Correlation is significant at the 0.05 level (2-tailed)*

3.7 In-vitro Anthelmintic Activity

In the present study the concentrated synthesized AgNPs was evaluated for *in vitro* anthelmintic activity by varying the concentration (50-250 mg/ 20 mL) with using Indian earth worm (*Pheretima posthuma*) as animal model. On comparison with standard drug synthesized AgNPs exhibited comparable anthelmintic activity at 250 mg/20 mL concentration with paralysis time 17.33±1.76383 (min) death time it was observed to be 34.66±1.76383 (min). Results were compared with standard drug Piperazine citrate which shows paralysis time 31.33±1.76383 (min) and death time 36.00±1.15470 (min) at 100 mg/20 mL concentration (Table 6).

Now a day's metal nanoparticles composed of copper, zinc, titanium, manganese, magnesium, silver and gold are gaining more attention due to their potential applications in various fields such as catalysis, photonics, optoelectronics, biological labeling and pharmaceutical applications. Among above mentioned nanoparticles, silver nanoparticles are gaining more interest due to their enormous application including biolabelling in optical receptors, catalyst in many chemical reactions and also possess different biological activities such as antibacterial, antifungal, antioxidant, antiviral and anti-inflammatory activity because of all these properties silver nanoparticles can play a significant role in the field of biology and medicine [5]. Silver nanoparticles can be synthesized by using different methods such as chemical, electrochemical, radiation, photochemical and biological methods [26-33]. Among above mentioned methods, the biological approaches are preferred way to synthesize nanoparticles due to their less toxic and eco-friendly nature. In case of biological methods, various biological sources were used for synthesis of silver nanoparticles such as bacteria, fungi, algae and plants. Among these biological sources, plants are gaining more importance because plants are provided with various secondary metabolites such as alkaloids, phenols, polyphenols, tannins and flavonoids which acts as capping and reducing agent in the formation of silver nanoparticles and also by using plant as biological source maintenance of culture and contamination can be avoided and also such methods are cost effective [34, 35].

In the present study, aqueous extract of *X. americana* leaves was used to synthesis of AgNPs from aqueous solution of $AgNO_3$. After the addition of aqueous extract of *X. americana* to $AgNO_3$, color of the reaction mixture changed due to reduction of the silver ions, which indicates the formation of silver nanoparticles. Within 15 min – 30 min the color of the reaction mixture changed from yellowish to brown color due to reduction of silver ions (Ag^+) into silver nanoparticles (Ag^0) [36]. Further confirmation and characterization of synthesized AgNPs was carried out by using UV-Vis spectroscopy. Generally UV-Vis spectroscopy is well recognized technique used to examine the size and shape of silver nanoparticles in aqueous solution [37, 38]. In the present study, synthesized AgNPs in aqueous suspension was subjected to UV-Vis spectroscopy (Hitachi 2900). The results revealed that λ_{max} sharp peak at 417 nm. Thus it confirmed the bioreduction of silver ions into AgNPs in the colloidal solution due to action of aqueous extract of *X. americana*. As the wavelength decreases the size of silver nanoparticles decreases it implies that synthesized AgNPs were smaller in size [39].The appearance of more than one peak is due to the formation of AgNPs with different shapes [40-42].

Formation of AgNPs was completed at 1 h of incubation as the incubation time increases large number of nanoparticles will formed and it causes the instability and aggregation of NP's which leads to the formation of larger sized nanoparticles [43, 44]. In the present study, reaction was carried out for 4hwith timely monitoring; sharp peak with highest absorbance was noticed at 1h with λ_{max} 421 nm. FTIR technique was used to identify the possible functional groups and biomolecules responsible for bioreduction of silver metal ions to silver nanoparticles [45]. In the present study, both plant extract and AgNPs were subjected to FTIR analysis. FTIR spectra of plant extract and AgNPs are illustrated in Figs. 5 and 6 respectively. It can be observed that FTIR spectra showed shifts in some peaks of aqueous extract of *X. americana* i.e 1520 cm^{-1}, 1446 cm^{-1}, 1215.17 cm^{-1}, 882.75 cm^{-1}, 826.82 cm^{-1} and 659.04 cm^{-1} with functional groups nitrocarbon compounds, alkanes, alkyl halides which were absent in FTIR spectra of AgNPs and responsible for synthesis of AgNPs. But in commonly some functional groups were present in both FTIR spectra of plant extract and AgNPs viz., phenols, aromatics, alcohol and alkyl halides with peaks at 3405.89 cm^{-1}, 3410.30 cm^{-1}, 1611.02 cm^{-1}, 1626.02 cm^{-1}, 1321.34 cm^{-1}, 562.78 cm^{-1} and 563.04 cm^{-1} which may acting as capping and reducing agents for synthesis of AgNPs.

Scanning electron microscope (SEM) helps to study morphological characteristics and size of AgNPs. In the present study, SEM results revealed that synthesized AgNPs were in irregular shapes with average size of 30 nm – 150 nm. EDX is used to determine purity and elemental composition of synthesized AgNPs. In the present study, EDX results showed that strong signal of metallic silver at 3 kev which is characteristic

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feature absorption of the metallic silver [46]. Biosynthesized AgNP showed various elemental compositions such as carbon, nitrogen, Si, oxygen with percentage 47.04%, 1.83%, 0.64% and 3.20% respectively and silver as major element with highest percentage 47.29%. It clearly confirms that silver nanoparticles are successively formed by the aqueous extract of *X. americana* [47].

Silver nanoparticles are having various application which includes biolabelling in optical receptors, catalyst in many chemical reactions and also possess different biological activities such as antibacterial, antifungal, antioxidant, antiviral and anti-inflammatory activity [6].

Reducing power of AgNPs is depending upon on reducing Fe^{+3} to Fe^{+2}. The reducing property of the AgNPs is closely associated with the capping agent loaded on AgNPs which acts as donor for giving proton atom in braking of free radical chain [48-53]. However in the present study it was observed that reducing power of AgNPs as well as standard ascorbic acid was increasing with increase in concentration. Maximum activity was observed at 500 µL with absorbance 1.1180±0.00300.

Hydrogen peroxide (H_2O_2) is an important reactive oxygen species due to its ability to penetrate through biological membranes however it may be toxic if it converted into hydroxyl radicals in the cell [54] and also it can inactivate few enzymes directly by oxidation of –SH groups. Antioxidant activity is determined and monitored based on the scavenging the H_2O_2. In the present study antioxidant activity was compared with standard ascorbic acid, on comparison it found that AgNPs exhibited highest scavenging activity with percentage 71.3967±0.11676 over ascorbic acid.

DPPH is a stable, nitrogen centered free radical which produces deep purple in methanol solution. The principle behind this assay is reduction of purple color methanolic complex in presence of antioxidant to produce yellow colored diphenyl-picryl hydrazine which is measured spectrophotometrically at 517 nm [55]. In the present study different concentrations of AgNPs exhibited variation in activity but maximum activity was observed with inhibition percentage 82.2633±0.34152 at 50 µg concentration which was comparable with standard ascorbic acid.

Inflammation is a prominent phenotype of various diseases such as rheumatoid arthritis, atherosclerosis and asthma, although inflammation is primarily a protective response against pathogens, toxins and allergens [56]. There are many synthetic drugs are available to treat inflammation but they have disadvantages because of their detrimental side effects on the gastrointestinal tract, kidneys and on the cardiovascular system and reappearance of symptoms after discontinuation [57, 58]. Silver nanoparticles from natural sources like plants getting more importance and they are more promising agent with less side effect. In the present study, *in-vitro* protein denaturation method was used in which synthesized AgNPs showed to be exhibit highest anti-inflammatory activity than the standard drug and all extracts with 82.8464 percentage inhibition of the protein Denaturation. Helminthes infections are also among the most common infections in humans, affecting a large proportion of the world's population in developing countries and produce global burden of disease and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia [59]. In the present study On comparison with standard drug synthesized AgNPs exhibited comparable anthelmintic activity at 250 mg/20 mL concentration with paralysis time 17.33±1.76383 (min) death time it was observed to be 34.66±1.76383 (min). Results were compared with standard drug piperazine citrate which shows paralysis time 31.33±1.76383 (min) and death time 36.00±1.15470 (min) at 100 mg/ 20 mL concentration.

4. Conclusion

The present study, successfully demonstrated the bioreduction of silver ions into silver nanoparticles by the aqueous leaves extract of *X. americana*. Preliminary identification of formation of AgNPs was confirmed by UV-Vis spectroscopy with maximum absorption peak at 417 nm. Average size of synthesized silver nanoparticles in SEM study was around 30 nm - 150 nm. Overall in summary EDX, TGA, DSC and FTIR spectroscopic techniques confirmed the formation of silver nanoparticles. The proposed method for synthesis of AgNPs is rapid and simple without use of hazardous or toxic chemicals. The newly synthesized AgNPs showed significant antioxidant, anti-inflammatory and anthelmintic activity in all performed assays. Further studies are needed for detailed characterization of the toxicity and mechanism involved with antioxidant activity of these particles. In future these AgNPs can have promising potential applications in drug formulation and biomedical application.

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