

THEVETIA PERUVIANA ROOTS EXTRACT MEDICATED GOLD NANOPARTICLES AND ITS UREASE INHIBITORY ACTIVITY

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ABSTRACT

Objective: The main objective of the current research work was synthesized of gold nanoparticles (AuNPs) of *Thevetia peruviana* aqueous extract, characterization, and screening for urease enzyme inhibitory activity.

Methods: AuNPs were synthesized by mixing 1 mM gold salt solution with *T. peruviana* aqueous extract without any reducing agents. The prepared AuNPs were characterized using UV-visible spectroscopy, Fourier transform infrared spectroscopy, and scanning electron microscopy techniques. The synthesized AuNPs were assessed for *in vitro* urease enzyme inhibitory activity at 0.2 μ g in comparison with the aqueous extract.

Results: In this finding, we synthesized the AuNPs of *T. peruviana* aqueous extract for the 1st time. The AuNPs exhibited significant stability at room temperature. The AuNPs showed significant urease inhibitory activity with IC_{50} 67.56 \pm 1.67 at 0.2 μ g as compared to aqueous extract which exhibited good activity with IC_{50} 39.21 \pm 1.32 μ g at 0.2 mg, against standard thiourea (IC_{50} =21.00 \pm 1.16). The formation of AuNPs correlates due to active phytochemical present in extract which is responsible for synthesizing NPs.

Conclusion: *T. peruviana* extract and prepared AuNPs are an outstanding urease enzyme inhibitor and are capable of making fine NPs. Application: The synthesized AuNPs of *T. peruviana* aqueous extract which significant urease inhibitory activity may allow us to discover NPs for potentially effective and safe nanoherbal therapy.

Keywords: *Thevetia peruviana*, Extract, Nanoparticles, Urease activity.

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INTRODUCTION

Thevetia peruviana belong to family Apocynaceae which is commonly known as yellow oleander, lucky nut, nerium oleander, as well as yellow bells. *T. peruviana* is a small tree whose height ranges from 1.3 to 2.3 m, its leaves are arranged spirally and linear and having length 13–15 cm [1]. The flowers are yellow in color having funnel like shape having 5 petals twisted spirally. *T. peruviana* (Fig. 1) is mostly grown for ornamental purposes. *T. peruviana* has been documented for various biological activities but also reported for its toxic effect [2]. The plant is normally utilized in local pharmaceuticals in tropical America and in tropical Asia. *T. peruviana* is used in traditional systems for cure of malarial fever, amenorrhoea, constipation, jaundice, hemorrhoids, skin scatter, as well as headaches. The principal medicinally dynamic mixes found in the plant are a range of cardiovascular glycosides of these glycosides; peruvoside has been investigated generally thoroughly. Among different glycosides, thevetin has viably been utilized clinically if there should be an occurrence of cardiovascular decompensation, in spite of the fact that its viable portion is somewhat near its poisonous dose. Thevetin A is far less intense than blend thevetin [2,3]. The strength of neriifolin as cardiovascular glycosides is just moderate. The latex is connected to rotted teeth to mitigate toothache and is utilized to treat chronic scores and ulcers. The bark has been documented for as febrifuge and an excellent antiperiodic. A tincture of the bark is used in the treatment of snake bite as well as malarial fever. The decoction of the leaves is taken to cure jaundice, fever, as well as a purgative for intestinal worms. The leaves sap is used as eye drops and nose drops to treat violent headaches. The seeds are also reported as a laxative and for cure of skin complaints. *T. peruviana* has also documented for antimicrobial and antitermite activities [4,5]. The main aim of current research was to synthesize gold nanoparticles (AuNPs) from *T. peruviana* aqueous extract and screen for urease inhibitory activity.

METHODS

Plant collection

The plant was collected in the winter season from ghazi. The plant specimen was identified by Dr. Muhammad Ilyas, Department of Botany University of Swabi and voucher specimen no UOS/bot-55 was placed in the department.

Extraction and preparation of solution

Plant roots were dried in shade for 2 months. Then, the dry weight was taken 5 kg and powdered it in simple mortar. The air-dried powdered of plant 1 kg was soaked in water as well as in methanol and was placed in a fuming hood for 14 days. The 18.8 g of aqueous extract obtained then collected in sample tubes. From these, 2 mg of extract was dissolved in distilled water to prepare stock solution. Similarly, 0.034 g of $HauCl_4$ was dissolved in distilled water to prepare 1 mM salt solution.

Green synthesis of AuNPs using aqueous extract

Two types of solutions were prepared: (i) Stock solution of extract and (ii) salt solution (1 mM). Stock solution was prepared by dissolving 5 mg of aqueous extract (weighted on digital balance (OHAUS) in 100 ml of distilled water and was stirred for 10–25 min and salt solution ($HauCl_4$ and distilled water) was prepared by dissolving 0.034 g of gold salt in 100 ml of distilled water and was stirred for 5 min. The gold and extract solutions were mixed in different ratios (1:1, 1:2, 1:3, 1:5, 1:7, 2:1, 2:3, and 2:5) in different vials and kept on stirring for 1–24 h. The color in the reaction mixture in each vial changed till 1:5. The color produced in these ratios was best and from beyond these ratios, there was no prominent color change. It was noted that the best nanoparticles (NPs) were synthesized in 1:2 and 1:3 solutions which were confirmed from color and ultraviolet (UV)-Vis spectrophotometer. The UV-Vis spectra showed a characteristic peak at 560–570 nm which indicated the formation of NPs (Fig. 2).

Urease inhibitory assay

The extract and prepared NPs of *T. peruviana* were assessed for urease inhibitory activity by following our reported method [3-7]. This experiment was performed using spectrophotometrically in 96-well plates. In this method, 5 μ L of crude extracts and 0.5 mM of synthesized NPs and 25 μ L urease catalyst (1 U/well) were hatched at standard condition as per reported procedure [8]. Furthermore, the substrate (urea) was incubated at standard condition, after that sodium nitroprusside and 1% w/v phenol, 70 μ L of alkali reagent (0.1% sodium hypochlorite, 45 μ L of phenol [0.005% w/v as well as 1% w/v phenol] as well as 0.5% w/v sodium hydroxide) were mixed. After that, the plates were reincubated for 1 h. The screening of urease was continuously recorded with hydrolysis of urea as well as the formation of ammonia (NH₃). The change in absorbance was noted at 630 nm on ELISA plate reader (USA).

RESULTS AND DISCUSSION

The current research study was performed to assess the urease inhibitory effect of *T. peruviana* crude extract and synthesize AuNPs. The synthesized NPs were characterized using advanced techniques including; UV-visible, Fourier transform infrared (FTIR) spectroscopy, and scanning electron microscope (SEM) technique. The formation of AuNPs is due to the secondary metabolites present in extract of *T. peruviana*. Furthermore, the extract and NPs were assessed for enzyme inhibitory potential which exhibited excellent activity in comparison to standard drug.

Synthesis of NPs

The green synthesis of AuNPs was done using plant extract of *T. peruviana* as stabilizing/reducing agent was carried out with progressive result as per standard methods [8,9]. To discover the improved ratio, reactions with different ratios (1:1, 1:2, 1:3, 1:5, etc.) of metal to ligand were carried out, keeping metal ratio constant and varying the ligand ratio. The best results, which were collected from different reactions, were 1:1 and 1:2 which had the sharpest absorption peak and selected for



Fig. 1: Image of *Thevetia peruviana*

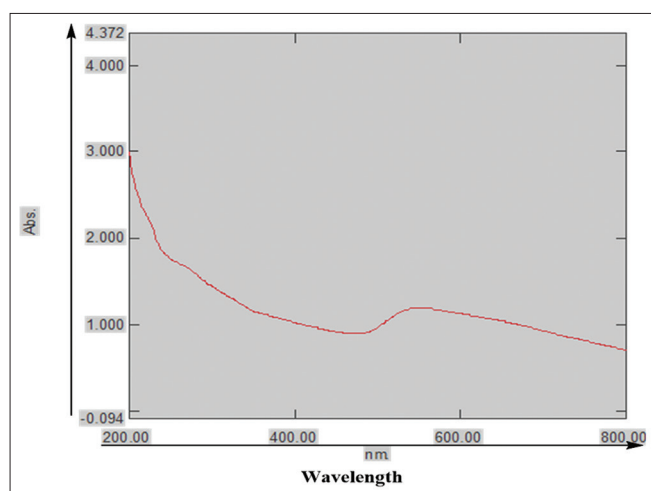


Fig. 2: Ultraviolet-visible spectra of AuNPs capped with *Thevetia peruviana*

further studies. UV-Vis result showed the formation of AuNPs because of a particular peak at 547 nm and 557 nm for 1:1 and 1:2, respectively, extract (Fig. 3).

The synthesis of AuNPs was confirmed by UV-Vis spectrophotometer. The UV spectrums of synthesized NPs were recorded at UV spectrophotometer for various ratios of reaction mixture. The spectrum was also measured for stock solutions as well as NPs. The first indications of formation on NPs from extract were color change and difference between stock solutions and prepared NPs. The NPs which have the most suitable graph were taken for next characterization.

FTIR analysis

In FTIR spectroscopy, we analyze the functional groups present in a compound. In general, FTIR spectroscopy contains two main regions, the region from 4000-1600 cm⁻¹ is called "Functional group" region though it cannot give the exact structure of a compound but when it is used with other evidence (physical or chemical), then it can be helpful. To confirm the synthesis of NPs, both the spectra of extract and NPs are correlate. The change occur in the spectra will confirm that the reaction between salt solution (HAuCl₄) and stock solution has occurred. When the NPs have become synthesized, then the FTIR spectra of NPs must be different from the spectra of extract.

Different functional groups have different vibrational and stretching frequencies. The bands obtained from FT-IR are used for the identification of functional groups of natural products present in the plant extracts [8,9].

FT-IR spectra guarantee extraordinary value due to its rapidity, simplicity, affectability, and low cost. One of the vital uses of the infrared spectroscopic examination is the diagnostic value in building up the presence of certain organic constituents in plants. FT-IR spectroscopy gives more detailed chemical data on the composition of samples because it measures the fundamental vibration. More recently, FT-IR has been introduced as a metabolic fingerprinting instrument for the plant sciences. In such an attempt, either the variation in intensity and frequency shift of some characteristic absorption bands can be of some use [8,9].

FTIR of plant extract

The peak is given in the range of 3251.98 cm⁻¹ which is a medium peak and shows N-H stretching and is assigned to aliphatic primary amine. The adjacent broad peak represents O-H stretching and is also a medium peak. The peaks in the region from 2933 to 2960 cm⁻¹ are assigned for C-H stretching of alkanes. The peaks in the region between

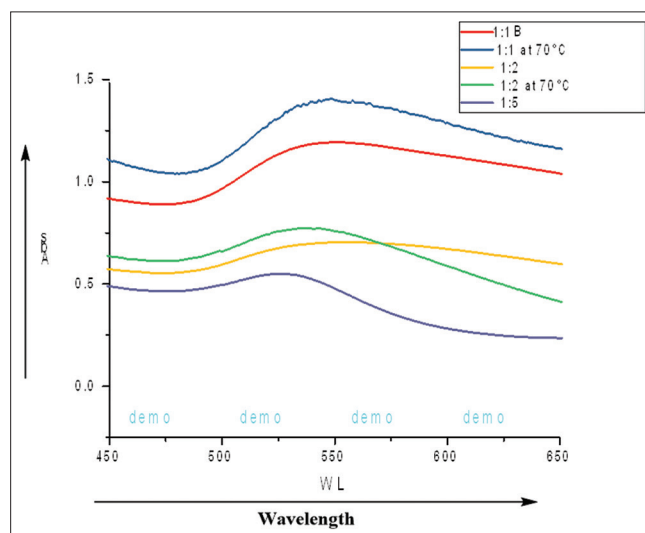


Fig. 3: Different colors of graphs of gold nanoparticles show different ratios of gold salt versus plant

1500 and 1550 cm^{-1} are assigned for N-O stretching specially in NO_2 group. The sharp, noisy, and medium peak in the range of 1415 cm^{-1} is assigned for O-H bending, especially present in carboxylic acids and alcoholic functional groups. The peaks in the region between 1312 and 1377 cm^{-1} are assigned for C-H bending in alkane, especially for methyl group (Fig. 4). The peaks present in the region between 1000 and 1400 cm^{-1} are assigned for O-H bending in carboxylic acids, alcohol, and phenol while the peaks from 1020 to 1250 cm^{-1} assigned for C-N stretching present in amines. The peaks in the region of 1026, 1053, and 1072 cm^{-1} are assigned for C-O and S=O, respectively, present in primary alcohol and sulfoxide.

FTIR spectra of AuNPs in land plant extract

The most peaks which were present in the FTIR of crude extract are vanish after the reaction because gold salt contains ions of gold and chloride which make bonds with the active/polar sides of the compounds (present in the extract) and the sites become blocked or change to another type of compounds.

Hence, after the reaction, the solution (which contains AuNPs) gives the spectra which are given below in the FTIR graph. The first peak in 3317 cm^{-1} region assigned for O-H functional group esp. present in

Table 1: Enzyme inhibitory activity of extracts and gold nanoparticles of *Thevetia peruviana*

Sample	% inhibition	IC ₅₀ μM
Extract	91.21	67.56 \pm 1.67
Gold nanoparticles	96.41	39.21 \pm 1.32
Thiourea	98.20	21.00 \pm 1.16

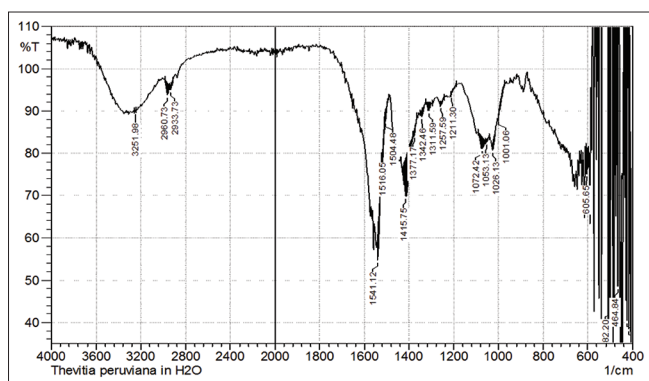


Fig. 4: Fourier transform infrared spectra of crude extract of *Thevetia peruviana*

alcohol. When this peak is sharp and strong, then it is assigned for N-H functionality present in amine salt. The region from 1540 to 1650 cm^{-1} is assigned for C=C stretching in cyclic alkene (Fig. 5). The very minor peak in the region 1310–1335 cm^{-1} (v) is assigned for sulfur compounds esp. S=O compounds. Another minor and very weak (v) peak in 1037 cm^{-1} is assigned for primary alcohols (C-OH str) (Fig. 6).

The difference between the two types of spectra which are given below is the difference in intensities of peaks although most spectra fall in the same region.

FTIR analysis was used for the characterization of the extract and the synthesized NPs (Figs. 3-5). Both the spectra of crude and AuNPs were compared to each other for the confirmation of NPs synthesis. The FTIR spectra of *T. peruviana* leaf extract before and after bioreduction show significant changes. Most of the bands that were present in the FTIR spectra of plant's extract were absent in the FTIR of NPs which confirmed that some metabolites and natural products are bonded to the gold through the active sites present in the natural products, that is, ---OH, -NH-, -SH, etc. Most of the spectra that were representing the active moieties in the compounds which are now bonded to the gold metal do not give the same spectra, that is, bands for aliphatic primary amines (mostly present in alkaloids), -OH stretching peak, and some other minor bands.

SEM analysis

SEM of AuNPs has confirmed its size. The size and shape of AuNPs were determined using SEM (JEM 2100, Jeol [CRL University of Peshawar]). Few drops of AuNPs solution were placed on the carbon grid and were allowed to dry before measurement. The SEM analysis shows that the fine NPs have synthesized. From the above image, the size of NPs that

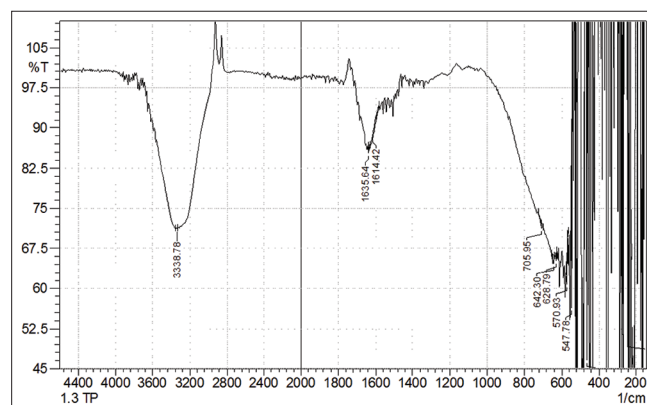


Fig. 6: Fourier transform infrared spectra of gold nanoparticles (1:3) of *Thevetia peruviana*

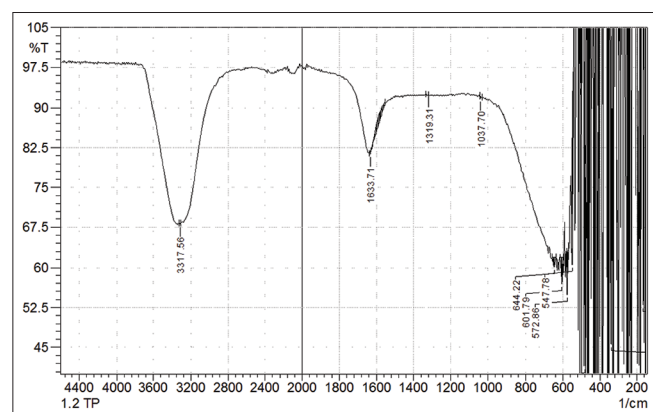


Fig. 5: Fourier transform infrared spectra of gold nanoparticles (1:2) of *Thevetia peruviana*

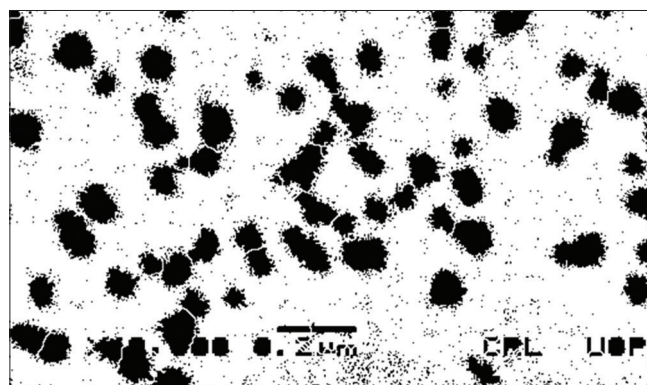


Fig. 7: Scanning electron microscope image of gold nanoparticles of *Thevetia peruviana*

have been measured was 100–120 nm, and these types of NPs fall in the category of fine NPs (Fig. 7). The size of NPs was assumed from the above scale given in the image, that is, 0.2 μm (200 nm).

Urease enzyme inhibitory activity

The crude extract and synthesized NPs were also assessed for *in vitro* urease activity. The results are given in Table 1.

The crude extract and synthesized NPs exhibited good urease inhibition activity. The active phytochemical present in extract is responsible for synthesized AuNPs. Urease enzyme inhibitory activity of the extract and NPs is displayed in Table 1, the extracts and NPs inhibit urease activity with an IC_{50} 67.56 \pm 1.67 and IC_{50} 39.21 \pm 1.32 μM while the IC_{50} of thiourea was 21.00 \pm 1.16 using thiourea. It is concluded that *T. peruviana* extract is an outstanding urease enzyme inhibitor and is capable of making fine NPs. The activities of both extract and NPs can be attributed to their coordinating capabilities with the metalcenter (i.e., nickel) of the enzyme. The greater activity of the extract and prepared NPs of *T. peruviana* can be conceived to be due to the some active groups present in the extract which can strongly bind with the active sites of the enzyme. This enzymatic study provides some useful information about the design of new inhibitors.

CONCLUSION

In this finding, we have established a green nanotechnological procedure to prepared AuNPs using *T. peruviana* extract which act as stabilizing agent as well as reducing agent by avoiding the usage of toxic and hazardous solvents. The prepared NPs were in the size of 100–120 nm which fall in the category of fine NPs. The FT-IR analysis showed the involvement of S=O, -OH, and C-OH in the synthesis of NPs. The excellent urease inhibitory activity may allow us to discover NPs for potentially effective and safe nanoherbal therapy.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

REFERENCES

1. Samal K, Sahu HK, Gopalakrishnakone P. Clinico-pathological study of *Thevetia peruviana* (yellow oleander) poisoning. *J Wilderness Med* 1992;3:382-6.
2. Rao MA, Venkata E, Visweswaram D. Effect of certain structural changes in cardiac glycosides of *Thevetia peruviana* on their toxicity. *J Mol Cell Cardiol* 1978;10 Suppl 1:86-8.
3. Rauf A, Bawazeer S, Naseer M, Alhumaydhi FA, Aljohani AS, Habib A, et al. *In vitro* α -glycosidase and urease enzyme inhibition profile of some selected medicinal plants of Pakistan. *Nat Prod Res* 2020;15:1-6.
4. Roberts DM, Southcott E, Potter JM, Roberts MS, Eddleston M, Buckley NA. Pharmacokinetics of digoxin cross-reacting substances in patients with acute yellow oleander (*Thevetia peruviana*) poisoning, including the effect of activated charcoal. *Ther Drug Monit* 2006;28:784-92.
5. Kareru PG, Keriko JM, Kenji GM, Gachanja AN. Anti-termite and antimicrobial properties of paint made from *Thevetia peruviana* (Pers.) Schum oil extract. *Afr J Pharm Pharmacol* 2010;4:87-9.
6. Rauf A, Uddin G, Raza M, Patel S, Bawazeer S, Hadda TB, et al. Urease inhibition potential and molecular docking of dihydroquercetin and dihydromyricetin isolated from *Picea smithiana* (wall) Boiss. *Biomed Res* 2017;28:10026-32.
7. Rauf A, Raza M, Saleem M, Ozgen U, Karaoglan ES, Renda G, et al. Carbonic anhydrase and urease inhibitory potential of various plant phenolics using *in vitro* and *in silico* methods. *Chem Biodivers* 2017;16:1-24.
8. Islam NU, Jalil K, Shahida M, Muhammad N, Rauf A. *Pistacia integerrima* gall extract mediated green synthesis of gold nanoparticles and their biological activities. *Arab J Chem* 2020;12:2310-9.
9. Khattak A, Ahmad B, Rauf A, Bawazeer S, Farooq U, Ali J, et al. Green synthesis, characterisation and biological evaluation of plant-based silver nanoparticles using *Quercus semecarpifolia* Smith aqueous leaf extract. *IET Nanobiotechnol* 2019;13:36-41.