ORIGINAL ARTICLE

Cytotoxic action of silver nanoparticles synthesized from *Phyllanthus fraternus* on hepatic and breast cancer cell lines: A green approach

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Abstract

Background: Silver nanoparticles (AgNPs) are an integral part of nanotechnology and setting up new trends in pharmaceutical field due to its wide therapeutic applications. **Aim:** In the present research, green synthesis of AgNPs using leaf extracts of *Phyllanthus fraternus* with an evaluation of their cytotoxicity activity against hepatic and breast cancer cell lines. **Materials and Methods:** AgNPs were used to characterized by ultraviolet (UV)-visible spectrophotometry, Fourier-transform infrared (FTIR) spectrophotometry, scanning electron microscopy (SEM) with elemental mapping, transmission electron microscope (TEM), and X-ray diffraction (XRD). *In vitro* cytotoxicity studied by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay method. **Results and Discussion:** AgNPs were identified by the change of color and their absorption at 420 nm measured by UV-visible spectroscopy, FTIR spectral analysis confirmed phenolic compounds presence, morphology and size visualized in SEM, and TEM used for the determination of size, shape, and light scattering analysis. Synthesized AgNPs were spherical in shape with size <50 nm. XRD analysis was affirmed the crystalline nature of metal particles. *In vitro* cytotoxic result showed an excellent half maximal inhibitory concentration value of 62.5 µg/mL and 125 µg/mL against hepatic cancer cell line (HepG-2) and breast cancer cell line (MCF-7). **Conclusions:** The current study reveals green synthesized AgNPs possess high cytotoxic action against HepG-2 and MCF-7 cell lines which suggested the potential therapeutic use of these AgNPs as alternative medicine for the treatment of hepatic and breast cancer cases.

Key words: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay hepatic cancer cell line 2, green, silver nanoparticles, breast cancer cell line, *Phyllanthus fraternus*

INTRODUCTION

Plant extract-mediated synthesized silver nanoparticles (AgNPs) are become a part of nanotechnology.[1] AgNPs as nanomedicine are set up new dimensions in pharmaceutical field due to its wide therapeutic applications.[2] Various numbers of reported uses of AgNPs including burn wounds. Nanoparticles prepared by physical and chemical ways such as thermal decomposition, reduction in solution, electrochemical, microwave, and sonochemical methods.[3]

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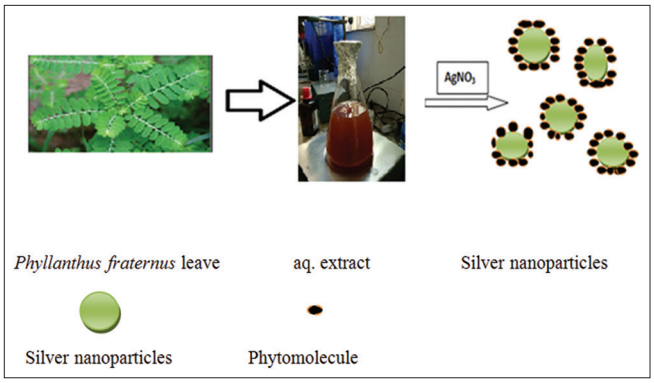

Figure 1: Schematic of synthesis of silver nanoparticles using *Phyllanthus fraternus*

Figure 2: (a) Synthesized silver nanoparticles (b) aq. extract (c) silver nitrate solution

Recently, biological method reported for synthesis of AgNPs by greenways using enzymes, microorganisms, and herbal plant extracts [Figure 1]. Biological method has various application over physical and chemical methods such as no requirement of heat, pressure, and temperature. Synthesis of AgNPs through biological methods is without using any harmful chemicals and reagents so they are cost-effective and eco-friendly.[4] Herbal plant extract arbitrated AgNPs synthesis is widely used recently due to its green, facile, economical, and safe.[5]

Phyllanthus fraternus (P.f.) (Family - Phyllanthaceae) commonly known as Bhuinanvalah in Hindi.[6] P.f. is used in jaundice, sores, fever, dysentery, diarrheal infections, and hepatoprotective activity. Conventionally, *Phyllanthus* holds a reputed position in Ayurvedic medicine. It has been various reported ethnomedicinal uses such as antioxidant, anticancer, and antipyretic.

Previous literature studies have reported about AgNPs as anticancer agent and their role as an anticancer agent could explore newer treatment therapy in the area of pharmaceuticals with help treatment of cancer.[7]

In this research work, we revealed green synthesis of AgNPs reducing by leaves extract of P.f. Extract-mediated synthesized metal nanoparticles, particularly characterized by ultraviolet (UV)-visible spectral analysis, Fourier transform infrared (FTIR), scanning electron microscopy (SEM), transmission electron microscope (TEM) with mapping, energy-dispersive X-ray (EDAX), and X-ray diffraction (XRD) techniques. AgNPs were confirmed by the change of color and surface plasmon resonance (SPR) peak confirmed by UV-visible spectroscopy, FT-IR spectra confirmed the presence of amine and phenolic compounds, and shape and size visualized by SEM and TEM. XRD used to affirm crystalline nature of particles. *In vitro* cytotoxicity studied by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method (cell viability method) against hepatic cancer cell lines (HepG-2) and breast cancer cell line (MCF-7). Programmed cell death or apoptosis is highly controlled by metal (silver [Ag]) nanoparticles through activated enzyme CASPASE-3 that irreversibly commit a cancerous cell to die.[8]

MATERIALS AND METHODS

Collection and Authentication

Fresh leaves of P.f.[6] were collected in rainy season from Jhusi, Allahabad (Uttar Pradesh), India. The voucher specimen TR No-097054 of P.f. authenticated by BSI, Allahabad (Uttar Pradesh), India.

Chemicals

Ag nitrate was procured from RENCHEM.

Preparation of Plant Extract

Leaves were washed, dried, and after grinding, about 100 g powdered leaves were mixed with 1000 ml double distilled water and heated for 30 min. Afterward, the extract was get filtered.[9] Preliminary phytochemical screening was performed to know about phytoconstituents present in leaves extract.

Preparation of Ag Nitrate Solution

Ag nitrate (1 mM solution) 1.6 g was dissolved in 1 l double distilled water.[10]

Synthesis of Nanoparticles

About 10% aq. extract of P.f. was mixed with 1 mM solution of $AgNO_3$ in 1:9 ratio and kept this mixture for continuous stirring for about 72 h at 25°C. After 72 h, color of mixture changed into dark brown color due to the formation

of AgNPs.[11] These nanoparticles were collected after centrifugation at 5000 rpm for 20 min.

Characterization of AgNPs

UV-visible spectroscopy

SPR observed by UV-visible spectrum. UV-visible spectral analysis was done by Perkin Elmer, Lambda 35.[12]

FTIR spectroscopy

FTIR is used to measure infrared absorption of the bioorganic molecules found on the surface of nanoparticles. FTIR ranges 800–4000 cm^{-1} using Shimadzu.[13,14]

Scanning electron microscope with elemental mapping

Surface morphology and topology of AgNPs confirmed by SEM and carried out by ZEISS instruments.[14]

TEM

Size and shape studied by TEM analysis and carried out by Oxford instruments.[15]

EDAX spectroscopy

Elemental analysis (EDAX) done by the help of Oxford instruments.[16]

XRD

XRD of AgNPs carried out using XPERT-PRO.[17]

Assessment cytotoxic activity on mcf-7 and hepg-2 cell lines by mtt assay method

Assay of cytotoxic action of P.f. mediated synthesized AgNPs[18] done by the help of MTT assay method (cell viability). Cancer cell lines (HepG-2 and MCF-7) were seeded (density of cells 5 × 103 cells/well) into separated plates and each plate had 96 wells. Cell lines were allowed to grow for about 24 h in 200 µl of Dulbecco's Modified Eagle Medium with 10% fetal bovine serum after the completion of 24 h. Media were removed and replaced with the different conc. of synthesized AgNPs ranging from 0.97 to 250 µg/ml. HepG-2 and MCF-7 cells were incubated for 48 h. Cells were incubated at 37°C for another 4 h after the addition of MTT (10 ml, 5 mg/ml). The medium was then removed and 200 µl of dimethyl sulfoxide added to each well as resultant a formazan was formed. Optical density of the formazan was read out using microplate reader at 570 nm. Cytotoxicity was calculated using software (GraphPad Prism-5). Obtained optical density value was subjected to sort out the percentage of viability[19] using the following equation:[20]

$$\text{Cell viability } (\%) = \frac{\text{Mean OD}}{\text{Control OD}} \times 100$$

RESULTS AND DISCUSSION

Phytochemical Analysis

Alkaloid, tannins, terpenoids, steroids, and saponins were present in leaves extract of P.f, while glycosides and flavonoids were found absent [Table 1].

Characterization of AgNPs

Physical appearance

Synthesis of AgNPs was physically confirmed by the color change light yellow to dark brown color, whereas no color was changed in 1 mM solution of Ag nitrate act as a control this was due to the reduction of Ag^{2+} into AgNPs [Figure 2].

UV-Visible Spectral Analysis

UV-visible spectra of P.f. leaves extract and biofabricated metal nanoparticles are shown in Figure 3. The maximum absorbance observed at 400–440 nm. UV-visible observations reported that SPR peak found at 420 nm.[1]

FTIR Spectral Analysis

FTIR spectra of AgNPs showed major absorption peaks at 504.35, 669.25, 1016.42, 1514.98, 1796.57, 2949.92, and

Table 1: Preliminary phytochemical screening

Chemical test	Extract
Alkaloids	+
Terpenoids	+
Glycosides	–
Flavonoids	–
Steroids	+
Phenolic compounds and tannins	+
Saponins	+

+: Positive, –: Negative

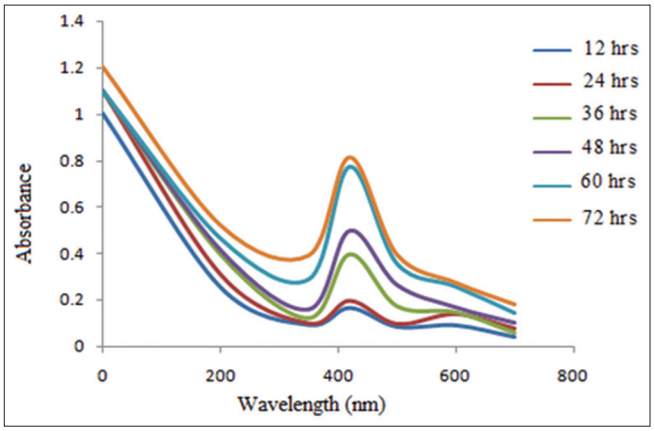

Figure 3: Ultraviolet-visible spectra

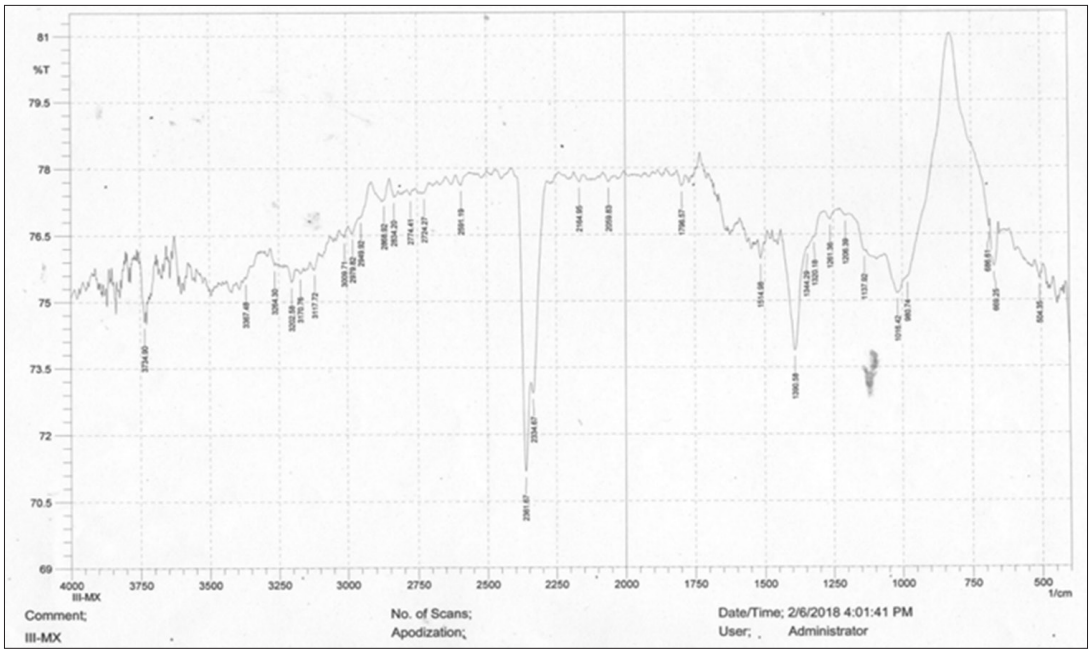

Figure 4: Fourier transform infrared spectra of silver nanoparticles

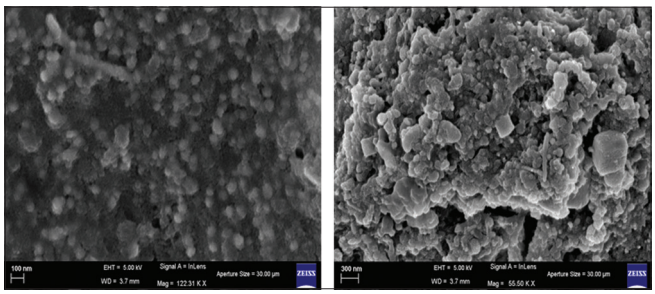

Figure 5: Scanning electron microscope images of silver nanoparticles

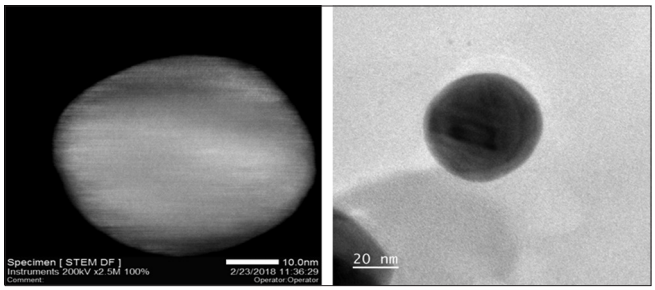

Figure 6: Transmission electron microscope images of silver nanoparticles

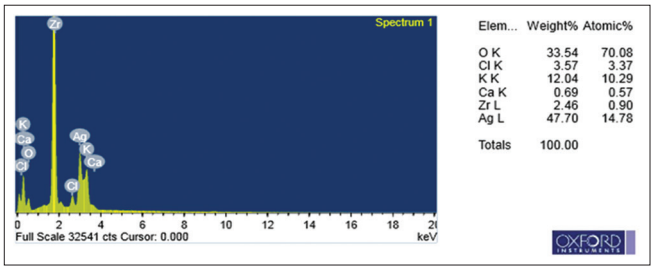

Figure 7: Energy-dispersive X-ray graph

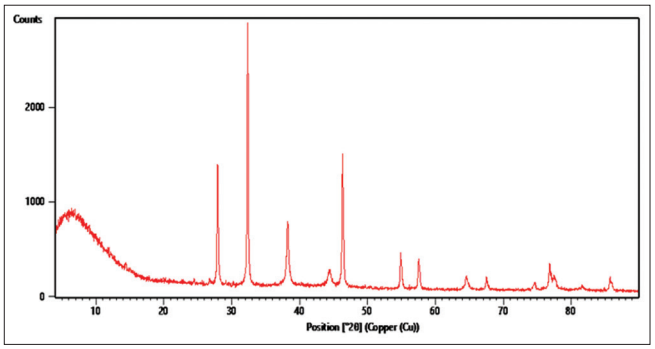

Figure 8: X-rays diffraction graph

3367.48 cm^{-1}, which confirms that the plant bioorganic molecules act as capping agents that were bound on nanoparticles surface. Absorption peak at 3367.48 cm^{-1} confirmed −OH stretching vibration, peak at 2949.92 cm^{-1} confirmed C-H stretching, confirmation of proteins by the amine N-H bending at the region of 1514.98 cm^{-1}, peak at 1796.57 cm^{-1} annex by CO functional groups, peaks at 669.25 cm^{-1} represented C-H stretching of the aromatic ring, and 504.35 cm^{-1} confirm OH bending of a phenolic group. FTIR spectra are shown in Figure 4.

SEM Imaging

SEM image showed that AgNPs were agglomerated spherical, rectangular, cubical, and triangular in shape with equal distribution rough surface and particle size range from 40 to 50 nm [Figure 5].[21]

Particle Size from TEM Analysis

AgNPs were spherical in shape and well dispersed with high surface area while some other was irregular in shape. Particle

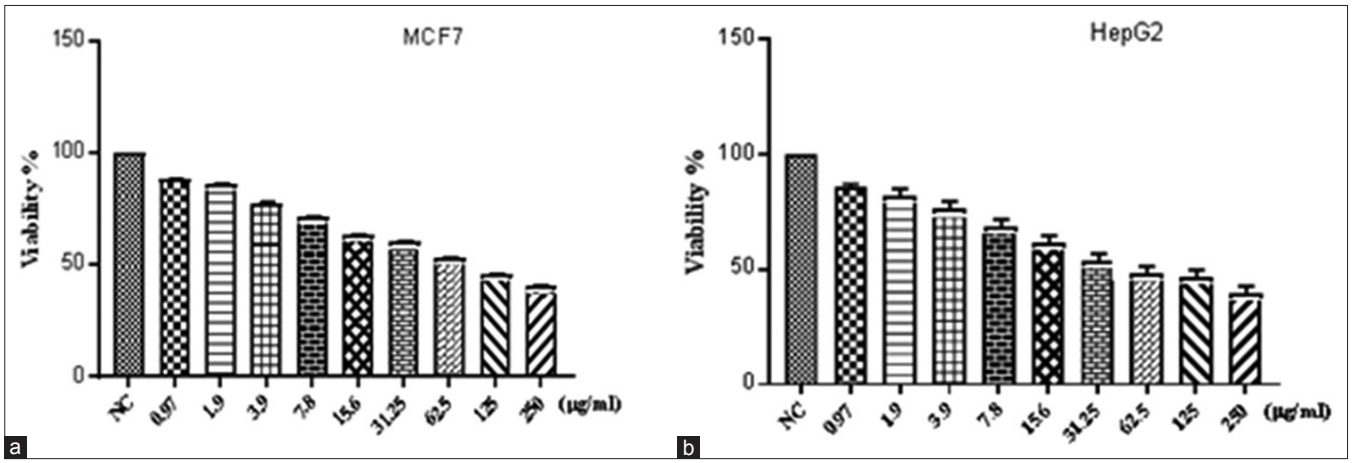

Figure 9: Bar diagrams. (a) Efficacy (% viability) of silver nanoparticles (AgNPs) on hepatic cancer cell line-2. (b) Efficacy (% viability) of AgNPs on breast cancer cell line-7

Table 2: Absorbance at different concentrations (HepG-2 cell line)

Conc. µg/ml	N.C	0.97	1.9	3.9	7.8	15.6	31.25	62.5	125	250
Absorbance	1.051	0.893	0.857	0.797	0.681	0.612	0.578	0.528	0.519	0.493
Absorbance	1.02	0.885	0.831	0.766	0.659	0.587	0.556	0.511	0.502	0.489

HepG-2: Hepatic cancer cell line, N.C: Normal control, Conc.: Concentration

Table 3: Absorbance at different concentrations (MCF-7 cell line)

Conc. µg/mL	N.C	0.97	1.9	3.9	7.8	15.6	31.25	62.5	125	250
Absorbance	1.035	0.909	0.877	0.794	0.721	0.654	0.611	0.569	0.519	0.498
Absorbance	1.052	0.927	0.878	0.82	0.743	0.676	0.621	0.587	0.524	0.501

N.C: Normal control, Conc.: Concentration, MCF-7: Breast cancer cell line

size ranging from 40 to 50 nm. This feature explained that phytoconstituents present in aq. extract of plant were effectively involved and affected the synthesis of AgNPs [Figure 6].[22]

EDAX Analysis

Metal nanoparticles of P.f. were observed in the graph obtained from EDAX analysis.[23] Chemical composition of Ag was found 47.40 wt% shown in Figure 9. This indicates the reduction of Ag^{2+} to Ag. EDAX spectra affirmed the presence of peak for elemental Ag at 3 keV. Oxygen and carbon peaks might be due to the presence of bioorganic compounds on the surface of AgNPs shown in Figure 7.

Degree of Crystallinity

XRD was carried out to confirm the crystalline structure and chemical composition of a metal particles.[24] Elemental Ag in metal nanoparticles confirmed by diffraction peaks. Graph of AgNPs crystal plane showed 2Q angles at the range of 38.68, 44.1, 64.11, and 77.4° corresponding to 111, 200, 220, and 222° affirmed the formation face-centered cubic Ag crystal shown in Figure 8.

Assessment of Cytotoxic Activity

Cytotoxicity analysis

Cytotoxicity action of the AgNPs was studied against the HepG-2 [Table 2] and MCF-7 cell line by MTT assay [Table 3].[20,25] Cytotoxicity effect on cancer cell lines was studied at different concentrations (0.97 µg/ml, 1.9 µg/ml, 3.9 µg/ml, 7.8 µg/ml, 15.6 µg/ml, 31.25 µg/ml, 62.5 µg/ml, 125 µg/ml, and 250 µg/ml). Half maximal inhibitory concentration (IC_{50}) of P.f. leaves extract-mediated synthesized AgNPs observed at concentration of 62.5 µg/ml against HepG-2 cell line and 125 µg/ml against MCF-7 cell line. This result showed that the minimum dose of AgNPs showed marked cytotoxic activity. The bar diagram represents the efficacy[26] of biofabricated AgNPs[8] against HepG-2 and MCF-7 cells at different concentrations [Figure 9].[27]

CONCLUSIONS

This research reports 100% green chemical process with simple, facile, and economical synthesis of AgNPs from leaves extract of P.f. Characterization techniques mainly

UV-visible and FTIR spectral analysis confirm the synthesis of nanoparticles. SEM and TEM images consolidate about spherical shape and particle size <50 nm. EDAX and XRD affirm the presence of Ag as an element. *In vitro* cytotoxic result on human HepG-2 and MCF-7 showed high cytotoxic action with an excellent IC_{50} value which suggested the potential therapeutic use of these AgNPs as alternative medicine for the treatment of hepatic and breast cancer cases.

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REFERENCES

1. Kumar V, Yadav SK. Plant-mediated synthesis of silver and gold nanoparticles and their applications. J Chem Technol Biotechnol 2009;84:151-7.
2. Mousavi B, Tafvizi F, Bostanabad SZ. Green synthesis of silver nanoparticles using artemisia turcomanica leaf extract and the study of anti-cancer effect and apoptosis induction on gastric cancer cell line (AGS). Artif Cells Nanomed Biotechnol 2018;46:499-510.
3. Anandalakshmi K, Venugobal J, Ramasamy V. Characterization of silver nanoparticles by green synthesis method using *Pedalium murex* leaf extract and their antibacterial activity. Appl Nanosci 2016;6:399-408.
4. Abraham J, Saraf S, Mustafa V, Chaudhary Y, Sivanangam S. Synthesis and evaluation of silver nanoparticles using *Cymodocea rotundata* against clinical pathogens and human osteosarcoma cell line. J Appl Pharm Sci 2017;7:55-61.
5. Kajani AA, Bordbar AK, Esfahani SH, Khosropour AR, Razmjou A. Green synthesis of anisotropic silver nanoparticles with potent anticancer activity using *Taxus baccata* extract. RSC Adv 2014;4:61394-403.
6. Sudha A, Jeyakanthan J, Srinivasan P. Green synthesis of silver nanoparticles using *Lippia nodiflora* aerial extract and evaluation of their antioxidant, antibacterial and cytotoxic effects. Resour Technol 2017;3:506-15.
7. Barabadi H, Ovais M, Shinwari ZK, Saravanan M. Anti-cancer green bionanomaterials: Present status and future prospects. Green Chem Lett Rev 2017;10:285-314.
8. Sreekanth TV, Pandurangan M, Kim DH, Lee YR. Green synthesis: *In vitro* anticancer activity of silver nanoparticles on human cervical cancer cells. J Clust Sci 2016;27:671-81.
9. Singh M, Velraj M, College V. *In vitro* evaluation of *Scindapsus officinalis* (ROXB.) Schott. Fruit for antioxidant potential. Afr J Basic Appl Sci 2009;1:83-6.
10. Medda S, Hajra A, Dey U, Bose P, Mondal NK. Biosynthesis of silver nanoparticles from *Aloe vera* leaf extract and antifungal activity against *Rhizopus* sp. and *Aspergillus* sp. Appl Nanosci 2015;5:875-80.
11. Xia QH, Ma YJ, Wang JW. Biosynthesis of silver nanoparticles using *Taxus* yunnanensis callus and their antibacterial activity and cytotoxicity in human cancer cells. Nanomaterials (Basel) 2016;6:E160.
12. Dasari PR, Anthony P. Synthesis, characterization of silver nanoparticles using *Rosa damascena* hips extract and antibacterial, anti-cancer activities of the synthesized Nanoparticles. Int J Pharm Sci Rev Res 2017;43:13-9.
13. Mukundan D, Mohankumar R, Vasanthakumari R. Comparative study of synthesized silver and gold nanoparticles using leaves extract of *Bauhinia tomentosa* Linn and their anticancer efficacy. Bull Mater Sci 2017;40:335-44.
14. He Y, Wei F, Ma Z, Zhang H, Yang Q, Yao B, *et al*. Green synthesis of silver nanoparticles using seed extract of: *Alpinia katsumadai*, and their antioxidant, cytotoxicity, and antibacterial activities. RSC Adv 2017;7:39842-51.
15. Bagherzade G, Tavakoli MM, Namaei MH. Green synthesis of silver nanoparticles using aqueous extract of saffron (*Crocus sativus* L.) wastages and its antibacterial activity against six bacteria. Asian Pac J Trop Biomed 2017;7:227-33.
16. Khalil MM, Ismail EH, El-Baghdady KZ, Mohamed D. Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. Arab J Chem 2014;7:1131-9.
17. Ahmed S, Saifullah, Ahmad M, Swami BL, Ikram S. Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract. J Radiat Res Appl Sci 2016;9:1-7.
18. Kaur K, Gupta R. Ethnobotanical and phytopharmacological review of *Scindapsus officinalis* ("Gajapippali"). Asian Pac J Trop Biomed 2017;7:78-85.
19. Kumar KK, Kumar BD, Punathil RR. Green Synthesis of silver nanoparticles using hydnocarpus pentandra leaf extract: *In vitro* cyto-toxicity studies against MCF-7 cell line. J Young Pharm 2018;10:16-9.
20. Devi JS, Bhimba BV, Ratnam K. *In vitro* anticancer activity of silver nanoparticles synthesized using the extract of *Gelidiella* Sp. Int J Pharm Pharm Sci 2012;4:710-5.
21. Buttacavoli M, Albanese NN, Di Cara G, Alduina R, Faleri C, Gallo M, *et al*. Anticancer activity of biogenerated silver nanoparticles: An integrated proteomic investigation. Oncotarget 2018;9:9685-705.

22. Ferdous N, Hridi SU, Hannan JM. Antinociceptive and hypoglycemic activities of *Scindapsus officinalis* (Roxb.) Schott in laboratory animals. Eur J Med Plants 2013;3:561-76.
23. Supraja S, Arumugam P. Antibacterial and anticancer activity of silver nanoparticles synthesized from cynodon dactylon leaf extract. J Acad Ind Res 2015;3:629-31.
24. Singh J, Singh T, Rawat M. Green synthesis of silver nanoparticles via various plant extracts for anti-cancer applications. Glob J Nanomed 2017;2:2-5.
25. Bonigala B, Kumar YV, Viswanath KV, Richardson PJ, Mangamuri UK, Poda S. Anticancer activity of plant mediated silver nanoparticles on selected cancer cell lines. J Chem Pharm Res 2016;8:376-81.
26. Selvarani S, Moorthi PV, Saranya P, Abirami M. Anti-cancer activity of silver nanoparticle synthesized from stem extract of *Ocimum kilimandscharicum* against hep-G2, liver cancer cell line. Kenkyu J Nanotechnol Nanosci 2015;12:1-7.
27. Suseela, V, Lalitha G. Cytotoxic effect of green synthesized silver nanoparticles using *Indigofera longeracemosa* on skin cancer SK MEL-28 cell lines. Int J Preclin Pharm Res 2015;6:118-25.

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