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Formulation and Evaluation of Silver Nanoparticles of Methanolic extract of *Solanum virginianum* L. for Antimicrobial and Antioxidant potential

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ABSTRACT

Nanotechnology can be termed as the characterization, Fabrication, exploration and application of nano sized materials where nano denotes extremely minute structures ranging 1-100 nm. Among the new towards for employ nanotechnology success in medicine, various nano particulates offer some special advantages as pharmaceutical delivery systems and image advancement agents *Solanum virginianum L*, (wild eggplant or nightshade plant), are a prickly herb, belongs to family Solanaceae. It has spines throughout the plant. Various phytoconstituents have been found, the major constituents is alkaloid. It has vital role in various traditional as well as medicinal uses for curing internal and external physiological disorders. This plant has also used for phytoremediation as it possess the ability to degrade carbofuran residues in rice field soil and therefore the plant species may further be investigated for its phytoremidial role. In the present study, AgNPs of plant *Solanum virginianum* was synthesized and characterized by UV-Vis spectrophotometer, FT-IR and SEM analysis. In-vitro anti-oxidant and anti-microbial investigations showed the impressive results. However the present investigation adverts that the AgNPs can be used to prepare and develop Nano-drug, new generation of antimicrobials, drug delivery systems, biosensors and different other applications such as Ag based dressing, Ag-coated medicinal device

Keywords: Silver nanoparticles, *Solanum virginianum l*, anti-microbial, antioxidants, antifungal

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INTRODUCTION

Nanoparticle, ultrafine unit with dimensions measured in nanometres (nm; 1 nm = 10−9 meter). Nanoparticles exist in the natural world and are also created as a result of human activities. Because of their submicroscopic size, they have unique material characteristics, and manufactured nanoparticles may find practical applications in a variety of areas, including medicine, engineering, catalysis, and environmental remediation. The Commission of the European Union endorsed a more-technical but wider-ranging definition natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm–100 nm.[1]

Due to the apparent resistance of bacteria towards antibiotics and metal ions, there is a necessary to engineer biocompatible, antimicrobial and functionalized substances, which can be successfully used. Different metallic nanoparticles, such as copper, gold, titanium and silver have been studied extremely. Metallic silver in thinly dispersed form shows special properties generally connect with the noble metals, such as electrical conductivity, chemical stability, catalytic activity along with other more specific ones like optical behavior, non-linear, anti-bacteriostatic effects etc. Metallic particles in the nano range show physical properties that are dissimilar from ion and bulk material. This construct them show additional properties such as high catalytic activity due to morphological structure with increased active surface[3].Silver is better choice in the field of living systems, bio- organisms and drugs amongst the numerous noble metals. Poisonousness of silver ion and its mixtures has been confirmed for microorganisms thus they are used in wound dressings and applied for the making of uncontaminated diet packaging material. The nanoparticles enter the cell, affecting injury by involving with phosphorus and sulfur enclosing compounds such as DNA and protein. In adding the AgNPs involvement with the enzymatic metabolism of O_2 successfully “suffocation” and destroys the specific microorganism.[4].

MATERIALS AND METHODS

Chemicals: Silver nitrate (AgNO3) and methylene blue are bought from Sigma-Aldrich Chemicals for this study. Dimethyl sulphoxide (DMSO) was purchased from Merck, India. The pH buffer tablets were purchased from Himedia. Nutrient Broth, Nutrient Agar, Muller Hinton Agar (MHA), Agar Agar and Sabouraud Dextrose Agar Media were purchased from Himedia Laboratories, Mumbai, India. The aqueous solutions were prepared with triple distilled water. All glassware's are washed with HNO_3 and distilled water and dried in oven.

Collection and Authentication of plant material:-The stem part of young healthy plantlets of Solanum virginianum, were collected in summer season from road sides area of Bhopal region, (M.P.) India. Herbarium of plant were prepared graciously and submitted to Division of Botany, Safia College of Science, Bhopal India, for verification. Plants were true by Dr. Zia-Ul-Hasan, Head, Department of Botany, Safia College of Science, Bhopal, and M.P. India. Plant authentication receipt numbers obtained were 324/Bot/Safia/18 for Solanum virginianum.

Methodology

A). Preparation of stem extract

The Crude stems of Solanum virginianum were extracted with petroleum ether, methanol and Chloroform by using maceration technique. For extraction of methanolic extract, 250g of powdered plant material was soaked in 600mL of methanol and kept at room temperature for 72 hours with periodical shaking. The solvent extract was filtered through muslin cloth and collected in a beaker and then filtrate was place on water bath for solvent evaporation. Finally greenish black, semisolid crude methanolic stem extract of plant *S. virginianum* obtained. For extraction of aqueous extract, 250g of powdered plant material was soaked in 450mL of water and kept at room temperature for 48 hours with periodical shaking. The solvent extract was filtered through muslin cloth and collected in a beaker and then filtrate was place on water bath for solvent evaporation. Finally brownish black, solid crude aqueous stem extract of plant *S. virginianum* obtained and percentage yield for each extract was calculated using following formula.

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Materialused}} \times 100$$

B).Qualitative phytochemical screening of extract

In order to identify various phyto-constituents namely alkaloids, terpenoids, glycosides, steroids, triterpenoids, flavonoids, carbohydrates, saponins and tannins in different extracts, phytochemical screening was performed using standard procedures [9.10]

C).Quantitative Phytochemical screening of Extracts

a) Estimation of total phenolic content (TPC):-The total phenolic content was determined by the spectrophotometric method[11]The Gallic acid was used as a reference standard for plotting calibration curve. In brief, aliquots of 20 to 100 µg/ml concentrations of Gallic acid were prepared in methanol. To 0.5 ml of methanolic extract (100µg/ml) and 0.5 ml all aliquots of gallic acid were mixed with 2 ml of Folin-Ciocalteu's phenol reagent. After 5 min, 4ml of a 7% Na_2CO_3 solution was added to the mixture followed by the addition of distilled water and mixed thoroughly. The mixture was kept in the dark for 30 min at room temperature, after which the absorbance was taken at 765 nm. TPC was determined from extrapolation of calibration curve of Gallic acid. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per g of dried extract.

b) Estimation of total flavonoid content (TFC):-Total flavonoid content was determined following a method by Park et al., (2008)[11]. Rutin solution of different concentrations (20-100 µg/ml) were prepare in methanol. The plant extract in methanol (100µg/ml) was also taken in a volumetric flask. 0.5ml of all aliquots and 0.5ml of extract was taken separately in test tube and 2ml of distilled water and subsequently with 0.15ml of 5% $NaNO_2$ was mixed. After 6 min, 0.15ml of 10% Alcl3 solution was mixed and further kept for 6min. Then 2 ml of 4% NaOH was added in to the mixture. Immediately distilled water was added to make up the volume up to 5ml and thoroughly mixed and kept for 15 min .The absorbance was measured against the reagent blank at 510 nm. The standard curve for was made using rutin and regression line plotted. The absorbance of extract was put in regressed line and total flavonoid was determined. Total flavonoid content was expressed as mg/g of rutin.

D).Preparation of Silver Nanoparticles (AgNPs)

Preparation of 1mM AgNO3 solution by One milimolar (mM) solution of AgNO3 (0.085gms) was prepared by dissolving in 500 ml distilled water (DW) and stored in amber colored bottle in cool and dry place. 500 mL of 1mM aqueous solution of AgNO3 solution was taken in Erlenmeyer flask and 75 mL of aqueous stem extract was added drop wise into it for bio reduction process at room temperature. The reaction mixture was allowed to stir at 200 rpm using magnetic stirrer till the solution was turned from yellow to dark brown indicating the formation of AgNPs. The reduced solution was centrifuged at 5000 rpm for 30 min to get clear supernatant. The supernatant was discarded and the particles obtained were centrifuged with water repeatedly to get pure nanoparticles [12]. Various formulations of silver nanoparticles (NP1, NP2, NP3, NP4 and NP5) were prepared by using *Solanum virginianum* extract and 01 nM silver nitrate solution. Different amount of extract from plant *Solanum verginianum* has been used to obtain the best morphology and size of nanoparticles. In the present study, increasing the amount of plant extracted to an increase in peak absorbance in UV/Vis spectrum. Literature showed that, larger quantities of extract reduced the particle size

[13-15].

Table 1:- Formulation Table

S. No	Formulation	Concentration of extract	Concentration of$AgNO_3$ Sol	Absorbance
1	NP1	100 mg/ml	01 mM	1.047
2	NP2	200 mg/ml	01 mM	0.601
3	NP3	300 mg/ml	01 mM	0.152
4	NP4	400 mg/ml	01 mM	0.228
5	NP5	500 mg/ml	01 mM	0.339

E).Characterization

a) Characterization of synthesized AgNPs by UV-spectrophotometer

The synthesized AgNPs (solution of 1 mg/mL in distilled water as a dispersive medium) were monitored using UV-Vis spectrophotometer (Systronics double beam spectrophotometer 2202, India) between the range of 300nm and 550nm. Distilled water was used as blank for UV-vis Spectrophotometer analysis.

b) Fourier Transform Infrared Spectroscopy (FTIR)

The infrared spectra for the plant extract and synthesized (formulation NP1) AgNPs were attained for the identification of functional groups in a (Perkin Elmer Spectrum 2, Germany) spectrophotometer IR affinity-1 by employing KBr pellet technique and registering amplitude waves ranging from 400 to 4000 cm−1.

C. SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using SEM machine (ZEISS-EVO MA 15, Japan). Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid.

F). Antioxidant assay (DPPH radical scavenging activity):

DPPH free radical scavenging potential of the AgNPs (formulation NP1) was determined using the modified Hanato method [16]. AgNPs and standard vitamin C were taken in different test tubes. In the above extracts, 1ml of freshly prepared DPPH (0.15%) dissolved in methanol was added and vigorous shaking. Finally, the solution was incubated in dark place for 30 min. The absorbance of stable DPPH was recorded at 517 nm. The DPPH control (containing no extract) was prepared using the same procedure (This method is based on scavenging of DPPH radical from the antioxidants, which produces a decrease in absorbance at 517 nm. When a solution of DPPH is mixed with a substance that can donate a hydrogen atom, the reduced form of the radical is generated accompanied by loss of colour. This delocalization is also responsible for the deep violet colour [17]. Free radical scavenging activity was expressed as the inhibitory percentage that was calculated using the equation of DPPH radical scavenging activity.The % Inhibition was calculated using following formula: Where, AC is absorbance of Control (DPPH radical + methanol) AS is the absorbance of sample (DPPH radical + sample AgNPs/ standard vitamin C).

$$\% \text{ Inhibition} = \left[\left(\frac{AC - AS}{AC} \right) \times 100 \right]$$

G). Antimicrobial activities of synthesized AgNPs (formulation NP1)

Antibacterial activity study Antibacterial study of different extract was carried out using agar well diffusion assay against selected bacterial strains. Various bacterial strains were used in the study like[18]. Proteous vulgaris (MTCC-742), *Pseudomonas aeruginosa* (MTCC-1036), *Bacillus subtalis* (MTCC-1205), Actinomysis spp.(MTCC-712),Cefixime was used as a standard. Nutrient agar Medium (NAM) was prepared by dissolving 28 g of the commercially available media (HiMedia) in 1L of DDW. Prepared medium (molten state) was poured into 100 mm petriplates (30 ml/plate) after autoclaving at 15 lbs pressure at 121 °C for 15 minutes. Commercially available medium (HiMedia) was prepared by dissolving 13 g in 1000 ml DDW. Prepared medium (molten state) was poured into culture tubes. 3-5 well isolated colonies of the same morphological type were selected from the NAM culture. Prepared AgNPs were diluted to the concentration of 50, 100, 200 and 250 mg/ml utilizing DMSO as the solvent. Nutrient agar media was prepared. Microbial strains grown in nutrient media were used. Microbial suspension of density 108 CFU/ml were used for inoculation on the Nutrient agar media. Four wells of 6 mm diameter and 5 mm depth were prepared on the solid agar in each plate using a sterile borer. Different concentrations of extracts, positive control (Cefaxime 10 µg/ml) and negative control (DMSO) were dispensed (50 µl). The plates were allowed to stand for 1 h at room temperature for diffusion of the extract and incubated at 37°C for 24 h. After 24 h, the zones of inhibition were measured using a digital Vernier caliper. All experiments were conducted in triplicate and the mean values of the diameter of inhibition zones ± standard deviations were calculated [17].

H). Antifungal activity

Antifungal study of prepared AgNPs was carried out using well diffusion assay. Following fungal strains were Sabouraud Dextrose Agar Mediawas prepared by dissolving 65 g of the commercially available media (HiMedia) in 1000ml of DDW. Prepared medium (molten state) was poured into 100 mm petriplates (25-30 ml/plate) after autoclaving at 15 lbs pressure at 121°C for 15 minutes. Sabouraud Dextrose Broth: Commercially available medium (HiMedia) was prepared by dissolving 30 g in 1000 ml DDW. AgNPs were diluted to the concentration of 50, 100, 200 and 250 mg/ ml utilizing DMSO as the solvent. Sabouraud dextrose agar media was prepared. Fungal strains (*Aspergillus niger* and *Candida crusi*) grown in Sabouraud broth were used. Microbial suspension of density 10^8 CFU/ml were used for inoculation on the Sabouraud dextrose agar media. Four wells of 6 mm diameter and 5 mm depth were prepared on the solid agar in each plate using a sterile borer. Different concentrations of extracts, positive control (Amphotericin B 10 µg/ml) and negative control (DMSO) were dispensed (50 µl). The plates were allowed to stand for 1 h at room temperature for diffusion of the extract and incubated at 28°C for 48 h. After 48 h, the zones of inhibition were measured using a digital Vernier caliper. All experiments were conducted in triplicate and the mean values of the diameter of inhibition zones ± standard deviations were calculated [19].

G). Acute oral toxicity:-The acute oral toxicity (as per OECD 423 guideline) of formulated silver nanoparticles (NP1) was performed and find the sign of toxicity at the dose for the further capsule formation.

RESULT AND DISCUSSION

A). Qualitative Phytochemical analysis

Plant extract of *Solanum virginianum* stems was subjected to preliminary phytochemical screening by qualitative chemical tests which provides knowledge about various phytoconstituents such as alkaloids, flavonoids, glycosides, saponins, sterols etc. present in crude plant extract (Table 2).

Table 2: (Phytochemical Study of *Solanum virginianum)*

Phytochemical constituent	Test	Petroleum Extract	Chloroform extract	Methanolic extract
Carbohydrates	Molisch'sTest	-	-	+
	Benedict's Test	-	-	-
	Fehling's Test	-	-	+
Alkaloids	Mayer's test	-	-	-
	Hager's test	-	-	-
	Wagner's test	-	-	-
Terpenoids	Salkowski test	-	+	+
	Libermann - Burchard test	+	+	+
Flavonoids	Shinoda test	-	-	+
	Alkaline reagent test	-	+	+
Tannins	$FeCl_3$ test	+	+	+
	Lead acetate test	+	+	+
	Gelatin test	+	+	+
Saponins	Foam test	+	+	+
Proteins and amino acids	Ninhydrin test	-	-	-
	Biuret test	-	-	-
Glycoside	Legal test	-	-	+
	Keller killani test	-	-	+
Fats and lipids	Spot test	-	-	+

[+ Presence, - Absent]

B). Qualitative Phytochemical analysis (Extraction yield, TPC, TFC)

The extraction yield of *Solanum virginianum* in methanol was found to be 14.3 ± 0.4% .Total phenolic content was estimated by using Folin-Ciocalteu reagent. The amount of phenolic compounds (mg/g GAE) in methanolic extract was determined from regression equation of calibration curve (y = 0.005x +0.055, R^2 = 0.971) and expressed in gallic acid equivalents (GAE) (Table 3 and Fig.1). Total phenolic content was found to be127.07mg/g expressed as gallic acid equivalents (GAE). It is well-known that flavonoid contain hydroxyl functional groups which are responsible for antioxidant effect in the plants. The content of flavonoid compounds (mg/g rutin) in methanolic extract was determined from regression equation of calibration curve (y = 0.001x+0.120, R_2 = 0.985) and expressed in rutin equivalents (QE) (Table 5&4 and Fig. 2).

Fig -1 Standard graph for TPC using Gallic acid

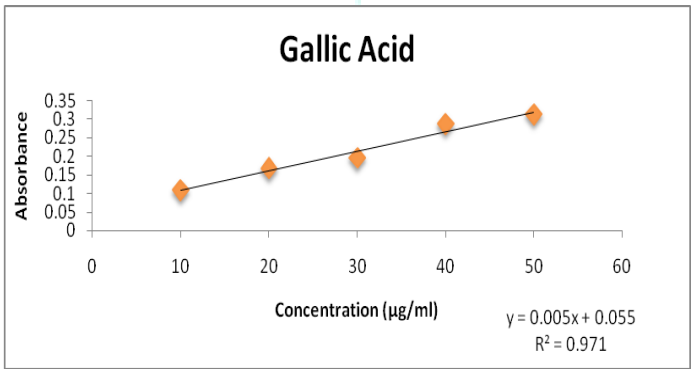

Table 3 (Total phenol content using Gallic acid as standard)

S. N.	Concentration (µg/mL)	Absorbance at 760 nm
1	20	0.108
2	40	0.167
3	60	0.195
4	80	0.286
5	100	0.312
6	Sample	0.690

Table 4 Total Phenolic and flavonoid Content Estimation

Parameter	Petroleum ether	Chloroform	Methanol extract
Total Phenolic Content (mg/g GAE)	9.27±0.115	91.47±0.115	127.27±0.231
Total flavonoid (mg/g Rutin)	8.33±0.577	37.67±1.528	88.33±0.577

Fig 2 Standard graph for TFC using Rutin as standard

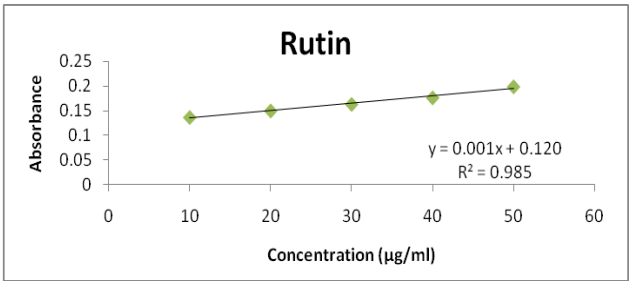

Table 5 Total Flavonoid content using Rutin as standard

S.N.	Concentration (µg/mL)	Absorbance at 510 nm
1	20	0.137
2	40	0.150
3	60	0.163
4	80	0.176
5	100	0.198
6	Sample	0.208

C). Formulation of Silver Nanoparticles (AgNPs)

The Wavelength of UV-Vis spectrum between 350-450 nm showed that the formation of silver nanoparticles reach the peak maxima. The specific characteristic peak for silver nanoparticles was due to the Surface Plasmon Resonance at near to 420 nm and Characterization by synthesized AgNPs initially showed yellowish color in aqueous solution due to excitation of surface plasma resonance of AgNPs. Then the color of the solution changed from yellow to brown on addition of plant extracts to silver nitrate. The color change indicated the formation of AgNPs. Further, it was confirmed with the wavelength of 411.2 nm recorded with the plant extract (Fig. .3). Since, this wavelength falls within the prescribed range, confirming the formation of AgNPs.Formulation NP1 showed the clear peak, so we selected the formulation NP1 for further studies.

D). FTIR

FTIR spectra of *S. virginianum* stem extract and synthesized AgNPs are shown in Table 6 and Fig 8 and 9. A strong peak was observed for stem extract and synthesized AgNPs around 3392-3273 cm^{-1} belongs to O-H stretch band H-bonded in alcohols or phenol groups. The weak band about 2924-2923cm^{-1}correspond to C-H stretch of alkanes groups [72]. The bands around 2172 to 2312 $cm^{-1}$and 1643 to1723 cm^{-1}related to C-NH+ stretch of Amine and C=O starch of amide I carboxylic group, respectively. The strong and medium peaks about 1410-1373cm^{-1} correspond to bending vibration of C-N of aromatic amine groups. The absorbance band at 1054-1063cm^{-1} indicates the presence of C-N stretch in aliphatic amines. The absorbance peak at 875-894cm^{-1} is corresponding to deformation of the C-H stretch in aromatic group. The weak absorbance bands at 583, 537 and 529 cm^{-1} are related to the Ag-O. The region between 400 and 600 cm^{-1} is corresponding to metal oxygen [19]. The FTIR spectra indicate that the functional groups O-H, H-, C-H, C-NH+, C=O, N-O and C-N could be responsible for the reduction and stabilizing of AgNPs. The results showed that the reducing some of the bio-organics of the stem extract such as proteins, flavonoids, phenols and polysaccharides bind to the Ag atoms are responsible for the synthesis and stabilization of AgNPs.

F). SEM

SEM image shows the morphological character, size and surface of the AgNPs synthesized (Fig 10). SEM microscopy which revealed that the AgNPs are around 40 to 60 nm in size with the mixture of many shapes i.e. rhombus and predominant spherical are clearly observed. SEM determination showed the formation of AgNPs, which were well dispersed.

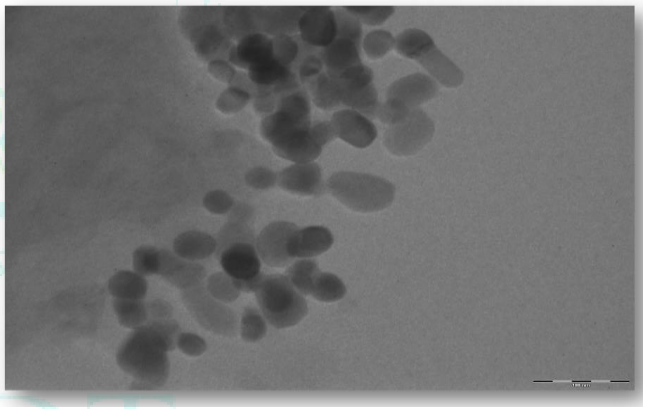

Fig 10: -SEM photograph of synthesized AgNPs)

G).Antioxidant activity

The evidenced the higher phenolic and flavonoid contents of extracts responsible for better antioxidant potential activity[14]. The antioxidant effectiveness in natural sources has been reported to be mostly due to phenolic compounds. The antioxidant activities of phenols are mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers. Flavonoid and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidant or free radical scavengers [19]The antioxidant activity of formulated silver nanoparticles was estimated by comparing the percentage inhibition of formation of DPPH radicals with that of ascorbic acid. Silver Nano particles showed moderate antioxidant activity when compared to ascorbic acid. The DPPH radical scavenging activity of silver nanoparticles increased with increase in concentration. The colour changes from purple to yellow after reduction, which can be quantified by its decrease absorbance at wavelength 517 nm. These results revealed that the silver nanoparticles are free radical inhibitor or scavenger acting possibly as primary antioxidants (Table 6.10 and Fig 6.12)

Fig 3

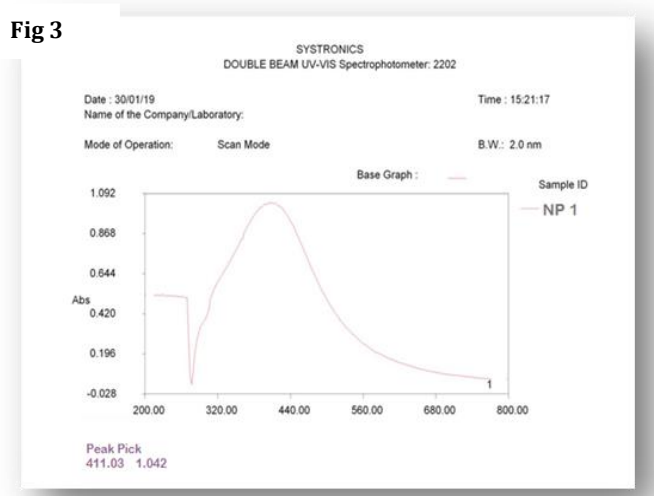

Fig 4

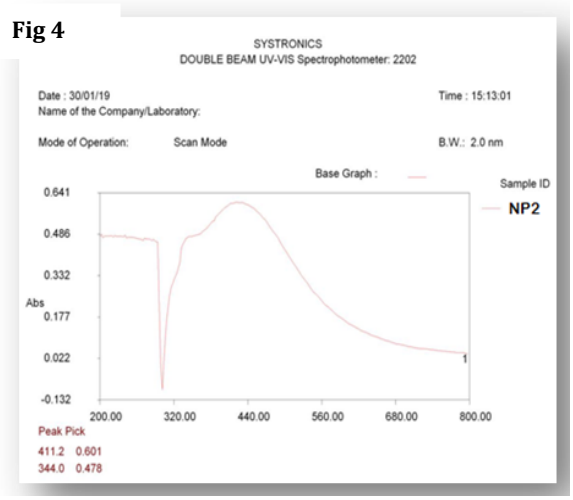

Fig 5

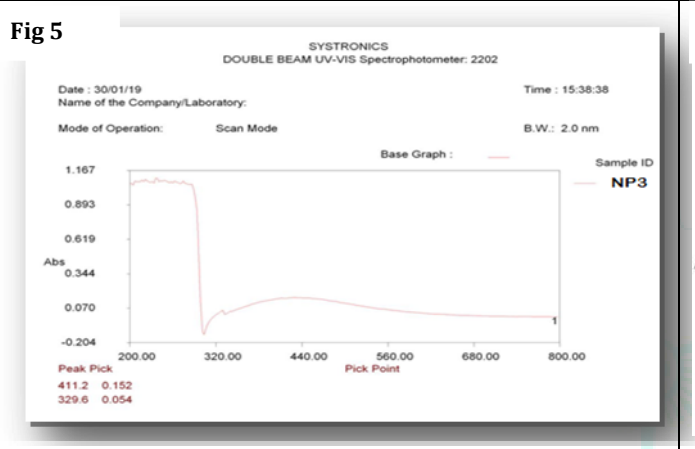

Fig 6

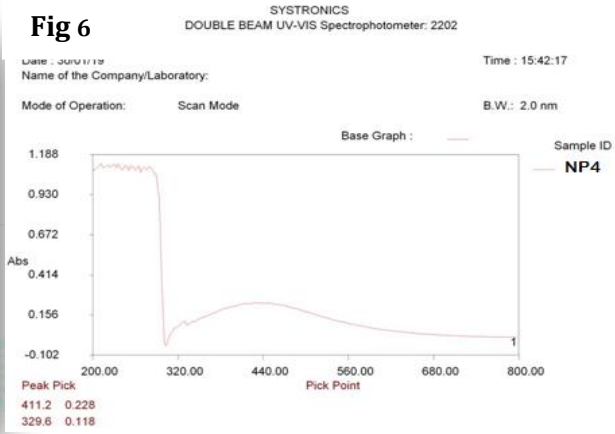

Fig 7

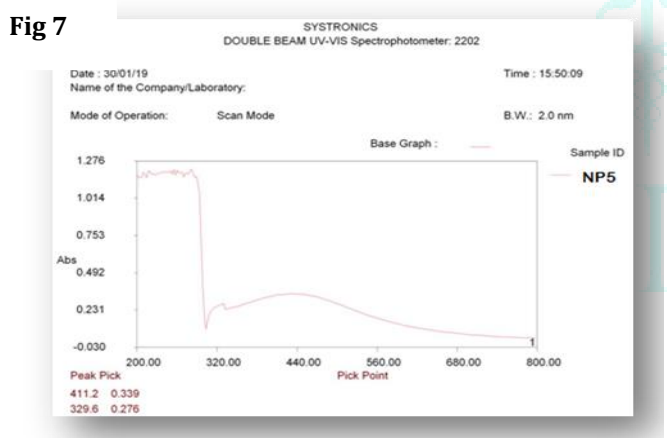

Fig 3 -UV-Vis spectra of synthesized AgNPs (NP1)

Fig 4 -UV-Vis spectra of synthesized AgNPs (NP2)

Fig 5 -UV-Vis spectra of synthesized AgNPs (NP3)

Fig 6 -UV-Vis spectra of synthesized AgNPs (NP4)

Fig 7 -UV-Vis spectra of synthesized AgNPs (NP5

Table 6: FTIR functional groups analysis for synthesized AgNPs

Wavenumber (cm-1)		Stretching	Functional groups
Stem Extract	AgNPs		
3392	3273	O-H	Alcohol and Phenol
2924	2923	C-H	Alkenes
2172	2312	C-NH+	Amines
1643	1723	C=O	Carboxylic
1410	1373	C-C	Aliphatic
1054	1063	C-O	Alcoholic, carboxylicacids, esters, ethers
875	894	C-N	Aromatics
583, 537	529	M-O	Ag-O

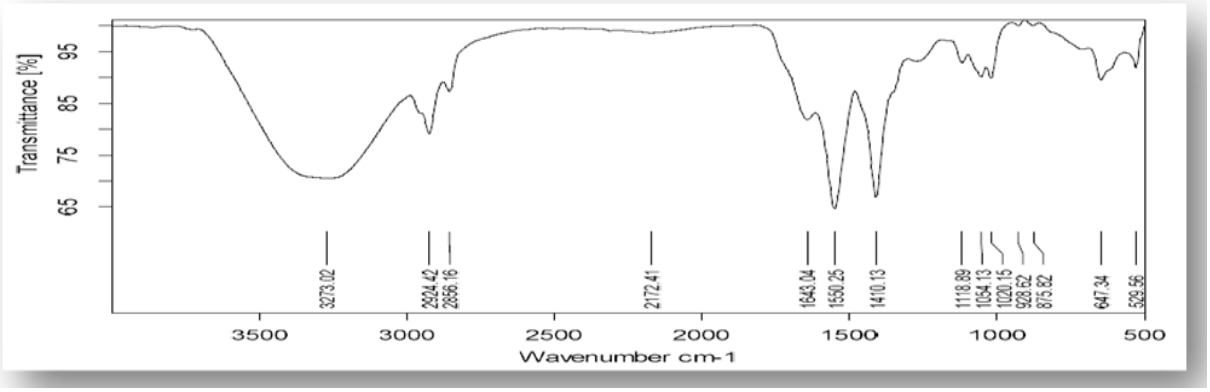

Figure 8 FTIR of stem extract of *S. Virginianum*

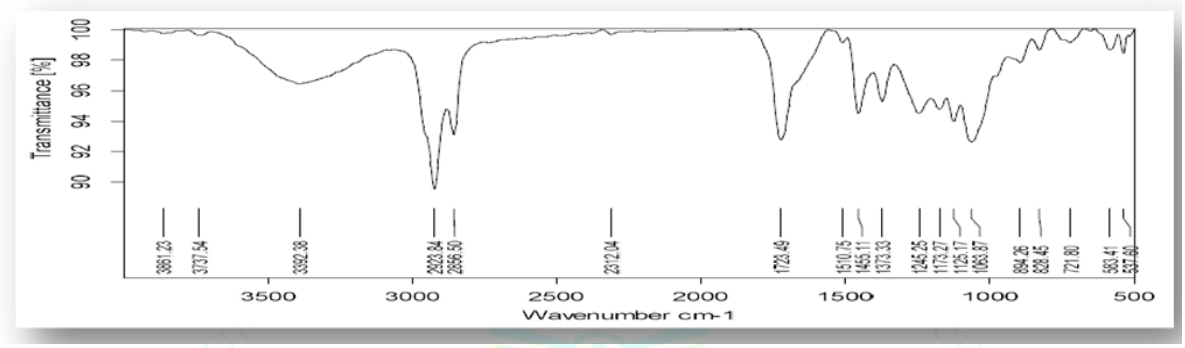

Figure 9. FTIR of synthesized AgNPs

Table 7 DPPH scavenging activity of AgNPs (%)

Concentration µg/ml	Plant extract	AgNPs(NP1)	Ascorbic acid
20	0.289	0.291	0.457
40	0.283	0.253	0.391
60	0.225	0.217	0.326
80	0.208	0.172	0.275
100	0.175	0.144	0.233
IC_{50}	30.37	25.07	17.60

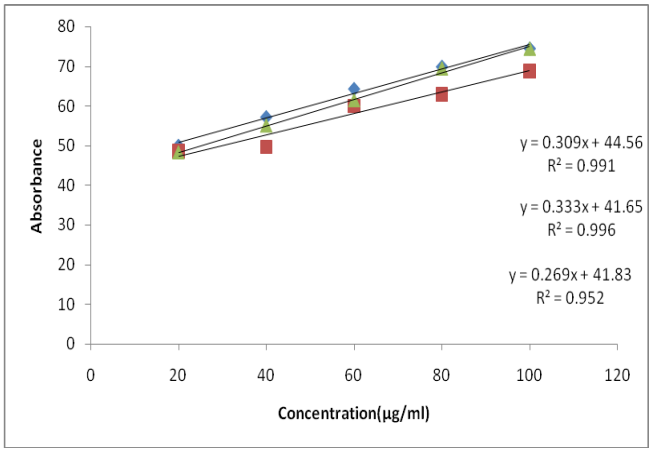

Fig 10 DPPH-free radical scavenging activity of synthesized AgNPs

(1) Antibacterial activity

S. virginianum plant is also possessed well antibacterial activity. To study the antibacterial property of plant extract, $AgNO_3$, AgNPs (15mL), Gram positive and Gram negative bacteria were used and standard antibiotic amoxicillin were used in this study. Inhibition zone against bacterial growth produced by AgNPs was compared to standard antibiotic Cefixime. Among Gram-positive bacteria, *B. subtilis* was inhibited to higher extent while others were inhibited to more or less similar extent. In case of Gram-negative bacteria, *P. aeruginosa* were susceptible to higher extent. From the table, it is could seen that synthesized AgNPs exhibit inhibition zone nearly close to standard antibiotic values (Table -8 & Fig 11).

(2)Antifungal activity

The data revealed that significant reduction in growth of test fungus *A. niger*, and *C. crusi* was observed with the extract, $AgNO_3$, synthesized AgNPs and standard Amphotericin B shown in Table 9 and Fig 12. Studies revealed that AgNPs may kill fungal spores by destructing the membrane integrity or may interact with phosphorus and sulphur containing compounds and their interaction may cause damage to DNA and protein which lead to cell death. In the present study reduction in colony diameter on prepared plates is an indication of antifungal activity of

AgNPs. *C. crusi* was inhibited to higher extent comparative to *A. niger*.

G). **Acute oral toxicity:-** The acute oral toxicity (as per OECD 423 guideline) of formulated silver nanoparticles (NP1) were performed and showed the no sign of toxicity at the dose of 2000mg/kg, Hence, 1/10th dose (200mg/kg) of the formulation was selected for the further capsule formation. The capsules of AgNP (NP1) was formulated and evaluated as per pharmacopieal procedure

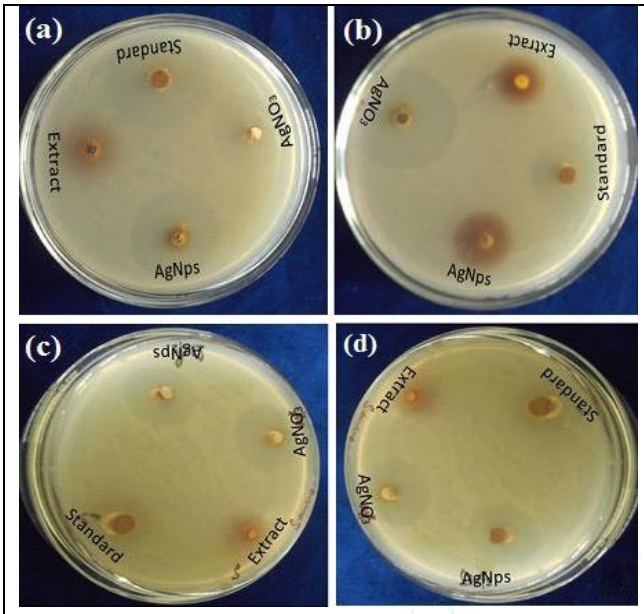

Fig-11 Inhibition zone of extract, AgNO3, AgNPs and antibiotic against(a) B. subtalis (b) Actinomysis spp (c) P. vulgaris (d) P. aeruginosa

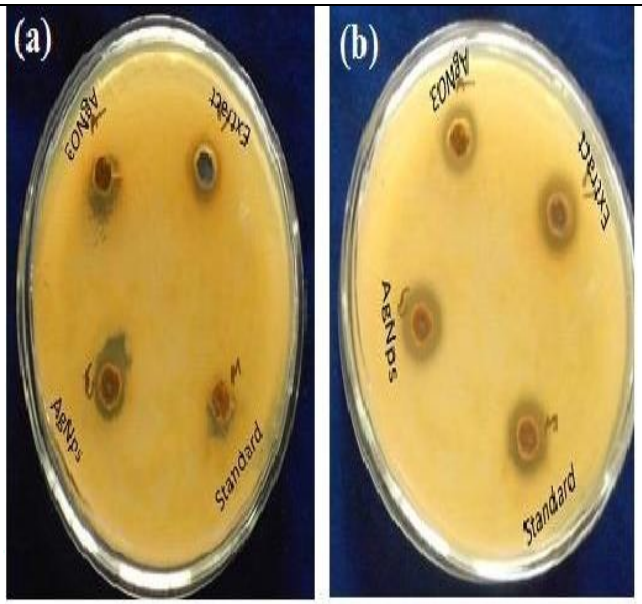

Fig-12 Inhibition zone of extract, AgNO3, AgNPs and antifungal against fungus (a)Aspergillus niger (b) Candida crusi

Table-8 Inhibition zone of extract, $AgNO_3$, AgNPs and antibiotic against four bacteria's

Bioactive agent	Zone of inhibition (Diameter in cm)			
	Bacillus Subtalis	*Actinomysis spp*	*Proteous vulgaris*	*Pseudomonas aeruginosa*
Extract	3.2	2.5	3.1	3.2
$AgNO_3$	3.3	2.4	2.5	2.5
AgNPs	3.8	3.1	2.6	2.8
Cefixime	4.1	3.9	3.8	3.8

Table -9 Inhibition zone of extract, $AgNO_3$, AgNPs and antibiotic against fungi

Bioactive agent	Zone of inhibition (Diameter, cm)	
	Aspergillus niger	*Candida crusi*
Extract	2.4	2.8
$AgNO_3$	2.3	2.6
AgNPs	2.6	2.8
Amphotericin B	2.6	3.0

CONCLUSION

The present investigation concluded that the green synthesis of AgNPs, using plant material as reducing and capping agent, having advantages such as, ease in availability, ecofriendly with which the process can be scaled up economic viability. Proteins, flavonoids and phenols may be responsible in the green synthesis of AgNPs. To elucidate precise mechanism and to comprehend the entire procedure behind green synthesis of silver NPs, further study is required. Owing to the rich biodiversity of plants, their potential for the synthesis of metal NPs is yet to be fully explored. A thorough understanding of biochemical mechanism involved in the plant mediated NPs synthesis is a prerequisite in order to make the approach economically more competitive and sustainable. However the present investigation adverts that the AgNPs can be used to prepare and develop nano-drug, new generation of antimicrobials, drug delivery systems, biosensors and different other applications such as Ag based dressing, Ag-coated medicinal devices.

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