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GREEN NANOTECHNOLOGY: SYNTHESIS OF SILVER NANOPARTICLES USING AQUEOUS LEAF EXTRACTS OF *Swertia* chirayita AND *Punica* granatum

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ABSTRACT

To prepare silver nanoparticles mediated by aqueous leaf extracts of *Swertia chirayita* and *Punica granatum*, the leaves of both plants were collected, washed and dried in shade and powdered. Using distilled water as media, aqueous extracts was obtained using Soxhlet extraction unit. The aqueous extracts were subjected to phytochemical screening to reveal the presence of alkaloids, flavonoids, phenols, saponins and tannins in the aqueous leaf extracts. The aqueous extract was then mixed with silver nitrate solution to obtain silver nanoparticles. These particles were then subjected to characterization using UV-Visible spectrophotometer, Scanning Electron Microscope, Fourier Transform Infrared Spectroscopy, Dynamic Light Scattering and Zeta potential analysis. The results confirmed that silver nanoparticles were formed within the nano-range (1 – 100 nm)

INTRODUCTION

Plants happen to be one of the richest source of bioactive chemicals in the world. They have great importance owing to their nutritive value and continue to be one of the major sources of medicines throughout the human history [1,2]. Researchers and academics are more and more interested in studying the antibacterial and therapeutic capabilities of plants since time immemorial. The plants have been playing a vital role in medicines and medicinal formulations [3,4]. The side effects of antimicrobial drugs available today compel the discovery of new pharmaco-therapeutic agents from medicinal plants [5,6]. In United States at least about 25% of pharmaceutical prescriptions contain plant-derived ingredient. There is a global inclination towards herbal medicines that repair and strengthen bodily systems and help fight microbes without posing any side effects to the body [7-9]. Today phytomedicine has developed as separate industry and is posing strong competition to the synthetic medicine industry. The ancient records of use of herbal medicine by Indian, Chinese, Egyptian, Greek, Roman and Syrian dates back to about 5000 years [10 – 12]. According to various reports currently around 80% of the world population depends on herbal medicines for their first line of defence[12].

Plants contain various phytochemicals, among these phytochemicals some potential beneficial are polyphenols, flavonoids, phytoestrogens, isoflavonoids, tannins, saponins, polyphenols, phytoesterols. All these phytochemicals have strong antioxidant and antimicrobial properties and impart other health benefits [13].

It is in the last decade when the exploration of medicinal properties of plant extracts attained its peak, nanotechnology came into scenario. Nanotechnology is a science, engineering, and technology which operates at nanoscale, *i.e.* equal to or below 100 nm. The concept behind the nanoscience and most properly nanotechnology started with a talk entitled 'There's plenty of room at the bottom' delivered by physicist Richard Feynman at an American Physical Society meeting at California Institute of Technology on December, 29, 1959. It is long before the term nanotechnology was used. His talk focused on process where scientists would be able to manipulate and control individual atoms and molecules. Later during his exploration, Professor Norio Taniguchi coined the term nanotechnology [14-15]**.** In the last few decade, the idea of synthesis of metallic nanoparticles mediated by extracts obtained (via polar and non-polar extraction media) from plant parts such as leaves, roots, fruit peals, flowers and whole plant etc started gaining grounds [16-19]**.** This idea gained importance and was explored widely by authors and researchers. Plant mediated nanoparticles can be synthesized using several metals such as silver [19], zinc [20], copper [21] iron [22] etc.

Swertia chirayita is an important medicinal herb available in India, Nepal and China. It is commonly known as Chireta in Hindi. *Swertia chirayita* grows at an altitude of 1200-3000 m and is available throughout the year. Its tea is consumed by

older people and people with type II diabetes. It has been reported to lower the blood glucose levels [19, 23].

Punica granatum is commonly known as pomegranate, is a fruit bearing deciduous shrub or a small tree, native to Asia. It has been used as traditional medicine in many countries for the treatment of dysentery, diarrhoea, helminthiasis, acidosis, hemorrhage.

In this work we report the synthesis and characterization of silver nanoparticles synthesized using aqueous leaf extracts of *Swertia chirayita* and *Punica granatum*.

MATERIALS AND METHODS

The general flow of materials and methods of this work is – Plant materials (leaves of *Swertia chirayita* and *Punica granatum*) were collected, dried and powdered. The powdered samples were used for extraction using aqueous media (Distilled water). The obtained aqueous extracts were then assessed for presence/absence of phytochemicals. The aqueous extracts were then used for synthesis of silver nanoparticles using $AgNO_3$ solution. The synthesized silver (nano) particles were subjected to Scanning Electron Microscope, UV-Visible Spectrophotometer, Fourier Transform Infra-red spectroscopy, Dynamic Light Scattering analysis for characterization of size, shape, stability etc. The details methodologies are as follows

2.1. Collection of plant materials

Fresh tender leaves of *Swertia chirayita* and *Punica granatum* were collected from Ranchi district (Coordinates 23.3441º N, 85.3096ºE) of Jharkhand state of India. The sample identification was done at the Department of Botany, Ranchi University, Ranchi and Department of Botany, St. Xavier's College, Ranchi.

2.2. Preparation of extracts

The leaves were washed with deionised water and disinfected with 0.1% $HgCl_2$ solution for 5 minutes and then dried in shade away from direct sunlight for 20 days. The dried leaves were grounded to fine powder with the help of electrical grinder and stored in dark bottles for further studies.

50 g of fine powder of leaves of *Swertia chirayita* and *Punica granatum* were separately subjected to Soxhlet extraction using distilled water as media for extraction. The extraction was continued for 72 hours. The obtained extracts were concentrated after filtration using rotary flash evaporator at 45ºC. The extracts were stored at room temperature in air tight black bottles for further studies [3, 25, 26].

2.3. preliminary phytochemical screening

Preliminary qualitative screening tests were conducted on the aqueous leaf extracts of *Swertia chirayita* and *Punica granatum* according to the previously published standards [27, 28]. Following the preliminary phytochemical screening the quantitative phytochemical screening was done

Determination of crude carbohydrate

For determination of crude fibre, 2 g of moisture free material were treated with 200 ml of 1.25% sulphuric acid. After filtration and washing, the residue was treated with 1.25% NaOH, filtered washed with hot distilled water and then with 1% HNO_3 and then again washed with distilled water. The residue was ignited and the ash weighed. Loss in the weight gives weight of crude fire [1, 29].

Determination of Protein and Nitrogen

Determination of protein and Nitrogen was done using Micro Kjeldahl method. 1g of sample of each plant was take in Pyrex digestion tube and 30 ml of concentrated sulphuric acid was carefully added, then 10 g potassium sulphate and 14 g copper sulphate were added. The mixture was placed on sand bath on a low flame just to boil the mixture. The solution was heated till it became colourless and clear. Then the solution was allowed to cool, and diluted with distilled water and transferred to Kjeldahl flask. Three or four pieces of granulated zinc and 100 ml of 40% caustic soda were added and the flask was connected with splash heads of distillation apparatus. Next 25 ml of 0.1 N sulphuric acid was taken in the receiving flask and distilled; the flask was then removed and titrated against 0.1 N caustic soda solution using Methyl Red Indicator for determination of nitrogen, which in turn gives the protein content [1, 30].

Determination of crude fat.

Crude fat were determined by extracting 1 g of moisture free plant materia of each plant leaf powder with petrol in a Soxhlet extractor by heating the flask on sand bath for about 1 hour. This petroleum extract (containing crude fat) was taken in a pre-weighed beaker (W_1) and petroleum was evaporated. The weight of beaker along with residual extract (Crude fat, W_2) was taken and crude fat content of the sample was calculated using the following formula [1, 29].

$$\% \text{ crude fat} = (W_2 - W_1)X\left(\frac{100}{S}\right)$$

Here S is the weight of the sample.

Determination of nutritive value

The nutritive value of leaves of *Swertia chirayita* and *Punica granatum* was determined using the following formula [31].

$$\text{Nutritive value(Cal/100g} = (4X \setminus \text{protein\%}) + (9X \text{ fat\%}) + (4x \text{ carbohydrate\%})$$

Alkaloid determination

Total alkaloid was determined by following previously published work of Harborne *et* al. (1998). The powdered sample was weighed in a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added. Beaker was covered and allowed to stand for 4 hours. Then the solution was filtered and the extract was concentrated on water bath to one quarter of original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The solution was then allowed to stand till settlement of precipitate. The precipitate was collected and washed with dilute ammonium hydroxide and filtered. Alkaloids were

Table 1 Results of preliminary phytochemical analysis of aqueous leaf extracts of *Swertia chirayita* and *Punica granatum*

Phytochemcials	Presence (+)/Absence (-)	
	Swertia chirayita (Aqueous leaf extract)	*Punica granatum* (Aqueous leaf extract)
Alkaloid	+	+
Flavonoid	+	+
Saponin	+	+
Tannin	+	+
Phenol	+	+

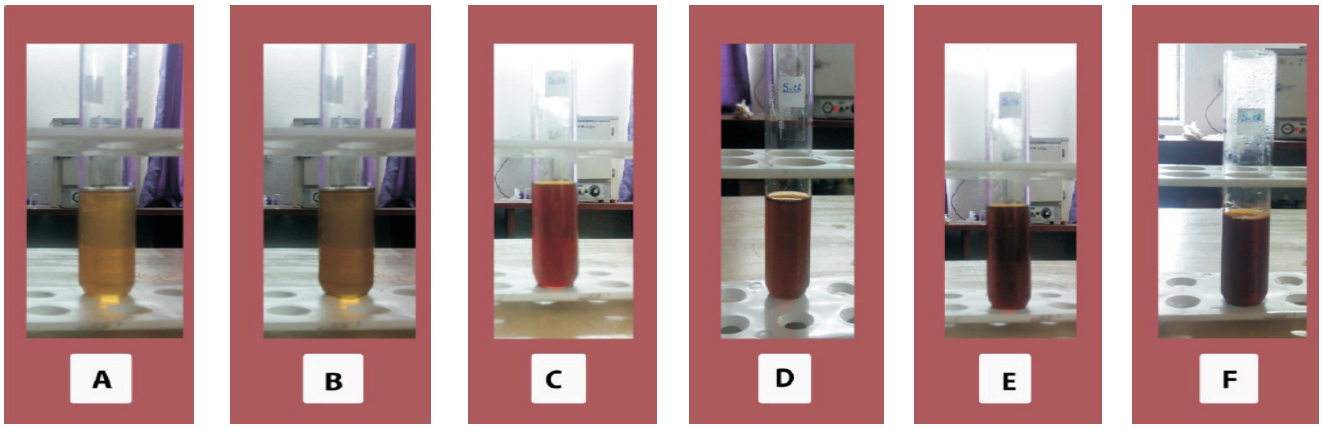

Fig. 1: Colour change from yellow to dark brown due to reduction of silver ions in case of aqueous leaf extract of *Swertia chirayita*

collected as residue and weighed after complete dryness then the percentage was calculated and expressed in mg/g of powdered sample [25, 28].

Tannin determination

The analyses of tannin content in the leaf powder was performed according to the International Pharmacopoea (2003) and work published by Helrich (1990). 25 ml of infusions of both the leaf extracts of *Swertia chirayita* and *Punica granatum* were taken separately and added into 1 L conical flask, then 25 ml of indigo solution and 750 ml distilled water were added. 0.1N aqueous solution of potassium permanganate was used. The blue coloured solution changed to green colour. Standard solution of Indigo carmine was prepared as following: 6g Indigo carmine was dissolved in 500 ml of distilled deionised water by heating , the solution was then cooled, then 50 ml of concentrated sulphuric acid was added, the solution was diluted to 1L and then filtered. The blank tests by titration of mixture of 25 ml indigo carmine solution and 75 ml distilled water were carried out [25].

Calculations: the tannin content was calculated as percentage and expressed as mg/g of plant extract (aqueous).

$$\text{Tannin\%} = \frac{(V - V_0)\ X\ 0.004157\ X\ 250\ X\ 100}{gX25}$$

Here V is the volume of 0.1 N aqueous solution of potassium permanganate for titration of the sample (ml), V_0 is the volume of 0.1N solution of potassium permanganate for titration of the blank sample (ml), 0.004157 is tannin equivalent in 1 ml of 0.1N aqueous sample taken for the analysis (g); 250 is the volume of volumetric flask, g is mass of the sample taken for analysis.

Table 2: Results of quantitative analysis of aqueous leaf extract of *Swertia chirayita* and *Punica granatum* (mean ± SD,n = 3)

Phytochemcials	Concentration (mg/ml)	
	Swertia chirayita (Aqueous leaf extract)	*Punica granatum* (Aqueous leaf extract)
Alkaloids	10 ± 1.2	3.075 ± 0.5
Flavonoids	60 ± 2.54	81.16 ± 3.25
Phenols	48 ± 2.36	5.02 ± 1.6
Saponins	30 ± 1.89	23.15 ± 2.38
Tannins	25 ± 0.8	38.5 ± 2.38
Carbohydrates	10.03 ± 1.8	20.01 ± 2.5
Lipids	4.23 ± 0.8	13.03 ± 1.5
Proteins	1.22 ± 0.4	1.02 ± 0.5

Table 3: Nutritive value of leaf of *Swertia chirayita* and *Punica granatum* (mean ± SD,n = 3)

Plant Leaves	Nutritive value(Cal/100g)
Swertia chirayita	83.07 ± 16
Punica granatum	201.39 ± 25.5

Saponin determination

Saponin content was determined as per the previously published work [25, 34]. 20g of each grounded sample was put into conical flask and 100 cm^3 of 20% aqueous ethanol was added. Then the flask was heated on a hot water bath for 4 hours, with constant stirring at about 55 C. The mixture was then filtered and residue was again extracted with 200 ml of 20% ethanol. The extract was then reduced to 40 ml on a hot water bath. The concentrate was transferred into a 250 ml separator funnel, then 20 ml of diethyl-ether was added followed by vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated for 4 to 5 times as desired. 60 ml of n-butanol was added. Now the n-butanol extracts wre washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the obtained ppt were dried in oven, weighed and saponin content was calculated and expressed as mg/g.

Phenolic compound determination

The amount of total phenol content in the aqueous leaf extracts of *Swertia chirayita* and *Punica granatum* was determined by Folin-Ciocalteu's reagent method [25, 35]. 0.5 ml of extract and 0.1 ml of 0.5N Folin-Ciocalteu's reagent were mixed and the mixture was incubated at room temperature for 15 minutes. Then 2.5 ml saturated sodium carbonate solution was added and further incubated at room temperature for 30 minutes. Then absorbance was measured at 760 nm. Gallic acid was used as a positive control. Total phenol values are expressed in terms of gallic acid equivalent (mg/g of extracted compound).

Flavonoid determination

Flavonoids were determined as per previously published work [25], 10g of each sample was extracted with 100 ml of 80% aqueous methanol repeatedly. The whole solution was filtered through Whatman Filter paper #42. The filtrate was later transferred into crucible and evaporated into dryness over a

water bath, the weight of the material and percentage quantity was calculated and expressed as mg/g of powder.

2.4. Synthesis of green nanoparticles

The term 'green' used here has dual meaning. Primary it means eco-friendly (green technology) of nanoparticle synthesis. Secondarily it also indicates the presence of chlorophyll in the leaves, thus the name 'green nanoparticles.' The silver nanoparticles were synthesized following the previously published works [3, 19, 22, 36-38]. For synthesis of silver nanoparticles 1 ml of aqueous leaf extracts of *Swertia chirayita* and *Punica granatum* were taken separately in 200 ml conical flasks. 99ml of 1mM aqueous silver nitrate solution was added. The mixture was allowed to stir for 2 hours at 90 ºC. during this duration the mixture was observed for colour change from pale yellow (initial) to dark brown (final). This colour change is well known visual confirmation, that the reaction is happening between the constituents of the extract and silver nitrate solution. The mixture was allowed to cooled down and after 2 hours, the mixture was centrifuged ad 15000 rpm for 15 minutes at room temperature. The supernatant was discarded, and sediment was washed three times with distilled water. The resultant black powder was dried overnight.

2.4. Characterization of synthesized nanoparticles

The colour change from pale yellow to dark brown while synthesis of silver nanoparticles is a well-known confirmation, that the phytoconstituents in the extract and the silver nitrate solution are reacting [25, 39,40], which leads to the formation of black powder of (nano) particles. These black powdered particles need to be characterized using various techniques (described later), to declare them as nanoparticles (size 1-100 nm), determine their shape, stability *etc.*

UV-Visible spectra analysis

The reduction of pure Ag^+ ions was monitored by measuring the UV-visible spectrum analysis of after 5 hr after diluting a small aliquot of the sample into Milli-Q water [19, 20, 38]. UV-visible spectral analysis was performed using Perkin Elmer, Lambda 25 UV-visible spectrophotometer (USA).

Scanning electron microscopy (SEM)

Scanning Electron Microscope (SEM) analysis was done using JEOL JSM-6390 LV (Japan) machine to observe the surface morphology of the silver nanoparticles. Thin films of the sample were prepared on a carbon-coated copper grid, extra solution was removed using a blotting paper and then the film on SEM grid was allowed to dry by putting it under mercury lamp for 5 minutes and was coated with gold using ion sputter.

Fourier transform infrared spectroscopy (FTIR) spectra analysis

FTIR analysis was carried out on IP Resting – 21 (Shimadzu) in the diffuse reflectance mode operated at a resolution of 4 cm^{-1} in the range of 4 to 400 cm^{-1} to evaluate the functional groups that might be involved in nanoparticles formation [19].

Dynamic light scattering (DLS)analysis

Dynamic light scattering and Zeta potential analysis of nanoparticles were carried on Malvern NanoZS (U.K.) to further confirm the size and distribution of nanoparticles and the stability of nanoparticles [19].

RESULTS AND DISCUSSION

Phytochemical screening

The results of preliminary phytochemical screen are presented as table 1. The preliminary phytochemical screening of aqueous leaf extracts of Swertia chirayita and Punica granatum showed the presence of alkaloid, flavonoid, saponin, tannin and phenol in both the extracts.

The quantitative phytochemical screening of aqueous leaf extract of Swertia chirayita (table 2) showed the presence of 10 ± 1.2 mg/ml of alkaloids, 60 ± 2.54 mg/ml of flavonoids, 48 ± 2.36 mg/ml of phenols, 30 ± 1.89 mg/ml of saponins, 25 ± 0.8 mg/ml of tannins; and the quantitative phytochemical screening of aqueous leaf extract of Punica granatum showed (table 2) showed the presence of 3.075 ± 0.5 mg/ml of alkaloids, 81.16 ± 3.25 mg/ml of flavonoids, 5.02 ± 1.6 mg/ml of phenols, 23.15 ± 2.38 mg/ml of saponins, 38.5 ± 2.38 mg/ml of tannins. Swertia chirayita is known mostly for its bitter taste [45]. This bitter taste can be attributed to the presence of saponins [46]. The saponins are reported to be anti-diabetic [47], ant cancerous [48] and antileishmanial [49]. Some of the beneficial roles of phytochemicals found in aqueous leaf extract of Swertia chirayita and Punica granatum are their antioxidant activity, antimicrobial activity, modulation of

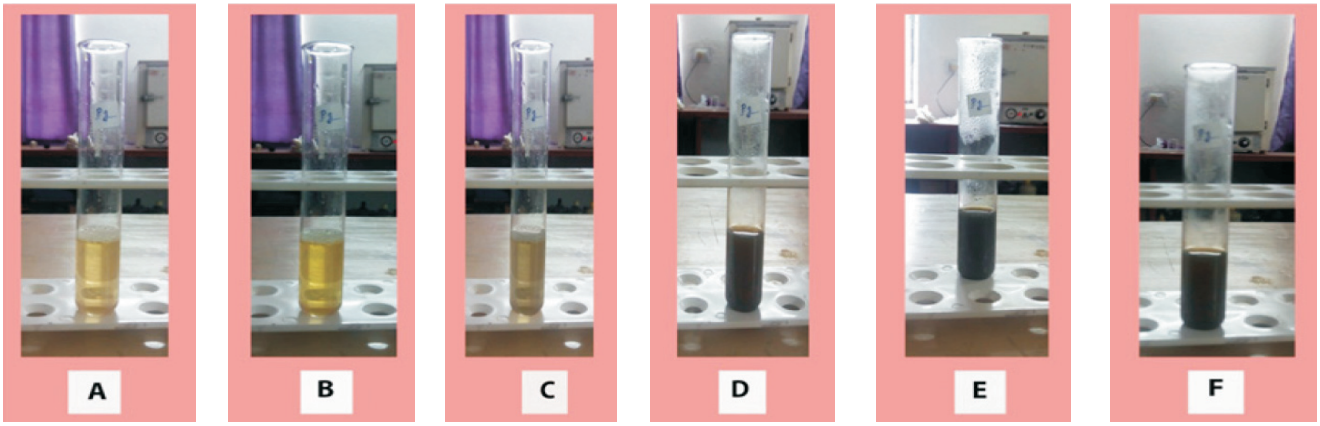

Fig. 2: Colour change from yellow to dark brown due to reduction of silver ions in case of aqueous leaf extract of Punica granatum

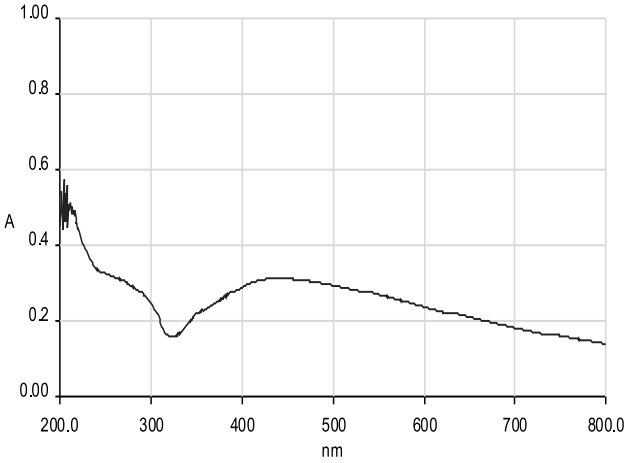

Fig. 3: result of UV-Vis analysis showing surface plasmon resonance peak at 450 nm

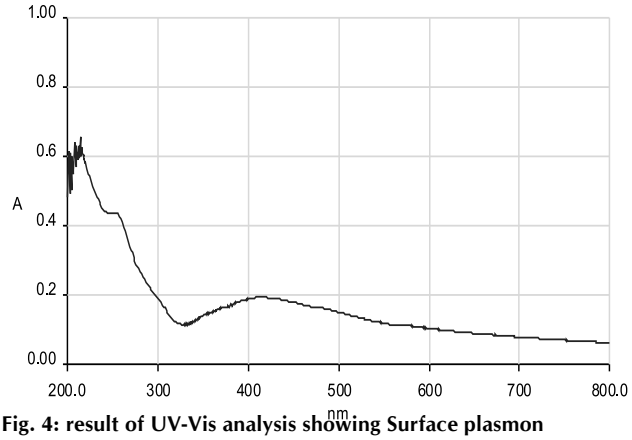

Fig. 4: result of UV-Vis analysis showing Surface plasmon resonance peak at255 and 420 nm

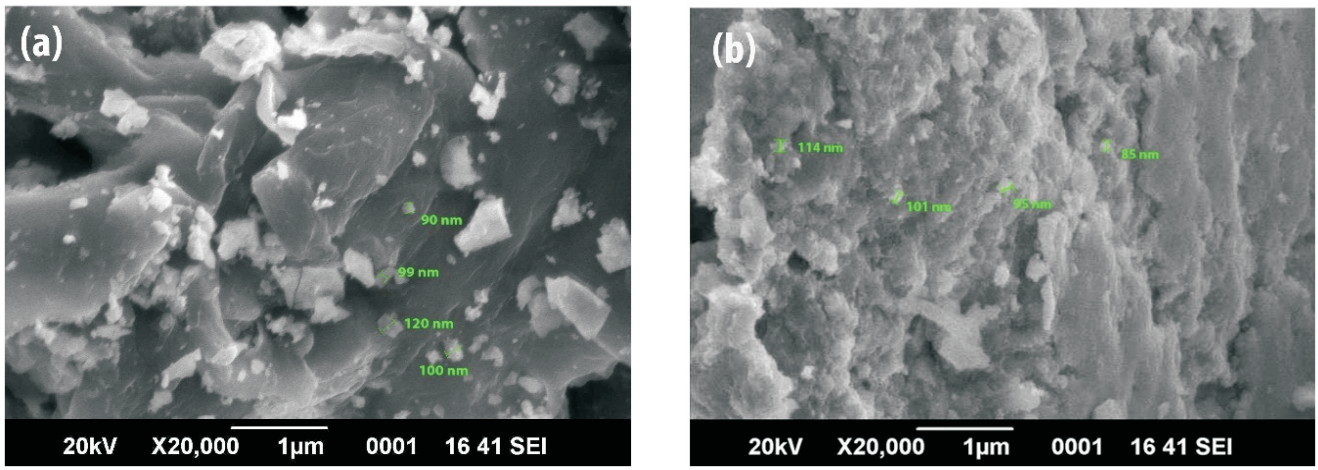

Fig. 5: microphotographs of SEM analysis of silver nanoparticles synthesized using aqueous extract of *Swertia chirayita*

detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism [50].

The nutritive value of the leaf powder of *Swertia chirayita* and *Punica granatum* is low, although the nutritive value of *Punica granatum* is higher than that of *Swertia chirayita*. The low nutritive value shows that the leaves cannot be used as food or fodder, although leaves of both the plants have good medicinal value and extracts can be used for medicinal formulations.

Synthesis of Nanoparticles

The silver nanoparticles were synthesized by mixing the aqueous leaf extract of *Swertia chirayita* and *Punica granatum* as stated in the materials and methods section. As soon as the extracts and silver nitrate solution were mixed, an initial paly yellow colour appeared, which may be due to the presence of free phytoconstituents in the extract. The mixture on incubation changed colour form pale yellow to dark brown over time due to reduction of silver ions by the phytoconstituents. The colour change from pale yellow to dark brown is reported as confirmation of formation of nanoparticles [41, 42, 50, 51 - 53]. The image showing gradual change in case of aqueous leaf extract of *Swertia chirayita* and aqueous leaf extract of *Punica granatum* is presented as figure 1 and figure 2 respectively.

Characterization of nanoparticles

The colour change as shown in figure 1 and figure 2 are confirmatory observation for reaction between silver nitrate and phytoconstituents. After the colour change ceased, a black powder was obtained by following the methodology stated in materials and methods section. The particles of the obtained black powder need to be characterized for size, surface morphology, capping agent, stability to declare them as particles falling in the range of 1 – 100 nm size.

UV-Visible spectroscopy analysis

As soon as the aqueous leaf extracts of *Swertia chirayita* and *Punica granatum* were mixed with the silver nitrate solution, a primary pale colour resulted. Gradual colour change was observed from pale yellow to dark brown over time due to reduction of silver ions. This colour change is reported as a confirmation of formation of nanoparticles [41, 42]. The results of UV-Visible spectroscopy analysis of silver nanoparticles synthesized using aqueous leaf extract of *Swertia chirayita* and *Punica granatum* showing Surface Plasmon Resonance (SPR) peaks is presented as figure 3 and figure 4 respectively. In case of nanoparticles synthesized using aqueous leaf extract

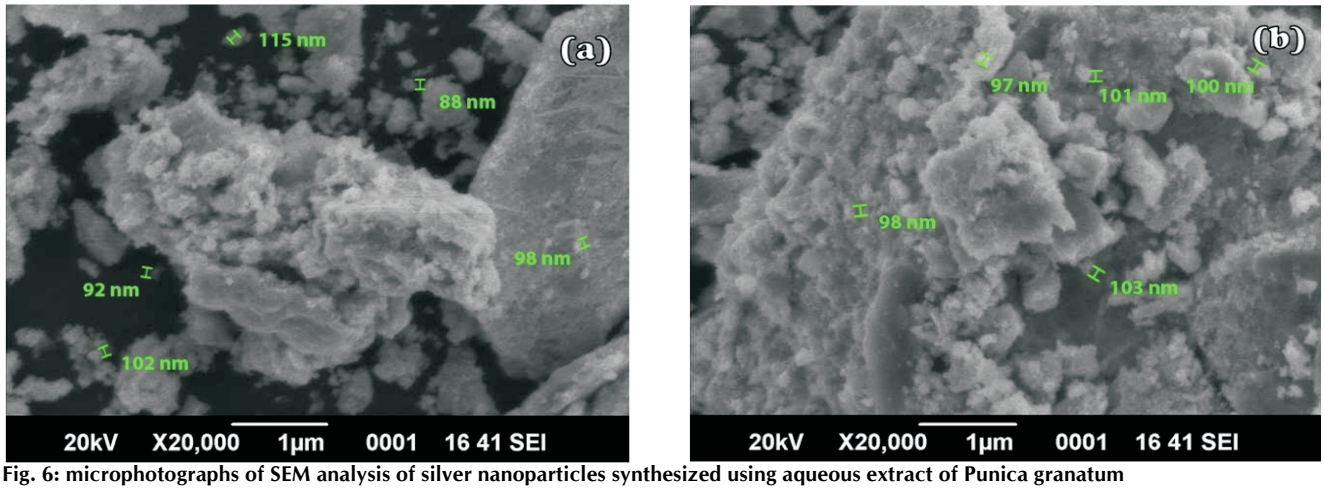

Fig. 6: microphotographs of SEM analysis of silver nanoparticles synthesized using aqueous extract of Punica granatum

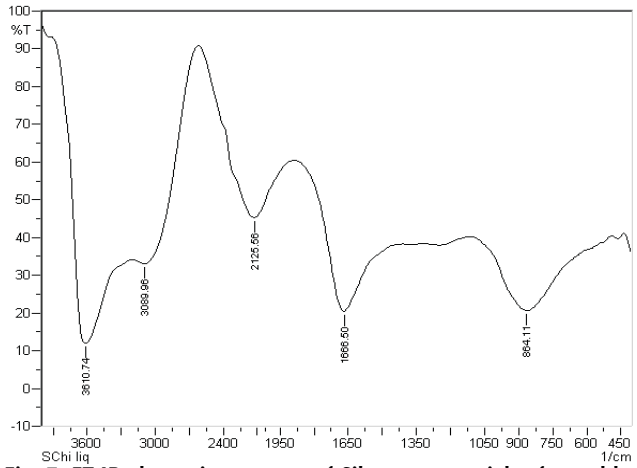

Fig. 7: FT-IR absorption spectra of Silver nanoparticles formed by reduction with aqueous leaf extract of *Swertia chirayita*

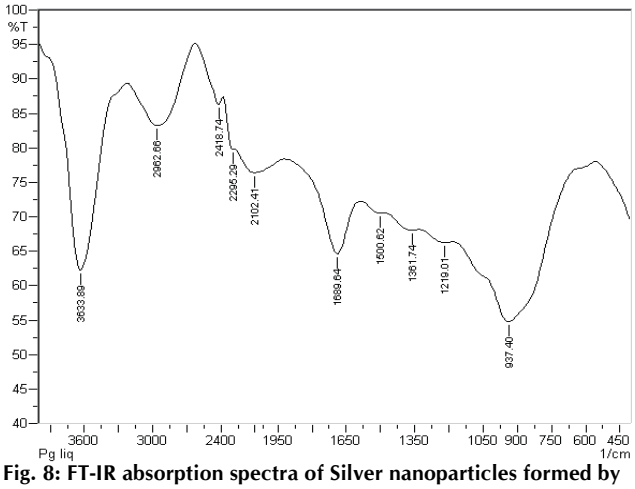

Fig. 8: FT-IR absorption spectra of Silver nanoparticles formed by reduction with aqueous leaf extract of *Swertia chirayita*

of *Swertia chirayita* the SPR peak was observed at 450nm. The nanoparticles synthesized using aqueous leaf extract of *Punica granatum* showed SPR peak at 255nm and 420nm. The review of literature reveals that the particles ranging from 1-100 nm show SPR peak between 300 – 500 nm [36, 54, 55]. Since the SPR peak in case of both the extracts is 450 nm and 420 nm, it indicates that the particles are in nano range and hence can be confirmed as nanoparticle.

SEM analysis of synthesized nanoparticles

Following the confirmation from the UV-Visible spectroscopy analysis, that the synthesized particles are in nano range, the nanoparticles were subjected to SEM analysis as per the methodology stated in the materials and methods section. The SEM micrographs obtained by SEM analysis were further studied with the help of software provided with the machine (JEOL JSM-9390 LV, Jeol, Japan), which helped in measuring the size of the nanoparticles on the display monitor. The SEM micrographs of silver nanoparticles synthesized using aqueous leaf extract of *Swertia chirayita* and *Punica granatum* are presented as figure 5 and figure 6 respectively. The SEM images reveals that the size of silver nanoparticles synthesized using aqueous leaf extract of *Swertia chirayita* ranged from 85 – 120nm with an average size of 101 nm, with spherical and cubical shape. The size of nanoparticles synthesized using aqueous leaf extract of *Punica granatum* ranged from 88-120 nm with an average size of 98.93nm.

Fourier Transform infrared spectroscopy (FT-IR) analysis

FTIR analysis of extracts is done to determine the role of phytoconstituents present in the plant extract as capping agent and functional groups [3, 19]. It is reported that the constituents (phytochemicals) present in the leaf extracts are responsible for reduction of silver nanoparticles [56]. These phytochemicals are identified by detecting the presence of their functional groups using FTIR [56-58]. The images showing the spectra obtained by the FTIR analysis of silver nanoparticles synthesized using aqueous leaf extract of *Swertia chirayita* and *Punica granatum* are presented as figure 7 and figure 8 respectively. Figure 7 reveals that the spectra of silver nanoparticles formed by reduction with aqueous leaf extract of *Swertia chirayita* exhibited broad transmission peaks at 3610.74 cm^{-1}, 3089.96 cm^{-1}, 2125.56 cm^{-1}, 1666.50 cm^{-1} and 864.11 cm^{-1}. The spectra observed were compared with reference values previously published. The fatty acid stretch was recorded at 1666.50 cm-1 [59, 60]. The present study confirms the presence of amines N-H (stretch) and C-N (stretch) characteristic peak at 864.11cm- 1 [59, 60]. The detection of alkaloids is confirmed by the presence of primary and secondary amines [59, 60]. Characteristic peak for hydroxyl

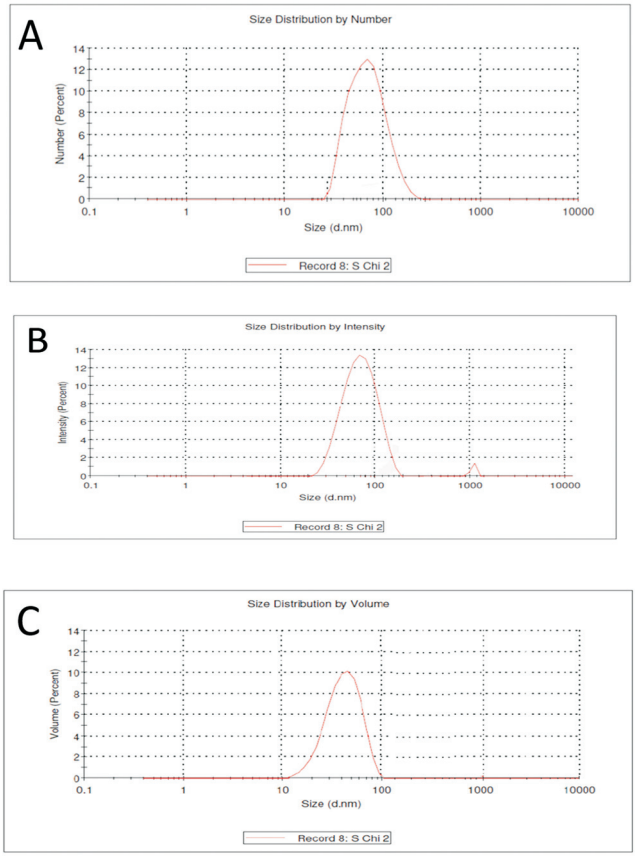

Fig. 9: A. size distribution by number of silver nanoparticles synthesized using aqueous leaf extract of *Swertia chirayita*. B. size distribution by intensity of silver nanoparticles synthesized using aqueous leaf extract of *Swertia chirayita*. C. size distribution by volume of silver nanoparticles synthesized using aqueous leaf extract of *Swertia chirayita*

compounds -OH (stretch) were obtained (3610.74 cm-1). Detection of hydroxyl groups is an indication of presence of flavonoids, alcoholic and phenolic compounds [59, 60]. The peak at 3089.96 cm-1 corresponds to the -CH stretching which represents the lipids [59, 61]. The peak at 2152.56 corresponds to the -C º C- stretching.

The spectra of silver nanoparticles formed by reduction of with aqueous leaf extract of *Punica granatum* exhibited broad transmission peaks at 3633.69 cm^{-1}, 2102.44 cm^{-1}, 1500.62 cm^{-1}, 1361.74 cm^{-1} and 937.40 cm^{-1} as revealed by figure 8. The spectra observed by FT-IR analysis was compared with reference value previously published by Coates [59]. The spectra showed broad transmission peak at 3633.69cm-1, which corresponds to hydrogen bonded hydroxyl group (O-H and H stretch) of alcohols and phenols. The 2102.44cm^{-1} peak corresponds to –SCN. The 1500.62cm^{-1} peak corresponds to C=C stretch, which represents alkenes, 1361.74cm^{-1} corresponds to sulphonates, and 937.40cm^{-1} corresponds to C=N stretch that represents aliphatic amines [59, 61, 62].

The result of FTIR analysis of silver nanoparticles synthesized using aqueous leaf extract of *Punica granatum* is represented as fig. 8. The spectra show broad transmission peaks at 3633.69 cm^{-1}, 2102.44 cm^{-1}, 1500.62 cm^{-1}, 1361.74 cm^{-1} and 937.40 cm^{-1}.

Dynamic light scattering (DLS) and Zeta Potential analysis

The dynamic light scattering (also known as static, Rayleigh or Multi-angle light scattering) provides a direct measure of particle size [43, 44]. The DLS analysis is used to further confirm the nano size of synthesized nanoparticles in terms of number, intensity and volume [19]. It measures the light scattered from a laser that passes through ta colloidal solution and by analyzing the modulation of the scattered light intensity as a function of time.

The results of light scattering are presented in terms of number, intensity and volume in fig. 9(a), fig. 9(b) and fig. 9(c) respec tively for silver nanoparticles synthesized using aqueous leaf extracts of *Swertia chirayita*. Fig. 9(a) shows one peak at 93.6 nm diameter of nanoparticles with percentage distribution of 100% the number distribution shows the number of particles in different size bins [63]. The number distribution graph thus reveals, that almost all 100 % particles formed had diameter of about 93.6 nm. Fig. 9(b) shows two peaks at 92.5 nm and 2120 nm with percentage intensity of 98.2% and 1.8% respectively. The intensity distribution describes how much light is scattered by particles of different size bins [63]. It shows that 98.2% of light was dispersed by nanoparticles whose average size was 92.5 nm. This shows that about 98.2% of the particles in the suspension had average size of 92.5mm. Fig. 9(c) shows two peaks at 78.2 nm and 2136 nm with percentage volume of 71.1 and 28.9 respectively. The volume distribution shows the total volume of particles in different size bins [63]. The volume distribution shows that bout 71.1% of total volume of nanoparticles formed had an average diameter of 78.2 nm.

The results of light scattering are presented in terms of number, intensity and volume in fig. 10(a), fig. 10(b) and fig. 10(c) respectively for silver nanoparticles synthesized using aqueous leaf extracts of *Punica granatum*. Fig. 10(a) shows two peaks at 113.5nm and 40.32 nm diameter of nanoparticles with number distribution of 0.1 % and 99.99%, the number distribution shows the number of particles in different size bins [63]. The number distribution graph thus reveals, that almost 99.9% of particles formed had diameter of about 113.5 nm. Fig.10(b) shows two peaks at 96.5 nm and 141.6 nm with percentage intensity of 91.0% and 9.0% respectively. The intensity distribution describes how much light is scattered by particles of different size bins [63]. It shows that 91.0% of light was dispersed by nanoparticles whose average size was 96.5 nm. This shows that about 91.0% of the particles in the suspension had average size of 96.5nm. Figure 10(c) shows two peaks at 85.6 nm and 24.76 nm with percentage volume distribution of 84.2% and 15.8% respectively. The volume distribution shows the total volume of particles in different size bins [63]. The volume distribution shows that about 100% of total volume of nanoparticles have their diameter in nano range, out of which 84.2% had size of 85.6nm and 15.8% particles had size of 24.76nm.

The zeta potential analysis is a technique for determining the surface charge of nanoparticles in solution (colloid). Nanoparticles have a surface charge that attracts a thin layer of ions of opposite charge to the nanoparticle surface. This double layer of ions travels with nanoparticle as it diffuses throughout the solution [64]. The electric potential at the

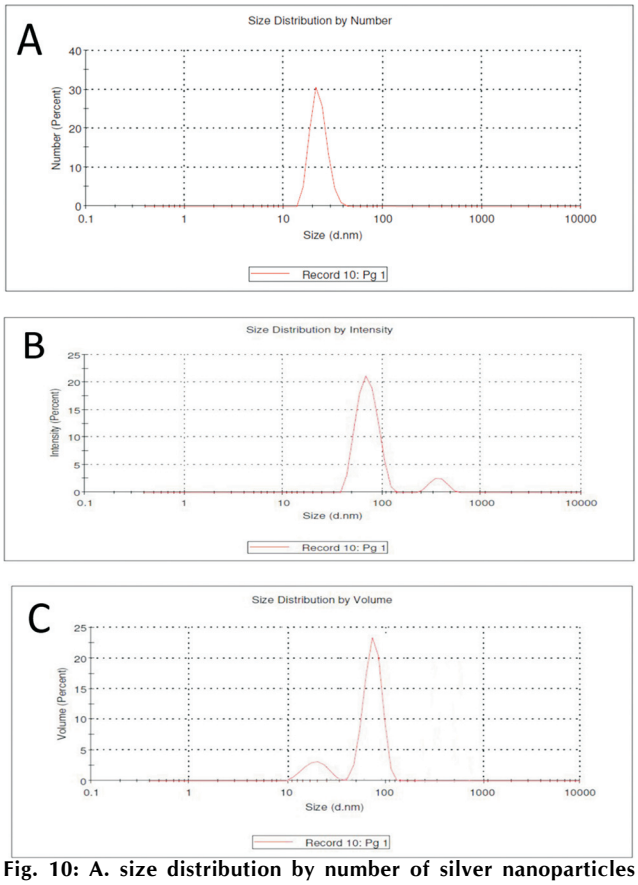

Fig. 10: A. size distribution by number of silver nanoparticles synthesized using aqueous leaf extract of *Punica granatum*. B. size distribution by intensity of silver nanoparticles synthesized using aqueous leaf extract of *Punica granatum*. C. size distribution by volume of silver nanoparticles synthesized using aqueous leaf extract of *Punica granatum*

boundary of the double layer is known as Zeta potential of the particle. The Zeta potential of particles has a value ranging from +100mV to -100mV [64]. Any nanoparticle with Zeta potential values in the range o +25mV to -25mV typically has high degrees of stability [19, 64]. The results of Zeta potential analysis of aqueous leaf extracts of *Swertia chirayita* and *Punica granatum* has been presented as fig. 11(a) and fig. 11(b) respectively, with peak of -15mV and -11.6mV respectively. The values thus obtained shows the efficiency on capping material in stabilizing the nanoparticle providing intensive negative charges that keep all the particles away from each other. This indicates that the nanoparticles synthesized using aqueous leaf extracts of *Swertia chirayita* and *Punica granatum* are stable in the solution.

CONCLUSION

The aqueous leaf extracts of *Swertia chirayita* and *Punica granatum* can be used for synthesis of silver nanoparticles within nanoscale range with strong stability. There are some reports stating that the nanoparticles synthesized using aqueous extracts of *Swertia chirayita* and *Punica granatum* has greater medicinal efficiency as compared to the extract alone. This indicates their future scope and immense exploration probabilities.

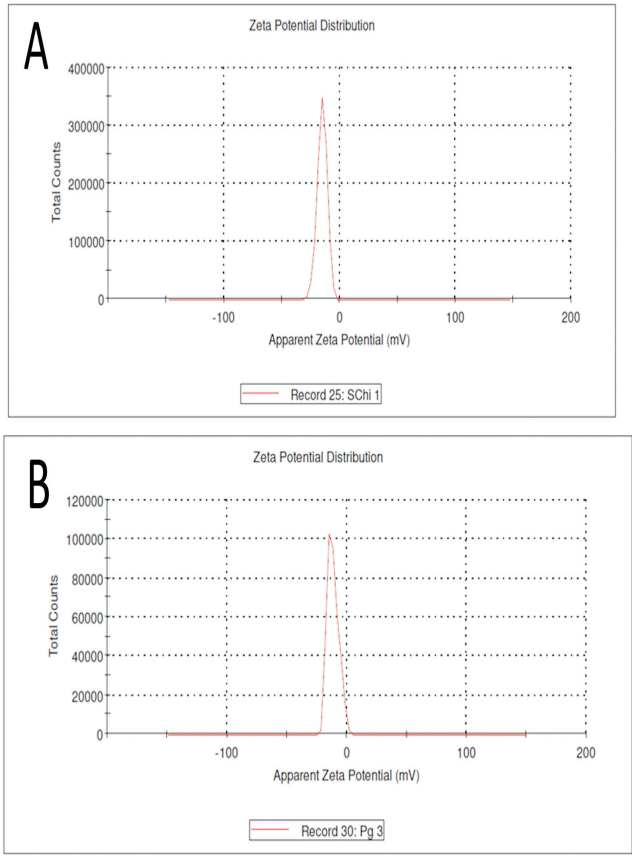

Fig. 11:A. Result of Zeta potential analysis of silver nanoparticles synthesized using aqueous leaf extract of *Swertia chirayita*. B. Result of Zeta potential analysis of silver nanoparticles synthesized using aqueous leaf extract of *Punica granatum*

Zeta Potential analysis

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