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# PHYTOSYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL INVESTIGATION OF GOLD NANOPARTICLES USING LEAVES OF *ACACIA MODESTA* WALL

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## Abstract

Aqueous leaves extracts of *Acacia modesta* Wall. was utilized to phyto-synthesize green AuNPs. The leaves of *A. modesta* were investigated to possess potential phytochemicals such as alkaloids, flavonoids, polyphenols, reducing sugars, tannins, saponins and steroids, which help in the bioconversion of $Au^{+3}$ to $Au^{o}$. The synthesized AuNPs were analyzed to be stable nano-crystalline spherical, having a size span of 20 – 100 nm. The $\lambda_{max}$ peak for AuNPs was observed at 550 nm, which manifests the precise fabrication of AuNPs. The sharp $Au^{o}$ peak, along with other organic elements, i.e., C, N, O, $Ca^{++}$, $Mg^{++}$, $Na^{+}$ and $Cl^{-}$ at variable intensities in EDX analysis, manifest phytochemicals in plant extracts have effectively reduced the ionic Au by acting as efficient biocapping and bioreducing agent. The pharmacological activities of these AuNPs in comparison to aqueous leaves extract manifest that AuNPs possess excellent antifungal activity against *F. solani* (92%), *F. oxysporum* (91%), *M. fur fur* (90%), *Penecillium* and *C. albicans* (89%). Moderate to good antibacterial activity was analyzed against *S. aureus* (62%) and *K. pnuemoniae* (53%). Less cytotoxic activity was observed against brine shrimp nauplii i.e. 6.67% at higest sample concentration i.e. 1000 µg/mL. Good anti-leshmanial activity was manifested by green AuNPs against *L. major*, showing IC50 value of 50.2 $\pm$ 0.01 respectively.

**Keywords:** Phytosynthesis, *A. modesta* Wall., Characterization, AuNPs, Antimicrobial, Antileshmanial, Cytotoxic

## INTRODUCTION

The concept of nano-biotechnology arises from combination of nano-sized ($10^{-9}$) materials that are utilized to benefit humanity. These nano-sized materials are currently exploited in the multiple fields of science including medicine and engineering (Lee & Moon, 2020; Prasad et al., 2021). It is basically referred as umbrella term, which unifies biomedicine with that of concepts of physics, chemistry and engineering. The applications of these nano-devices range from the sphere of cosmetology to that of chemotherapy (S. P. S. Yadav et al., 2021).These nano-devices may be incorporated in diverse commodities such as diagnostic procedures, conductive inks, imaging devices, fertilizers and pesticides and even biosensors (Madkour, 2019). In past few years, metal nanoparticles were chemically and physically formulated. The predominant targeted metals were gold, silver, titanium, platinum and copper. Among the mentioned, silver were profusely investigated to possess their unique antimicrobial property (Kumar et al., 2016). Later on, availability of gold salts has garnered scientist's attention across the globe as gold ion acts as excellent antimicrobial, antioxidant, anti-inflammatory antiviral and anti-cancer agents (Krishnani et al., 2022; S. Yadav et al., 2021). The gold salts are also conjugated with anti-inflammatory drugs such as aurothiomalate to speed up treatment of rheumatoid arthritis. Consumption of colloidal gold under WHO permissible limits can prevent osteoporosis, wrinkles and bone deformation in old age (Arora et al., 2010; S. Yadav et al., 2021). Gold alloys are also used as laminations to device antimicrobial stents and pace makers, laser equipment, surgical scissors and bandages to treat and prevent multiple infections (Alahmad, 2022; Babbar et al., 2022). The metallic gold salts were effectively reduced to gold nanoparticles (AuNPs) in presence of chemical reducing agent. The employment of these reducing agents including sodium borohydride, ethylene diamine tetra-acetic acid (EDTA) and sodium citrate were highly toxic to human health and environment sustainability. So, in order to tackle the situation, green synthesis of gold nanoparticles was introduced in order to bio-reduce gold ion ($Au^{+3}$) to gold nanoparticles ($Au^{o}$) (Singh et al., 2015; Zayadi & Bakar, 2020). Green synthesis is present day introduced technology, which employs natural reducing properties of plants and micro-organism (bacteria, fungi and algae) to convert $Au^{+3}$ to $Au^{o}$. In comparison to micro-organisms, plant parts or whole plants are preferred bio-reducing agents due to profuse availability, quick conversion of nanoparticles and effortless purification steps (DESHPANDE). Phytosynthesis or plant mediated synthesis of AuNPs exploit plant natural phytochemicals particularly polyphenols to bio-reduce and bio-cap $Au^{+3}$ to $Au^{o}$. The bio-manufactured AuNPs were reported previously to possess exclusive therapeutic activity against microbial infections, aging, cancers and cosmetic ailments (Saraswat, 2022). The AuNPs production using plant material have proven to be user and environment friendly method, because it omits the utilization of toxic chemicals and high energy inputs such as heat and pressure (Eckelman et al., 2008). Along with it, the rationale behind effective therapeutic properties of these phyto-synthesized AuNPs is considered to be the synergism of medicinal phytochemicals with that of gold ions plus fabricated nano-size structures which could penetrate easily in the biological membranes and imparts its medicinal value (Mahakham et al., 2016). Phytosynthesized AuNPs have opened a promising dimension in the field of medicine for drug delivery approach (Unterlass, 2016). The monolayer of AuNPs can aid to transport the drug to the targeted sites such as nucleic acids and proteins. The AuNPs can also be conjugated with the selected drug using polyethylene glycol or physically adsorbed to the surface using ionic bonds. The transport of the payloads is investigated to occur in presence of in-vivo (pH, cellular enzymes and glutathione) and in-vitro (temperature) stimuli (Su, 2017). Pakistan is a country which is rich in medicinal plant flora. According to documented data, approximately 5000 plants possess medicinal properties. Out of 5000, 600 medicinal plants are exploited by herbalists to treat many diseases (Labiad et al., 2020). As an under-developed country, the population mostly depends on the herbal medicine formulations to treat the affliction (Chaachouay et al., 2022). Likewise, Acacia modesta Wall. is known to be effective medicinal plant to remediate

chronic inflammation, menstrual cramps, fever, injuries and even cancers (Atiya et al., 2022) . The plant is locally called as Palosa and Phulai, which belongs to Family Mimosaceae. The whole plant including flowers, fruits, back, stem, roots and leaves possess medicinal phytochemicals, which imparts certain medicinal activity. In Pakistan, the plant is widely found in all provinces including Khyber Pukhtoon Khuwa (KPK) (Qadri, 2012). In Ayurveda, the fruit and leaves concentrates were used to treat bacterial wound infections, constipation, fatigue and chronic dry cough (Kumar et al., 2012). The soft twigs are chewed and tapered to brush like form, to clear oral plaque. The twig-like-brush is locally termed as 'Miswak'(Munir et al., 2020). Considering the medicinal properties of *A. modesta*, the current research investigation was plotted in order to biologically reduce, produce and stabilize AuNPs using aqueous leaves extracts of the plant. Then comparative assessment of AuNPs and aqueous leaves extract was preceded for antimicrobial, anti-leishmanial and cytotoxic activity.

## MATERIALS AND METHODS

### 3.1. Collection of Leaves

Leaves of *Acacia modesta* were procured from Forest Institute, Peshawar. The identification of sample was conducted by Prof. Dr. Farrukh Hussain, Director at Institute of Biological Sciences, Sarhad University of Science and Information Technology, Peshawar.

### 3.2. Phyto-chemical Analysis of Leaves of *A. modesta*

The selected leaves of *A. modesta* was investigated for their active phytochemicals mainly alkaloids, polyphenols, flavonoids, reducing sugars, tannins, steroids and saponins following protocol reported by (Lakshmibai et al., 2015). These phyto-chemicals act as natural reducing and capping agents that aid to fabricate green AuNPs.

### 3.3. Preparation of Aqueous Leaves Extract

The collected leaves of *A. modesta* were cleaned and washed using distilled water. Then it was subjected to shade drying at room temperature. The dried leaves were then powdered using electric grinder. The net weight of the powdered leaves was 5 kg. From the 5 kg leaves powder, 25 gm was boiled in a sterile flask containing 500 mL distilled water. After 30 minutes of boiling, the extract was filtered off using Whattman filter paper No. 1. Finally, aqueous extract of *A. modesta* was procured in the form of filtrate, which was then stored in blue cap sterile bottles for further use (Ahmad et al., 2016).

### 3.4. Phyto-synthesis of AuNPs

90 mL $AuCl_3$ solution (1mM) was reacted with 10 mL of aqueous leaves extract in a sterile conical flask. The reactant concoction was then subjected to shaking water bath for 1 hour at 75 $^oC$. At the end of incubation time, the color change from yellowish green to dark blackish brown indicates the precise production of AuNPs. The bio-reduced AuNPs were then dried using traditional spread drying method utilizing hot plate at $\leq$50 $^oC$. The dried AuNPs were then scratched and purified at 12,000 rpm using ultra-centrifuge. The supernatants were then discarded and purified AuNPs in form of pellets were then collected and dried in hot air oven at $\leq$50 $^oC$ (Amina et al., 2021).

### 3.5. Spectroscopic Characterization of Phyto-synthesized AuNPs

#### 3.5.1. Ultravoilet-Visible Spectrophotometery

The λmax peak absorbance of the fabricated AuNPs was analyzed UV-Vis spectrophotometer (UH4150AD) under the spectrum range of 400 – 600 nm (Ahmad et al., 2016)

#### 3.5.2. Scanning Electron Microscopy (SEM)

Surface area, functional stability and structure of AuNPs were analyzed by using SEM (JSM–5920, Japan). The thin layer test sample was applied on the copper grid, which was dried using mercuric

lamp. Then micrographs of AuNPs were observed at 1000X, 5000X and 10,000X magnifications (Lee & Moon, 2020)

### 3.5.3. Transmission Electron Microscopy (TEM)

Detailed investigation of internal structure and size of AuNPs was estimated by using TEM (Techni-G2-300kV, USA). Minute quantity of test sample was applied and air dried using aluminium stubs. Then the 2-D micrographs were observed at 1000X, 5000X and 10,000X magnifications (Lee & Moon, 2020)

### 3.5.4. Energy Dispersive X-Ray Spectroscopy (EDX)

For elemental analysis, EDX (INCA-200) spectrophotometer was used. For the purpose, minute quantity of test sample was added on copper grids, which was then analyzed for presence of various organic and reduced gold particle spectroscopic peaks at variable intensities (Lee & Moon, 2020)

### 3.5.5. X-Ray Diffration Measurements (XRD)

Crystal lattice structure of phyto-synthesized AuNPs was analyzed using XRD spectrophotometer (JDX–3532, Japan). The 2θ value at variable intensities was estimated to analyze the average size of the particles according to Beer Lambert Law (Lee & Moon, 2020).

### 3.6. Pharmacological Analysis of AuNPs

Comparative pharmacological analysis was conducted for green A uNPs and aqueous leaves extract by following protocols for antibacterial, antifungal, antileshmanial and cytotoxic assays.

### 3.6.1. Antibacterial Assay

For antibacterial assay of AuNPs and crude aqueous leaves extract of *A. modesta*, well diffusion method was followed (Lee & Moon, 2020). First, 3 mg/mL stock solution was formulated using aseptic DMSO. Then 100 µL working solution, from the stock was added to 6 mm wells prepared on sterile nutrient agar plates. Further, test bacterial broth cultures were seeded on the petri-plates. The culture plates were left undisturbed in laminar flow hood for at least 30 minutes to diffuse the test samples into the media. Finally, all the culture plates were incubated at 37ºC for 24 hours in upright position. Sterile DMSO was used as negative control while for positive control standard drug Amoxicillin was used. After 24 hours of incubation, percent inhibition was estimated by computing the zone of inhibition using following formula;

$$\%\ \text{Inhibition} = \frac{\text{Zone of Inhibition of test Sample (mm)}}{\text{Zone of Inhibiton of Standard (mm)}} \times 100$$

### 3.6.2. Antifungal Assay

Slant agar dilution method (Ahmad *et al*., 2017) was followed to analyze antifungal potentials of AuNPs and crude aqueous leaves extracts. First 24 mg/mL stock solution of the test samples was prepared in sterile DMSO. The SDA media was then supplemented with 70 µL working solution of AuNPs and crude aqueous extracts. The media containing test tubes were then placed in slanting position to solidify. The selected pathogenic fungal species were then inoculated on SDA supplemented media. All the test tubes were then sealed and incubated in fungal incubator at 28ºC for 1 week. For standard negative control, sterile DMSO was used while for positive control standard antifungal drug Miconazole was used. At end of incubation period, percent inhibition was estimated using the formula given below;

$$\%\ \text{Inhibition} = \frac{\text{Linear growth of fungi in test sample (mm)}}{\text{Linear growth of fungi in Standard (mm)}} \times 100$$

### 3.6.3. Cytotoxic Assay

For cytotoxic assay of AuNPs and crude aqueous leaves extracts Brine shrimp lethality assay (Shaheen & Rahman, 2019) was followed. In a minute glass tank brine solution was introduced along with shrimp eggs. It was allowed to hatch in 48 hours with continuous light source. After hatching, 30 nauplii were subjected to reaction mixture of 10 mL brine solution and test samples. Variable test sample dilutions i.e. 10, 100 and 1000 µg/mL was prepared in analytical grade methanol for the analysis. Standard drug Etoposide was used as positive control. After 24 hours, the viability of nauplii was observed and percent mortality was estimated by using formula given below.

$$\% \text{ Mortality} = \frac{\text{Number of shrimps survived in test}}{\text{Number of shrimps introduced}} \times 100$$

### 3.6.4. Antileshmanial Assay

For anti-leshmanial assay of AuNPs and crude aqueous leaves extracts were 96- well serial dilution protocol (Abdelwahid *et al.*, 2019) was followed against *Leishmania major*. Macrophages cell line (J774) was added to 96 well micro-titer plate containing DMEM supplemented medium. The media was supplemented with 10% fetal bovine serum. Approximately, $1.5 \times 10^5$ cells were subjected to each well. Immediately, *L. major* promastigotes were added to each well, and incubated at 23 ºC for 4 hours. Then, the monolayer was washed with warm HBSS solution to remove non-adherent cells. Further multiple dilutions i.e. 1 - 100 1 µg/mL of AuNPs and crude leaves extract were added. All the test samples were then incubated for 72 hours at 23 ºC. The wells were then washed and replaced with 500 µL Schneider's medium, supplemented with 10% FBS and 2% human urine. Again, the reactants were incubated at 23 ºC for 72 hours. Finally, amastigotes were estimated microscopically.

## RESULTS

### 4.1. Phyto-chemical Analysis of Leaves of *A. modesta*

From the phytochemical analysis of leaves of *A. modesta*, it was manifested that polyphenols, flavonoids and rerducing sugars are present in higher amounts. While the alkaloids, tannins, steroids and saponins are present in moderate quantity. The results are summarized in Table 4.1. The presence of these phytochemicals promotes the green synthesis of AuNPs in cost effective and ecofriendly manner due to their easy availability, less purification steps and brisk production. It is also manifested that these plant compounds possess therapeutic properties which can act in conjunction with $Au^{+3}$, to increase their medicinal potency in least doses.

**Table 4.1.** Phytochemical analysis of *A. modesta* (leaves)

| PHYTO-CHEMICALS | PRESENCE (+) / ABSENCE (-) |
|---|---|
| Alkaloids | ++ |
| Flavonoids | +++ |
| Polyphenols | +++ |
| Reducing Sugars | +++ |
| Tannins | ++ |
| Steroids | ++ |
| Saponins | +++ |

**Key: + :** Present in less amount, **++ :** Present in moderate amount, **+++ :** Present in higher amounts

### 4.2. Spectroscopic Characterization of Phyto-synthesized AuNPs

#### 4.2.1. Ultravoilet-Visible Spectrophotometery

From UV-Vis spectrophotometer analysis, it was manifested that the λmax absorbance for AuNPs lies at 550 nm, which supports the accurate synthesis of AuNPs. In comparison, λmax absorbance peak was deficient for aqueous leaves extract of *A. modesta*. The results are summarized in Fig. 4.1 and Fig. 4.2 respectively.

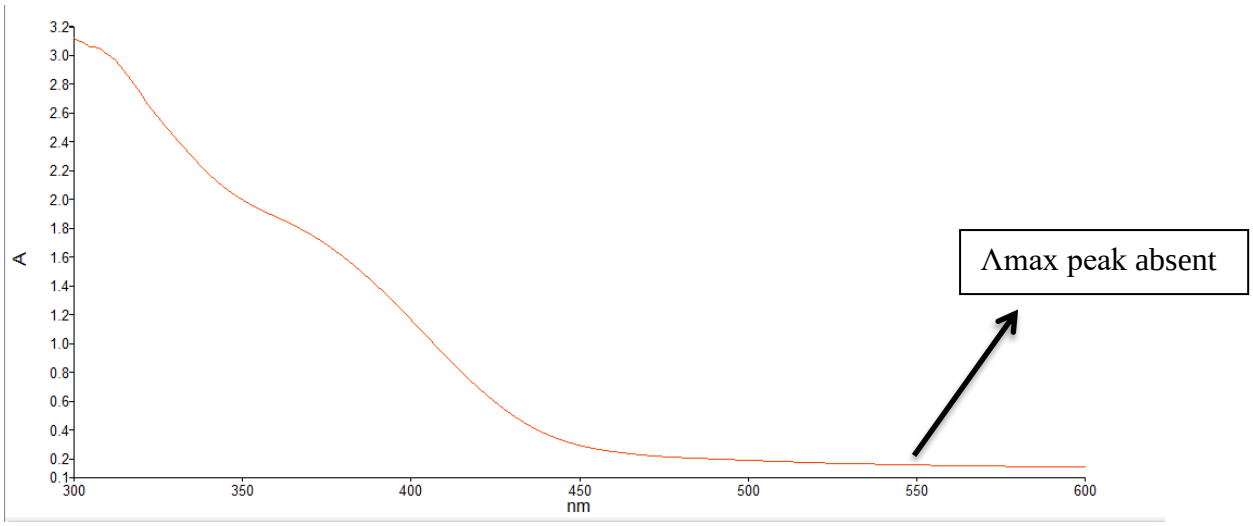



**Fig. 4.1.** UV-Vis analysis of aqueous leaves extract

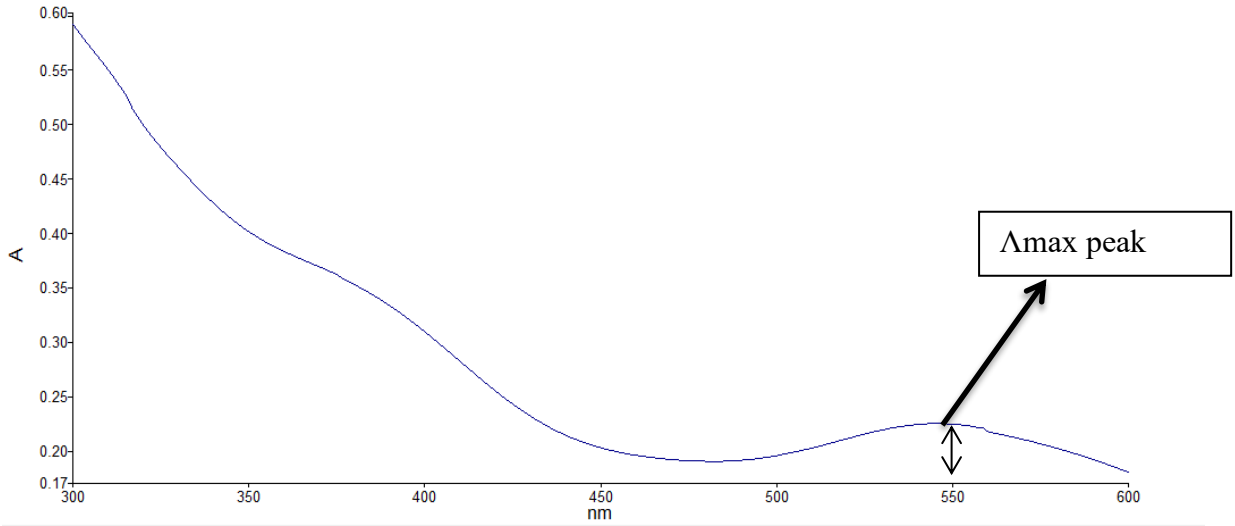
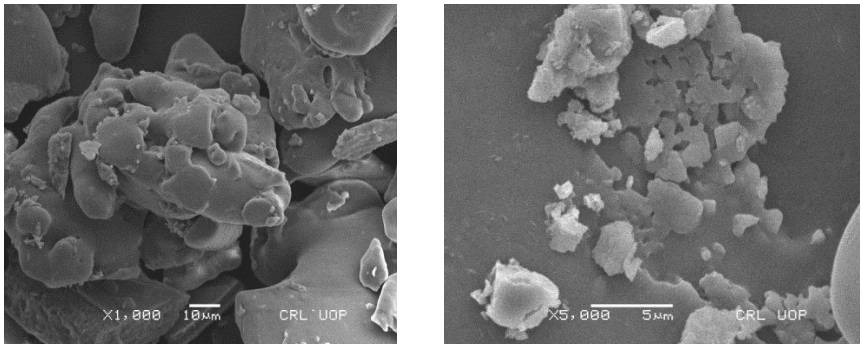



**Fig. 4.2.** UV-Vis analysis of green AuNPs

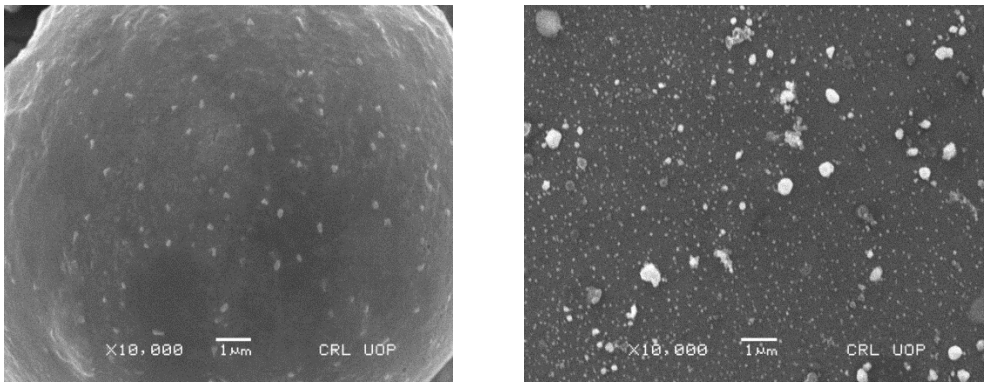
### 4.2.2. Scanning Electron Microscopy (SEM)

From SEM investigation, it was analyzed that aqueous leaves extract of *A. modesta* showed uneven and poly-dispersed morphology. While in comparison, biosynthesized AuNPs possessed unique mono-dispersed and spherical morphology. Little irregular and triangular morphology were also observed. The nano-spherical AuNPs were observed to be stable and about 1 µm in diameter. The SEM images of aqueous leaves extract and green AuNPs are displayed in Fig. 4.3 (a , b) and Fig. 4.4 (a , b).




**(a)** **(b)**

**Fig. 4.3 (a , b).** SEM analysis of aqueous leaves extract

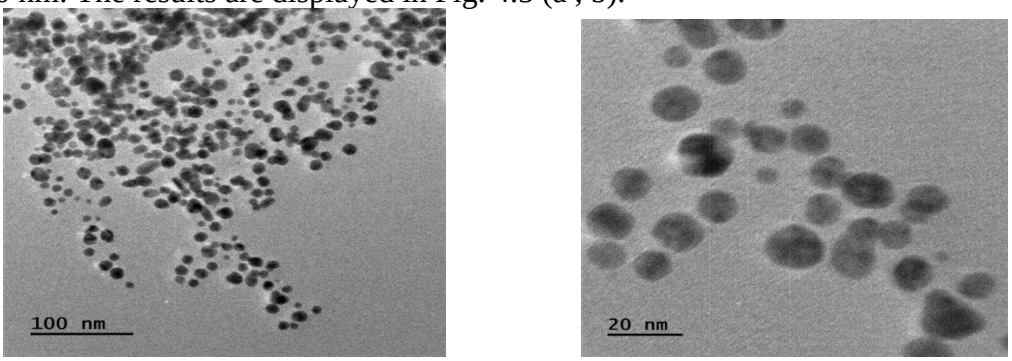



**(a)** **(b)**

**Fig. 4.4 (a , b).** SEM analysis of green AuNPs

### 4.2.3. Transmission Electron Microscopy (TEM)

From detailed TEM analysis, it was observed that the bio-reduced AuNPs are mostly spherical in shape. A small number of irregular morphologies were also observed which could be due to agglomeration of nano-spheriods. The average size of these nano-spherical AuNPs were in the range of 20 – 100 nm. The results are displayed in Fig. 4.5 (a , b).




**(a)** **(b)**

**Fig. 4.5 (a , b).** TEM analysis of green AuNPs

### 4.2.4. Energy Dispersive X-Ray Spectroscopy (EDX)

From EDX analysis, it was testified that the green AuNPs were precisely reduced by aqueous leaves extract of *A. modesta*. The sharp peak of $Au^o$ in reduced form supports the efficient strategy of phyto-synthesis. In comparison, the absence of $Au^o$ peak in aqueous leaves extract shows that plant extract have the capability to naturally reduce to ionic gold to particle nature. Along with $Au^o$, other organic elements such as carbon, nitrogen, potassium, oxygen, calcium, sodium, chlorides and magnesium were also observed. The results of elemental analysis are summarized in Fig. 4.6 and 4.7.

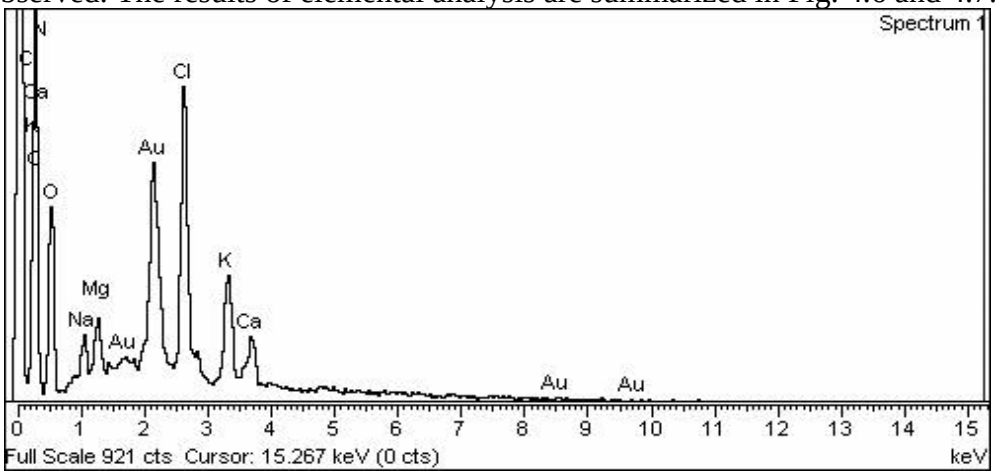



**Fig. 4.6.** EDX analysis of green AuNPs

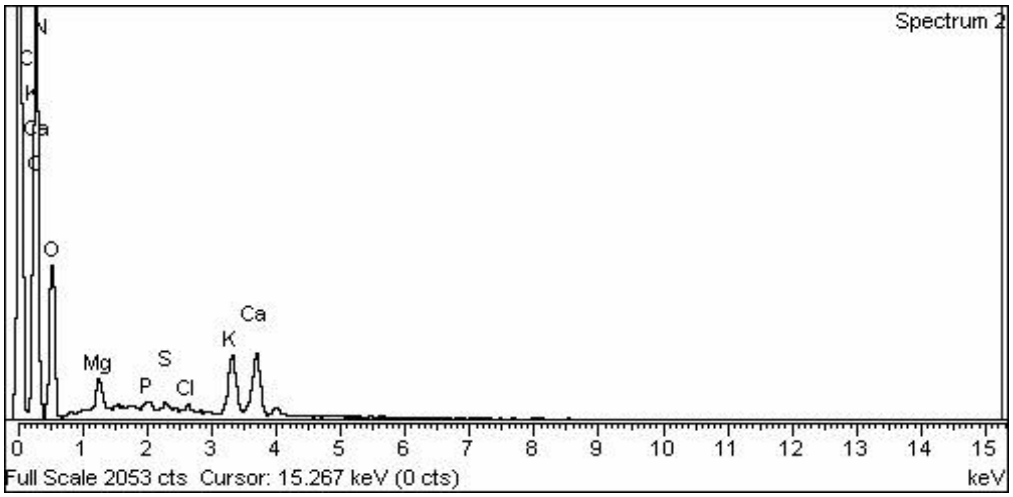



**Fig. 4.7.** EDX analysis of aqueous leaves extract

### 4.2.5. X-Ray Diffraction Measurements (XRD)

From XRD analysis, it was observed that sharp 2 theta peaks at variable intensities manifest that the phyto-synthesized AuNPs were crystalline structures. While in comparison, the deficient 2 theta peaks at variable intensities manifest that aqueous leaves extract of A. modesta is amorphous structure. The XRD results are summarized in Fig. 4.8 and 4.9.

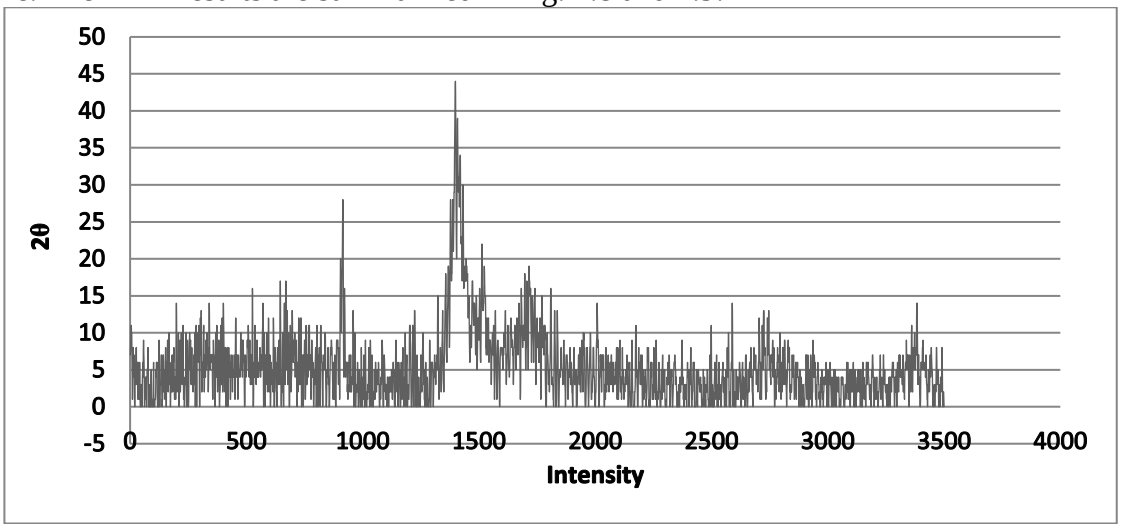



**Fig. 4.8.** EDX analysis of green AuNPs

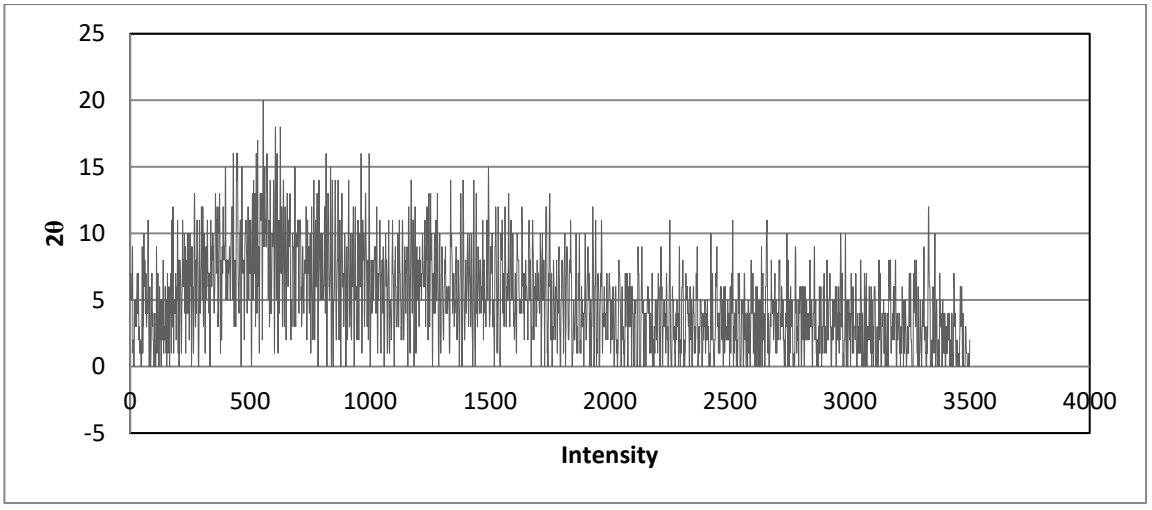



**Fig. 4.9.** XRD analysis of aqueous leaves extract

### 4.3. Pharmacological Analysis of AuNPs

From the comparative pharmacological assays of AuNPs and aqueous leaves extracts, following results were observed;

#### 4.3.1. Antibacterial Assay

From comparative antibacterial assay of aqueous leaves extract and AuNPs, it was analyzed that AuNPs possess good bactericidal activity against *S. aureus* (62%). Moderate bactericidal activity was observed against *E.coli* (41%), *S. pyogenes* (44%) and *K. pneumonia* (53%). *P. aeruginosa* (21%) and *S. typhi* (28%) were poorly inhibited by AuNPs. In contrast, aqueous leaves extract manifested low activity against all test bacterial species and *P. aeruginosa* was resistant to it. The results are summarized in Fig. 4.10.

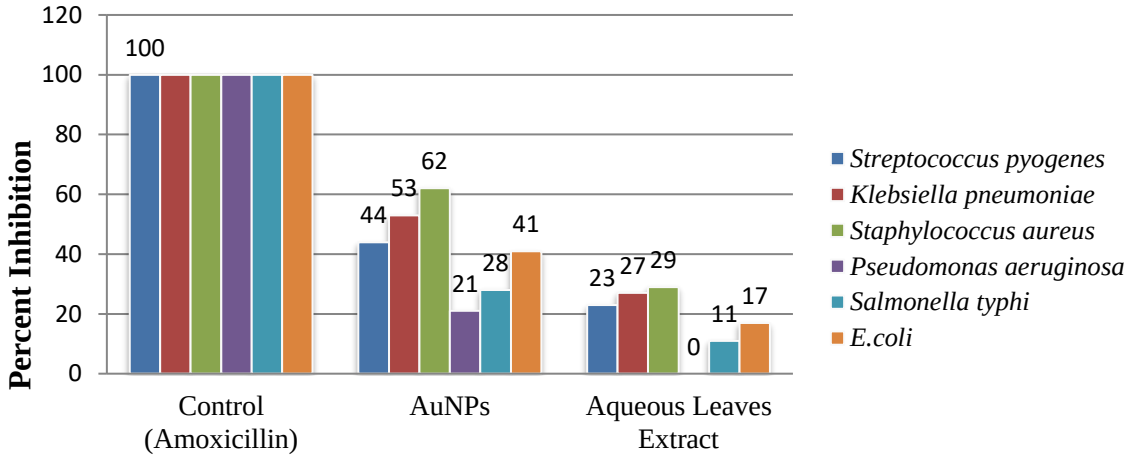



**Fig. 4.10.** Comparative antibacterial assay of AuNPs and crude aqueous leaves extract from *A. modesta*

#### 4.3.2. Antifungal Assay

From comparative antifungal assay it was analyzed that AuNPs excellently inhibited all test fungal growth. Highest percent inhibition was observed against *F. solani* (92%), *F. oxysporum* (91%) and *M. furfur* (90%). Similar growth inhibition pattern was manifested by *C. albicans* and *Penecillium* spp. i.e. 89%. Good antifungal activity was observed against *T. harizanum* (75%) and *A. flavus* (74%). In contrast, the aqueous leaves extract showed moderate to good results against all test fungal species. The highest inhibitory activity was observed against *Penecillium* spp. (69%) and *F. oxysporum* (67%). While *A. flavus* was poorly inhibited i.e. 29%. The results are summarized in Fig. 4.12.

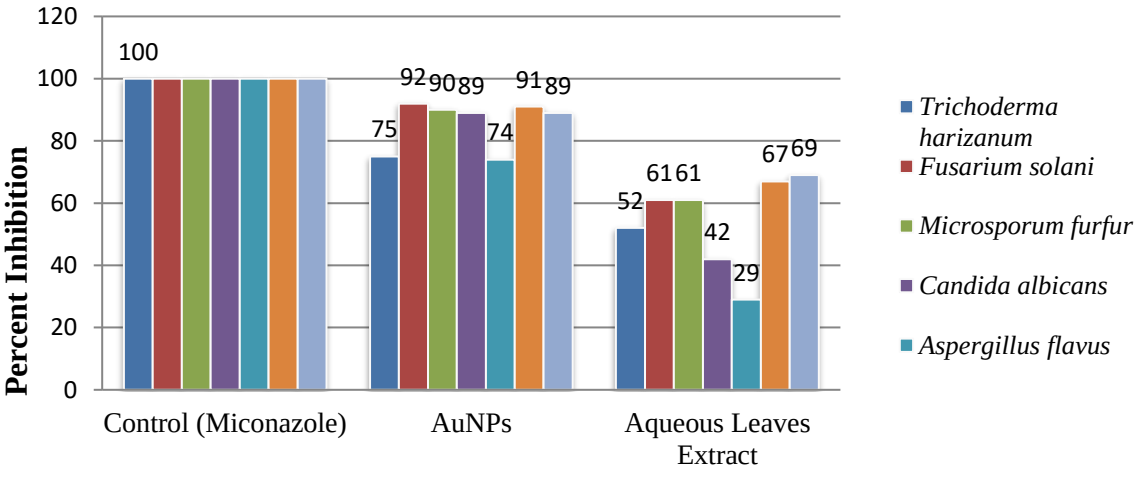



**Fig. 4.11.** Comparative antifungal assay of AuNPs and crude aqueous leaves extract from *A. modesta*

### 4.3.3. Cytotoxic Assay

From the comparative cytotoxic assay, it was analyzed that AuNPs even at highest sample concentration i.e. 1000 µg/mL does not showed cell toxicity and remained inactive against brine shrimp nauplii. In contrast, at highest sample concentration, aqueous leaves extract showed less cell toxicity i.e. 30% against the brine shrimp nauplii. Standard drug, Etoposide at least concentration 7.5 µg/mL, showed 70% cell mortality as compared to the test samples. The results are summarized in Fig. 4.13.

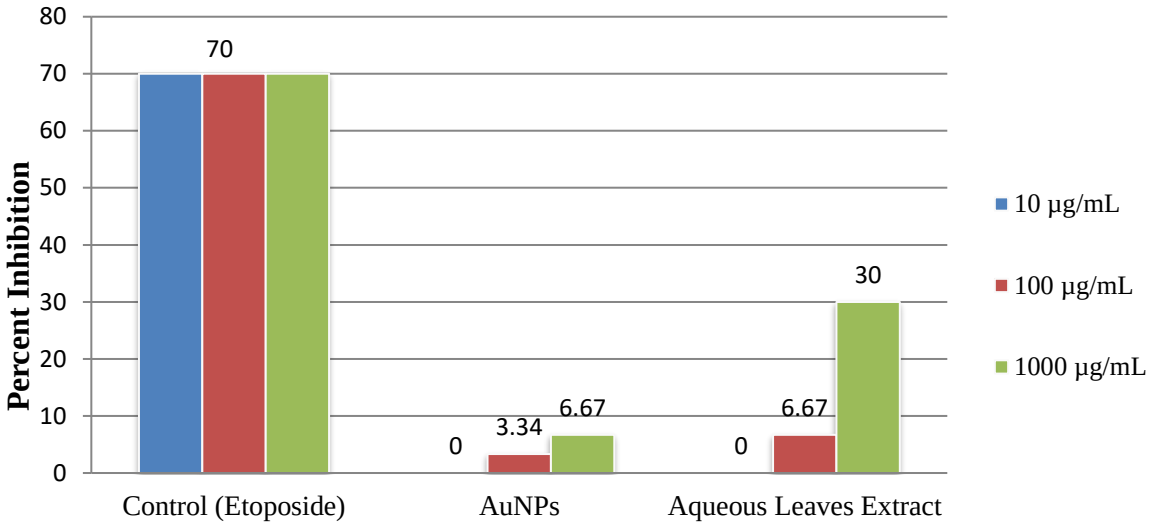



**Fig. 4.12.** Comparative cytotoxic assay of AuNPs and crude aqueous leaves extract from *A. modesta*

### 4.3.4. Anti-leshmanial Assay

From comparative anti-leshmanial assay, it was analyzed that promastigotes transition to amastigotes can be efficiently inhibited by AuNPs at sample concentration ≥ 50 µg/mL. The IC50 value for AuNPs was observed as 50.2 ± 0.01. Below the stated concentration, the sample remained inactive against *L. major*. The activity can be analogue of standard anti-leshmanial drug Miltefosine, which shows $IC_{50}$ value approximately 42.6 ± 0.42. In contrast, aqueous leaves extracts of *A. modesta* remained inactive against proliferation of *L. major* even at highest sample concentration i.e. ≥ 100 µg/mL, thus cannot be exploited as anti-leshmanial drug.

## DISCUSSION

Nanobiotechnology or nanobiology is modish sphere of science, which focuses on manipulation of atoms to mould into desired product. Currently, nano-biologist have integrated multiple fields such as engineering, agriculture and therapeutics with that of nanotechnology, to device efficient commodities such as biofertilizers, targeted biosensor and medicines, diagnostic tools and even nano-infused coatings (Marzi et al., 2022). There are efficient chemical and physical methods which could assist in mass-fabrication of these nanostructures. But most of these protocols are highly toxic, expensive and non-budget friendly (Khan et al., 2019). Switching to green method using microbes, plants and enzymes is a favorable advancement to the sphere (Gour & Jain, 2019). In previous years, metal nanoparticles are bio-reduced from their ionic state to particle nature using plant extracts produce excellent outcomes. These phyto-synthesized metal nanoparticles particularly silver, gold, zinc and copper have been employed in many nanodevices to benefit mankind (Dappula et al., 2023). Gold nanoparticles synthesized by using plant extracts are preferred over conventionally methods because it is eco-friendly, cost effective having excellent medicinal properties (Santhosh et al., 2022). Many plants are investigated to produce AuNPs such as *Aloe vera, Bauhinia purpurea, Cinnamomum camphora, Dodonaea genus, Musa acuminata colla, Mentha longifolia* and *Emblica officinalis*. These nano-gold particles were characterized to be stable crystalline in structure having size range of

10 – 100 nm. The AuNPs were spherical and oval in morphologies which could tend to agglomerate after longer incubation period (Khanna et al., 2019). Similar to the current study, leaves of *A. modesta* possess phenolic compounds and reducing sugars which mediate the economic and ecofriendly synthesis of AuNPs. The morphology of AuNPs were similarly to that of previously fabricated AuNPs i.e. mostly spherical in shape having size range of 20 – 100 nm. Few irregular shapes were also testified, which could be due to agglomeration phenomenon. Recently, it has been investigated that green AuNPs possess tremendous therapeutic benefits that ranges from cosmetic improvement to remediating chronic cancers. It was previously investigated that aqueous leaves extracts of *Opuntia ficus-indica, Anacardium occidentale, Bauhinia tomentosa,Chenopodium album, Dracocephalum kotschyi* and *Camellia sinensis* have potentials to biosynthesize AuNPs. These AuNPs were observed to lie in the range between 10 – 200 nm. (Siddiqi et al., 2018). These fabricated nanoparticles were investigated to act in synergism with that of plant extracts to treat many bacterial infections. The AuNPs were found to be active against E. coli, Staphylococcus aureus, Pseudomonas and Salmonella species (Nadeem *et al.*, 2017). Similar to current study, leaves mediated AuNPs of *A. modesta* were observed to be active against *Staphylococcus aureus, Klebsiella pneumonia, E.coli* and *Streptococcus* species. The green AuNPs were observed previously to efficiently possess antifungal properties. It was previously evidenced that AuNPs synthesized using aqueous leaves extract of *Punica granatum, Pistacia integerrima* and *Nepenthes khasiana* can inhibit the mycelial growth of *Candida albicans, Aspergillus flavus* and *Microsporum gypseum* (Amina et al., 2021). The similar to current study, AuNPs fabricated using aqueous leaves extracts of *A. modesta* can inhibit the fungal proliferation *T. harizanum, F. solani, M. furfur, F. oxysporum, Penecillium* and *Aspergillus flavus.* Leaves mediated AuNPs from *Maytenus royleanus* were also investigated to curb leishmanial infections (Ahmad et al., 2015). Similar to current findings, the AuNPs from *A. modesta* was active against leishmanial parasite. The $IC_{50}$ value was observed to be $50.2 \pm 0.01$ against *L. major*. Green method for synthesis AuNPs was evidenced from previous studies to be ecofriendly and user friendly. It was also testified to exhibit aced medicinal potentials with least side effects. (Husen et al., 2019) researched that green AuNPs that were synthesized using aqueous leaves extracts of *Acacia nilotica* and *Olea europaea* were inactive against normal cell lines at higher sample concentrations. The various histopathological studies showed AuNPs pose least side effects and thus exhibit therapeutic potency in smaller doses. Similar to current findings, cytotoxic study against healthy cell line showed that AuNPs were non-toxic even at highest sample dilution i.e. 1000 µg/mL. Hence, the findings can help in formulations of novel drugs which could act as smart medicine to treat multiple afflictions.

## Conclusion

From the current research investigation, it was concluded that leaves of *A. modesta* have the potential phyto-chemicals that could mediate the active synthesis of AuNPs by acting as natural reducing and capping agent.The fabricated AuNPs were analyzed to be crystalline nano-spherical having size range of 20–100 nm. In comparison to crude aqueous leaves extract from *A. modesta*, AuNPs exhibited excellent antimicrobial and anti-leshmanial activity. The AuNPs are active against all test bacterial and fungal species. Excellent anti-leshmanial activity was evidenced which was similar to Miltefosine. Least cytotoxic activity was evidenced even at 1000 µg/ mL. The findings can be exploited in medical fields to formulate novel therapies.

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