PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(4): 43-51
https://doi.org/10.14719/pst.3031

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RESEARCH ARTICLE

Green synthesis, characterization and biological activity analysis of silver nanoparticles from commercially available root powder of *Picrorhiza kurroa* Royle ex Benth.

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OPEN ACCESS

ARTICLE HISTORY
Received: 21 October 2023
Accepted: 10 May 2024

Available online
Version 1.0 : 02 September 2024
Version 2.0 : 01 October 2024

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
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Negi N, Agrawal S, Bhandari N, Negi P, Saklani P. Green synthesis, characterization and biological activity analysis of silver nanoparticles from commercially available root powder of *Picrorhiza kurroa* Royle ex Benth. Plant Science Today. 2024; 11(4): 43-51. https://doi.org/10.14719/pst.3031

Abstract

Picrorhiza kurroa Royle ex Benth., a medicinal plant native to the alpine regions of the Himalayas, is renowned for its diverse therapeutic properties. Recently, green-synthesized silver nanoparticles have gained attention for their potential in pharmaceutical and industrial applications. In this study, silver nanoparticles (Pk-AgNPs) were synthesized using commercially available root powder of *P. kurroa*. The objective was to evaluate their potential biomedical applications by assessing their antioxidant properties and cytotoxicity. The Pk-AgNPs were characterized using Scanning Electron Microscopy (SEM) and Fourier-Transform Infrared Spectroscopy (FT-IR), which revealed their morphology and functional groups. The Total Antioxidant Capacity (TAC) of methanolic root extract of *P. kurroa* (PkRE) and the Pk-AgNPs was determined using the Phosphomolybdenum method. The results indicated that the antioxidant activity of PkRE was higher than that of the Pk-AgNPs. Moreover, the activity increased with concentration, ranging from 20 µg/mL to 120 µg/mL, demonstrating their capability to eliminate harmful free radicals. The cytotoxicity of PkRE and Pk-AgNPs was evaluated using the MTT assay against the SV40 T-antigen-containing HEK293T cell line, derived from human embryonic kidney 293 cells and the L929 cell line, a mouse fibroblast cell line. The results demonstrated dose-dependent cytotoxicity. L929 cells remained 100 % viable at concentration up to 200 µg/mL for PkRE and 100 µg/mL Pk-AgNPs. In contrast, HEK293T cells didn't show 100 % viability even at a concentration of 50 µg/mL for both samples. This study highlights the potential use of PkRE and Pk-AgNPs as antioxidants. However, the cytotoxicity findings suggests that they may be harmful to humans if consumed orally, though they could be suitable for use in other organisms. This paves the way for further research into the application of AgNPs synthesized from *P. kurroa* in biomedical and pharmaceutical domains as well as in veterinary sciences.

Keywords

Picrorhiza kurroa ; SEM; FT-IR; antioxidant; TAC assay; cytotoxicity; MTT assay

Introduction

Nanotechnology refers to the manipulation or arrangement of matter at the atomic and molecular levels within the size range of 1 and 100 nm. Nanoparticles exhibits enhanced electrical, optical and magnetic properties (1) and an increased proportion of surface atoms. As a result, nanoparticles

have a significantly greater mass of surface particles compared to microparticles, with chemical reactivity estimated to be approximately 1000 times higher (2). The remarkable physical and chemical capabilities of nanoparticles can lead to novel discoveries and commercially valuable developments, benefiting society (3).

Nanoparticles can be categorised into 2 groups based on their composition: metallic and non-metallic (4). Metallic nanoparticles include inorganic metals, metal oxide cores and metal-associated particles such as aluminium, gold, iron, copper, silver, cerium, manganese, zinc, nickel, titanium oxide and quantum dots. Non-metallic nanoparticles, on the other hand, encompasses ceramic, carbon, silica and biological macromolecule-based nanoparticles (5). Metallic nanoparticles are utilized in various sectors, including medicinal sciences, electronics, optical science, drug-gene delivery, ambient cosmetics, energy conservation and as catalysts in the chemical industry, thus increasing their popularity in manufacturing (6).

Silver nanoparticles (AgNPs), with sizes below 100 nm, are of particular interest due to their potent antibacterial, antifungal, antiviral and anti-inflammatory agents (7). Additionally, AgNPs have been investigated for various applications, including as a coating and fiber graft material, a surface modification agent, a tool gel preparation and other uses (8).

A diverse range of methods has been employed to create AgNPs due to their biocidal properties. Conventional physical and chemical synthesis methods, such as lithography, pyrolysis, laser ablation, sol-gel techniques, chemical vapor deposition and electro-deposition are both expensive and hazardous. These methods require multiple reactants, including toxic reducing agents and stabilizing agents, to prevent nanoparticle agglomeration (7).

Traditional physical and chemical approaches to silver nanoparticle synthesis have several limitations, including low yield, difficulty in producing AgNPs with well-defined size (9), the use of hazardous solvents and precursor chemicals and the formation of toxic by-products. As science advances, more environmentally friendly methods for synthesizing metal nanoparticles have been discovered. In green synthesis, metal salt are converted into nanoparticles using a biological plant extracts. The bioactive substances in the extract act as *in vitro* capping and reducing agents (10).

Nanotechnology, combined with green chemistry, has shown promising potential for developing novel products that benefit human health, the environment and various industries (11). Green-synthesised silver nanoparticles offer several environmental advantages. Firstly, green synthesis methods reduce the need for toxic and hazardous chemicals commonly used in chemical synthesis, minimizing environmental impact and ensuring the safety of both humans and ecosystems (12). Additionally, these methods have minimal energy requirements and generate less waste compared to traditional chemical methods. Furthermore, green synthesis utilizes renewable resources such as plant extracts, microorganisms and other bio-regenerable materials (11). These methods are cost-effective and sustainable, making them viable alternatives for large-scale production of silver nanoparticles (13). Furthermore, green -synthesised silver nanoparticles have demonstrated biocompatibility, indicating that they are well-tolerated by living organisms and do not cause harmful side effects when utilized in various applications. Various biological resources, such as plants, yeast, fungi, algae, bacteria and viruses, can be employed for nanoparticles synthesis through biological methods. Among these resources, plant leaf and root extracts enable faster reduction processes due to their composition of compounds like alkaloids, flavonoids, saponins, steroids, tannin and phenol. These compounds have the ability to convert Ag+ ions into Ag^0 states, thereby facilitating the formation of silver nanoparticles (7).

Picrorhiza kurroa Royle ex Benth. (Family: Scrophulariaceae), commonly known as 'kutki' or 'kadu' by locals, is a medicinal plant indigenous to the alpine Himalayan regions, ranging from Kashmir to Sikkim at altitudes of 2700 m to 4500 m above sea level (14). This plant is renowned for its efficacy in treating recurrent fevers, nausea, anorexia and jaundice. In addition to possessing anti-inflammatory, antioxidant and immunomodulatory properties, its hepatoprotective action is particularly noteworthy, contributing significantly to its medicinal value (15).

The current study investigates the antioxidant and cytotoxic activities of the methanolic extract of commercially available *P. kurroa* root powder as well as silver nanoparticles synthesized from this extract using green methods. The choice of commercial root powder of *P. kurroa* for green synthesis of silver nanoparticles is based on its numerous pharmacological properties. This root powder contains a variety of bioactive compounds such as iridoid glycosides, unsaturated sterols/triterpenes, cucurbitacins and polyphenols (16), which efficiently facilitates the conversion of silver ions into nanoparticles without the use of toxic chemicals or harmful reducing agents (17). This study aims to shed light on the applications of silver nanoparticles synthesized from the root powder, thereby facilitating the effective utilization of this plant.

Materials and Methods

Sample Collection and Preparation of Methanolic Extract of Commercial P. kurroa Root Powder (PkRE)

The root powder of *Picrorhiza kurroa* was purchased from the local market of Srinagar, in the Pauri-Garhwal region of Uttarakhand. A total of 25 g of the purchased root powder of *P. kurroa* was homogenized in 100 mL methanol and maintained at 150 rpm and 37 °C in an orbital shaker for 24 h. The extract obtained from this mixture was filtered using Whatman filter paper and subsequently stored at 4 °C for further use.

Green Synthesis of Silver Nanoparticles by Methanolic Extract of Commercial P. kurroa Root Powder (Pk-AgNPs)

The plant extract and 5 mM silver nitrate solution were mixed in a ratio of 1:4, thoroughly combined, and then left to stand in a dark location for 24 h or until the mixture turned dark brown. Subsequently, the solution was centrifuged for 10 min at 10000 rpm. The supernatant was discarded and the remaining pellet was suspended in ethanol. This mixture underwent centrifugation again for 10 min at 10000 rpm. The ethanol washing step was repeated twice. The final pellet obtained was suspended in a few drops of ethanol and placed on a watch glass to air drying. The synthesized powdered silver nanoparticles were then carefully removed from the watch glass using a clean brush (18).

Characterization of Green Synthesized Silver Nanoparticles

Scanning electron microscopy (SEM) was employed to examine the morphology of green silver nanoparticles synthesised from the commercially available root powder extract of *P. kurroa*. The biological compounds involved in the synthesis of Pk-AgNPs were analyzed by Fourier-Transform Infrared Spectroscopy (FT-IR). These analysis were conducted at the CSIR-National Botanical Research Institute (NBRI), Lucknow, Uttar Pradesh. For FT-IR analysis, a Shimadzu Spectrometer was utilized to analyse the sample within the wavelength range of 4000-400 cm^{-1}, with a resolution of 4 cm^{-1}. To prepare the sample, KBr was mixed with the sample to create a thin sample pellet. The pellet was then formed by pressing it with a Hydraulic Pellet Press and subsequently subjected to FT-IR analysis.

Analysis of Total Antioxidant Capacity (TAC) via Phosphomolybdenum Assay

Different concentrations of the methanolic plant extract and nanoparticles were prepared (20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, 100 µg/mL and 120 µg/mL) and placed in test tubes. The volume was adjusted to 1000 µL (equivalent to 1 mL) using methanol. To all the test tubes, 3 mL of the reagent solution was added. A reaction mixture without a sample or standard served as the blank. Subsequently, the test tubes were placed in a water bath and incubated at 95 °C for 90 min. After cooling to room temperature, the absorbance at 695 nm was measured and compared to a control. The outcomes were expressed as Ascorbic Acid Equivalents per gram extract (AAEC) (19).

Cytotoxicity Assay

The assay was conducted in the laboratory of CSIR-National Botanical Research Institute (NBRI), Lucknow, Uttar Pradesh (20).

The cytotoxicity assay, originally introduced by Mosman in 1983 and utilizing MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), was employed with minor adjustments in concentration. Prior to the experiment, a phosphate-buffered saline (PBS, pH 7.2) solution containing a fresh stock solution of MTT at a concentration of 5 mg/mL was prepared. SV40 T-antigen-containing HEK293T cell line, derived from human embryonic kidney 293 cells, and the L929 cell line, consisting of mouse fibroblast cells were seeded at a density of (1 x 10^4) cells each and cultured overnight in individual wells of a 96-well flat-bottom cell culture plate. Additionally, as mentioned previously, the cells were subsequently subjected to 8 distinct concentrations of the PkRE and Pk-AgNPs (50, 100, 200, 400, 750, 1000, 1500 and 2000 µg/mL). Control cells were simultaneously cultured in regular media without any extract and subjected to the same conditions for comparison.

After the treatment duration, 10 µL of MTT stock solution, equivalent to one-tenth of the total medium volume in each well, was added, followed by a 3 h incubation period under standard culture conditions. Following the incubation period, the medium was aspirated and 200 µL of Dimethyl Sulfoxide i.e., (DMSO) from Sigma Aldrich was added to each experimental well. This solution was then incubated at room temperature for 20 min. Finally, the absorbance intensity was measured at a wavelength of 550 nm using an ELISA plate reader calibrated to 492 nm to detect MTT activity (Biotek, PowerwaveXS2). The formula used to calculate the growth inhibition % is as follows:

$$\% \text{ cell inhibition} = 100 - [(At - Ab)/(Ac - Ab)] \times 100$$

where At denotes the absorbance of the sample, Ab denotes the absorbance of the control and Ac denotes the absorbance of the blank.

Statistical Analysis

All experiments were conducted in triplicates and expressed as Mean ± Standard error. The correlation analysis between the sample concentration and AAEC values in the Phosphomolybdem assay was performed using Microsoft office 365.

Results and Discussion

In the present study, silver nanoparticles were prepared utilizing commercially available root powder extract of *P. kurroa* and their bioactivity was assessed. The synthesis of silver nanoparticles involved the use of silver nitrate ($AgNO_3$) as the reducing agent and the commercially available root powder extract of *P. kurroa* as the capping agent. Subsequently, the green-synthesized silver nanoparticles underwent characterization and were evaluated for their biological activities using a diverse range of tools and techniques.

Methanolic Extract of Commercial Root Powder of Picrorhiza kurroa

The objectives of extraction are to obtain extracts with the highest biological activity and to maximize the yield of target compounds (21). Various solvents, including methanol, water, ethanol and acetone can be utilized to extract bioactive chemicals such as phenolics, alkaloids, flavonoids and terpenoids from plant material, depending on the plant sample and the specific compounds to be isolated (22). Methanolic extraction is suggested to yield the highest extraction efficiency (23). This can be attributed to the high solubility of bioactive compounds in methanol compared to other solvents, owing to their similar polarity

(24).

In this background, a methanolic extract of commercial *P. kurroa* root powder at a concentration of 10 mg/mL was prepared.

Green Synthesis of Silver Nanoparticles

The change in color of the solution from light yellow to colloidal brown served as visual confirmation of the synthesis of silver nanoparticles (Fig. 1(A) and (B)). The bioactive components present in the commercial root powder extract of *P. kurroa* played a significant role in reducing metallic ions from their oxidation state, Ag^{+} to Ag^{o} (25). Additionally, the bioactive components of the methanolic root extract also aided in preventing the agglomeration of the freshly produced silver nanoparticles by stabilizing them. The observed color change resulted from the excitation of surface plasmon vibrations in the silver nanoparticles, induced by the absorption of energy from light waves by the free electrons of the nanoparticles. This phenomenon, known as surface plasmon resonance (SPR), is unique to AgNPs (26). From a 1000 mL solution of methanolic extract of commercial root powder of *P. kurroa* mixed with $AgNO_3$ in the ratio of 1:4, 13 mg of silver nanoparticles were obtained. The color of the silver nanoparticles was observed to be black, as depicted in the given Fig. 1(C).

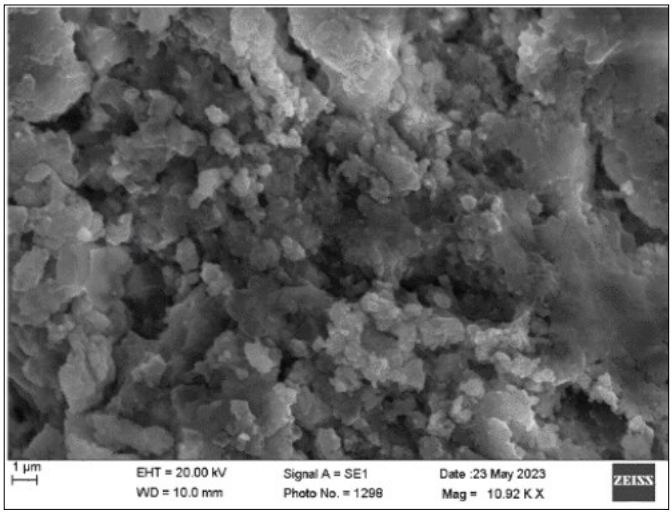

Fig. 2. SEM image of Pk-AgNPs.

Fig. 1. (**A**) PkRE and $AgNO_3$ immediately after mixing, (**B**) Solution of PkRE and AgNPs after photoreduction, (**C**) Green synthesized Pk-AgNPs.

Characterization of Silver Nanoparticles

Scanning Electron Microscopy (SEM)

The morphology of green-synthesized AgNPs using the methanolic extract of commercial root powder of *P. kurroa* was examined through scanning electron microscopy analysis, utilizing a Zeiss SEM analyzer operating at 20 kV with a working distance of 7.5 mm (Fig. 2). The analysis revealed that the silver nanoparticles are uniformly distributed and spherical in shape. The presence of larger silver nanoparticles in the image is likely due to the aggregation of smaller nanoparticles. Similar observation have been reported by other researchers, who also noted that nanoparticles tend to have a spherical shape and are often found in clusters (27, 28).

Fourier-Transform Infrared Spectroscopy (FT-IR)

FT-IR is a chemical analysis technique that investigates the relationship between infrared light intensity and wavelength (expressed as wave number). This method is used to determine the characteristics of molecules associated with nanoparticles in plant extracts (29).

FTIR analysis results reveals the existence of phenols, alkanes, secondary alcohols, aromatic amines, carboxylic acids, nitro compounds, fluoro compounds and halo compounds (Table 1 and 2). All these compounds are secondary plant metabolites (30, 31).

In the current study, the shift in peak values (Fig. 3) (A) and (B) in PkRE and Pk-AgNPs from 3309.85 to 3369.64, 2924.09 to 2850.79, 1691.57 to 1720.5 and 1629.85 to 1597.06, indicates that the functional groups, such as alcohols, carboxylic acid, aldehyde and amine respectively are responsible for the synthesis of AgNPs. This confirms the role of secondary metabolites in the biogenic synthesis of AgNPs. Similar results were observed in studies conducted on various extracts of *Sansevieria cylindrica* and methanolic leaf extract of *Momordica balsamina* (32, 33). Another study demonstrated a shift in the absorption peak associated with OH groups from a higher value in plant extract to a lower value in green synthesized AgNPs, suggesting that the OH groups participate in the reduction of Ag^{+} to Ag^{0} (34).

Table 1. Peak values representing compound classes in PkRE.

Serial No.	Absorption range (cm^{-1})	Peak values (cm^{-1})	Possible Functional group	Compound Class
1.	3550 - 3200	3309.85	O-H stretching	Alcohol
2.	3300 - 2500	2924.09	O-H stretching	Carboxylic Acid
3.	1710 – 1685	1691.57	C=O stretching	Conjugated Aldehyde
4.	1650 – 1580	1629.85	N-H bending	Amine
5.	1550 – 1500	1512.19	N-O stretching	Nitro Compound
6.	1342 – 1266	1273.02	C-N stretching	Aromatic Amine
7.	1400 – 1000	1014.56	C-F stretching	Fluoro Compound
8.	850 – 550	763.81	C-Cl stretching	Halo Compound

Table 2. Peak values representing compound classes in Pk-AgNPs.

S. No.	Absorption range (cm^{-1})	Peak values (cm^{-1})	Possible Functional group	Compound Class
1.	3550 – 3200	3369.64	O-H stretching	Alcohol
2.	3000 – 2800	2918.30	N-H stretching	Amine Salt
3.	3300 – 2500	2850.79	O-H stretching	Carboxylic Acid
4.	1740 – 1720	1720.50	C=O stretching	Aldehyde
5.	1650 – 1580	1597.06	N-H bending	Amine
6.	1385 – 1380	1382.96	C-H bending	Alkane
7.	1275 – 1200	1228.66	C-O stretching	Alkyl Aryl Ether
8.	1250 - 1020	1172.72	C-N stretching	Amine
9.	1250 - 1020	1068.56	C-N stretching	Amine

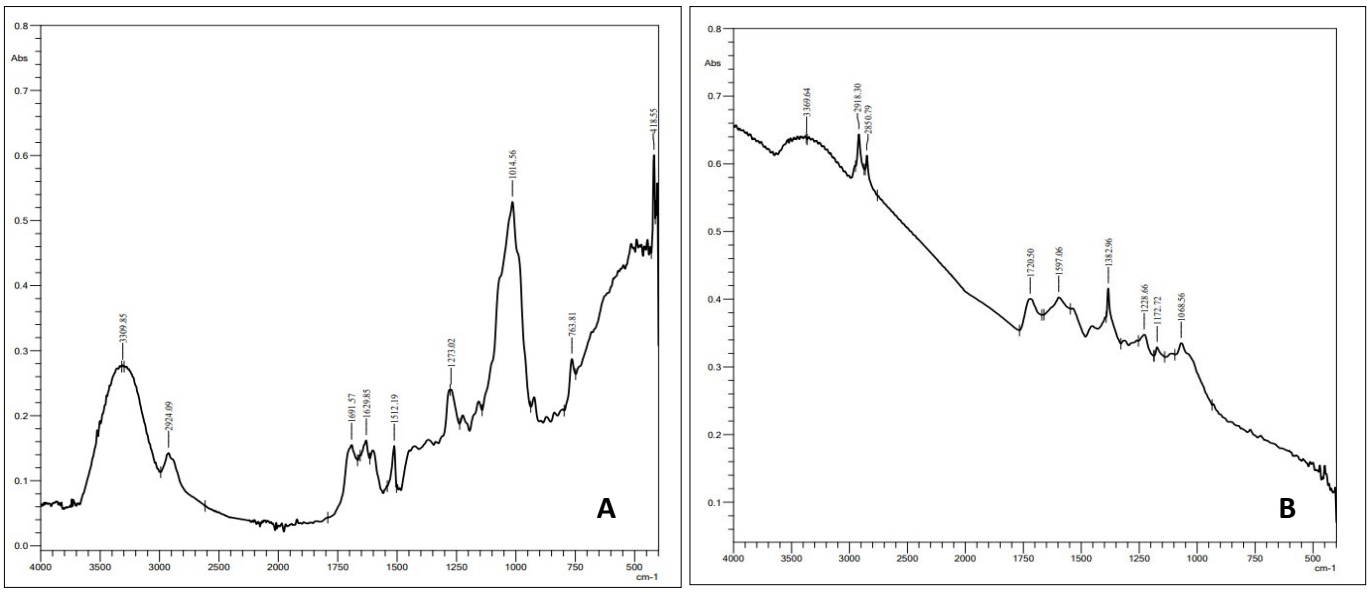

Fig. 3. (A) FT-IR spectrum of PkRE, (B) FT-IR spectrum of Pk-AgNPs.

Total Antioxidant Capacity (Phosphomolybdenum Assay)

The phosphomolybdenum assay is a critical antioxidant test conducted in a controlled environment to measure the total antioxidant capacity (TAC) of plant extracts. The fundamental principle of this assay is that any extract or substance containing antioxidants can reduce Mo (VI) to Mo (V), leading to the formation of green phosphate Mo (V). Ascorbic acid, a well-known antioxidant, was used as the reference standard to estimate the TAC of the commercial root powder extract and Pk-AgNPs. The TAC was quantified as the Ascorbic Acid Equivalent Concentration (AAEC) (35).

The antioxidant capacity of the methanolic extract of commercial *P. kurroa* root powder (PkRE) and Pk-AgNPs was evaluated and found to increase with higher concentrations of PkRE and Pk-AgNPs, ranging from 20 µg/mL to 120 µg/mL. This result indicates that the antioxidant capacity of both PkRE and Pk-AgNPs is concentration-dependent. Fig. 4 shows the standard curve of ascorbic acid.

At 20 µg/mL, PkRE exhibited the lowest total antioxidant capacity (TAC) of 12.27 ± 0.09 mg AA g^{-1} extract, while at 120 µg/mL, it had the highest TAC of 24.02 ± 0.75 mg AA g^{-1} extract. Conversely, Pk-AgNPs showed a minimum TAC of 10.62 ± 0.6 mg AA g^{-1} extract at 20 µg/mL and a maximum TAC of 10.98 ± 1.12 mg AA g^{-1} extract at 120 µg/mL. (Fig. 5, Table 3). Both PkRE and Pk-AgNPs demonstrated a positive correlation with the concentration of the sample. The statistical correlation analysis between the sample concentration and AAEC of PkRE is 0.98, while for Pk-AgNPs, it is 0.94, indicating a strong correlation in both cases.

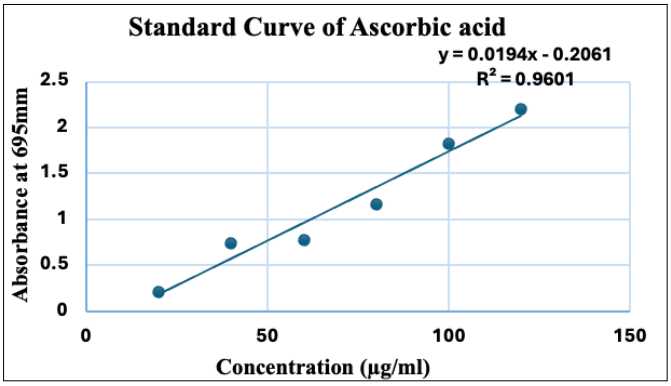

Fig. 4. Regression line produced by standard, Ascorbic Acid.

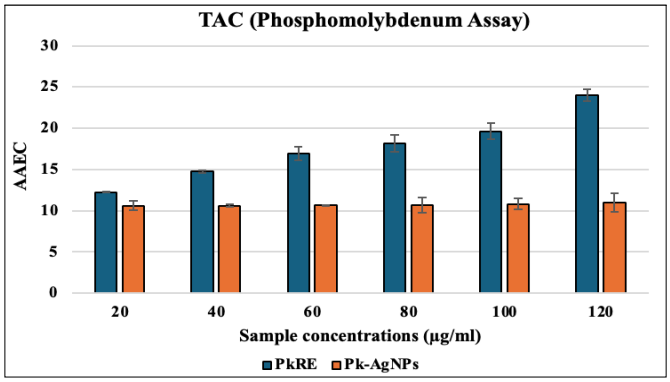

Fig. 5. Comparative Analysis of AAEC values of PkRE and Pk-AgNPs at different concentrations.

Table 3. Total Antioxidant Capacity (AAEC) of PkRE and Pk-AgNPs (mg AA g^{-1}).

Sample concentration	PkRE	Pk-AgNPs
20	12.27 ± 0.09	10.62 ± 0.6
40	14.75 ± 0.13	10.62 ± 0.15
60	16.96 ± 0.8	10.67 ± 0.01
80	18.20 ± 1.04	10.73 ± 0.92
100	19.64 ± 0.95	10.83 ± 0.7
120	24.02 ± 0.75	10.98 ± 1.12

In the present study, the antioxidant potential of the root powder extract (PkRE) was observed to be higher compared to the silver nanoparticles (Pk-AgNPs). This could be attributed to the presence of a wide range of secondary metabolites in the root extract, whereas only a few major secondary metabolites are present on the surface of the silver nanoparticles, contributing to their antioxidant properties (36). Similar results have been reported in other studies, where plant extracts demonstrated higher antioxidant potential than the AgNPs synthesized from them (37).

Cytotoxic Activity Evaluation

In the present study, the effect of *P. kurroa* root extract (PkRE) and their AgNPs (Pk-AgNPs) on the cell viability and cytotoxicity of the SV40 T-antigen-containing HEK293T cell line, derived from human embryonic kidney 293 cells and the L929 cell line, a mouse fibroblast cell line were investigated using the MTT assay. The MTT assay (3-(4,5-dimethyl -2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide) is widely employed as a colorimetric method to determine cell densities (38).

L929 Cell Line

In this study, it was found that PKRE and Pk-AgNPs exhibited concentration-dependent cytotoxicity in the L929 cell line. PkRE maintained 100 % cell viability up to a concentration of 200 µg/mL, whereas Pk-AgNPs maintained 100 % cell viability up to 100 µg/mL. At a concentration of 1500 µg/mL for Pk-AgNPs, the full extent of cell toxicity was observed (Fig. 6, 7; Table 4). In prior studies, nanoparticles synthesized from *Anthemis atropatana* extract also

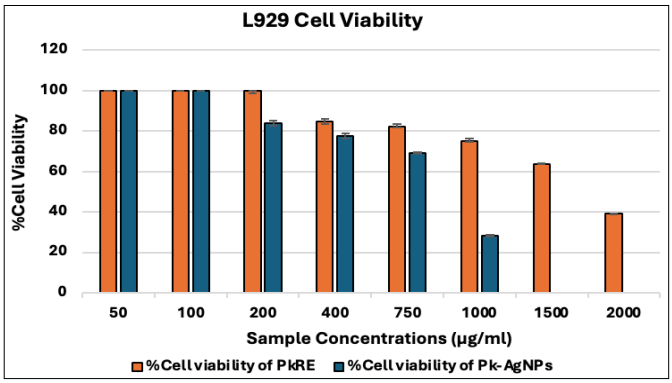

Fig. 6. Comparing L929 cell line viability towards different concentrations of PkRE and Pk-AgNPs.

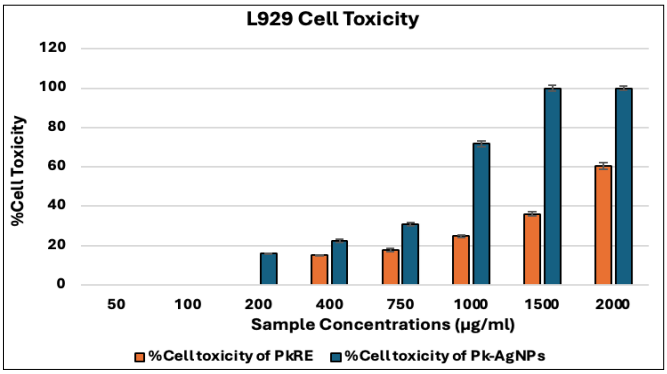

Fig. 7. Comparing toxicity of PkRE and Pk-AgNPs towards L929 cell line.

Table 4. Cell viability and toxicity shown by L929 cell lines towards different concentrations of PkRE and Pk-AgNPs.

Concentration (µg/mL)	% Cell toxicity of PkRE	% Cell viability of PkRE	% Cell toxicity of Pk-AgNPs	% Cell viability of Pk-AgNPs
50	0.00 ± 0.00	100 ± 0.00	0.00 ± 0.00	100 ± 0.00
100	0.00 ± 0.00	100 ± 0.00	0.00 ± 0.00	100 ± 0.00
200	0.00 ± 0.00	100 ± 0.00	15.99 ± 0.22	84.01 ± 1.3
400	15.14 ± 0.15	84.86 ± 0.99	22.47 ± 0.9	77.53 ± 1.16
750	17.85 ± 0.76	82.15 ± 1.21	30.87 ± 0.86	69.13 ± 0.5
1000	24.85 ± 0.53	75.15 ± 1.1	71.75 ± 1.5	28.25 ± 0.29
1500	36.13 ± 1.14	63.87 ± 0.27	100 ± 1.41	0.00 ± 0.00
2000	60.60 ± 1.6	39.14 ± 0.11	100 ± 1.01	0.00 ± 0.00

demonstrated dose-dependent cytotoxicity in L929 cells, with around 5 % cell viability at 100 µg/mL (39). Additionally, cytotoxicity analysis of synthesized *Nyctanthes arbortristis* AgNPs against these cell lines showed 83 % cell viability at a concentration of 250 µg/mL (40). These results indicate that the efficacy of AgNPs synthesized from *P. kurroa* is superior to that of many other previously studied green-synthesized nanoparticles.

HEK293T Cell Line

In the case of the HEK293T cell line, cell viability was observed to be 87.29 % at a 50 µg/mL concentration of PkRE and 93.26 % at a 50 µg/mL concentration of Pk-AgNPs (Fig. 8, 9; Table 5). Previous studies have shown that silver nanoparticles derived from *Brachychiton populneus* exhibited 51.92 % viability of HEK293 cells at a concentration of 100 µg/mL (41). Similarly, a study on AgNPs synthesized from *Mangifera indica* seeds demonstrated a negative correlation between cell viability and the concentration of AgNPs, showing approximately 80 % cell viability at 100 µg/mL (42).

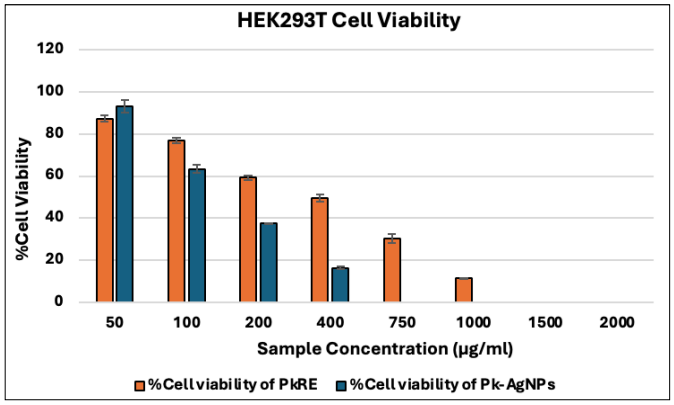

Fig. 8. Comparing HEK293T cell line viability towards different concentrations of PkRE and Pk-AgNPs.

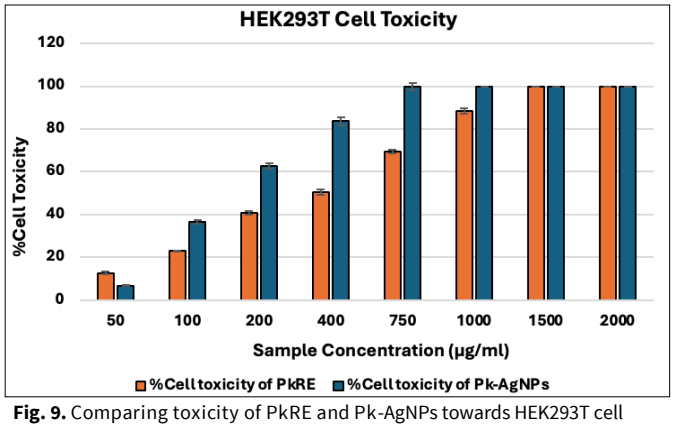

Fig. 9. Comparing toxicity of PkRE and Pk-AgNPs towards HEK293T cell line.

The results indicate that Pk-AgNPs exhibit differential cytotoxic effects on human and mouse cell lines. L929 cells demonstrate greater tolerance to Pk-AgNPs compared to HEK293T cells, indicating that even at high concentrations, AgNPs are not lethal to mouse fibroblast cells. This finding suggests the potential use of these AgNPs as a drug delivery system for animal models, owing to their small size and high surface area. Additionally, their significant antioxidant and cell viability properties highlight potential applications in imaging, as they may be able to detect and visualize oxidative stress in the body as well as in veterinary health and other therapeutic purposes.

Table 5. Cell viability and toxicity shown by HEK293T cell lines towards different concentrations of PkRE and Pk-AgNPs.

Concentration (µg/mL)	% Cell toxicity of PkRE	% Cell viability of PkRE	% Cell toxicity of Pk-AgNPs	% Cell viability of Pk-AgNPs
50	12.71 ± 0.5	87.29 ± 1.5	6.74 ± 0.24	93.26 ± 3.1
100	22.92 ± 0.16	77.08 ± 1.27	36.64 ± 0.85	63.36 ±1.95
200	40.72 ± 0.9	59.28 ± 0.97	62.60 ± 1.3	37.40 ± 0.37
400	50.41 ± 1.4	49.59 ± 1.6	83.62 ± 2.0	16.38 ± 0.64
750	69.54 ± 0.78	30.46 ± 2.1	100 ± 1.7	0.00 ± 0.00
1000	88.62 ± 1.26	11.38 ± 0.18	100 ± 0.00	0.00 ± 0.00
1500	100 ± 0.00	0.00 ± 0.00	100 ± 0.00	0.00 ± 0.00
2000	100 ± 0.00	0.00 ± 0.00	100 ± 0.00	0.00 ± 0.00

Conclusion

The present study on the commercially obtained root powder of *Picrorhiza kurroa* Royle ex Benth. and the silver nanoparticles synthesized from it through bio reduction revealed successful nanoparticles formation under the given conditions. Both PkRE and Pk-AgNPs exhibited antioxidant properties, with PkRE demonstrating higher activity. The cytotoxicity analysis indicates that the cytotoxic effects of PkRE and Pk-AgNPs varied between different cell lines and were dose-dependent. Consequently, these substances are not safe for direct consumption, as they may be harmful to the body. Further research is necessary to develop safer methods for synthesizing AgNPs and to thoroughly assess the potential risks and benefits of using these nanoparticles. Such studies are essential to ensure that AgNPs can be safely and effectively utilized as nanomedicines or in other industries.

Acknowledgements

The authors are thankful to the Department of Biotechnology, HNBGU, Srinagar for providing the laboratory facilities.

Authors' contributions

NN and SA contributed equally to the study. NN, SA and PS conceptualised the study. NN and SA carried out the exper - iments. NB assisted in the literature survey and experimentation. PN assisted in statistical analysis. PS, NN and SA wrote and finalized the manuscript. SA and NN contributed equally to the study. SA, NN and PS conceptualised the study. SA and NN carried out the exper- iments. NB assisted in the literature survey and experi- mentation. PN assisted in statistical analysis. PS, SA and NN wrote and finalized the manuscript.

Compliance with ethical standards

Conflict of interest: There is no conflict of interest with anyone either within the institute or outside the institute.

Ethical issues: None.

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