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Cytotoxicity Property of Biologically Synthesized Gold Nanoparticles from Aqueous Leaf Extract of *Calotropis procera* (Apple of Sodom) on MCF-7 Cell Line

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Authors' contributions

This work was carried out in collaboration between the authors. Author OKS did the study design, wrote the protocol and first draft of the manuscript. Author DIS did the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The use of gold nanoparticle in drug delivery has emerged as a promising avenue to reduced toxicity and frequency of dosage while maintaining therapeutic effects and biocompatibility. Therefore, the possibility of developing eco-friendly metallic gold nanoparticles is evaluated. To achieve this, aqueous leave extracts of *Calotropis procera* was used to synthesis gold nanoparticles and its cytotoxic effect was investigated. The gold nanoparticles (AuNPs) produced were characterized using Ultra Violet–Visible spectroscopy, Zeta-sizer nano, High Resolution Scanning Electron Microscopy (HRSEM), Energy-Dispersive X-ray (EDAX) spectroscopy and Fourier Transmission Infrared (FTIR) spectroscopy. The cytotoxic ability of the synthesized gold nanoparticles was evaluated on MCF-7 cell using MTT assay. The result of Ultra Violet–Visible spectroscopy showed development of gold nanoparticle reaction at 550 nm of Surface Plasmon

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Resonance and average particle size of 45 nm was confirmed using nano Zeta-sizer. EDAX profile result suggested the presence of gold at 2.30ke while FTIR result confirms the presence of biomolecules serving as reducing and capping agents on the synthesized gold nanoparticle with a strong signal at 3426 cm of the hydroxyl group of alcohol or phenol. The cytotoxic effect of the synthesis gold nanoparticles shows cell viability decreased as the concentration of AuNPs increased from 0.156 mg to 5 mg with an IC_{50} of 0.312 mg/l. In conclusion, this study demonstrated the bioreductive capability of aqueous leaf extract of *Calotropis procera* to produced gold nanoparticle and its cytotoxicity effect on MCF-7cell line.

Keywords: Biosynthesis; gold nanoparticles; characterization; Calotropis procera extract; cytotoxicity; MCF-7 cell line.

1. INTRODUCTION

Nanotechnology is a platform technology which enhances the use of gold nanoparticles in the area of medical applications especially as a drug carrier for targeted drug delivery. Gold nanoparticles aids drug molecules to conjugate or exchange with a second organic molecule (the capping agent or the ligand of the AuNP), which complement the entire drug delivery system thereby distributing drugs to diseased organs, tissues or cells, in order to improve target drug delivery. An optimal nanodrug delivery system ensures that the active drug is available at the site of action at the correct time and duration, and their concentration above the minimal effective concentration (MEC) and below the minimal toxic concentration (MTC).

It is historically known that chemical reducing agents such as hydrazine, sodium citrate and sodium borohydride can be used as a way of producing metallic nanoparticles that have uniform suspensions. Some of these chemicals are hazardous to human health; therefore, there is a need to use natural materials which are safe, nontoxic, and environmentally-friendly.

Plants have been reported to rich in a wide variety of phytochemical metabolites that can be group into two: primary and secondary metabolite. The primary metabolite comprises of common sugars, amino acids, proteins and chlorophyll while secondary metabolite consists of glycosides, alkaloids, saponins, phenolic compounds, terpenes steroids, anthraquinone etc [1,2].

Calotropis procera (common name: Apple of Sodom) is a shrub or small tree, which has become a serious weed in pastures and overgrazed range lands. It is found in coastal dunes, roadsides, watercourses and disturbed urban areas. *C. procera* holds a reputed position as a medicinal plant in different systems of medicine in India. It has been reported that all parts possess valuable medicinal properties such as the whole plant used as an alexipharmic, cures leprosy, ulcers, spleen and liver diseases The juice as anthelmintic, laxative, and cures piles; the root bark for diaphoretic, and cures asthma and syphilis. Also, the flowers have been reported to be an analgesic, astringent, and cure for inflammations and tumors [3].

Therefore, in this study, the use of *Calotropis procera* aqueous leaves extract in the biosynthesis of gold nanoparticles and its cytotoxicity was investigated.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Reagents and chemicals

All reagents were of analytical grade and obtain from Sigma Chemicals.

2.1.2 Collection and identification of plants

Mature leaves of *Calotropis procera* were collected from plantations within Minna City of Niger State of Nigeria. The leaves of the plant were sent to the International Institute for Tropical Agriculture Ibadan and also to the National Biotechnology Institute of Nigeria, Ibadan for identification.

2.2 Methods

2.2.1 Sample preparation

The leaves were removed from the whole plant and then washed, air dried for fourteen days at

the research laboratory of the Department of Biochemistry of the Federal University of Technology Minna, Niger state at room temperature. After the period of drying the leaves were collected and then milled to powder form and kept under safe condition.

2.2.2 Determination of phytochemical constituents

Adopting the methods of Trease and Evans [4] and Sofowora [5], the aqueous leave extracts of *Calotropis procera* were tested for the presence of the phytochemical constituents.

2.2.3 Extract preparation

The plant leaf broth solution was prepared by taking Five grams of thoroughly washed and finely cut leaves powder in 100-mL Erlenmeyer flask with 100 mL of sterile distilled water and then boiled for 5 min before finally filtered using Whatman filter paper. The solution was stored at 4°C and used within a week.

2.2.4 Characterization of gold nanoparticles with ultraviolent visible spectrophotometry

Ten milliliters (10 mL) of leaf broth was added to 190 mL of 1 mM aqueous $HAuCl_4$ solution for the reduction of Au3+ ions. The reduced chloroauric acid was monitored using a UV-VIS Spectrophotometer (UV-1800 Shimadzu) with slit widths of 0.5, 1.0, 2.0, and 5.0 nm. The spectrum was scanned from 300 nm to 800 nm.

2.2.5 Nano zetasizer

The particle size and distribution for the Gold nanoparticles were measured using dynamic light scattering (DLS) equipment (Zetasizer, malvem) malven zeta sizer equipped with 20mV He-ne laser (633 nm) and operated at an angle of 90°.

2.2.6 High Resolution Scanning Electron Microscope (HRSEM)

The synthesized gold nanoparticles were freeze-dried into a pellet and mounted on the copper stub. The images were studied using Scanning Electron Microscope (SEM), HITACHI (model: S-3400N) with secondary electron detectors at an operating voltage of 30 kV according to Singh, [6].

2.2.7 Energy dispersive X-ray spectroscopy (EDAX)

EDAX of the reduced gold nanoparticle was done on S-3400N, Hitachi instrument according to Singh [6].

2.2.8 Fourier Transform Infrared (FTIR) spectroscopy

The synthesized gold nanoparticles solution was centrifuged at 15,000 rpm for 15 min, and the pellets were washed with deionized water to get rid of the free proteins/enzymes that were not capped on the gold nanoparticles. Thereafter, the purified suspension was freeze-dried to obtain a dry powder. The dried powder was analyzed using FTIR. The samples were dried and ground with KBr pellets and analyzed on a thermo Nicolet model 6700 spectrum instrument. A disk of 50 mg of KBr was prepared with a mixture of 2% finely dried samples and then examined under infra-red spectrometer. Infrared spectra were recorded in the region of 500-4,500 cm-1.

2.2.9 MCF-7 cell culture

Four milliliters (4 ml) trypsin was added to the T-75 flask. The flask was placed in an incubator for 5 minutes until the cells detached. Six milliliters (6 ml) of media were then added to diluted trypsin. The mixture was pipetted out of the flask and put in 15 ml centrifuge tube and centrifuged for 4 min at 650 g. While centrifuge was spinning, an appropriate volume of fresh media was pipetted into the new T-flasks. Cells were suspended in 10 ml of media and swirled to mix. The resulting mixture in T-Flask was then placed into the incubator.

2.2.10 Cytotoxicity studies

The cultures were removed from the incubator into the biosafety cabinet. Each vial of MTT [M-5655] was reconstituted to be used with 3 ml of medium or balanced salt solution without phenol red and serum. The reconstituted MTT in an amount equal to 10% of the culture medium volume was added. The cultures were returned to the incubator for 2-4 hours. After the incubation period, the cultures were removed from the incubator and the resulting formazan crystals were dissolved by adding 2 µl of MTT solubilization solution [M-8910] equal to the original culture medium volume.

The mixtures were gently mixed in a gyratory shaker to enhance dissolution. Occasionally, titration was required to completely dissolve the MTT formazan crystals. The culture was then left to incubate for 72 Hours and later read with UV Spectrophotometer at 490 nm [7].

Calculation of Percentage Growth Inhibition IC_{50}

The percentage growth inhibition was calculated using the method of Sanjay (10).

% cell inhibition= 100-{(At-Ab)/ (Ac-Ab)} x100

Where,

At= Absorbance value of test compound
Ab= Absorbance value of blank
Ac=Absorbance value of control

3. RESULTS

3.1 Phytochemical Composition of Plants

The aqueous extract *Calotropis procera* contained alkaloids, tannins, flavonoids, saponins, glycosides, phlobatannins and Steroids while anthraquinones were absent (Table 1).

Table 1. Phytochemical composition of *Calotropis procera*

Phytochemicals	*Calotropis procera*
Anthraquinons	-
Alkaloids	+
Tannins	+
Flavanoids	+
Saponins	+
Steroids	+
Glycosides	+
Phlobatannins	+

Key: Present + *Absent -*

3.2 Biosynthesis of Gold Nanoparticles Using Aqueous Leaf Extract of *Calotropis procera*

Plate 1c shows that gold chloride solution is yellow in colour before reaction and Plate 1b shows that the aqueous leaf extract of *Calotropis procera* is slightly red in colour. Plate 1a shows a reddish colloidal dispersion formed within three minutes after the reaction between the aqueous leaf extract of *Calotropis procera* and the gold chloride solution resulting in the formation of gold nanoparticles

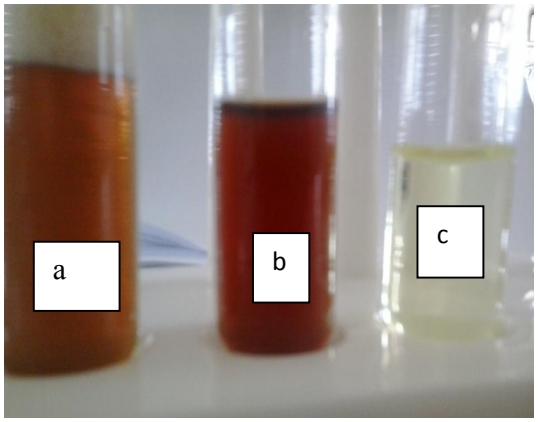

Plate 1. a- Gold nanoparticles synthesized, b- Aqueous extract of *Calotropis procera* c- Tetra auric chloride solution (Gold Chloride solution)

3.3 UV-Visible Spectrophotometric Analysis of the Gold Nanoparticles

The UV- Visible spectra indicated a strong Surface Plasmon Resonance which is clearly visible at a peak of 550 nm.

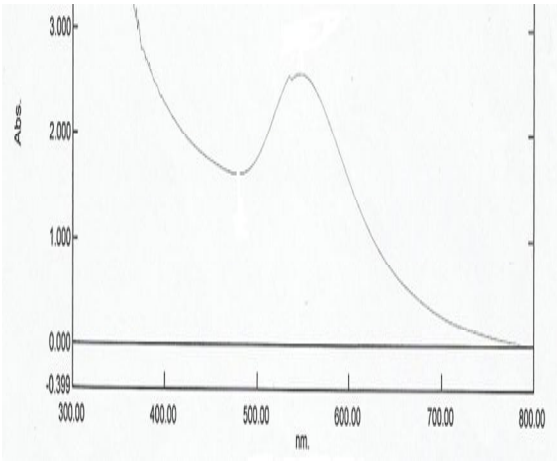

Fig. 1. UV-VIS spectra of gold nanoparticles synthesized by the use of aqueous leaf extract of *Calotropis procera*

3.4 Zeta-Sizer Analysis of the Gold Nanoparticles

Fig. 2 show average particle peak size of 45 nm. With intensity volume percentage of 13(%).

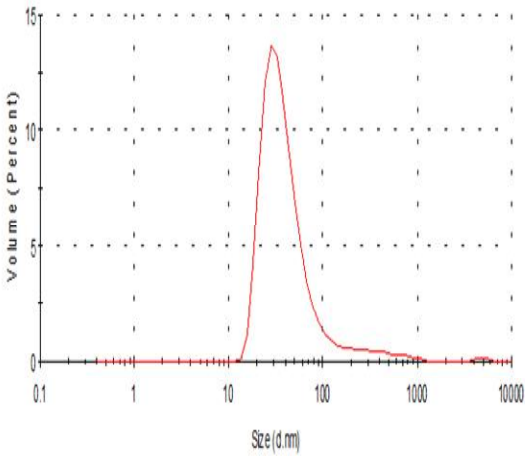

Fig. 2. Zeta sizer nano analysis of gold nanoparticles synthesized from aqueous leaf extract *Calotropis procera*

3.5 Energy-Dispersive X-ray Spectroscopy of Gold Nanoparticle

EDAX spectrometry confirmed the presence of gold with as shown in Fig. 3. The EDAX profile for gold nanoparticles synthesized using the aqueous leave extract showed strong gold atom signals at around 1.90, 2.30, 8.30, 9.80, 10.30, 11.50 and 13.40 keV.

3.6 HRSEM Images of Nanoparticles

Surface morphology of gold nanoparticles showed the particles were spherical in shape with some in aggregated forms.

3.7 FTIR Spectra of Synthesized Gold Nanoparticles

The Fourier transmission infra-red (FT-IR) spectroscopy of Gold Nanoparticles synthesized under optimized showed strong signals at 3426.55 cm $^{-1}$ corresponding to the hydroxyl group arising from alcohols or phenolic compounds and a weak band at 1641.48, 1427.37 and 1093.67cm^{-1}.

3.8 The Cytotoxic Result of Gold Nanoparticles

The cytotoxic result of Gold Nanoparticles synthesized using the aqueous leaf extract of *Calotropis procera* as shown in Table 2. This indicated that the cell viability decreased as concentration of Gold Nanoparticles increased from 0.156 mg to 5 mg with calculated IC_{50} at 0.312 mg/l.

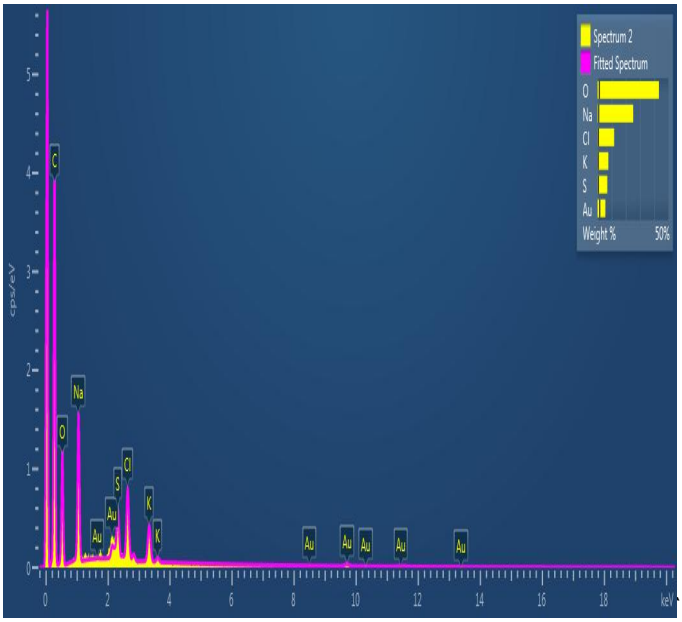

Fig. 3. The EDAX profile for gold nanoparticles synthesized from the aqueous leaf extract of *Calotropis procera*

4. DISCUSSION

Several researches have shown that Gold Nanoparticle-based technologies are becoming promising approaches in delivering drugs or DNA into cells [8] and gold ion in solution is readily reduced and precipitated out as gold metal by any reducing agent. The reducing agent is oxidized and dissolved allowing the gold to be displaced from the solution and recovered as a solid precipitate.

Also, previous studies of Shankar and others have shown that there was a rapid synthesis of gold nanoparticles with neem (*Azadirachta indica*) leaves and sundried C. *campphora* leaves. They attributed the reduction and stabilization of gold nanoparticles to a water-soluble heterocyclic compound found in the plants [9,10]. The absence of anthraquinones in *Calotropis procera* in this study is in agreement with Inavova et al. [11].

The presence of tannins as phytochemical constituents in the aqueous extracts of this plants suggest their ability to reduce metallic gold to its nanoparticle size as suggested by Sivaraman et al. [12].

It has been reported that tetra Auric chloride ion $[AuCl_4]^-$ shows an absorption spectra band before and after reduction. The surface plasmon resonance absorption spectra band of range 500-600 nm has been reported for gold nanoparticles by Umesh et al. [13]. Therefore, the appearance of a characteristics band in the visible region at 550 nm band (Fig. 1) of biosynthesized gold nanoparticle from aqueous leaves extracts of *Calotropis procera* is in agreement with Umesh et al. [13].

The morphological image from Scanning Electron Microscopy (Plate 2) shows that the gold nanoparticles synthesized from *Calotropis procera* aqueous leaves extract are spherical in shapes which are examples of shapes reported for gold nanoparticles [14]. The Fourier Transform infrared Spectroscopy (Fig. 4) shows strong bands at 3426.55 cm $^{-1}$ indicating hydroxyl group of alcohol or phenol and a weak band at 1641.48, 1427.37 and 1093.67cm^{-1} indicating stretch vibration of normal aliphatic groups. This is an additional evidence to confirm that phenolic phytochemicals are the reducing agents responsible for the reduction of gold chloride to the synthesized gold nanoparticles as also reported by [15].

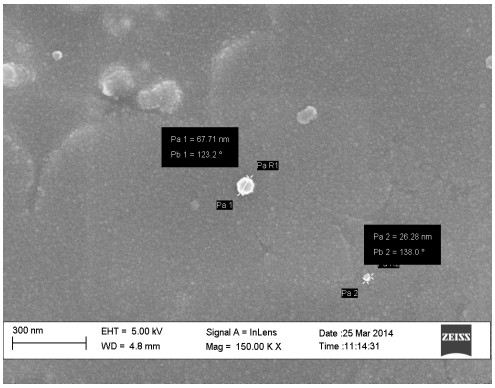

Plate 2. Morphological visualization of synthesized gold nanoparticles

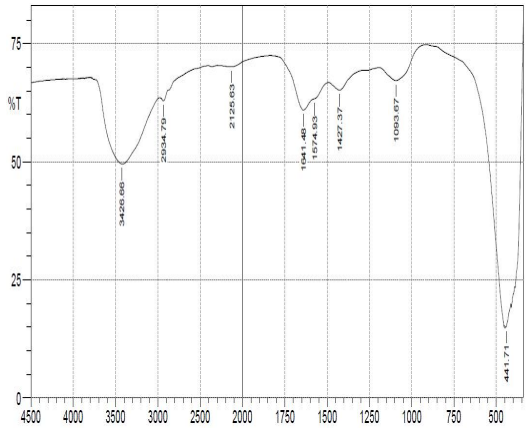

Fig. 4. FTIR spectra of gold nanoparticles synthesised from aqueous leaf extract of *Calotropis procera*

Table 2. Determination of cytotoxicity by MTT assay using gold nanoparticles from *Calotropis procera*

Plant extracts	Concentration (mg/ml)	Absorbance	% inhibition	IC_{50}	R^2
Calotropis procera	0.156	0.98	37		
	0.312	0.89	50	0.312	0.754
	0.625	0.654	63		
	1.25	0.445	69		
	2.5	0.233	72		
	5	0.09	77		

From the particle size Fig. 2, the biosynthesized gold nanoparticles make up 13% of the aqueous leaf extract of *Calotropis procera* which showed a distribution size range of between 12 to 100 nm with an average size of 45 nm. This shows that colloid gold suspension is polydisperse with a significant maximum which is in correlation with the findings Lapresta et al. [16].

The Energy-Dispersive X-ray Spectroscopy (EDAX) profile shown in Fig. 3 confirms the presence of gold with optical adsorption peak observed at approximately 2.30 keV (Fig. 3), which is typical of adsorption of gold Nano crystallites due to surface plasmon resonance. This extract showed similar observation to other plant species as earlier reported [17-19].

The EDAX profile of gold nanoparticles presents a strong signal for gold with very strong oxygen, sodium, sulphur, chlorine and potassium peaks, which further confirmed the presence of synthesized gold nanoparticles.

High magnification of HRSEM images recorded during this study showed that biologically synthesized gold nanoparticles at the end of reaction with extract of leaves *Calotropis procera* were predominantly spherical in morphology. A large quantity of spherical gold nanoparticles with thin smooth ends on the exterior of the nanoparticles was seen confirmed (Plate 2). Aggregation of semispherical nanoparticles was also confirmed which shows the inability of biomolecules to act as protecting agents. The images from Plate 2 shows that the particles are not highly monodisperse but seem non-agglomerated. This may be due to the presence of some bio-organic compounds in the plant extract that can act as a ligand which effectively stabilizes the formed gold nanoparticles [16].

The FTIR spectra corresponds to the hydroxyl group arising from alcohols and phenolic compounds, secondary amine, amide bond of proteins, an asymmetric deformation of CH_3 from alkenes and C–N stretching vibrations of the amide bond has been reported by Szymanski and Erickson [19] and Colthrup et al. [20]. Therefore, the FT-IR spectrum of biosynthesized gold nanoparticle (Fig. 4) of this study is in agreement with previous studies.

This indicates that biomolecule compounds from the leaf extract are responsible for the bio reduction process as well as capping agents on the synthesized gold nanoparticles.

From the Table 2, the % growth inhibition increased with increasing concentration. Therefore, the overall study showed that the aqueous extract used in this study has potential activity on MCF-7 cell line. The gold nanoparticles synthesized using the aqueous leaf extract of *Calotropis procera* has a higher cytotoxic effect.

5. CONCLUSION

This study has demonstrated the bio-reductive capability of aqueous leaf extract of *Calotropis procera* on tetra gold chloride which may be attributed to the presence of a phenolic compound of the leaf extract. The shape of the gold nanoparticle was confirmed by SEM to be spherical shape with UV spectra of a sharp peak at 550 nm confirming synthesis of Gold nanoparticle with an average size of 45 nm using Zeta sizer. The cytotoxicity activity shows a dose dependent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Yusuf KO, Ekanem JT. Studies of phytochemical constituents and anti-trypanosomal properties of fermented wheat germ and garlic bulbs extract on *Trypanosoma brucei* – Infected rats. Journal of Medicinal Plants Research. 2010;4(19):2016-2020.
2. Habtermariam S, Gray AI, Waterman RG. A new anti-bacterial sesquiterpene from

Premma oligotricha. Journal of Natural Product. 1993;5(1):140–143.

3. Thakker JN, Patel JN, Kothari IL. *Fusarium oxysporum* derived elicitor induced enzymological changes in banana leaves against *Fusarium* wilt disease. Journal of Mycology and Plant Pathology. 2007;37:510–513.
4. Satyavani K, Ramanathan T, Gurudeeban S. Plant-mediated synthesis of biomedical silver nanoparticles by using leaf extract of *Citrullus colocynthis*. Research Journal of Nanoscience and Nanotechnology. 2011; 1:95–101.
5. Trease GE, Evans WC. Pharmacognosy. 13th (ed). ELBS/Bailliere Tindall, London. 1989;345-6,535-6,772-3.
6. Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd., Nigeria. 1993;289-300.
7. Singh M, Kalaivani R, Manikandan S. Facile green synthesis of variable metallic gold nanoparticle using *Padina gymnospora*, a brown marine macroalga. Applied Nanoscience. 2013;3:145–151.
8. Mossman T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 1983;10:55-63.
9. Sanjay P, Nirav G, Ashok S, Anand S. *In-Vitro* cytotoxicity activity of *Solanum nigrum* extract against HeLa cell line and Vero cell line. International Journal of Pharmacy and Pharmaceutical Sciences. 2009;1(1):38-46.
10. Shankar SS, Rai A, Ahmad A, Sastry M. Biosynthesis of silver and gold nanoparticles from extracts of different parts of the geranium plant. Applied Nano Science. 2004;1:69–77.
11. Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, Wang H, Wang Y, Shao W, Hong NJ, Chen C. Biosynthesis of silver and gold nanoparticles by novel sundried leaf. Nano Technology. 2007;18(10):105104-105115.
12. Inavova D, Gerova T, Chervenkov T, Tankova. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. Journal of Ethnopharmacology. 2005; 96(1):145-150
13. Sivaraman SK, Elango I, Kumar S, Santhanam V. A green protocol for room temperature synthesis of silver nano-particles in seconds. Current Science. 2009;9(7):1055-1059.
14. Umesh KP, Birendra KB, Padmalochan N. Green synthesis and characterization of gold nanoparticles using onion (*Allum cepa*) extract. World Journal of Nano Science and Engineering. 2011;1:93-98.
15. Tomar A, Garg G. Short review of gold nanoparticles. Global Journal of Pharmacology. 2013;7(1):34-38.
16. Jae YS, Hyeon-Kyeong, Beom SK. Biological synthesis of gold nanoparticles using *Magnolia kobus* and *Diopyros kaki* leaf extracts. Process Biochemistry. 2009;44:1133-1138.
17. Lapresta-Fernanández A, Fernandéz A, Blasco J. Nanoecotoxicity effects of engineered silver and gold nanoparticles in aquatic organisms. Trends in Analytical Chemistry. 2012;9:40-59.
18. Narayanan B, Sakthive IN. Coriander leaf mediated biosynthesis of gold nano-particles. Material Letter. 2008;62:4588–4590.
19. Colthrup NB, Daly LH, Wiberley SE. Introduction to infrared and Raman spectroscopy. Academic Press, San Diego, Califonia. 1990;1–73.
20. Szymanski HA, Erickson RA. Infrared Band, Plenum Press, New York. 1970;2.

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