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In vitro Membranous activity of Biosynthesized Gold Nanoparticle from Aqueous Leave Extract of *Nelsonia canescens*

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Authors' contributions

This work was carried out in collaboration between the authors. Author OKS did the study design 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

Nano drug delivery technologies have advantages of transporting the drug to target site to better enhance its effectiveness, bioavailability and reduces side effects with dose frequency. In this study, the reducing capability of *Nelsonia canescens* for gold nanoparticle synthesis and its membranous activity on MCF-7 cell have been investigated. The morphology, particle size and the functional group of the bio- reducing agent of the synthesized gold nanoparticle were investigated using High-Resolution Scanning Electron Microscopy (SEM), Zeta-sized nano and Fourier Transmission Infrared (FTIR) spectroscopy. The biosynthesis gold nanoparticle showed a strong surface plasmon resonance at 537.5 nm with an average size of 50nm. SEM image showed the morphology of the biosynthesized gold nanoparticle to be spherical with the possibility of aggregation. While FTIR confirms the reducing and capping agents of the synthesized gold Nanoparticle as hydroxyl group of alcohol or phenol with a strong signal at 3417.98 cm^{-1}. The *in vitro* membranous activity shows concentration-dependent with an IC_{50} of 0.455 mg/l. Therefore, it

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could be concluded that *Nelsonia canescens* have the bioreductive capability to produce gold nanoparticle and *in vitro* membraneous activity on MCF-7 cell line.

Keywords: Bioreduction; gold nanoparticle; characterization; Nelsonia canescens; MCF-7 cell; drug delivery.

1. INTRODUCTION

Nanoparticles are particles whose size ranges from 1-100 nm and have completely novel or advanced properties due to their high ratio of surface area to particle size as opposed to their larger scale counterparts. They have wide applications in the field of biomedicine such as delivery of pharmaceuticals, *in vitro* and *in vivo* diagnostic as well as nutraceuticals [1,2,3]. There has been immense interest in targeted drug delivery as one of the possibility to address the problem of toxicity and resistance to some of the current pharmaceutics and biotechnology [4]. Metallic nanoparticles are the subject of research efforts as new platforms for the target-specific delivery of therapeutic agents [5]. Gold nanoparticles (AuNPS) in particular are an excellent candidate for drug delivery due to their unique properties enabling the transport and release of the therapeutic agents to the target site [5].

Gold nanoparticles have been reported to be synthesized chemically using chemicals such as sodium citrate, sodium borohydride and also hydroquinone reduction of chloroauric acid ($HAuCl_4$) [5]. They can also be synthesized by biological methods using microorganisms, enzymes, and plant extracts. Currently, biological methods have been used as an eco-friendly alternative to chemical methods [6]. Using plants for nanoparticle synthesis have advantages over other biological processes because it eliminates the process of maintaining cell cultures and is suitable for large-scale nanoparticle synthesis [7].

Nelsonia canescens is a herbaceous species that often grows in disturbed and open habitats. This species is also an agricultural weed (i.e., rice and oil palm plantations) reducing crop yield by competing with crop plants for common resources such as water, mineral nutrients, and space. Considering that *N. canescens* plants are not especially attractive and not used in the horticultural trade, their wide geographic distribution across the tropics show that this species is a very agile disperser.

The genus *Nelsonia* is usually treated in the subfamily *Nelsonioideae* within the family of *Acanthaceae*. This subfamily has been repeatedly shown to be monophyletic and to comprise the basal lineage among clades of *Acanthaceae*. A molecular phylogenetic study suggested that only a single species of *N. canescens* should be recognized [8]. According to these authors, the variation in vegetative traits likely reflected plasticity rather than distinct species and they doubted the validity of recognizing more than one highly variable species. The ethanolic extract of the leaves of *N. canescens* has been reported to possess analgesic, anti-inflammatory anti-ulcer activities [9,10]. Also, the activities have been attributed to the present of polyphenol compounds including tannins and flavonoids [11]. However, in this study, the reducing capability of *Nelsonia canescens* for gold nanoparticle synthesis and its in vitro membranous activity on MCF-7 cell are investigated.

2. MATERIALS AND METHODS

2.1 Plant Sample

The leaf of *Nelsonia canescens* were collected from plantations within Minna city of Niger State of Nigeria and identified at Biological Sciences Department, Federal University of Technology, Minna, Niger State.

2.2 Plant Preparation

Fresh leaves of *Nelsonia canescens* were washed with clean water and then air-dried for 15 days at room temperature to prevent the destruction of thermo- labile constituent of the plant by direct sun rays. The leaves were then milled into a coarse powder.

2.3 Qualitative Phytochemical Screening

The qualitative phytochemical analysis was carried out as described by Trease and Evans, [12] and Sofowora [13] to confirm the presence of alkaloids, saponins, flavonoids, cardiac

glycosides, tannins, steroids, phlobatannins, terpenoids and anthraquinones.

2.4 Synthesis of Gold Nanoparticles

One hundred milliliters of distilled water was added to 5 g of milled plant in an Erlenmeyer flask and then boiled for 5mins after which it was filtered, and then 0.5 ml of the plant extract was added to 9.5 ml of 1mM aqueous $HAuCl_4$ solution for the reduction of Au^{3+} ions according to the method described by [7].

2.5 Characterization of Biosynthesized Gold Nanoparticle

The UV-spectroscopy measurements of the $HAuCl_4$-Plant extract solution was carried out using UV-1800 Shimadzu spectrophotometer with various peaks present over a range of 300-800 nm.

The hydrodynamic diameter of the nanoparticles in solution was determined by dynamic light scattering (DLS) with the help of a Zetasizer 3000 (Malvern Instruments, UK) using an argonion laser beam at a wavelength of 550 nm and a scattering angle of 90º.

The levels of functional groups were evaluated using Fourier transform infrared (FTIR) spectroscopy. The biosynthesized gold nanoparticle was freeze-dried into pellet and washed with deionized water to get rid of the free proteins/enzymes that were not capped on the gold nanoparticles. Thereafter, the samples were dried and ground with KBr pellets and analyzed on a thermo Nicolet model 6700 spectrum instrument. The images were studied using Scanning Electron Microscope (SEM), HITACHI (model: S-3400N) with secondary electron detectors at an operating voltage of 30 kV. Energy Dispersive X-ray Spectroscopy (EDAX) of the reduced gold nanoparticle was done on S-3400N, Hitachi instrument according to Singh [14].

2.6 Cell Line

The human cancer cell lines, MCF-7 (breast cancer) were obtained from Polio Laboratory, College of Medicine, University College Hospital, University of Ibadan, Nigeria.

2.7 MCF-7 Cell Culture

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

2.8 Cytotoxicity Studies

The cultures were removed from the incubator into the bio safety 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, tritration was required to completely dissolve the MTT formazan crystals. The culture was then left to incubate for 72 Hours and the absorbance was latter measured at 490 nm using a microplate spectrophotometer (BioTek, Power Wave XS). The proliferation rate and inhibitory rate of the cells were calculated with the following formulas of Sanjay method [15].

Proliferation rate (PR) %= *(*Abs sample/Abs control)×100

Inhibitory rate (IR) % = 100−PR

3. RESULTS

3.1 Phytochemical Composition of Plants

The aqueous extract of *Nelsonia canescens* showed the presence of tannins, flavonoids and glycosides while anthraquinones, alkaloids, saponins, phlobatannins and Steroids were absent (Table 1).

3.2 UV-visible Spectrophotometric Analysis

The biosynthesized gold nanoparticle of aqueous leaf extract of *Nelsonia canescens* (plate A) analyzed with Ultraviolet-Visible spectroscopy

showed a sharp peak at 537.5 nm (Fig. 1) when scanned within the range of 300 – 800 nm.

Table 1. Phytochemical composition of *Nelsonia canescens*

Phytochemicals	*Nelsonia canescens*
Anthraquinons	-
Alkaloids	-
Tannins	+
Flavanoids	+
Saponins	-
Steroids	-
Glycosides	+
Phlobatannins	-

Key: + Present; - Absent

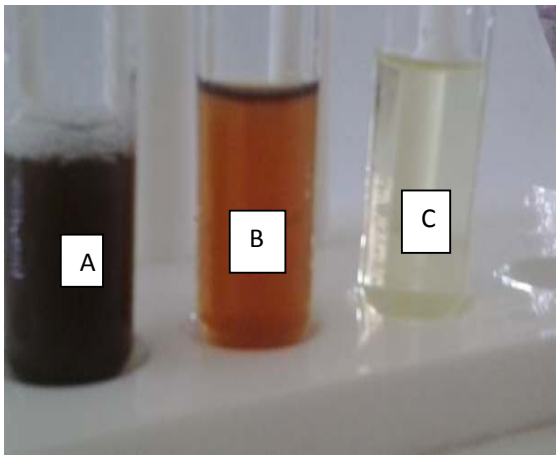

Plate 1. A- Gold Nanoparticle synthesized B- Aqueous extract of *Nelsonia canescens* C- Tetra Auric chloride solution (Gold chloride solution)

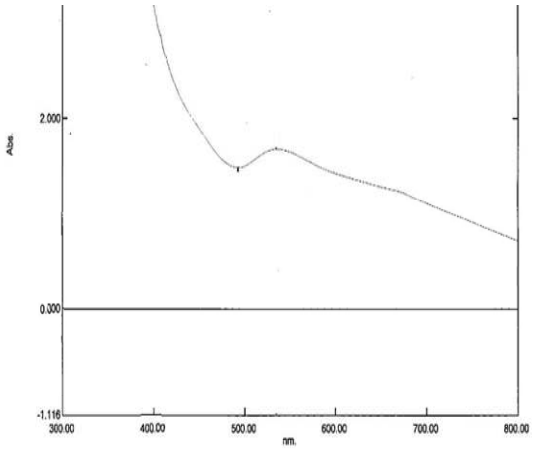

Fig. 1. UV-VIS spectra of gold nanoparticle synthesized by the use of aqueous leaf extract of *Nelsonia canescens*

3.3 Zeta-Sizer Analysis of Gold Nanoparticle Synthesized Using the Aqueous Leaf Extract of *Nelsonia canescens*

The Zetasizer measurement of biosynthesized AuNPs of aqueous leaf extract of *Nelsonia canescens* showed particle sizes ranging from 15 nm to 125 nm with optimum the particle size of 50 nm occupies fourteen percent of the entire volume of the sample analyzed (Fig. 2).

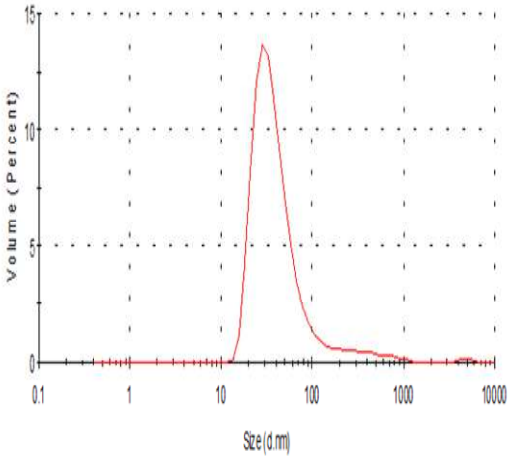

Fig. 2. Zeta Sizer spectra of gold nanoparticle synthesized from aqueous leaf extract of *Nelsonia canescens*

3.4 Energy-Dispersive Xray Spectroscopy Analysis of Gold Nanoparticle Using Aqueous Leaf Extract of *Nelsonia canescens*

The bio-synthesized Gold Nanoparticle from the aqueous leave extract of *Nelsonia canescens* was investigated using EDAX and confirmed the presence of gold with no contaminants as shown in Fig. 3. The vertical axis shows the number of X-ray counts and the horizontal axis shows energy in keV. The maximum optical adsorption peak was observed at approximately 2.30 keV and addition signals for carbon at 0.20, oxygen 0.50, sodium 1.0, chlorine 2.60 and potassium 3.30 and 3.50 KeV, (Fig. 3).

3.5 High-Resolution Scanning Electron Microscopy Analysis of Gold Nanoparticle Using Aqueous Leaf Extract of *Nelsonia canescens*

HRSEM image showed that the biosynthesized AuNPs were almost spherical in shape with the possibility of aggregation.

3.6 FTIR of Gold Nanoparticle Synthesized Using Aqueous Leaf Extract of *Nelsonia canescens*

The FT-IR spectra of synthesized AuNPs s under optimized conditions indicated the presence of biomolecules on the surface of AuNPs. The FT-IR spectra *of Nelsonia canescens* showed strong signals at 3417 cm^{-1} and weak signals at 2931, 2345 and 1645 cm^{-1} (Fig. 5).

3.7 Cytotoxicity of Gold Nanoparticles Synthesized Using the Aqueous Leaf Extract of *Nelsonia canescens*

The cytotoxicity result of gold nanoparticles synthesized using the aqueous leaf extract of *Nelsonia* is shown in Table 2. This indicates that the cell viability decreased as the concentration of Gold Nanoparticles decreased from 5 mg to 0.156 mg respectively and the calculated IC_{50} was 0.455 mg/l.

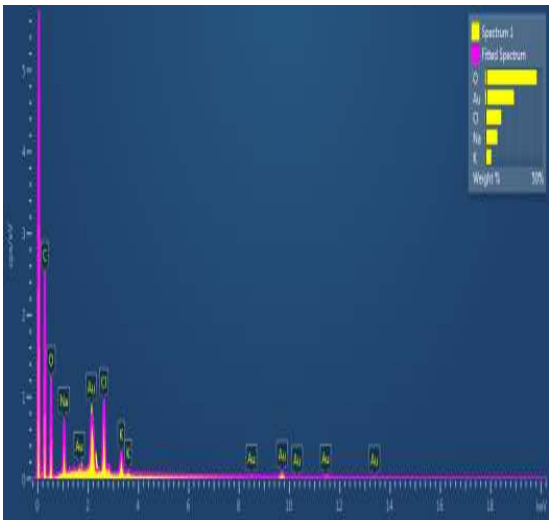

Fig. 3. The EDAX profile for gold nanoparticle synthesized from the aqueous leaf extract of *Nelsonia canescens*

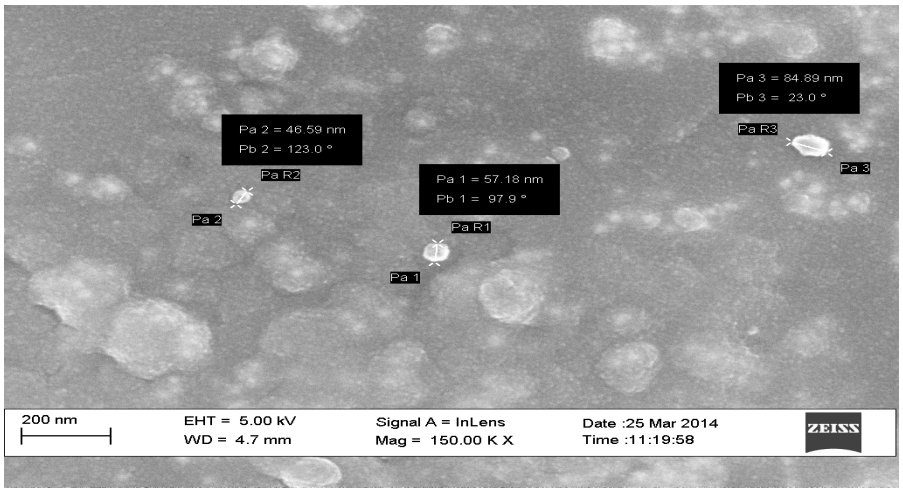

Fig. 4. HRSEM images of gold nanoparticle synthesized from aqueous leaf extract of *Nelsonia canescens*

Table 2. Determination of cytotoxicity by MTT assay using gold nanoparticles of *Nelsonia canescens*

Plant extract	Concentration (Mg/ml)	Absorbance	% inhibition	IC_{50}	R^2
Nelsonia camprestris	5	0.09	90		
	2.5	0.233	80		
	1.25	0.445	75		
	0.625	0.654	65		
	0.312	0.89	54	0.455	0.8749
	0.156	0.98	30		

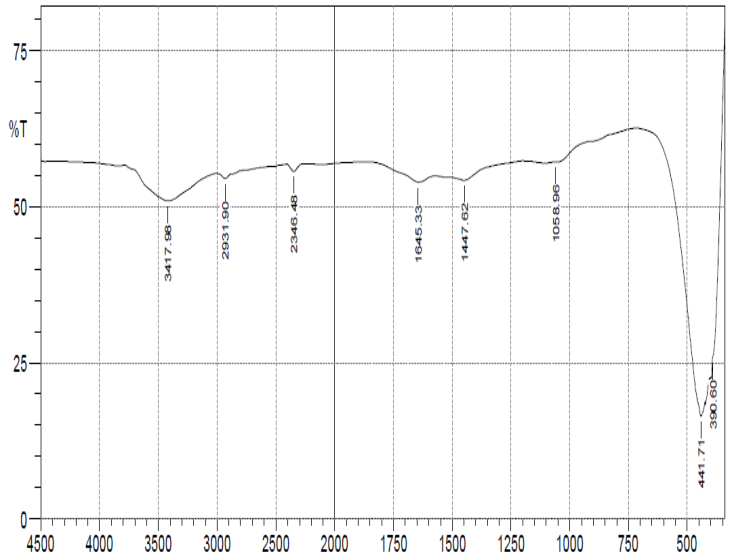

Fig. 5. FTIR spectra of gold Nanoparticle synthesized from extract of *Nelsonia canescens*

4. DISCUSSION

The qualitative phytochemical constituents of the aqueous leaf extract of *Nelsonia canescens* are Tannins, Flavonoids, and glycosides as shown in Table 1. The presence of the phenolic compounds including tannins and flavonoids (Nacoulma, 1996) which are known to possess antioxidant activities (Aderogba et al. 2005; Badami et al. 2003; Motalleb et al. 2005) in the aqueous extracts of the plant suggests its reducing capability and therapeutic purposes as antioxidant properties. Therefore, it could be concluded that the phenolic compounds: tannins present in the leaf extract of *Nelsonia canescens* have the ability to cap the gold nanoparticle by ionic interaction and thereby stabilizing them [16].

The colour change of gold chloride (light yellow) (Plate 1C) to dark brown color (Plate 1A) on the addition of plant extract (Plate 1B) indicating the formation of gold nanoparticles by *Nelsonia canescens* leaf extract. It has been reported that colour changes arise due to excitation of surface plasmon vibrations in the gold metal nanoparticles [17].

The UV–Visible spectrophotometer analysis of dark green color solutions (Plate 1A) shows a strong plasma resonance at 537.5nm for gold nanoparticle (Fig. 1).

The biosynthesized gold Nanoparticle was further characterized with zeta sizer which indicates the optimal average particle size of 50 nm (Fig. 2).

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 [18,19,20].

The EDAX profile of gold nanoparticles shows the presence of gold and other element such as carbon from the plant, and other elements (oxygen, sodium, chlorine and potassium) which may be from the grid used as reported by Akinsiku et al. [21].

High magnification of HRSEM images recorded during this study showed that biologically synthesized gold nanoparticles at the end of reaction with extract of leaves *Nelsonia canescens*were predominantly spherical in morphology (Fig. 3). Aggregation of semispherical nanoparticles was also confirmed which shows the inability of biomolecules to act as protecting agents for aggregation. The images from Fig. 3 show 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 [22].

The FTIR spectra of gold nanoparticle show bands 3417 and weak signals at 2931 and 2345 cm^{-1} (Fig. 4). The band at 3417 cm^{-1} corresponds to the O-H stretch of strong broad of alcohols or phenols and 2931 cm^{-1} corresponds to alkane (C-H) strong stretching group while 2345 cm^{-1} has no identified functional group. The highest absorption peak 3417 cm^{-1} confirms that the hydroxyl group (O-H) is responsible for the reducing property of the *Nelsonia canescens* leaf extract and this functional group is a reflection of the presence of the polyphenolic compound [23]. The polyphenolic compound of the plant extract has been reported to have antioxidant [10], analgesic, anti-inflammatory anti-ulcer activities [7,8]. Tannin has been reported as a reducing agent which silver nitrate to its nanoparticle size [24].

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. Therefore, the gold nanoparticles synthesized using the aqueous leaf extract of *Nelsonia canescens* has a cytotoxic effect which is dose/ concentration dependent.

5. CONCLUSION

This study has demonstrated the bio-reductive capability of aqueous leaf extract of *Nelsonia canescens* 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 a spherical shape with UV spectra of a sharp peak at 537.5 nm confirming the synthesis of Gold nanoparticle with an average size of 50nm using Zetasizer. The cytotoxicity activity shows a dose dependent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Duncan R. The dawning era of polymer therapeutics. Nature Review Drug Discovery. 2003;2(5):347-60.
2. De Jong WH, Geertsma RE, Roszek B. Nanotechnology in medical application. Possible risks for human health Report 265001002/2005. National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands; 2005.
3. ESF. European Science Foundation Policy Briefing, Scientific forward look on Nanomedicine IREG Strasbourg, France, ISBN2-912049-520; 2005.
4. Giljohann DA, Seferos DS, Daniel WL, Massich MD, Patel PC, Mirkin CA. Gold nanoparticles for biology and medicine. Angew. Chem. Int. Ed. Engl. 2010;49: 3280–3294.
5. Manson J, Kumar D, Meenan BJ, Dixon D. Polyethylene glycol functionalization gold nanoparticles, the influence of capping density on stability in various media. Gold Bull. 2011;44:99-105.
6. Umesh KP, Birendra KB, Padmalochan N. Green synthesis and characterization of Gold nanoparticles using onion (*Allumcepa*) extract. World Journal of Nano Science and Engineering. 2011;1:93-98.
7. 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.
8. McDade LA, Daniel TF, Kiel CA, Borg AJ. Phylogenetic placement, delimitation, and relationships among genera of the enigmatic Nelsonioideae (Lamiales: Acanthaceae). Taxon. 2012;61(3):637-651(15)
9. Owoyele BV, Oloriegbe YY, Balogun EA, Soladoye AO. Analgesic and anti-inflammatory properties of *Nelsonia canescens* leaf extract. Journal Ethnopharmacology. 2006;99:153-156.
10. Owoyele BV, Akozoriand FA, Soladoye AO. Anti-ulcer Effect of Leaf Extracts of

Nelsonia canescens in Rats. RPMP – Chronic and Common Diseases II. 2006; 18:345-349.
11. Ouattara Nabèrè, Hilou Adama, Guenné Samson, Konaté Kiessoum, Zerbo Patrice, Meda Nâg-Tiero Roland, Compaoré Moussa, Kiendrébeogo Martin, Millogo F. Jeanne, Nacoulma O. Germaine. Antibacterial and Phytochemical studies of three *Acanthaceae* species used in Burkina Faso traditional medicine. Journal of Applied Pharmaceutical Science. 2013; 3(05):049-055.
12. Trease GE, Evans WC. Pharmacognosy. 13th (ed). ELBS / Bailliere Tindall, London. 1989;345-6, 535-6, 772-3.
13. Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd., Nigeria. 1993;289-300.
14. Singh M, Kalaivani R, Manikandan S. Facile green synthesis of the variable metallic gold nanoparticle using *Padina gymnospora*, a brown marine macroalga. Applied Nanoscience. 2013;3:145–151.
15. 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.
16. Singh HP, Gujral SS, Sharma SK, Sharma RK. Tannic acid: A natural source to tailor nano crystalline silver particles of different morphologies as antibacterial agent. Advanced Material Letters. 2015;6(12): 1043-1049.
17. Mulvaney P. Surface Plasmon spectroscopy of nanosized metal particles. Langmuir. 1996;12(3):788–800.
18. Narayanan B, Sakthive lN. Coriander leaf mediated biosynthesis of gold nanoparticles. Material Letter. 2008;62: 4588–4590.
19. Colthrup NB, Daly LH, Wiberley SE. Introduction to infrared and Raman spectroscopy. Academic Press, SanDiego, Califonia. 1990;1–73.
20. Szymanski HA, Erickson RA. Infrared Band, Plenum Press, New York, 2 Volumes; 1970.
21. Akinsiku AA, Ajanaku KO, Adekoya JA, Dare EO. Green synthesis, characterization of silver nanoparticles using *Canna indica* and *Senna occidental* is leaf extracts. International Conference on African Development Issues (CU-ICADI) 2015: Materials Teclmology Track. 2015;154-157.
22. 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.
23. Varshneya R, Bhadauria S, Gaur MS. Biogenic synthesis of silver nanocubes and nanorods using sundried *Stevia rebaudiana* leaves. Advanced Material Letters. 2010;1(3):232-237.
24. Adeyemi SO, Sulaiman FA. Evaluation of metal nanoparticles for drug delivery systems. Journal of Biomedical Research. 2015;29(2):145-149.

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