Characterization and Biological Evaluation of Gold Nanoparticles Synthesized from *Taverniera nummularia* DC Crude Extract

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Abstract

The current study was aimed to synthesize and characterize *Taverniera nummularia* crude methanolic extract gold nanoparticles and to explore their biological potential. The gold nanoparticles synthesis was carried out by using previously reported protocol. The characterization of the nanoparticles was done by using various techniques including UV-Visible, FT-IR spectroscopy and XRD techniques. The extract assisted gold nanoparticles were synthesized at a wavelength of 520nm. The UV-vis spectroscopy of synthesized gold nanoparticles showed distinguishing peak at 520 nm indicated the formation of nanoparticles. Different functional groups were identified by using FT-IR spectroscopy. The XRD was utilized to find crystallographic planes of the face-centered cubic gold crystals. The average size of the synthesized gold nanoparticles obtained was calculated to be 38.57 nm. The synthesized nanoparticles were also subjected for their biological potential determination and significant antimicrobial activities were noted.

Keywords: VU spectroscopy, FTIR, SEM, antimicrobial, *T. nummularia*

1: Introduction

The synthesis of plant extract loaded gold nanoparticles is a method which is eco-friendly in nature and it facilitates the nanoparticles synthesis in "one pot process". This is due to the fact that plant extract acts as a capping and reducing agent for the synthesized nanoparticles[1-3]. Many

plants contain a complex structure of four flavonoids units [4], glucose, saponins, starch and alkaloids[5-8]. Most of the compounds, particularly flavonoids are related with metal complexation. Many plant extracts assisted nanoparticles have been reported as a good antibacterial and anti-mycotic agent against for both gram-positive, gram-negative pathogens, and as well as against fungi[9]. Their role as antiprotozoal agents is also well established[10]. They have a promising role in the treatment of skin related ulcers etc. As the plant extracts possess polyphenols, their role as an antioxidant agent by themselves is well established[11-13]. The plant extract loaded gold nanoparticles' optical properties are not only dependent on their size, shape etc. but also on the nature of the solvent in which they are dissolved. The optical properties are also dependent on the nature of stabilizing agents which acts as a capping agent[14-16].

Similarly, the catalytic properties of gold nanoparticles have been exclusively reported by the researchers[17-19]. Due to their catalytic properties, gold nanoparticles are being under use in many applications including contaminated water cleaning etc[20]. The genus *Taverniera* is a perennial and is branched in nature. The size varies from 1 to 2feets. It has trifoliated leaves. Leaves of *T. nummularia* are used as dressing for wounds smeared on the exterior on swelling, sore and abscesses. Patients suffering from throat infections use this plant as dye. Coughing can also be treated by its root's decoction.[21] The current work was assessed to synthesize and characterize *T. nummularia* extract loaded gold nanoparticles and to assess their biological potential. The current research is eco-friendly and cost effective.

2: Materials and Methods

2.1 Collection and Extraction of Plant

The plant *Taverniera nummularia* was collected from district Bannu, Khyber Pakhtoonkhwa Pakistan. Dr. Faizan ullah, Department of Botany, UST Bannu identified and confirmed the plant

as *Taverniera nummularia*. Voucher specimen was deposited at the herbarium of the university. The plant was shade dried and dried plant material was grinded into fine powder. The powdered plant material was then soaked into 80% aqu. methanol. After 5days, the material was filtrated and the filtrate was concentrated by using a rotary evaporator (Buchi Rota vapor R-200) at room temperature. The crude plant extract was weighed (200g) and kept at 4^{o}C for further process.

3.2. Extract Solution Preparation and Nanoparticles Synthesis

The plant mediated synthesis of gold nanoparticles was carried out by using general protocol. The synthesis was done by dissolving 2g methanolic dry extract of plant with 100 mL methanol. The dissolving was done by using hot plate magnetic stirrer for a period of 30 minutes at 50^{o}C. After cooling at ambient temperature, 5mL of extract was added in 45mL 1mM aqueous $HAuCl_4$ solution. The solution was kept for 2Hrs and synthesis of gold nanoparticles was observed visually by color change to ruby red. The solution was kept for 2 Hrs and synthesis of gold nanoparticles was observed visually by color change to ruby red.

2.3 UV-Visible Spectra

A double-beam (Shimadzu, UV1700) UV-Visible spectrophotometer was incorporated to get the extract's UV-Vis spectrum, in a measurement range of 200–400 nm, with a scan rate of 240 nm/min. "AuTm SPR" was monitored between 250 and 875 nm.

2.4 FTIR- spectrometry

The plant's extract and the FTIR were obtained by an FT-IR instrument (IR Prestige-21, Shimadzu 400-4000 cm^{-1}) by using a dense sample. The spectra were obtained on transmittance mode at a resolution of 2 cm^{-1}, from 4500 to 500 cm^{-1}.

2.5 X-ray Diffraction

The x-ray diffraction analysis was carried out for the measurement of size and structure exploration of the synthesized gold nanoparticles. The loading of the synthesized gold nanoparticles was done in x-ray diffractometer (PANalytical) that was operational at 4000 Volts and a current of 20mA. 2θ angle from 20° to 80° was used for scanning purpose at 0.02°/min, with 2θ time constant. The crystal structure of all materials was refined in order to get atom's accurate position.

2.6 SEM analysis

SEM analysis of the synthesized gold nanoparticles was obtained by using JEOL, Japan: Model MJSM5910 machine (Centralized Resource Laboratory, Peshawar).

2.7 Antimicrobial activities

The synthesized gold nanoparticles were assessed for their antibacterial activity using standard method. *Klebsiella pneumonia*, *Staphlococcus aureus* and *Serratia marcescens* were used in this activity. Similarly, the synthesized gold nanoparticles and extract numerous concentrations were assessed for their fungicidal potential using standard procedure.[22] Various fungal strains *Aspergillus niger, Aspergillus flavus,* and *Penicillium notatum* were used in antifungal activity.

2.8 Antioxidant activities

The synthesized gold nanoparticles were assessed for their antioxidant activities. In all three antioxidant activities, the various concentrations (50, 100, 150, and 200µg/mL) of the synthesized gold nanoparticles, extract and ascorbic acid were used.

DPPH radicals scavenging activity was assessed using standard method. [23]

ABTS radicals scavenging activity was carried out by using standard protocol.[24]

The"H_2O_2 (Hydrogen peroxide) radicals scavenging activity of the synthesized gold nanoparticles was assessed by the standard method.[25]

2.9 Cytotoxic Activity Using Brine Shrimp Lethality Test (BSLT)

Cytotoxic activity of the synthesized gold nanoparticles was evaluated according to the reported procedure.[26]

3: Results and Discussion

3.1 Synthesis

Synthesis of plant assisted gold nanoparticles by using as a capping agent was started just after few minutes of mixing both the extract solution with gold salt. Color reaction was perceived at regular breaks during which $HAuCl_4$ solution was transformed into ruby red. The color change confirmed the synthesis of gold nanoparticles. Further confirmation was done with the help of UV-visible spectroscopy. Based on literature data, the peak at 540 nm confirmed the synthesis of gold nanoparticles.[7]

3.2 UV-Visible Spectroscopy

The production of plant assisted gold nanoparticles was established by exposing the samples to UV-Visible spectroscopy. The "UV-Visible spectroscopy" of synthesized gold nanoparticles displayed characteristic peaks at 520nm that confirmed the gold nanoparticles synthesis. Once nanoparticles are synthesized, polyphenolic compounds in plant are absorbed on their surface providing solidity to nanomaterials. The results are shown in (Fig # 1).

3.3 Fourier Transform Infrared (FTIR) Spectroscopy

During this study, the methanolic crude extract's FTIR spectra was scrutinized before and after the production of gold nanoparticles and this was done in order to quantify the plausible functional groups for the formation of gold nanoparticles. The purpose of the analysis through FT-IR was the identification of the entities present in the extract that were accountable for metal ion reduction. The topping agent gives stability to the sample solution. The spectra displayed wide-ranging bands at 3400, 3000 and 1600 cm^{-1}, which signifies the occurrence of hydroxyl, CH unsaturated and carbonyl groups. The FTIR spectra of extract and gold nanoparticles are cited in (Fig # 2 and Fig # 3) respectively.

3.4 X-ray diffraction analysis

The XRD of synthesized gold nanoparticles is mentioned in the (Fig # 4). The plant extract pattern shows not a single peak assigned to crystalline structure. A general peak that was positioned at 22.56^{o} which could be assigned to organic material in plant extract. Upon addition of gold salt into the extract solution, the peak shifted to 38.5^{o}.

The plant extracts loaded gold nanoparticles pattern presented powerful peaks at 38.35°, 44.28°, 65.12° and 78.16° that could be accredited to 111, 200, 220, and 311 crystallographic planes of the face-centered cubic gold crystals, correspondingly. It is also predicted that the width in the peaks might also ascend from the local crystal defects. Origin software was used to analyze the data.

3.5 SEM Imaging

SEM investigation method was assimilated to measure the size of the synthesized gold nanoparticles as presented in (Fig # 5) SEM microscopy used. SEM grids that were prepared by

means of sample powder on a grid coated with copper. A lamp was used to dry the grids. The typical size of the produced gold nanoparticles attained was calculated to be 38.57nm."

3.6 Anti-bacterial activity of gold nanoparticles

The particular mode of action for antibacterial action of nanoparticles is not fully cleared however, several researchers have diverse school of opinions. Some of the investigators attribute the antibacterial activity of nanoparticles is due to their large surface area which provide enough space for microbial attachment with their surface. The study was steered to evaluate the possible antibacterial potential of synthesized gold nanoparticles against two Gram-negative bacterial strains *Serratia marcescens* and *Klebsiella pneumoniae* and one Gram-positive strain *Staphylococcus aureus*. The highest inhibition was shown by the synthesized gold nanoparticles against *Staphylococcus aureus* at the concentration of 150µg/ml which is 29.76±0.53. The next highest inhibition was shown against *Staphylococcus aureus* at the concentration of 100µg/ml which is 21±0.53 as compare to the simple crude methanolic crude extract. The comparative investigation of both crude methanolic extract and synthesized gold nanoparticles reveals that the antibacterial potential of the extract is enhanced with synthesized gold nanoparticales. These conclusions supported the ideas of other researchers that the increase in the activity is due to large surface area of gold nanoparticles. The results are displayed in (Table # 2).

3.7 Anti-fungal activity of gold nanoparticles

Antifungal activity of the extract and synthesized gold nanoparticles was assessed using varying concentrations of the extract and synthesized gold nanoparticles. Terbinafine use was carried out as positive and unadulterated DMSO as a negative control. The antifungal activity assessment exhibited inhibition of the strains *Aspergillus niger, Penicillium notatum* and *Aspergillus flavus* in a "concentration dependent way". The maximum doze of the extract and synthesized gold

nanoparticles inhibited all the fungal strains expressively. The highest inhibition was shown by synthesized gold nanoparticles synthesized from crude extract of *T.nummularia* plant against *Aspergillus niger* at the doze of 150µg/ml which is 46.2 ± 1.29. The next highest inhibition was shown against *Penicillium notatum* at the doze of 100µg/ml which is 41.66 ± 1.08 as compare of simple methanolic crude extract of the same plant. The results are displayed in (Table # 1).

3.8 DPPH activity of extract and synthesized gold nanoparticles.

The outcome of this radical scavenging assay (Fig # 6) displayed radical scavenging ability of the plant as well as synthesized gold nanoparticles. The foraging action of the extract was established to be amplified in growing concentrations as (150 < 100 < 150 < 200µg/mL).

3.9 ABTS activity of extract and synthesized gold nanoparticles.

The radical scavenging potential of extracts and gold nanoparticles using ABTS evaluation technique is built on making of a greenish/blueish ABTS cations that is suitable for the two hydrophilic and lipophilic antioxidant systems. The outcome of this study showed that the extract possessed radicals hunting potential in a concentration dependent manner. 150 and 200µgm/mL concentrations presented a momentous antioxidant action in contrast to positive control. The results are stated in (Fig # 7).

3.10 Hydrogen peroxide activity of extract and synthesized gold nanoparticles.

H_2O_2 augmented quantities in the human are transformed into free radicals and results in producing harmful effects on the body. Due to the reason aforementioned, both the extract and nanoparticles are judged for their strength to hunt these reactive species. The results presented realistic stalking ability of the extract in a concentration reliant way in contrast to standard (ascorbic acid). The outcomes are stated in the (Fig # 8).

3.11 Cytotoxic activity of extract and synthesized gold nanoparticles.

The activity is dependent on the toxic effects of synthesized nanoparticles and extract on unicellular organisms i.e., brine shrimps that are grown under meticulous settings. Followed by shrimps emerging, they were moved to tubing system having water of sea salt and numerous concentrations of the plant extract and nanoparticles. After 24 hrs, the effects were assessed and found an inverse relationship between the shrimps survival and samples concentrations. The results are mentioned in (Fig # 9)."

Conclusions

The current experimental facts supported that *Taverniera nummularia* could be helpful in providing an environmentally friendly and rapid route of synthesis of gold nanoparticles which can be used in many clinical applications. FT-IR analysis showed that crude methanolic extract of plant *T.nummularia* exhibited the important functional groups which were responsible for the synthesis of AuNPs. XRD analysis showed face-centered cubic gold crystal structure and the average size was calculated to be "38.57nm" of plant base AuNPs. SEM analysis showed cubic gold crystal morphology and aggregation properties of plant base AuNPs. The antimicrobial activities of synthesized gold nanoparticles showed significant antibacterial and antifungal potential. The antioxidant activities of synthesized gold nanoparticles showed significant free radical scavenging potential. It is also concluded that the synthesized gold nanoparticles using *T. nummularia* as a capping agent showed significant cytotoxic activity, hence shows anticancer effects. It is therefore, recommended to evaluate *T. nummularia* potential against these studies using more in-vivo investigations for its probable use in new therapeutic agent.

Figures and Tables

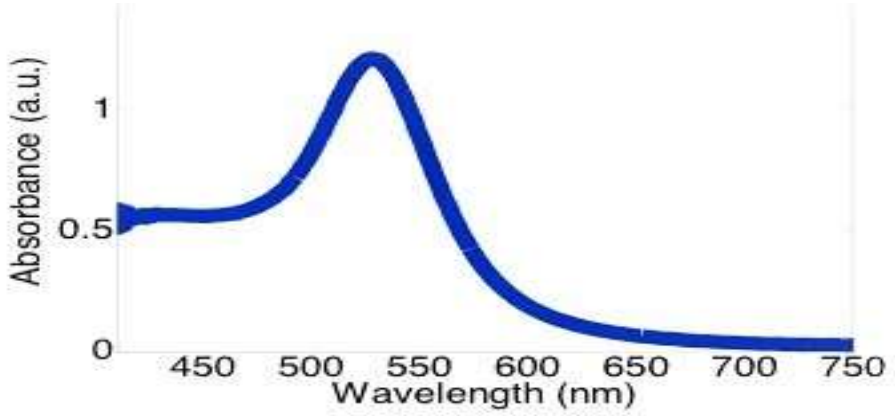

Figure 1. UV-Visible spectrum of the synthesized gold nanoparticles.

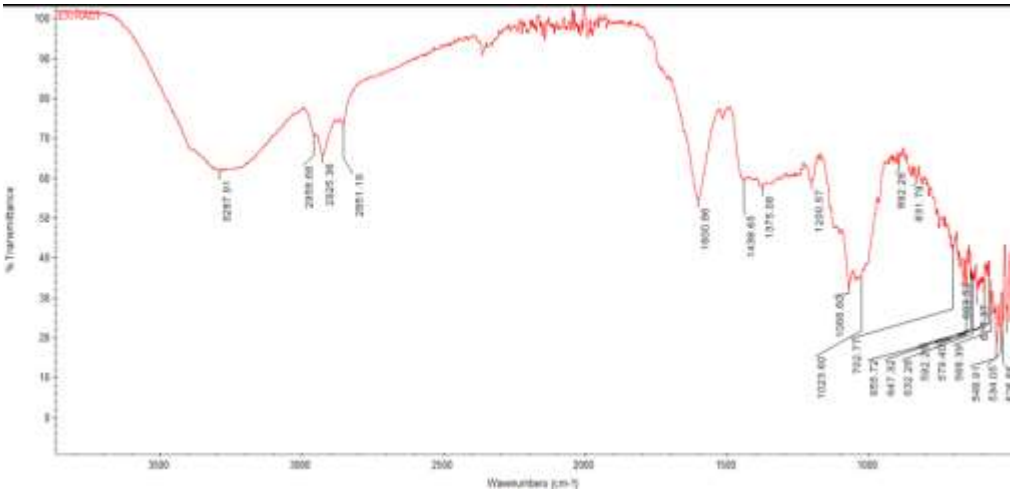

Figure 2. FTIR spectrum of crude methanolic extract of *T.nummularia.*

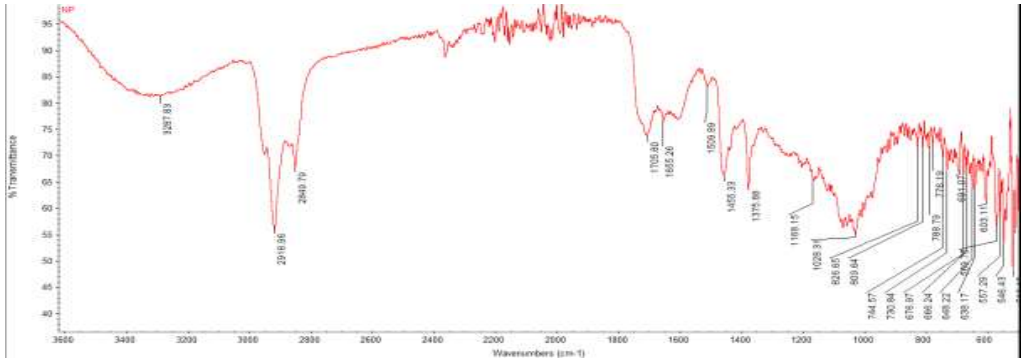

Figure 3. FTIR spectrum of synthesized gold nanoparticles from *T.nummularia.*

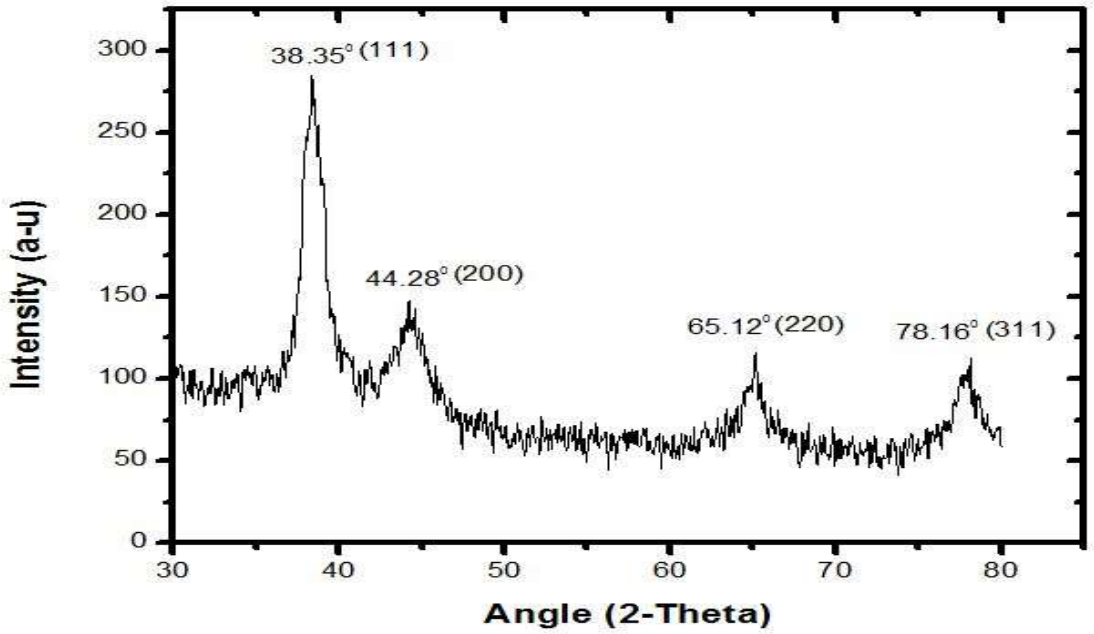

Figure 4. X-ray diffraction spectrum of synthesized gold nanoparticles from *T.nummularia.*

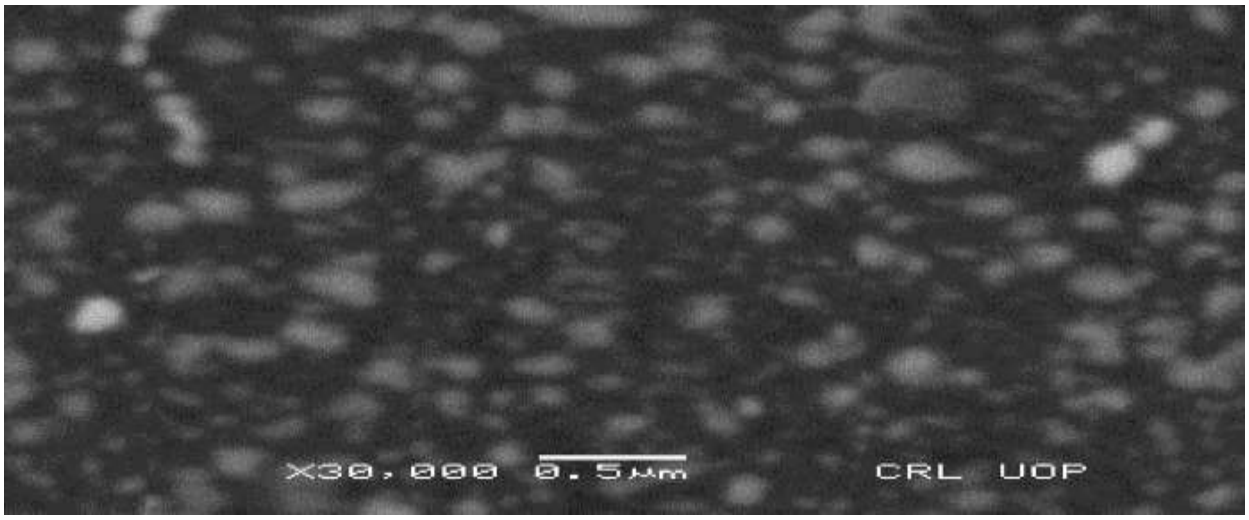

Figure 5. SEM image of the synthesized gold nanoparticles from T.nummuaria.

Standard (100µg/ml)	***Aspergillus niger***	***Aspergillus flavus***	***Penicillium notatum***		
Terbinafine	73.66±1.69	68.33±2.05	70.6±1.72		
Fungal Strains	**Sample**	**DMSO**	**50µg/ml % inhibition**	**100µg/ml % inhibition**	**150µg/ml % inhibition**
Aspergillus niger	AuNPs	0	36.2±1.17	38.6±1.84	46.2±1.29
	Extract	0	21.5±0.74	25.4±1.14	32±2.44
Aspergillus flavus	AuNPs	0	28.3±2.05	33.26±1.26	38.6±1.24
	Extract	0	20.33±2.05	26.3±1.58	31.06±1.35
Penicillium notatum	AuNPs	0	35.36±0.71	41.66±1.08	44.6±1.15
	Extract	0	24.5±1.34	32.6±1.06	38.3±1.24

Table 1. showing the comparative Antifungal potential of crude methanolic extract and synthesized gold nanoparticales of *T.nummularia*.

Standard (100µg/ml)	***Serratia marcescens***	***Klebsiella pneumonia***	***Staphylococcus aureus***		
Levofloxacin5mg/mL	40.4±0.163	48.3±0.081	32.56±0.124		
Bacterial Strains	**Sample**	**DMSO Inhibition Zone**	**50µg/ml Inhibition Zone**	**100µg/ml Inhibition Zone**	**150µg/ml Inhibition Zone**
Serratia marcescens	AuNPs	0	14.7±0.81	17±0.1	22.3±0.081
	Extract	0	6.62±0.008	15±0.081	19.5±0.20
Klebsiella pneumonia	AuNPs	0	15±0.32	18.5±0.41	26.3±0.96
	Extract	0	10.86±0.24	16.9±0.78	19.33±1.24
Staphylococcus aureus	AuNPs	0	11.5±0.57	21±0.53	29.76±0.53
	Extract	0	8.7±0.82	18.2±0.95	22.46±0.61

Table 2. Showing the comparative Antibacterial potential of crude methanolic extract and synthesized gold nanoparticales of *T.nummularia*.

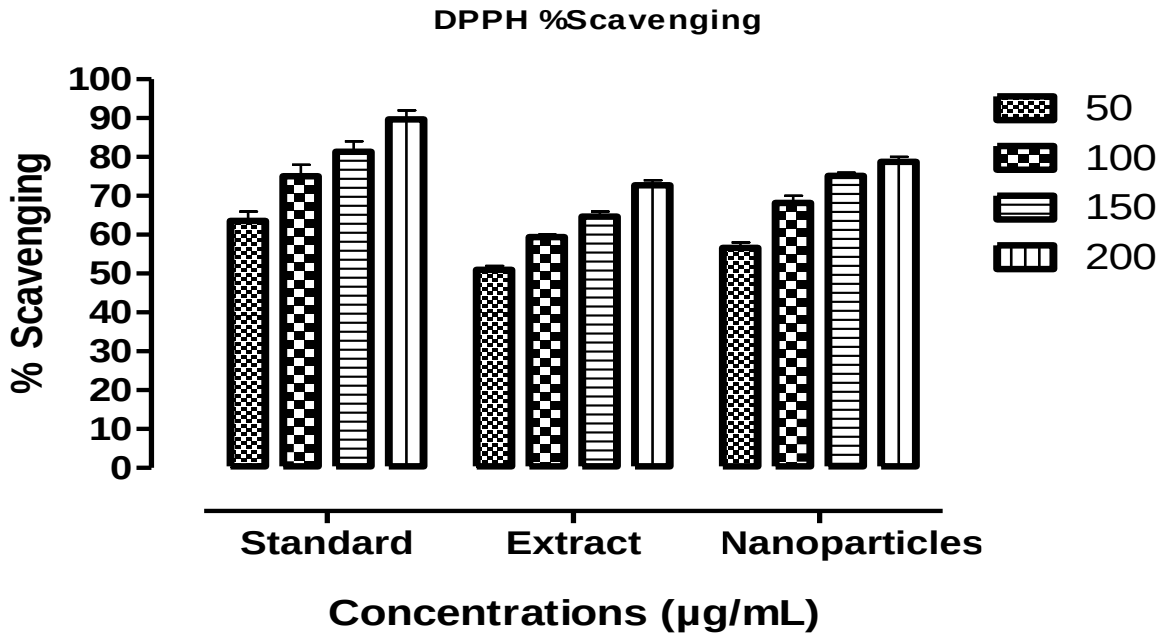

Figure 6. DPPH radical scavenging activity of extract and nanoparticles

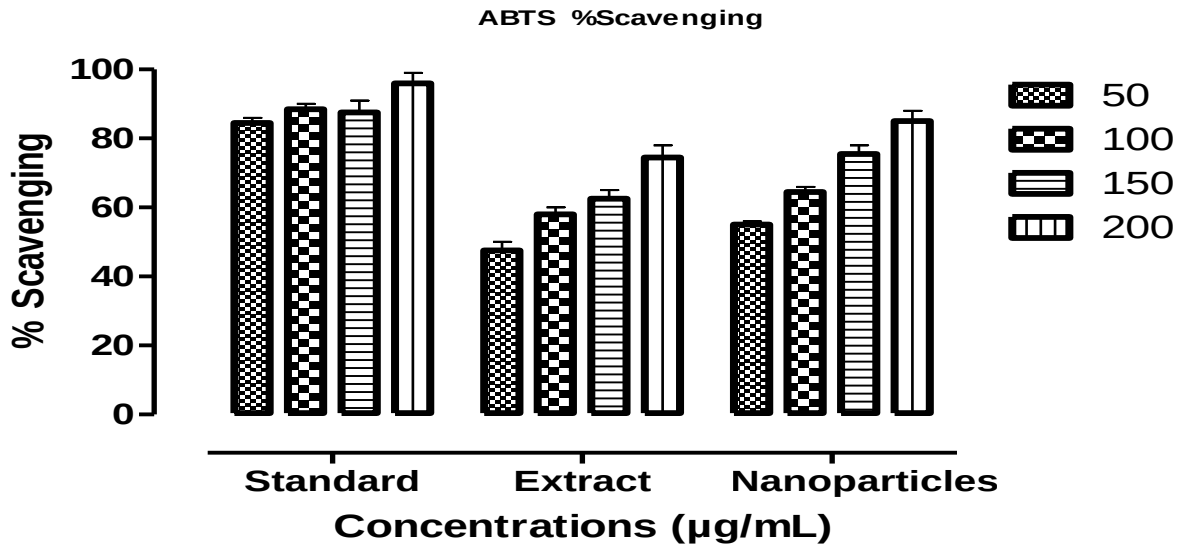

Figure 7. ABTS radical scavenging activity of extract and nanoparticles

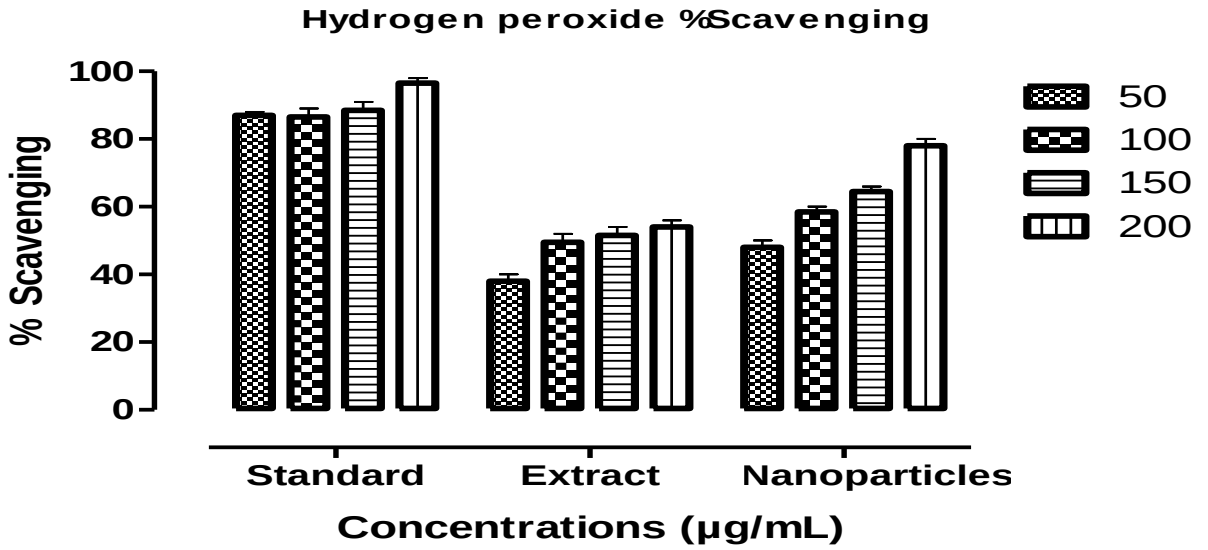

Figure 8. H_2O_2 radical scavenging activity of extract and nanoparticles

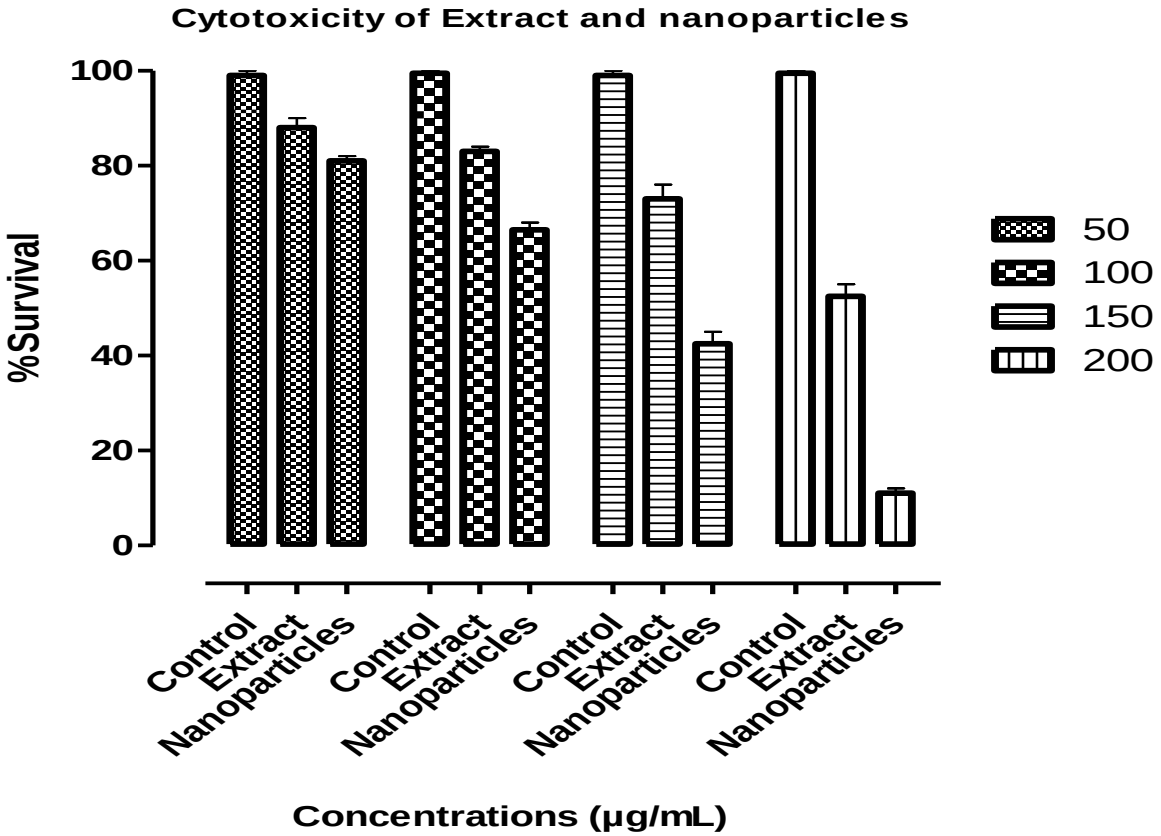

Figure 9. Cytotoxic activity of extract and synthesized gold nanoparticles.

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