

Research Article

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Green synthesis of silver nanoparticles via *Taxus wallichiana* Zucc. plant-derived Taxol: Novel utilization as anticancer, antioxidation, anti-inflammation, and antiurolithic potential

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Abstract: Throughout history, the utilization of plant products as medicinal remedies has been widespread, with numerous modern drugs finding their origins in the plant kingdom. Taxol, derived from *Taxus* species, stands out as an exceptional and highly potent anticancer medication. In this study, we present a rapid one-pot synthesis method for silver nanoparticles (AgNPs) using the leaves of *Taxus wallichiana* Zucca in the presence of sunlight. The synthesized

AgNPs were comprehensively characterized using X-ray diffraction, transmission electron microscopy, and HPLC Q-TOF. The AgNPs were further investigated for their antioxidative, anticancer, anti-inflammatory, and antiurolithic properties. The anticancer activity was assessed through a sulforhodamine B assay conducted on the MDA-MB-231 human breast carcinoma cell line and SiHa human cervical cancer cell line. The findings of this study reveal the impressive antioxidative, anticancer, anti-inflammatory, and antiurolithic characteristics exhibited by AgNPs synthesized from leaf extracts. This research highlights an environmentally friendly and cost-effective approach to producing AgNPs by utilizing plant extracts as reducing agents, underscoring the immense potential of natural resources in advancing nanotechnology and its applications.

Keywords: silver nanoparticle, *Taxus wallichiana*, applications, anti-inflammation, anticancer

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1 Introduction

The utilization of Himalayan yews for medicinal purposes dates back several millennia. Indigenous communities residing near the forests possess valuable traditional knowledge regarding the use of plants, and the medicinal practices of the region, including Ayurveda, Amchi, Han Chinese, and Unani, have been influenced by the healing properties of Himalayan medicinal plants. The yew, in particular, has long been employed in traditional medicine to address fevers and alleviate painful inflammations. Decoctions, herbal teas, and juices derived from the yew have been utilized to treat ailments such as colds, coughs, respiratory infections, indigestion, and epilepsy [1]. For centuries, the utilization of plants in the treatment of illnesses caused by microorganisms, including infectious diseases, has been prevalent. Medicinal

plants are rich in a diverse array of phenolic compounds, such as flavonoids, stilbenes, phenols, coumarins, lignin, and tannins, which exhibit a wide range of biological effects, including antimicrobial and antioxidant properties [2].

Cancer remains a prominent global cause of mortality, and contemporary medical practices involving chemotherapy and radiation therapy carry recognized risks. In the realm of modern medicine, certain plant-derived substances, including vincristine, vinblastine, and Taxol, have exhibited noteworthy anticancer attributes. *Taxus baccata* L., in particular, has demonstrated long-standing efficacy in the treatment of cancer [3]. Moreover, noble metal nanoparticles (NPs), like silver, exhibit remarkable antibacterial characteristics, possess expansive reactive surfaces, and have been extensively utilized in various fields such as catalysis, electronics, optics, environmental studies, and biomedicine [4]. Compared to other yews, the Himalayan yew boasts a remarkable medicinal background, distinct for its absence of toxic taxine, which is found in European *T. baccata*. This plant possesses therapeutic qualities that have proven effective in treating indigestion and epilepsy [5,6] and possesses analgesic, antipyretic, and anticonvulsant properties [7]. The plant also contains bioactive molecules, including nematocidal compounds [8], and is traditionally used to treat high fever, pain, and inflammation [9]. Tinctures derived from Himalayan yew plants are employed as herbal remedies for various ailments such as headaches, dizziness, weak or irregular pulses, cold extremities, diarrhea, and intense biliousness. Additionally, the leaves of the plant possess emmenagogue and antispasmodic properties [10]. In recent years, there has been a growing interest in the synthesis of silver nanoparticles (AgNPs) through easy, sustainable, cost-effective, and eco-friendly approaches [11]. AgNPs have demonstrated promising potential in various areas, including anticancer, anti-inflammatory, and wound healing properties, as well as antimicrobial characteristics [12]. While methods like electro-irradiation [13], laser-mediated [14], and photochemical treatment are commonly used for AgNPs synthesis, they are often expensive and environmentally toxic. In contrast biological-based methods utilizing aqueous plant or microbial extracts are preferred. The biological process of synthesizing AgNPs using plant extracts offers several advantages, such as lower toxicity, compatibility, longer synthesis time, and a narrow particle size distribution [11]. Plant extracts contain numerous secondary metabolites that serve as reducing agents, playing a crucial role in the capping and stabilization of AgNPs. Notably, medicinal plants like *Peaonia emodi* [15], *Althaea rosea*, *Swertia chirata*, *Bergenia stracheyi*, and *Solanum xanthocarpum* possess secondary metabolites of pharmaceutical

importance [16]. AgNPs have been successfully synthesized from different parts of these plant species, including roots, leaves, flowers, and fruits, which hold significant pharmaceutical value [17]. The anticancer activity of AgNPs derived from plants has been demonstrated against a variety of cancer cell lines, including breast cancer cell lines MCF-7 and MDA-MB-231, and brain cancer cells U251 [18]. The compound has also been reported to be effective against human hepatoma SMMC-7721 cells [19].

In this study, we synthesize AgNPs through a one-pot method and characterize them using X-ray diffraction (XRD), transmission electron microscopy (TEM), and HPLC techniques. We also evaluated the anticancer, antioxidant, antiurothilic, and anti-inflammatory properties of these AgNPs derived from *Taxus wallichiana*. Notably, this is the inaugural investigation showcasing the application of *T. wallichiana* aqueous leaf-based AgNPs in combating MDA-MB-231 human breast carcinoma and SiHa human cervical cancer cell lines for their anticancer activity.

2 Materials and methods

2.1 Materials

Silver nitrate, ascorbic acid, and 2,2-diphenylpicrylhydrazyl (DPPH) were sourced from Sigma Aldrich, Muller Hilton media (Hi-Media) and were employed without any supplementary treatment. All experiments were conducted using double distilled water. Whatman filter paper number 1 was used for the filtration of plant extract. Sigma-Aldrich was the source of paclitaxel ($\geq 97\%$), while analytical grade ethanol and acetonitrile, as well as dimethyl sulfoxide, and ammonium acetate were purchased from Fisher Scientific.

2.2 Methods

2.2.1 Preparation of leaf extract for HPLC analysis

About 0.5 g of *T. wallichiana* leaf powder was accurately weighed and placed in 15 mL polypropylene (PP) tubes. About 4 mL of ethanol was added to each tube and vortexed briefly, then allowed to stand for 15 min to ensure proper extraction, and vortexed once more. Subsequently, sonication was performed using a K615HTDP water bath-type sonicator at a frequency of 50 Hz for 5 min, with a pulse rate of 2:1. After sonication, the samples were subjected to centrifugation using a Bio-era Microcentrifuge at 3,000 rpm for 20 min. The resulting supernatant was

carefully transferred to a clean 15 mL PP tube. This process was repeated twice, with each supernatant being collected and combined into a clean 50 mL PP tube. The extract was then diluted four-fold using ethanol, with 250 μL of the sample mixed with 750 μL of ethanol. Finally, the diluted extract was stored in HPLC vials for analysis.

2.2.2 Preparation of leaf extract for the synthesis of AgNPs

The preparation of the crude extract from the leaf material was conducted following the previously described method [11], with slight modifications. The leaf extract was filtered using Whatman filter paper number 1 to obtain a clear solution, which served as the stock solution.

2.2.3 Synthesis of AgNPs

In the first step, a 10 mL of stock solution of the leaf extract was gradually mixed with 1 mM of silver nitrate solution (90 mL). The resulting mixture was thoroughly stirred and exposed to sunlight for a duration of 5 min, leading to a transformation from a colorless solution to brown. Subsequently, the solution was subjected to centrifugation at 10,000 rpm for 15 min, following the methodology outlined in ref. [12]. To eliminate any traces of biological matter, the resulting pellets were washed with ethanol and subsequently dispersed in Milli-Q water, adhering to the procedure described in ref. [13].

2.2.4 Characterization of AgNPs

The presence of AgNPs was confirmed using a UV-visible (UV-Vis) spectrophotometer Systronic 2203 [14]. The crystal structure of AgNPs was investigated using XRD, employing a BRUKER D8 Advance diffractometer operating at 40 kV, 40 mA, and Cu $\text{K}\alpha$ radiation at 2θ angle. The morphology of AgNPs was examined using TEM with a Philips CM 200, in addition to TEM analysis.

2.2.5 Quantitative screening

2.2.5.1 LC-Q-TOF-MS

In order to prepare the crude alcoholic extract, powdered leaf samples were sonicated in 95% ethanol using an Ultrasonicator made by Sonics. After the crude alcoholic samples were extracted, they were dissolved in 0.9 mL of alcohol and 0.1 mL of 0.1% formic acid. The leaf extract was analyzed using liquid chromatography-tandem mass spectrometry

(LC-Q-TOF-MS 6540, ultra-high definition [UHD]). The mass spectrometer was equipped with an ESI Jet Stream source.

2.2.5.2 LC parameter

The mobile phase consisted of 0.1% formic acid in water and 1.1% formic acid in acetonitrile, flowing at a rate of 0.6 $\text{mL}\cdot\text{min}^{-1}$. Chromatographic separation was performed using a Reprosil C18 column from Dr Maisch, Germany. The mobile phase composition started at 10% B and gradually increased to 100% B over 14 min, before returning to the initial state. The setup included an auto-sampler, well plate, vacuum degasser, and thermostat column compartment. Compound identification utilized the LC-Q-TOF-MS 6540 (UHD) with a jet stream ESI source.

2.2.5.3 Q-TOF parameter

The experiment utilized an LC system with a dual electrospray jet stream Agilent Q-TOF, operating in positive ion mode. The following parameters were used: capillary voltage of 4,000 V, nebulizer pressure of 45 psi (N_2), gas temperature at 32.5°C, drying gas flow rate of 8 $\text{L}\cdot\text{min}^{-1}$, nozzle voltage of 1,000 V, the fragmented voltage of 150 V, skimmer voltage of 65 V, and a mass range of 100–1,700 m/z . Additionally, a sheath gas flow rate of 11 $\text{L}\cdot\text{min}^{-1}$ and a sheath gas temperature of 350°C were employed. Data acquisition was performed using the Agilent Mass Hunter software.

2.2.5.4 Chromatographic analysis

An Agilent 1260 series HPLC system was used for chromatographic analysis. It consisted of a quaternary pump, vacuum degasser, column oven, photodiode array detector, and EZChrome software. The separation employed an Agilent 1260 Infinity C-18 column (5 μm X-bridge, 250 cm length, 4.6 mm diameter). Two mobile phases, A (water) and B (acetonitrile), were delivered to the column in a gradient fashion at a flow rate of 0.8 $\text{mL}\cdot\text{min}^{-1}$. The column temperature was maintained at 30°C. Analysis was performed at 30°C with a detection wavelength of 231 nm. Each sample was injected at a volume of 1 μL .

2.2.5.5 Quantitation and statistical analyses

The EZChrome software was used for HPLC analysis, including quantitation and statistical analyses. A calibration curve with three replicates was generated by plotting the peak area of paclitaxel against its corresponding amount. Paclitaxel concentrations in the samples were determined by fitting their peak areas into the calibration curve equation. UV detection at 231 nm was employed, and external calibration curves using paclitaxel standards were utilized for the determination of paclitaxel concentrations.

2.2.6 Antioxidant studies

The scavenging activity of antioxidants was assessed using DPPH radicals. Plant extracts at various concentrations were mixed with DPPH and either AgNPs or a test sample. After incubation, the absorbance at 517 nm was measured to calculate inhibition percentages. Thin-layer chromatography (TLC) dot blots with DPPH were used to investigate antioxidant activity. AgNPs were applied to a silica gel-coated plate, followed by the introduction of DPPH. White spots indicated radical scavenging. Hydrogen peroxide scavenging activity was evaluated by measuring absorbance at 230 nm after incubation with test fractions. The scavenging abilities were calculated using a formula. The ferric reducing power assay examined the reduction capacity of synthesized NPs. Extracts, phosphate buffer, and potassium ferricyanide were incubated, followed by centrifugation. The reaction was stopped, and the supernatant was mixed with distilled water and FeCl_3 . Ascorbic acid served as the reference compound.

2.2.7 Anti-inflammatory studies

In this study, we examined the anti-inflammatory properties of AgNPs produced from *T. wallichiana*. To evaluate their effects, we investigated their ability to inhibit protein denaturation and used the human red blood cell (HRBC) membrane stabilization method. Various concentrations of AgNPs were introduced into water containing 0.1% bovine serum albumin. The pH of the solution was adjusted to 7 using an acidic solution. Subsequently, the sample mixtures were incubated at 37°C for 20 min, followed by an additional 20 min at 51°C. We measured the turbidity of the samples using spectrophotometry at a wavelength of 660 nm.

2.2.8 Studies on the potential of *T. wallichiana* NPs to prevent kidney stone formation

2.2.8.1 Calcium oxalate crystal nucleation inhibition assay (turbidity method)

We investigated the antiurolithiatic properties of *T. wallichiana* NPs by assessing their ability to inhibit calcium oxalate crystal formation and aggregation. The turbidity method was used to measure crystal formation inhibition. Solutions of calcium chloride and sodium oxalate were prepared in a pH 6.5 buffer. *T. wallichiana* NPs and cystone standard solutions ($100\text{--}500\text{ }\mu\text{g}\cdot\text{mL}^{-1}$) were added to the calcium chloride solution, followed by the introduction of a sodium oxalate solution. The resulting solution was stirred at 37°C, and the induction times were compared

to determine the nucleation rate. For the evaluation of crystal aggregation inhibition, a spectrophotometric assay was employed. Calcium oxalate monohydrate crystals were generated from potassium chloride and sodium oxalate solutions. The crystals were diluted and incubated in the presence or absence of *T. wallichiana* AgNPs and Cystone at concentrations ranging from 100 to $500\text{ }\mu\text{g}\cdot\text{mL}^{-1}$. The percentage inhibition of aggregation was calculated using the same formula as the turbidity method.

2.2.9 Anticancer studies

The anticancer potential of *T. wallichiana* AgNPs was investigated against SiHa cervical cancer and MDA-MB-231 breast cancer cell lines. The cell viability was assessed using the Sulforhodamine B assay. Cancer cells were cultured and incubated with varying concentrations of AgNPs (10, 20, 40, and $80\text{ }\mu\text{g}\cdot\text{mL}^{-1}$) for 48 h. After fixation and staining, the percentage of growth inhibition was calculated by comparing the absorbance of test wells to control wells, following the methodology described in ref. [20].

3 Results and discussion

3.1 UV-Vis spectroscopy analysis

Figure 1 illustrates the validation of the reduction process from silver ions to AgNPs, which was confirmed through

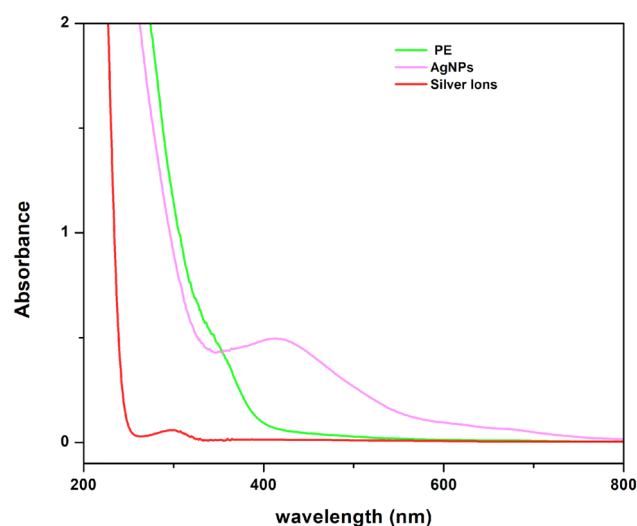


Figure 1: UV-Vis spectrum of *T. wallichiana* crude extract, AgNO_3 , 5 min of exposure to sunlight of AgNPs.

UV and visible spectroscopy. Typically, AgNPs exhibit a brown color in water and display peak absorption in the range of 420–450 nm when stimulated by specific light wavelengths. Upon the introduction of the plant extract into a 1 mM silver nitrate solution, a noticeable change was observed, transforming the initially colorless solution into a dark brown solution. Analysis of the UV-Vis spectrum, covering the range of 200–1,100 nm, revealed the absorption spectra of the AgNPs formed in the reaction mixture. Notably, a prominent absorption peak at 440 nm was observed in the absorbance readings [21–23].

3.2 XRD analysis

The synthesized AgNPs, after undergoing multiple rounds of centrifugation and washing for purification, were dried and subjected to analysis using an X-ray diffractometer. X-ray powder diffraction is a rapid analytical technique that provides valuable information regarding the dimensions of the unit cell. In Figure 2, the XRD pattern of AgNPs is depicted, revealing characteristic peaks observed at 2θ values of 38.11° , 44.27° , 64.42° , 77.47° , and 82.0° . These peaks can be assigned to the respective planes of (111), (200), (220), (311), and (222) based on the JCPDS 04-0783.

3.3 TEM

In Figure 3, the particle size and shape are characterized using TEM images. The images revealed that the NPs had a nanoscale size, with an approximate diameter of 50 nm.

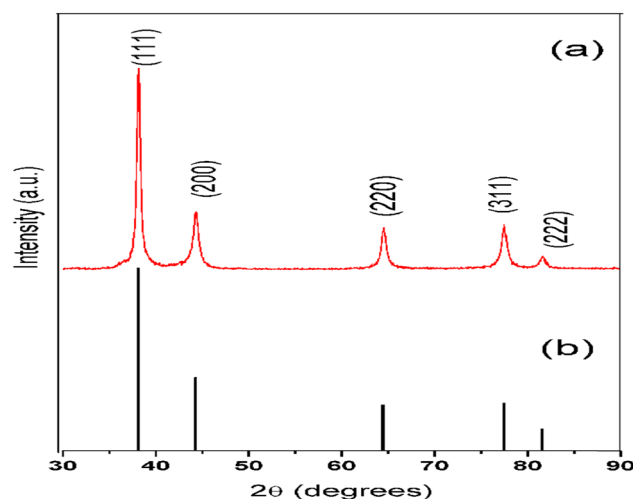


Figure 2: (a) XRD pattern for synthesized AgNPs and (b) pattern from JCPDS file No. 04-078.

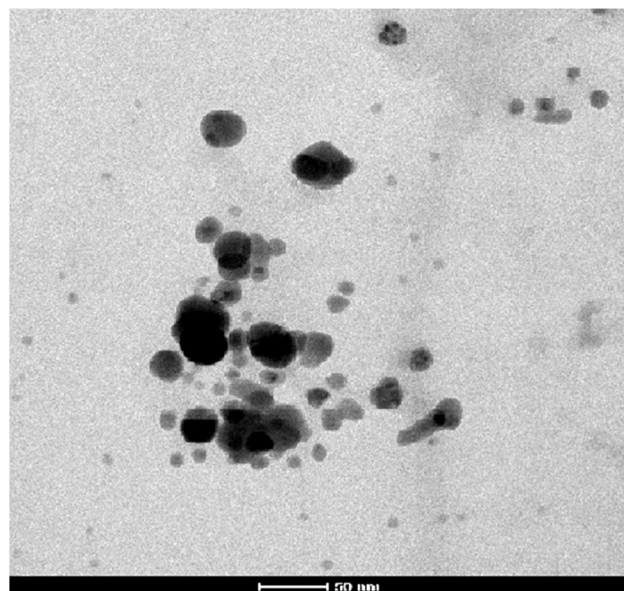


Figure 3: TEM images of AgNPs synthesized from dried leaves of *T. wallichiana*.

The NPs appeared mostly spherical, displaying smooth edges. Overall, the NPs exhibited a circular shape and were uniformly dispersed at an appropriate distance from one another.

3.4 HPLC-Q-TOF-MS

The precise concentration of paclitaxel was determined through HPLC-UV assessment using a combination of UPLC-Q-TOF/MS analysis, purpose screening, and quantitative analysis. In the target MS/MS mode, disintegration spectra were obtained for selected compounds to elucidate their likely structures. During the non-target screening, three compounds with m/z values of 737.23, 712.32, and 585.27 were identified, which could potentially impact the quantitation by HPLC-UV. Notably, the highest concentration of compound 5 was discovered in the needles of *T. baccata* cv. Repandens, representing 0.02% of the dry weight.

3.5 High-performance liquid chromatography

In order to quantify Taxol and identify its presence in the leaf of *T. wallichiana*, purified samples (20 μ L) were subjected to HPLC analysis using an Agilent (1260 Infinity) system equipped with a C18 column (250 mm $\times$ 4.6 mm, 5 μ m X-Bridge water), as depicted in Figure 4. The elution

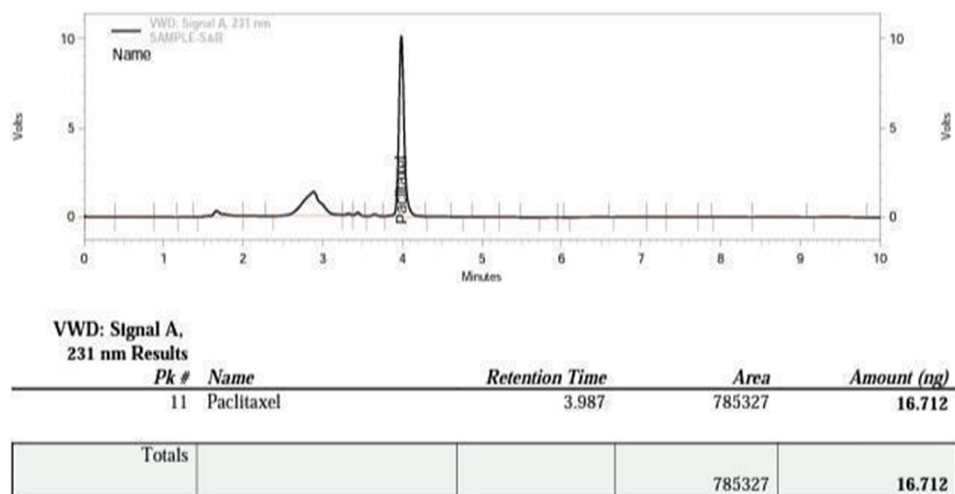


Figure 4: Method validation of paclitaxel.

of Taxol from the column was monitored at 231 nm using a UV detector and EZChrome software, employing an isocratic flow rate of $0.8 \text{ L} \cdot \text{min}^{-1}$ with an mobile phase consisting of $\text{MeOH}:\text{AcN}:\text{H}_2\text{O}$ (20:40:40 v/v). The quantification of Taxol was performed by comparing the known quantities of authentic Taxol with the measured quantities using a standard curve. Additionally, the UV spectra at 231 nm of the HPLC-purified Taxol were compared with those of authentic Taxol to confirm their similarity. The analysis verified the presence of 16.712 ng of Taxol in the leaf samples.

To validate the HPLC method for paclitaxel, a method validation study was conducted. The validation process encompassed various parameters, including robustness, accuracy, recovery, precision, and linearity, to ensure the suitability of the developed method for the intended purpose. A series of solutions were prepared using a stock solution of paclitaxel, and the linearity responses were

evaluated across concentration ranges spanning from 100 to $600 \mu\text{g} \cdot \text{mL}^{-1}$, as demonstrated in Figure 5.

3.6 Antioxidation activity

3.6.1 DPPH method

The scavenging activity of AgNPs against free radicals was evaluated using the DPPH radical as a substrate, with concentrations ranging from 100 to $500 \mu\text{g} \cdot \text{mL}^{-1}$. The results indicated significant scavenging activity by AgNPs at different concentrations. At 100, 200, and $400 \mu\text{g} \cdot \text{mL}^{-1}$, AgNPs exhibited free radical scavenging activities of 45.91%, 53.06%, and 36.73%, respectively (Figure 6). As a positive control, ascorbic acid demonstrated 95% inhibition at $500 \mu\text{g} \cdot \text{mL}^{-1}$.

The antioxidant properties of *T. wallichiana* were found to be potent, and its reducing power was observed. When reacting with antioxidant molecules, DPPH radicals are scavenged through hydrogen donation, resulting in a reduction in their absorbance and a visible color change from purple to yellow. DPPH is commonly employed as a substrate to assess the antioxidative activity of antioxidants. Based on these findings, it can be concluded that AgNPs produced using *T. wallichiana* possess antioxidant properties by donating hydrogen [15].

3.6.2 TLC profiling method

In this study, we investigated the ability of AgNPs to scavenge light purple molecules using the dot blot method (Figure 7). The percent scavenging activity of AgNPs was

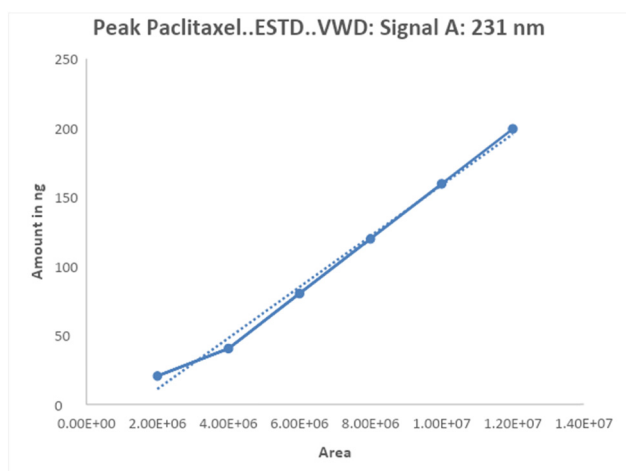


Figure 5: Linearity plot of paclitaxel by the proposed method.

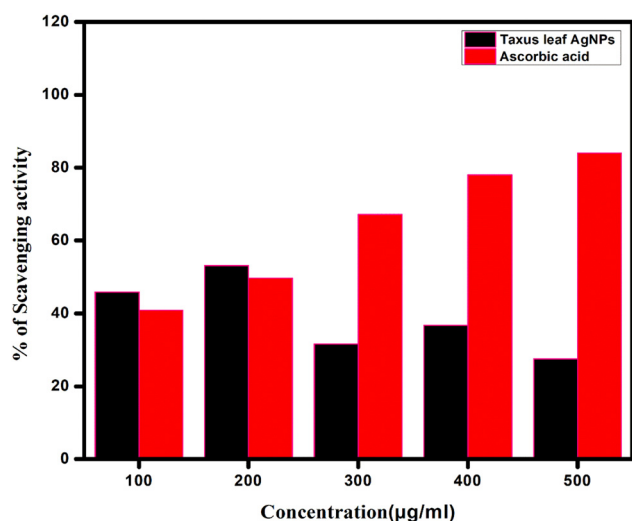


Figure 6: DPPH scavenging activity of *T. wallichiana* at different concentrations.

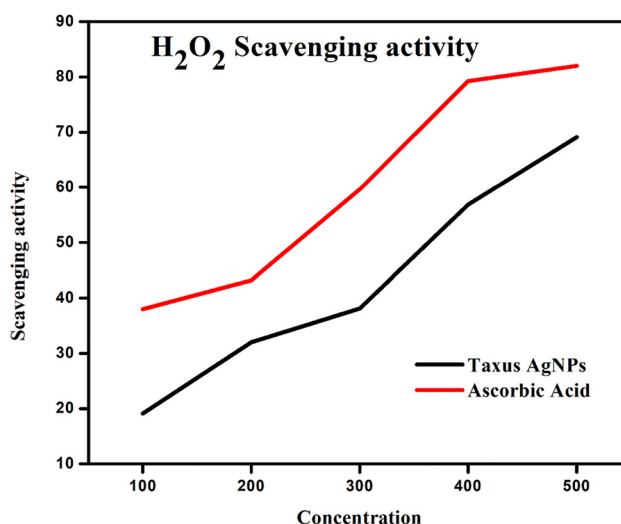


Figure 8: Radical scavenging activity of *T. wallichiana* at different concentrations.

determined through spectrophotometric analysis at various concentrations (Figure 8b). To assess the antioxidant activity of the crude plant extract qualitatively, we employed TLC, which offers a simple and efficient approach. In order to confirm the presence of antioxidant compounds in the extracted AgNPs, a TLC qualitative antioxidant assay was conducted. When the TLC plate was sprayed or dipped in DPPH solution, yellow antioxidant spots emerged against a purple background. Furthermore, the detection of Taxol was achieved by heating sulfuric acid with Vanillin (1% w/v), resulting in a dark grey to blue spot after 24 h. The obtained results were compared with the 2016 TLC Atlas drug for reference. The study observed yellow staining for compounds exhibiting antioxidant activity, while the remaining portion of the TLC plate displayed purple staining. A TLC plate sprayed with DPPH displayed a yellow color band

and a blue band at different RF values (RF value of 0.65 in Figure 7a and RF value of 0.33 in Figure 7b), indicating potent antioxidant activity. These results tentatively identified the two compounds as phenolic compounds possessing potent antioxidant properties. The objective of this research was to establish a biological fingerprint capable of identifying active compounds in complex samples, and the analysis of thin-layer chromatography revealed the presence of various biomolecules in the extract.

3.6.3 H₂O₂ scavenging activity

Hydrogen peroxide is naturally present in various environments, including water, plants, bacteria, food, and the human body, albeit at low concentrations. Within living

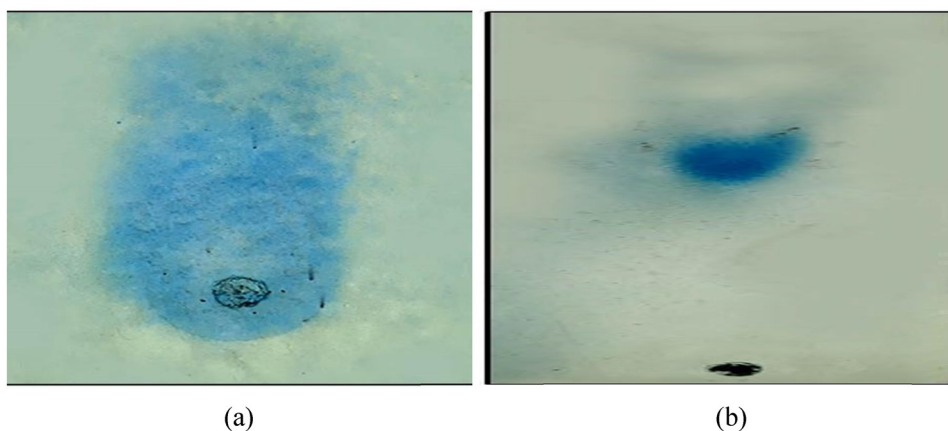


Figure 7: The TLC-DPPH assay of *T. wallichiana* AgNPs after spraying DPPH: (a) *Taxus* leaf extract after DPPH spray show retention factor of 0.65 and (b) AgNPs with *Taxus* after DPPH spray show retention factor of 0.33.

organisms, certain oxidizing enzymes like superoxide dismutase can generate this compound, which gradually oxidizes different substances [24]. The study demonstrated that *T. wallichiana* AgNPs, at a concentration of $500 \mu\text{g}\cdot\text{mL}^{-1}$, were capable of scavenging 69.12% of hydrogen peroxide, whereas ascorbic acid exhibited a hydrogen peroxide scavenging activity of 82.00% at the same concentration. These findings confirm the efficacy of *T. wallichiana* AgNPs as hydrogen peroxide scavengers, as depicted in Figure 8. Additionally, the research explored the impact of *T. wallichiana* AgNPs on neutralizing hydrogen peroxide radicals to prevent the oxidative deterioration of substrates.

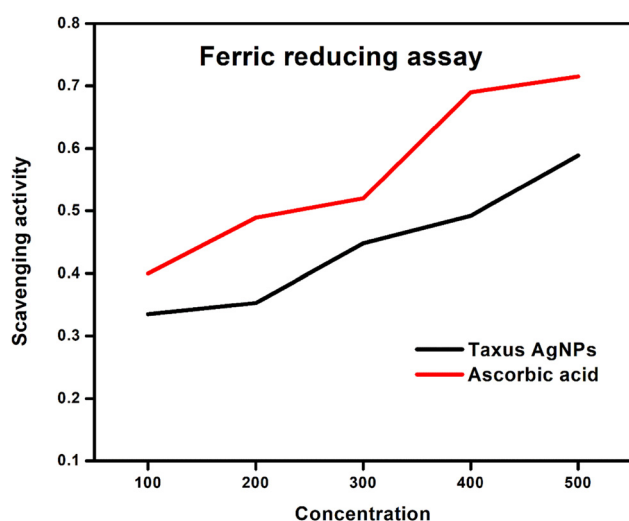


Figure 9: Ferric reducing power assay at different concentrations of *T. wallichiana* AgNPs.

3.6.4 Ferric-reducing power assay

The ferric-reducing power assay is a method used to assess the ability of antioxidants to convert Fe^{3+} /ferricyanide to its ferrous form. As the reducing power increases, the absorbance also increases, indicating the need for higher doses of *T. wallichiana* AgNPs [25]. The results of the ferric-reducing antioxidant content test conducted on *T. wallichiana* AgNPs are presented in Figure 9. To investigate the anti-inflammatory activity of *T. wallichiana* AgNPs, denaturation and hemolysis of proteins were examined *in vitro*. Table 1 demonstrates that both AgNPs and sodium diclofenac can inhibit protein denaturation, with AgNPs exhibiting greater effectiveness in reducing inflammation. Protein denaturation triggers inflammatory signals and aggregation due to the loss of quaternary structure [26]. Previous research indicating the inhibition of albumin denaturation by AgNPs further supports their anti-inflammatory properties [27].

Additionally, the study investigated the membrane-stabilizing effects of *T. wallichiana* AgNPs by evaluating hypotonicity-induced hemolysis of HRBCs. The results in Table 1 indicate an increasing inhibition of heat-induced and hypotonic hemolysis with higher concentrations of AgNPs. This evaluation focused on the membrane stabilization effect as excessive fluid accumulation in cells can lead to membrane rupture and hemolysis [28].

Inhibition of protein denaturation is a well-known mechanism for suppressing various inflammatory mediators [29]. Different drugs, such as sodium diclofenac, phenyl butanone, and salicylic acid, exhibit varying degrees of anti-denaturation effects depending on their dosage [30].

Table 1: Heat-induced and hypotonicity studies of the effect of AgNPs on HRBC membrane hemolysis of erythrocytes

Treatment(s)	Concentration ($\mu\text{g}\cdot\text{mL}^{-1}$)	Absorbance at 560 nm		% Inhibition of hemolysis	
		For heat-induced studies	For hypotonicity-induced studies	For heat-induced studies	For hypotonicity-induced studies
Control	—	0.32	0.33	—	—
<i>T. wallichiana</i> AgNPs	100	0.29**	0.41**	9.37	24.24
	200	0.39**	0.30**	21.87	36.36
	300	0.22*	0.20*	31.25	39.39
	400	0.20*	0.50**	37.5	51.51
	500	0.10NS	0.56**	68.75	69.69
Sodium diclofenac SE	100	0.08	0.13	75	60.60
CD	5%	0.043	0.063		
CD	1%	0.112	0.162		
		0.175	0.255		

SE, standard error; CD, critical difference.

* Indicates the heat induced and hypotonicity is minimum.

** Indicates the heat induced and hypotonicity is maximum.

Natural compounds derived from plants have also shown comparable anti-inflammatory properties to synthetic drugs [31].

In vitro tests for screening anti-inflammatory drugs typically involve inhibiting denaturation, a common cause of rheumatoid arthritis [32]. Nonsteroidal anti-inflammatory drugs, which block prostaglandin production and relieve pain, are commonly used. Anti-inflammatory agents achieve this by inhibiting COX enzymes or protecting lysosomal membranes from degradation [30]. Most anti-inflammatory drugs have been found to stabilize erythrocyte plasma membranes, preventing heat and hypotonicity-induced hemolysis.

This study found that concentrations of *T. wallichiana* AgNPs ranging from 100 to 500 $\mu\text{g}\cdot\text{mL}^{-1}$ protected human RBC membranes from lysis during inflammation, potentially through their stabilizing effect, which prevents the leakage of fluids and serum proteins from tissues induced by inflammatory mediators [33]. The membrane-stabilizing effect suggests that AgNPs could serve as an anti-inflammatory compound by protecting red blood cell membranes from hemolysis [34].

3.7 *In vitro* antiurolithiatic studies

3.7.1 Nucleation assay (turbidity method) and aggregation assay

The demand for herbal medicines in developed nations is on the rise due to their effectiveness, safety, and minimal side effects in primary healthcare. Unlike allopathic medicines that target specific aspects of a disease, herbal

therapies have demonstrated success in addressing various stages of stone pathophysiology. Renal stones, which commonly consist of CaOx, occur as a result of urine supersaturation, followed by the processes of crystallization, nucleation, growth, and aggregation. By preventing supersaturation or intervening in the later stages of crystallization, the development of lithiasis can be averted.

In this study, the inhibition of CaOx crystallization at each phase was achieved using AgNPs synthesized from *T. wallichiana* leaf extract. Both the plant extracts and AgNPs exhibited inhibitory effects on kidney stone development by impeding the processes of nucleation and aggregation (Figure 10). The AgNPs derived from *T. wallichiana* may contain phytochemicals that hinder the growth of CaOx crystals. Increasing the dosage of the plant extract resulted in greater inhibition of CaOx crystal growth, with the highest percentage of inhibition observed at a concentration of 500 $\mu\text{g}\cdot\text{mL}^{-1}$. Figure 11 illustrates the aggregation of AgNPs following treatment with *T. wallichiana* extract at different concentrations. The test drug showcased significant anti-lithiasis properties, exhibiting a higher percentage of inhibition compared to the standard drug at the same concentration. These findings indicate that the test drug holds promise as a potential alternative treatment for kidney stones.

3.8 Anticancer activity

The AgNPs synthesized from *T. wallichiana* leaf extract exhibited superior anticancer activity compared to

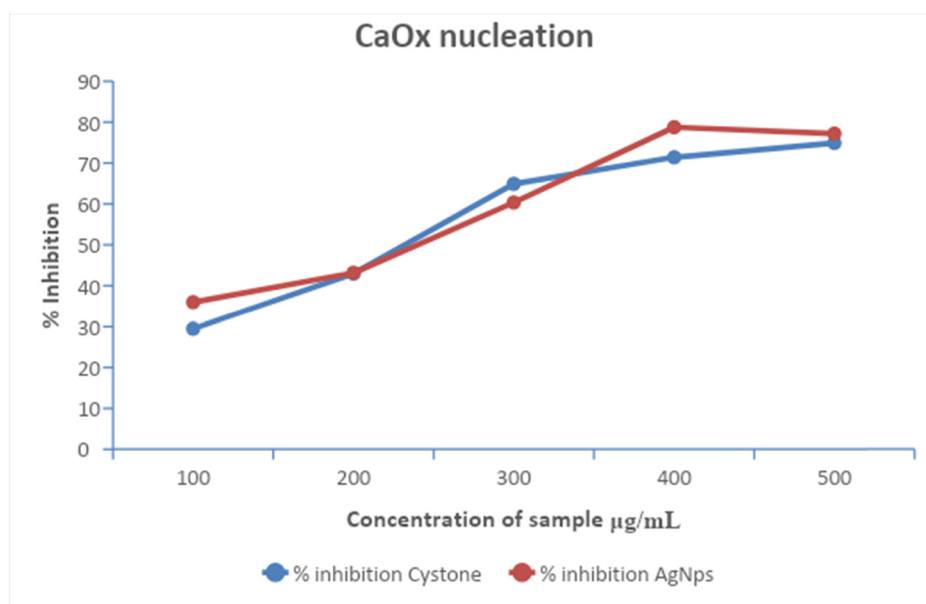


Figure 10: CaOx nucleation in AgNPs and *T. wallichiana* extract at different concentrations.

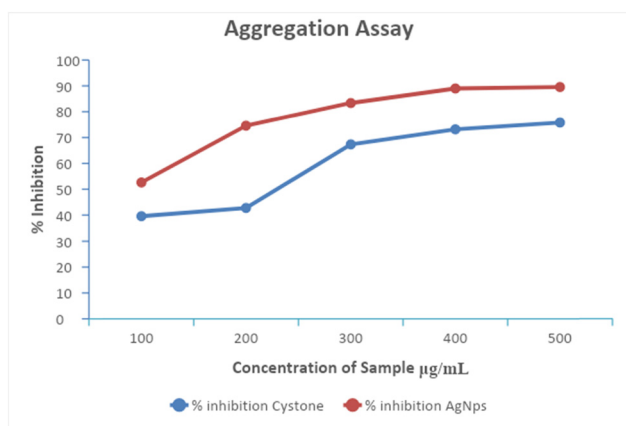


Figure 11: AgNPs aggregation after treatment with *T. wallichiana* extract at different concentrations.

Table 2: Anticancer activities of AgNPs and control (ADR)

Samples	Human cervical cancer cell line SiHa			
	Drug concentration (µg·mL ⁻¹)			
	10	20	40	80
AgNPs	99.5	103.6	106.3	39.5
ADR	-51.2	-27.6	-34.5	-23.1
Human breast cancer cell line MDA-MB-231				
AgNPs	103.5	91.3	69.8	14.3
ADR	-48.0	-55.6	-56.7	-49.9
LC 50 TGI GI50 studies				
	Human cervical cancer cell line SiHa			
	LC 50	TGI	GI50	
AgNPs	NE	NE	78.6	
ADR	NE	<10	<10	
Human breast cancer cell line MDA-MB-231				
AgNPs	NE	92.2	53.0	
ADR	NE	<10	<10	

LC50: concentration of drug causing 50% cell kill; GI50: concentration of drug causing 50% inhibition of cell growth; TGI: concentration of drug causing total inhibition of cell growth; ADR: Adriamycin, positive control compound; NE: not evaluated.

Adriamycin (ADR), as shown in Table 2. The experimental groups were compared with the ADR group, which served as a positive control (Table 2). The growth inhibition (GI50) was calculated by multiplying $[(Ti - Tz)/(C - Tz)]$ by 100, resulting in a 50% inhibition of net proteins. To calculate the total growth inhibition (TGI), Ti was calculated as Tz

LC50 (the concentration of the drug that leads to a 50% reduction in measured protein after treatment), indicating a net loss of 50% of cells:

$$[(Ti - Tz)/Tz] \times 100 = -50 \quad (1)$$

4 Conclusion

In this study, a comprehensive range of analytical techniques was employed to assess the characteristics of AgNPs synthesized utilizing plant extracts and to quantify the concentration of paclitaxel in *T. wallichiana*. The outcomes confirmed the successful synthesis of AgNPs exhibiting a smooth spherical shape and identified functional groups that contribute to the reduction and capping of AgNPs. Moreover, the study demonstrated the remarkable antioxidant and anti-inflammatory activities of AgNPs derived from *T. wallichiana*. These findings collectively indicate the promising biomedical and pharmaceutical applications of AgNPs produced through plant extracts. The study provides significant insights into the properties and potential uses of AgNPs, underscoring their relevance in diverse research fields.

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