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ORIGINAL ARTICLE

Green Synthesis and Antioxidant Analysis of *In vivo* Leaf and *In vitro* Callus of *Tephrosia villosa*

V. Ranjitha, K. Kalimuthu, V. Chinnadurai, Y. Sharmila Juliet, M. Saraswathy[1]

Plant Tissue Culture Division, PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore, [1]Department of Biological Sciences, Vidhyasagar Women's College of Education, Chengalpattu, Tamil Nadu, India

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ABSTRACT

Background: *Tephrosia villosa* (Leguminosae) is an important medicinal plant widely used in traditional Indian medicine. To the best of our knowledge, there is no report on this field. **Objective:** The aim of this study was to synthesize silver nanoparticles (SNPs) using *T. villosa in vivo* leaf and *in vitro* callus extract and characterization of SNPs. **Materials and Methods:** The synthesized SNPs were characterized by ultraviolet (UV) visible spectrum, Fourier transform infrared (FT-IR), X-ray diffraction (XRD), scanning electron microscopic (SEM), and Energy-dispersive X-ray spectroscopy (EDX). The *in vitro* antioxidant activities of the synthesized nanoparticles were studied by 1, 1-diphenyl-2-picryl-hydrazyl radical scavenging activity, hydrogen peroxide assay, nitric oxide radical scavenging activity, and hydroxyl radical scavenging activity. **Results:** The *in vivo* leaf SNPs have absorbance peak at 740 nm, and callus SNPs have absorbance peak at 305 nm for reaction at room temperature. The FTIR results also showed interaction between the plant extract and Ag-NPs due to the similarity in the peak patterns. The four and nine diffraction peaks were obtained from leaf and callus SNPs, respectively, through XRD studies. The obtained SEM image confirms the presence of polymorphic nanoparticles such as spherical, ellipsoidal, and some irregular shaped. The EDX results showed that Ag-NPs display an absorption peak at 3 keV, indicating the presence of the element silver. **Conclusion:** It is confirmed that both the *in vivo* leaf and *in vitro* callus SNPs revealed high level of antioxidant activity in *in vitro* system.

Key words: Green synthesis, hydrogen peroxide, nanotechnology, radical scavenging and tephrosia

SUMMARY

- This study was performed to synthesis silver nanoparticles (SNPs) from *Tephrosia villosa in vivo* leaf and *in vitro* callus. Characterization of SNPs and its antioxidant potential through DPPH radical scavenging activity, hydrogen peroxide assay, nitric oxide radical scavenging activity and hydroxyl radical scavenging activity. The characterization of *in vivo* leaf and *in vitro* callus SNPs showed similar peak patterns, presence of polymorphic nanoparticles and element silver. Both the SNPs showed significant antioxidant activity when compared to the control. Due to similarities of SNPs towards antioxidant activity, the callus can be replaced the wild plant for commercial or pharmaceutical purposes.

Abbreviations used: $AgNO_3$: Silver Nitrate; mg: Mille gram; ml: Mille litter; mM: Micro molar; µg: Micro gram.

Correspondence:

Dr. K. Kalimuthu,
Department of PG and Research in Botany,
Plant Tissue Culture Division,
Government Arts College (Autonomous),
Coimbatore - 641 018, Tamil Nadu, India.
E-mail: k_kalimuthu@rediffmail.com

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INTRODUCTION

Nanotechnology is an emerging area with interdisciplinary research, particularly in biotechnology.[1] It is an important technical tool for development of eco-friendly, reliable methodology for the synthesis of nanoscale material using biological source. Nanosilver is an additive in Indian Ayurveda medicine due to its ability to counter the disease spreading organisms. Due to attractive physiochemical properties, the silver nanoparticles (SNPs) play a vital role in the field of biology and medicine.[2] SNPs are reported to possess antifungal,[3] anti-inflammatory, and antiviral activity.[4]

Plant-mediated nanoparticles (green synthesis) under biological synthesis are environmental friendly, cost-effective, devoid of toxic chemicals, zero-energy consumption, less time consumption, and nonrequirement of any kind of stabilizers. Green originated SNPs are highly suitable for pharmaceutical and other biomedical applications.[5] Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as provide natural capping agents. Moreover, use of plant extracts also reduces the cost of microorganisms, isolation and culture media, enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms.

Tephrosia villosa (Leguminosae) is widely used in traditional Indian medicine as a treatment for dropsy and diabetes.[6] The taxon is also used as green manure in Coffee and Heave Plantations and as a shade crop in tea plantations.[7] Roots, leaves, fruits, and twigs of *T. villosa* showed significant activity against *Culex quinquefasciatus* larvae.[8] *T. villosa* leaves showed reduction in glucose level and pancreatic cell regeneration in alloxan-induced diabetes in the presence of 20 (29)-lupen-3-one a compound.[9,10] Four new retinoid were isolated from seeds, and dehydroxyrotenoid and lupenone were isolated from whole plant.

The present study aimed that the synthesis of SNPs using *in vivo* leaf and *in vitro* callus extract of *T. villosa* characterized of SNPs and evaluated its antioxidant activity.

MATERIALS AND METHODS

Plant collection and authentication

The plant of *T. villosa* (L.) pers (Fabaceae family) was collected from Nambiyur, Tamil Nadu, India and it identifies was further authenticated by Botanical Survey of India (BSI) Southern Region, Coimbatore, India. Reference number is BSI/SRC/5/23/2016Tech/207.

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Preparation of plant extract

The powdered *T. villosa* leaf and callus was extracted using 100 ml of ethanol for each sample using the Soxhlet extractor for 14 h.[11] The extracts were filtered through Whatman No. 1 filter paper to remove all undissolved matter including cellular materials and other constitutions that are insoluble in the extraction solvent and stored at 4°C used for further experiments.

Biosynthesis of silver nanoparticles

Aqueous solution of 1 mM $AgNO_3$ was prepared and used for the synthesis of SNPs. A volume of 10 ml of *T. villosa* ethanol extract of leaf and callus is mixed with 90 ml of $AgNO_3$ for the synthesis of SNPs. The formation of SNPs of leaf extract was confirmed by color change from green to yellowish brown and for callus white to dark brown by ultraviolet-visible (UV-Vis) spectroscopy.

Characterization of silver nanoparticles synthesis

Ultra violet-visible spectrum analysis

The color change in reaction mixture (metal ion solution + plant extract) was recorded through visual observation. Synthesized SNPs were confirmed by sampling the aqueous component of 2 h after reaction, and the absorption maxima were scanned by UV-Vis spectrophotometer at the wavelength of 200–800 nm.

Lyophilization of silver nanoparticles

The solution containing different nanoparticles were lyophilized through Lyophilizer. The samples were centrifuged at 10,000 rpm for 15 min, and the reaction was carried out thrice. After the reaction period supernatant was discarded and collected the pellet. The collected pellet was freeze dried. The lyophilized samples were kept in the freezer at 4^0C for further analysis.

Fourier-transform infrared analysis

Fourier-transform infrared (FT-IR) spectroscopy analysis of the SNPs of *in vivo* leaf and *in vitro* callus was carried out through the potassium bromide (KBr) pellet (FTIR grade) method in 1:100 ratio and spectrum was recorded using Jasco FT/IR-6300 FT-IR spectrometer equipped with JASCO IRT-7000 Intron Infrared Microscope using transmittance mode operating at a resolution of 4 cm^{-1} (ALPHA FTIR Spectrophotometer, Bruker, Germany).

X-ray diffraction studies

X-ray diffraction (XRD) analysis was done using instrument RICA KU ULTIMA. The crystalline structure of the biosynthesized SNPs was investigated through XRD technique using X-ray powder diffract meter. The SNPs dispersion was placed on a glass slide, and the solution (ethanol) was allowed to evaporate, to get a thin film of SNPs. This thin film was subjected to XRD operating between 10° and 80° with the scanning rate of 2° per minute.

Scanning electron microscopic analysis

Scanning electron microscopic (SEM) analysis was done using instrument QUANTO 250 SEM. 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 was allowed to dry by putting it under a mercury lamp for 5 min.

X-ray spectroscopy analysis

Energy-dispersive X-ray spectroscopy (EDX) analysis was carried out for the detection and confirmation of elemental silver. Very small amount of the sample was drop coated onto carbon film and analyzed for the composition of the synthesized nanoparticles.[12]

Antioxidant studies of silver nanoparticles

1, 1-diphenyl-2-picryl-hydrazyl radical scavenging method

Preparation of test solutions

21 mg of the leaf and callus SNPs of *T. villosa* was weighed and dissolved in distilled separately to a solution of 21 mg/ml concentration. Each of these solutions was serially diluted separately to obtain the final concentration ranging from 1,000 to 1.953 µg/ml.

Preparation of standard solution

Twenty-one milligram of each ascorbic acid and rutin were separately dissolved in 2 ml of dimethyl sulfoxide DMSO_ to get 21 mg/ml concentration. This solution was serially diluted with dimethyl sulfoxide to get lower concentrations.

Method

The assay was carried out in a 96-well microtiter plate. To 200 µl of 1, 1-diphenyl-2-picryl-hydrazyl DPPH_ solution, 10 µl of each of the sample or the standard solution was added separately in well of the microtiter plate. The final concentration of the sample and standard solution used are 1000 to1.953 µg/ml. The plates were incubated at 37°C for 20 min, and the absorbance of each well was measured at 490 nm, using ELISA reader against the corresponding test and standard blank and the remaining DPPH was calculated. IC_{50} (Inhibition Concentration) is the concentration of the sample required to scavenging 50% of DPPH free radicals.[13]

$$\% \text{ of Inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Nitricoxide radical inhibition activity

Preparation of test and standard solution

Twenty-one milligram of each of the leaf and callus SNPs of *T. villosa* and standard such as ascorbic acid and rutin were separately dissolved in 2 ml of DMSO to get 21 mg/ml concentration. This solution was serially diluted with dimethyl sulfoxide to get lower concentrations.

Method

The reaction mixture (6 ml) containing SNP (10 mM, 4 ml) and 1 ml of DMSO was incubated at 25°C for 90 min. After incubation, 0.5 ml of the extraction mixture containing nitrate was removed and 1 ml of sulfanilic acid reagent was added, mixed well, and allowed to stand for 5 min for the completion of diazotization, then 1 ml of NEDD was added, mixture, and allowed to stand for 30 min in different light at room temperature. The absorbance of these solutions was measured at 540 nm using ELISA reader against corresponding blank solution. IC_{50} value obtained is the concentration of the sample required to inhibit 50% NO radical.[14,15]

Scavenging of hydrogen peroxide radicals

Preparation of test and standard solution

Thirty milligram of each leaf and callus SNPs of *T. villosa* and the standards (Ascorbic acid and Rutin) were accurately weighted and separately dissolved in 10 ml of methanol. This solution was serially diluted with methanol to obtain the lower dilutions.

A solution of H_2O_2 (20 mM) was prepared in phosphate buffer saline (PBS), (pH 7.4). Various concentrations of 1 ml of the extracts or standard in methanol were added to 2 ml of H_2O_2 solution in PBS. The absorbance was measured at 230 nm, after 10 min against a blank solution that contained extracts in PBS without H_2O_2.[16]

Hydrogen radical scavenging activity by P-nitrosodimethyl aniline

Preparation of test and standard solution

Thirty milligram of each leaf and callus SNPs of *T. villosa* and standard such as ascorbic acid and rutin were weighted and dissolved separately in 5 ml of DMSO. The solutions were serially diluted with DMSO to obtained lower dilution.

Ferric chloride (0.1 mM, 0.5 ml), EDTA (0.1 mM, 0.5 ml), ascorbic acid (0.1 mM, 0.5 ml), H_2O_2 (2 mM, 0.5 ml), and pNDA (0.01 mM, 0.5 ml) in phosphate buffer pH 7.4 (20 mM) were added and various concentrations of extracts or standard (0.5 ml) were made a final volume of 3 ml. Sample blank is prepared by adding 0.5 ml sample and 2.5 ml of phosphate busffer pH 7.4. Absorbance was measured at 440 nm.

RESULTS

Silver nanoparticle synthesis

The *T. villosa* plant leaf and callus extract were mixed with aqueous solution of 1 mM $AgNO_3$ solution led to the appearance of yellowish brown and dark brown color indicates the synthesis of SNPs. The complete the synthesis of SNPs was signified by the color change from after 15 h incubation at room temperature, and there was no significant change afterward.

Characterization of silver nanoparticles

The synthesized biogenic SNPs were subjected to determine their size and morphological characteristics with the help of UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), XRD analysis, energy dispersive X-ray analysis (EDX), and Scanning electron microscope studies (SEM).

Ultraviolet-visible spectroscopy

The UV-Vis spectrophotometer proved to be very useful technique for the characterization of *in vivo* leaf and *in vitro* callus extracts after the formations of SNPs. UV-Vis absorption spectrum of the SNPs has been monitored and recorded by taking reading at regular intervals as shown in Figures 1 and 2. The leaf SNPs have absorbance peak at 740 nm and callus SNPs have absorbance peak at 305 nm for reaction at room temperature. A remarkable expansion of peak around 200 nm to 800 nm point toward that the particles are polydispersed.

Fourier transform infra-red

FTIR spectroscopy analysis was carried out to know the functional groups accountable for the reduction of silver ions and capping of the bioreduced SNPs synthesized by *T. villosa* leaf and callus extracts. Figures 3 and 4 represent the FTIR spectrum of the leaf and callus extracts SNPs. The spectrum of leaf extract shows the intense peaks at 3896.21, 3741.90, 3626.17, 3556.74, 3456.44, 3417.86, 3394.72, 3332.99, 2947.23, 2908.65, 2360.87, 1643.35, 1535.345, 1072.42, 910.40, 825.53, and 740.62 in the region of 4000 cm^{-1}–750 cm^{-1} whereas the callus extract, the intense peaks in FTIR patters observed between 4000 cm^{-1} and 500 cm^{-1} clearly shows the stretching vibrations for C = O,-C-N,-C-NH2,-C-H and C = C functional groups. Here, the bands are 3383.20, 2922.81, 2854.55, 1629.80, 1382.65, 1032.06, 492.80, 459.93, and 430.47 cm^{-1}. The absorption bands at 1538/cm and 1382/cm of leaf and callus extracts represent the characteristics of N-H group of amines. The bands observed at 1643/cm and 1629/cm indicates the stretching vibration of alkenes (–C = C) and alpha, beta-unsaturated aldehydes, and ketones (C = O) the absorption spectra above 3417/cm and shows the presence of O-H and N-H stretching vibrations, respectively.

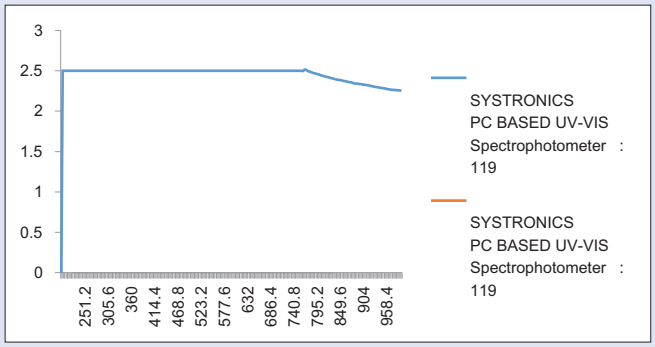

Figure 1: UV–vis absorption spectrum of in vivo leaf SNPs of *Tephrosia villosa*

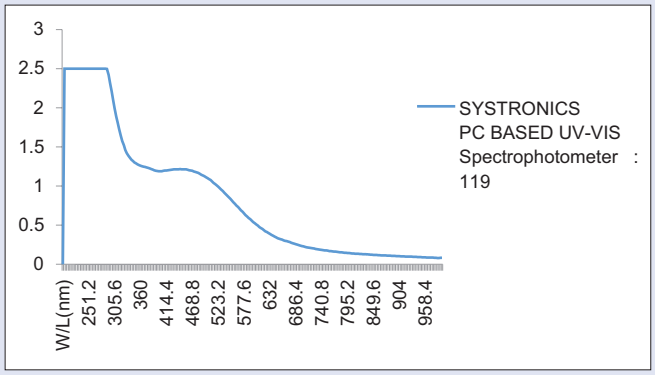

Figure 2: UV–vis absorption spectrum of *in vitro* callus SNPs of *Tephrosia villosa*

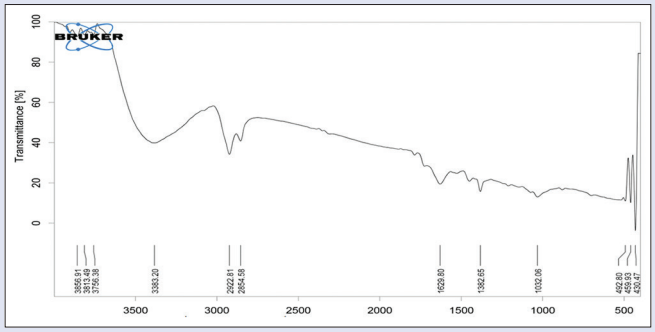

Figure 3: Graphs obtained from FTIR analysis of *in vivo* leaf SNPS of *Tephrosia villosa*

X-ray diffraction studies

XRD powder diffraction is the method to analyze the crystalline phase, orientation, and the grain size of SNPs. XRD diffract gram showing the intense peaks in the whole spectrum of 2ɵ values are represented in Figures 5 and 6. In XRD of leaf extract, SNPs exhibited intense peak in the whole spectrum of 2ɵ value ranging between 0 and 80. The four intense diffraction peaks at 18.6°, 27.74°, 32.12°, and 76.65° corresponding to four diffraction factors of silver. Whereas in callus extract SNPs XRD diffract gram showed nine diffraction peaks at 23.5°, 27.83°, 32.27°, 44.3°, 46.28°, 54.9°, 57.52°, 76.74°, and 85.78°.

Scanning electron microscopic

The size and shape of the SNPs of leaf and callus extracts were ascertained using SEM. The obtained SEM image confirms the presence

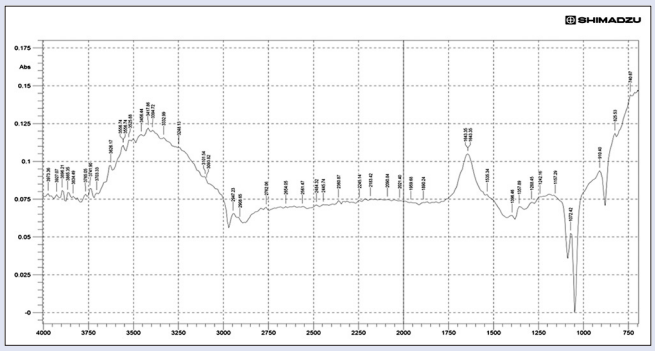

Figure 4: Graphs obtained from FTIR analysis of *in vitro* callus SNPs of *Tephrosia villosa*

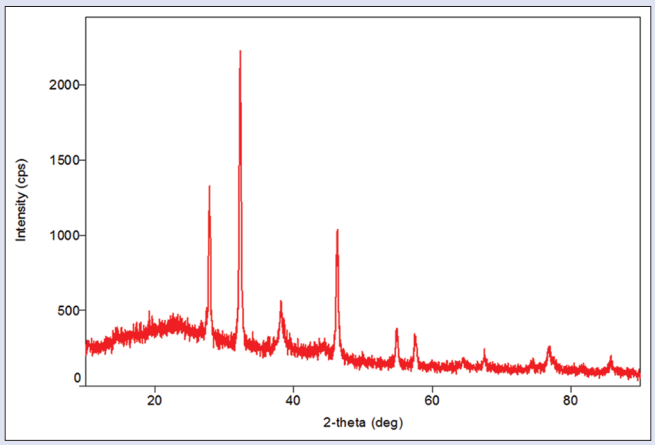

Figure 6: Typical XRD pattern of SNPs using *in vitro* callus extract of *T. villosa*

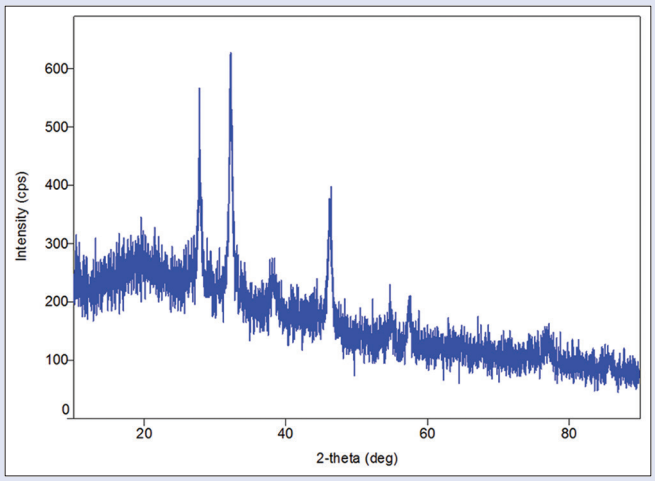

Figure 5: Typical XRD pattern of SNPs using *in vivo* leaf extract of *T. villosa*

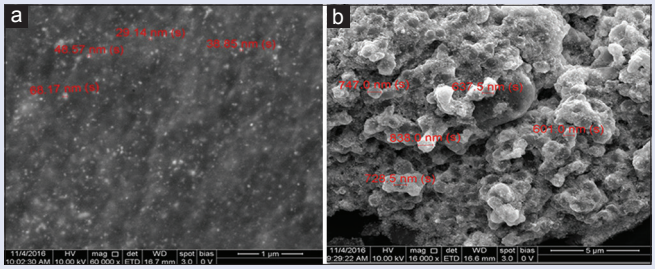

Figure 7: SEM images of silver nanoparticles. (a) *In vivo* leaf and (b) *In vitro* callus

of polymorphic nanoparticles such as spherical, ellipsoidal, and some irregular shaped [Figure 7]. It also shows that the SNPs are not aggregated monodisperse in nature. The size of the SNPs was about 29 nm to 68 nm and 601–838 nm in leaf and callus SNPs, respectively [Figure 7].

X-ray spectroscopy

The synthesized leaf and callus extracts SNPs were analyzed through EDX for the detection and confirmation of element silver. Figures 8 and 9 show the typical EDX pattern and spectra of SNPs obtained from leaf and callus extracts. In both the leaf and callus extract, SNPs strong optical absorption peaks were observed approximately at 3kev.

Antioxitant activity

1, 1-diphenyl-2-picryl-hydrazyl assay

The result for DPPH assay and graphical representation of *T. villosa in vivo* leaf and *in vitro* callus extracts was presented. *In vivo* leaf extract, SNPs had significant scavenging effect in all the concentrations. It can be see that the *in vivo* leaf extract SNPs was showed the higher inhibition percentage 51.4 at 31.25 µg/ml concentration followed by 50.0% at 62.5 µg/ml concentration [Figure 10]. Increasing in the concentration from 125 µg/ml to 1000 µg/ml decreased in the inhibition percentage. *In vitro* callus extract, SNPs at 1.95 µg/ml to 1000 µg/ml concentration showed no effect or inactive.

The IC_{50} value was found to be 60.23 ± 0.13,9.51 ± 0.22 and 14.5 ± 0.29 in *in vivo* leaf extract SNPs, ascorbic acid, and rutin, respectively [Table 1].

In vivo leaf explant possessed a good radical scavenging capacity. There is no scavenging activity against *in vitro* callus extract of SNPs.

Hydrogen peroxide assay

The hydrogen peroxide assay results obtained from *in vivo* leaf and *in vitro* callus extract SNPs and its graphical representations were presented in Figure 11. Among the seven concentration of *in vivo* leaf extracts SNPs examined (1.95 µg/ml to 125 µg/ml) the highest inhibition percentage 51.2 and 54.5% was observed at 15.65 µg/ml and 7.81 µg/ml against *in vivo* leaf and *in vitro* callus extracts SNPs, respectively.

Increasing in the extract concentration decreases the percentage inhibition. This is followed by 44.6 and 50.4% at 7.80 mg/ml and 3.90 mg/ml against *in vivo* plant and *in vitro* callus extracts SNPs. The IC_{50} value analysis was found to be 11.09 ± 0.12, 2.71 ± 0.10, 16.55 ± 0.44 and 8.58 ± 0.17, 16.55 ± 0.44 and 8.58 ± 0.17 for *in vivo* leaf and *in vitro* callus SNPs, ascorbic acid, and rutin, respectively [Table 1].

Nitric oxide radical scavenging activity

The results obtained for the nitric oxide scavenging assay and the graphical representation was shown in Figure 12. In the present study, *in vivo* leaf and *in vitro* callus extracts SNPs of *T. villosa* at the concentration of 15.65, 31.25, 62.5, 125, and 250 µg/ml were studied in the nitric oxide radical scavenging activity. Almost all the concentration of both the extracts have good inhibition percentage. Among these concentrations, the more inhibition percentage 50.57 and 52.84 were observed at 15.65 µg/ml concentration, respectively. The second more inhibition was observed (50.50, 51.21) at 31.25 µg/ml concentration. The IC_{50} value calculated using the linear regression analysis was found to be

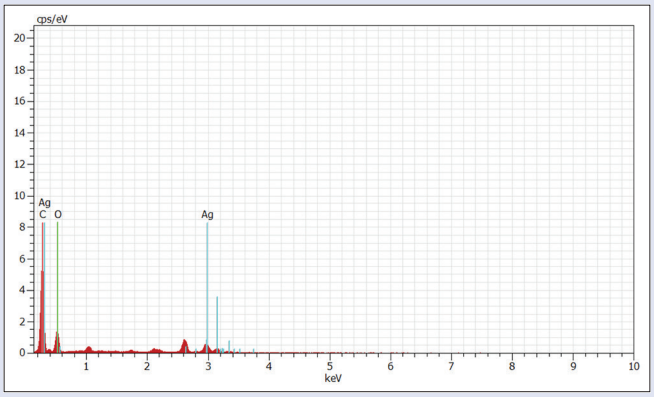

Figure 8: Typical EDX pattern of SNPs using *in vivo* leaf extract of *T. villosa*

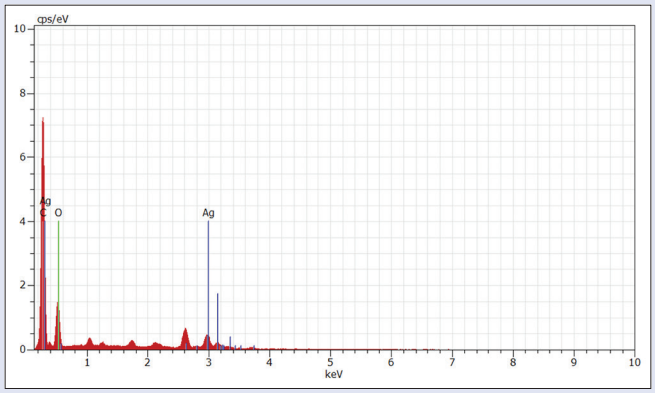

Figure 9: Typical EDX pattern of SNPs using *in vitro* callus extract of *T. villosa*

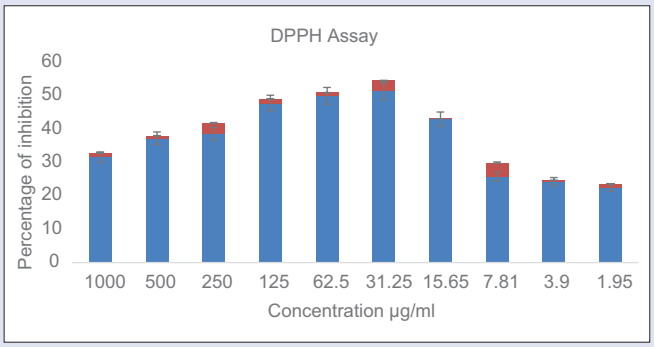

Figure 10: Percentage of inhibition of DPPH activity of *in vivo* leaf extract SNPs of *Tephrosia villosa*

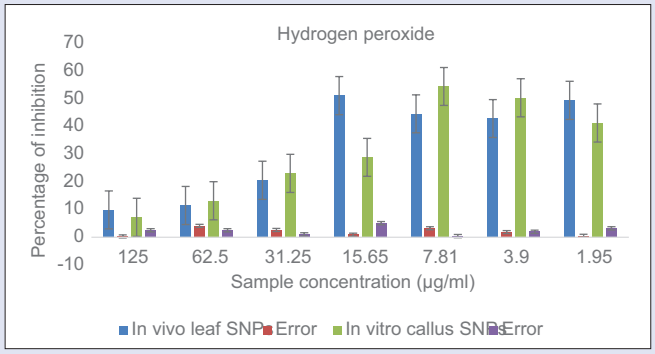

Figure 11: Percentage of inhibition of Hydrogen peroxide activity of *in vivo* and *in vitro* ethanol extracts SNPs of *Tephrosia villosa*

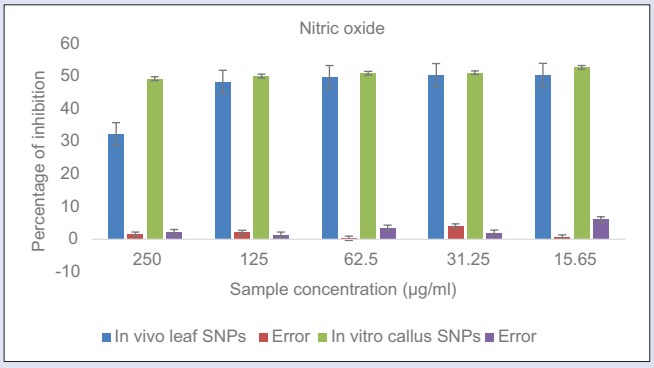

Figure 12: Percentage of inhibition of Nitric oxide assay of *in vivo* leaf and *in vitro* callus ethanol extracts SNPs of *Tephrosia villosa*

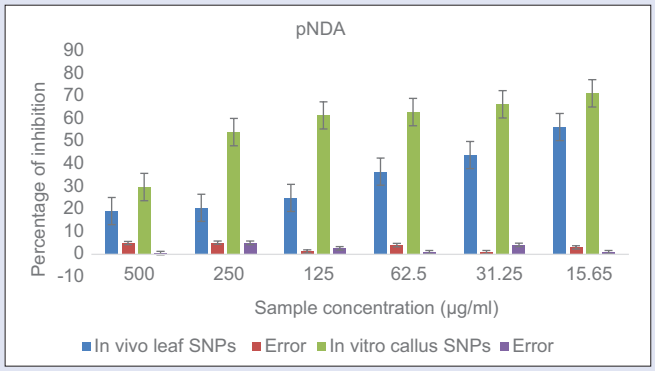

Figure 13: Percentage of inhibition of Hydroxyl Radical Scavenging by p-NDA (p-nitrosodimethyl aniline) method of in vivo leaf and *in vitro* callus ethanol extracts SNPs *Tephrosia villosa*

34.79, 150.2, >1000, 69.57 µg/ml for *in vivo* leaf extract SNPs, *in vitro* callus extract SNPs, ascorbic acid, and rutin, respectively.

Hydroxyl radical scavenging activity

The values of hydroxyl radical scavenging activity by pNDA method of *in vivo* leaf and *in vitro* callus extract SNPs of *T. villosa* and its graphical representation was presented in Figure 13. As per the result, SNPs showed the higher percentage inhibition of 56.5 and 71.5 at the concentration of 15.65 µg/ml. This is followed by 44.1 and 66.6% at 31.25 µg/ml concentration, respectively. The IC_{50} values *in vivo* and *in vitro* plant extract SNPs, ascorbic acid, and rutin was 20.03, 362.5, 16.55, and 14.5 µg/ml, respectively [Table 1].

DISCUSSION

Medicinal plants are the most exclusive source of life-saving drugs for the majority of the World's population. Medicinal plants represent a vast potential resource for anticancer compounds. The anticancer activity of medicinal plant-derived compounds may result from a number of mechanisms, including effects on cytoskeletal proteins that play a key role in cell division, inhibition of DNA topoisomerase enzymes, antiprotease or antioxidant activity, stimulation of the immune system, etc., The value of medicinal plants lies in the potential access to extremely

Table 1: IC_{50} values of Different method of antioxidant activity of *in vivo* leaf and *in vitro* callus SNPs of *Tephrosia villosa*

Antioxidant methods (IC50 values* ± SEM# (µg/mL)				
Sample	**DPPH**	**Nitric Oxide**	**H_2O_2**	**pNDA**
In vivo Sample	60.23±0.13	34.79±0.07	11.09±0.12	20.03±0.15
In vivo Sample	Inactive	150.2±0.19	2.71±0.10	362.5±0.16
Standards				
Ascorbic acid	9.51±0.22	>1000	16.55±0.44	16.55±0.44
Rutin	14.5±0.29	69.57±0.15	8.58±0.17	14.5±0.29

complex molecular structures that would be difficult to synthesize in the laboratory.[17]

T. villosa, an medicinally important plant, was selected for the present study to standardize the protocol for rapid multiplication, synthesis of SNPs from *in vivo* leaf and *in vitro* callus, to analyze secondary metabolites, to test the antioxidant activity to confirm the SNPs characters through UV spectroscopy, FTIR, XRD, SEM, and EDX.

Due to the reduction of silver ion, the appearance of brown color in the reaction vessels suggested the formation of SNPs. The *T. villosa* leaf and callus SNPs samples were mixed in the aqueous solution of the silver ion complex. The appearance of yellowish brown color during this process could be due to the excitation of surface plasma vibrations in SNPs. To observe the size and shape of SNPs, the UV-Vis spectroscopy could be used maximum in the range of 400–500 nm.[3,18] Surface plasmon resonance is an unusual phenomenon exhibited by SNPs due to the oscillation of the conducting metal surface electrons in resonance with nonparticulate radiation.[19] The recorded data show the green synthesized SNPs study exhibited strong absorption spectrum at 305 nm.

The spectra with single maximum absorption band at 305 nm are supportive of the spherical shaped SNPs. A remarkable expansion of peak at around 200 and 800 nm point toward that the particles are polydispersed. Biosynthesis of SNPs was affects by various factors including concentration of plant extracts and silver, temperature, and exposure time.[20,21] The result of UV-Vis absorption of the present study was in accordance with the reports of Rajathi and Sridhar[22] in *T. purpurea*.

The bioreduced SNPs synthesized by *T. villosa* leaf and callus extracts SNPs were analyzed through FTIR to determine the functional groups responsible for the reduction of Ag + ions to Ag° nanoparticles. The FTIR spectra revelled that the presence of various functional groups like secondary alcohol (O = H, stretching, H-bonded) Alkane (C-H stretching) Alkene and aromatic (C = C stretching), aldehyde C = C-H stretching) acid, and ester (C-O stretching and alkyl halide (C-Br stretching), respectively.

The peaks at 3427 cm^{-1} and 882 cm^{-1} indicate the presence of OH group and aromatic ring C-H vibrations, respectively, shows the involvement of free catching.[23] The assignment at 1643.3 and 1629.8 cm^{-1} of leaf and callus extracts SNPs, respectively, corresponds to the amide bond of proteins obtained due to the carbonyl stretch in protein. The presence of amino acid and proteins in carbonyl group has the strong ability to bond to metal, indicates that protein could possibly from the metal nanoparticles.[24] The present FTIR results suggest that protein nanoparticle integration occurs by free amino group and interaction of carboxyl groups. Previous reports also indicate that the proteins stabilize nanoparticles through precipitation, thereby preventing agglomeration.[25,26]

To analyze the crystalline phase orientation and the grain size of the SNPs, the XRD powder diffraction profile of green synthesized *T. villosa* extracts SNPs were studied. The peaks observed in XRD profile revealed that the sample contains a mixed structure of SNPs. In the XRD diffract gram, many unusual peaks were seen. This may be due to the crystallization of the bioorganic phases occurs on the crystallization of the SNPs.[27,28] The XRD pattern of the present study clearly shows that the synthesized SNPs were crystalline in nature similar to the earlier studies.[23,26,29]

The SNPs obtained from leaf and callus of *T. villosa* were subjected to SEM to characterize the size, shape, and morphology. In the present study, the morphology of SNPs were mainly spherical and size was about 29–68 nm and 601–838 nm in leaf and callus SNPs, respectively. This is in agreement with the shape of SPR band in the UV-Vis-spectra.[30] These particles were also found to be similar to that of Nanoparticles synthesized from *Achyranthes bidentata*.[29]

EDX analysis revealed the complete inorganic composition of the synthesized SNPs. The elemental analysis of biologically synthesized nanoparticles was done through EDX. Synthesized in both extracts SNPs study optical absorption peaks were obtained at 3Kev, which is typical for absorption of metallic SNPs.[31] Similar results were also observed in SNPs of *Achyranthes bidentata* aqueous leaf extract.[29]

Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from substances to an oxidizing agent. The oxidation reaction can produce free radicals, which starts chain reaction that damage cells. Antioxidant terminates these chain reactions by removing free radicals intermediates and inhibits other oxidation reactions by being oxidizing themselves.

Antioxidants can scavenge reactive oxygen species that may cause damage to DNA, protein and lipids. In addition, by affecting cyclooxygenase-2 enzyme or inhibiting oncogene expression, the antioxidants may suppress cancer cells.[32] Third most common cancer in both males and females is colorectal cancer accounts for about 9% of cancer deaths each year. Due to higher percentage of colon cancer and promoting medicinal plants as potential source of new remedies, as they are the most exclusive source of life-saving drugs for the majority of the World's populations.[17]

In vivo, leaf SNPs of *T. villosa* significantly scavenged the DPPH radical. The DPPH assay was widely used as the model system to investigate the scavenging activity of several natural compounds such as extract of plants in a relatively short time.[33] DPPH radical is scavenged by antioxidants through the donation of proton, forming the reduced DPPH, illustrated a significant decrease in the concentration of DPPH radical due to the scavenging ability of the extracts. The scavenging activity of the *in vivo* leaf SNPs of *T. villosa* was increased with the increase in the concentration of sample.

The result obtained in the present study may be attributed to several reasons through scavenging of H_2O_2 or by changing/converting Fe^{3+}/Fe^{2+}.[34] The reducing capacity of compound may serve as significant indicator of its potential antioxidant activity.[35] An increase in the absorbance revealed that the reducing power of *in vivo* leaf and *in vitro* callus SNPs was increased in dose-dependent manner. The antioxidant activity has been reported to be the concomitant development of reducing power.[36,37]

The ability of extracts to reduce Fe^{3+} to Fe^{2+} may be attributed to the donation of hydrogen from the plant extract. It is evident that high number and position of hydroxyl group in phenolic compounds possess rich antioxidant activities. Phenolic compounds are highly responsible for antioxidant activity.[38] In the presence of hydroxyl and H molecules, DPPH is reduced to yellow. Here, source of hydrogen molecules is phenolic compound. Traditional medicines are active in treating various diseases, which may be due to free radicals scavenging property.

Flavonoid and tannins are the phenolic compounds responsible for the antioxidant potentials of medicinal plants.[39] Superoxide anion is oxygen-centred radical with selective activity. It can reduce certain iron complexes such as cytochrome. Various scientists reported this availability of steroids, terpenoids, flavonoids, and phenolic compounds tannins. In *T. villosa,* SNP from all these observations it is confirmed that both the *in vivo* leaf and *in vitro* callus SNPs revealed high level of antioxidant activity in *in vitro* system.

CONCLUSION

We have developed a simple green chemistry approach for the synthesis of cost-effective and Environmentally, friendly SNPs by *T. villosa* leaf and callus extracts that demonstrate the multifunctional activities of bio-synthesized SNPs. Surprisingly, we have observed the similar nature of both SNPs toward antioxidant activity, that indicates the future application as antioxidant agent. For the commercial purpose instead of using wild plant parts, we can use callus. This will reduce the pressure on wild plant collection and also conserve the plant species.

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Conflicts of interest

There are no conflicts of interest.

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