

Green Synthesis of Silver nanoparticles using *Scindapsus officinalis* (Gajpipli): *In-vitro* cytotoxic activity against HepG-2 & MCF-7 cancer cell lines

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ABSTRACT

Background: Green synthesis of silver (Ag) nanoparticle was performed with the fruit extract of *Scindapsus officinalis* and test against HepG-2, MCF-7 cancer cell lines. These biosynthesized metal nanoparticles have a potential of therapeutic use as an alternative medicine for the treatment of hepatic & breast cancer cases.

Results : AgNPs were identified by change of color & their absorption at 340 nm measured by UV-visible spectroscopy, FTIR spectral analysis confirmed phenolic compounds presence, morphology & size visualized in SEM, TEM used for determination of size, shape & light scattering analysis. Synthesized silver nanoparticles were spherical in shape and size less than 50 nm. XRD analysis was affirmed the crystalline nature of metal particles. *In-vitro* cytotoxic result showed an excellent IC₅₀ value of 155.81 µg/ml and 114.74 µg/ml against HepG-2 & MCF-7 cell lines.

Conclusion: The current study reveals green synthesized AgNPs possess high cytotoxic action against HepG-2 & MCF-7 cell lines which suggested the use of silver nanoparticles as a medicine to treat hepatic & breast cancer.

Keywords: Silver nanoparticles, *Scindapsus officinalis*, MTT assay, HepG-2, MCF-7

1. Background

Green synthesized Silver nanoparticles (AgNPs) is an integral part of nanotechnology.(Kumar and Yadav, 2009) Research work on silver nanoparticles is set up new trends in pharmaceutical field due to its wide therapeutic applications.(Mousavi et al., 2018) Various papers reported application of silver nanoparticles including burn wounds treatment by cream or ointments. Silver nanoparticles prepared by various ways such as thermal decomposition, photochemical reductions in micelles, reduction in solutions, chemicals, electrochemical, microwave, sonochemical methods.(Anandalakshmi et al., 2016) Biological method reported recently for green synthesis of AgNPs using enzymes, microorganisms & herbal plant extracts (Fig.1). Synthesis of AgNPs through biological methods is without using any harmful chemicals & reagents so they are cost effective & eco-friendly.(Abraham et al., 2017) Biological method has various application over traditional methods (Physical & chemical methods) such as no requirement of heat, pressure & temperature. Herbal plant extract arbitrated silver nanoparticles synthesis is widely used recently because eco-friendly, safe & cost-effective.(Kajani et al., 2014)

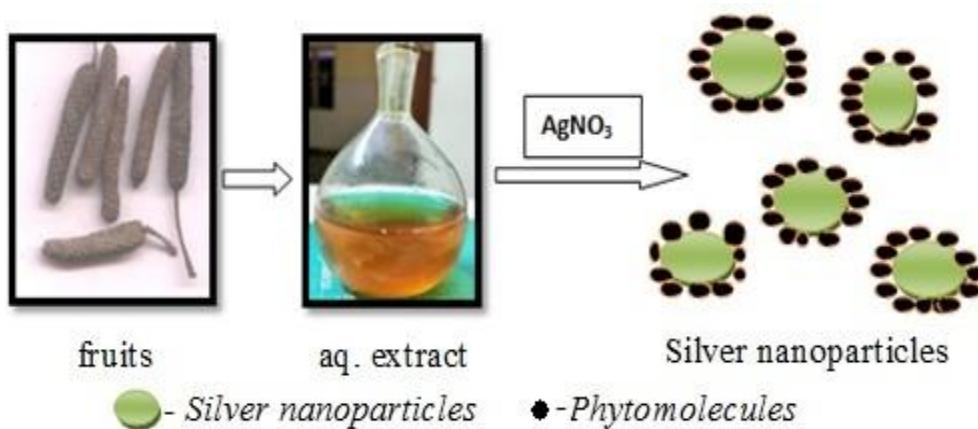


Fig.1. Schematic of synthesis of silver nanoparticles using *Scindapsus officinalis*

Scindapsus Officinalis (Family – Araceae) commonly known as Gajpipli in hindi. *S. Officinalis* is large, epiphytic, stout, perennial climber with adventitious roots. Fruits of *S.*

Officinalis mainly contains oil, Sterols, mixture of sugar and two glycosidic substance Scindapsinidine-A, Scindapsinidine-B, rhamnose, fructose, glucose, xylose and polyphenols. Traditionally Gajpippi holds a reputed position in ayurvedic medicine system and its used in chyawanprash as a active ingredient. It has been various reported ethanomedicinal uses like antioxidant, anticancer, diarrhea, worm infestation, antipyretic.

Previous literature studies has reported about silver nanoparticles as anti-cancer agent & their role as an anti-cancer agent could explore newer treatment therapy in the area of pharmaceuticals with help treatment of cancer.(Barabadi et al., 2017) In present research work, we reported first time green synthesis of AgNPs by reducing the Ag^{2+} (silver ion) of silver nitrate by the aq. fruit extract of *Scindapsus officinalis*. Synthesized metal nanoparticles particularly characterized by UV spectral analysis, FT-IR, SEM, TEM with mapping, EDAX, XRD techniques. (Ferdous et al., 2013) Silver nanoparticles was identified by change of color & absorption peak confirmed by UV-visible spectroscopy, FT-IR spectra confirmed latency of phenolic compounds, morphology & size visualized in SEM, TEM used for determination of size, shape & light scattering analysis. (He et al., 2017) XRD used to affirm crystalline nature of particles. (Supraja and Arumugam, 2015) Synthesized AgNPs from fruit extracts of *Scindapsus officinalis* showed 50% inhibition of the cell viability of hepatic cell , Breast cancer cell lines. (Suseela, V; Lalitha, 2015) *In-vitro* result on cell lines showed an exceptional cytotoxic action in term of IC_{50} value. (Sreekanth et al., 2016)

Apoptosis or programmed cell death is highly regulated by silver nanoparticles (Singh et al., 2017) through activated enzyme CASPASE-3.Family of enzyme caspases are the main component of apoptosis that irreversibly commit a cancer cell to die.(Fig.2)

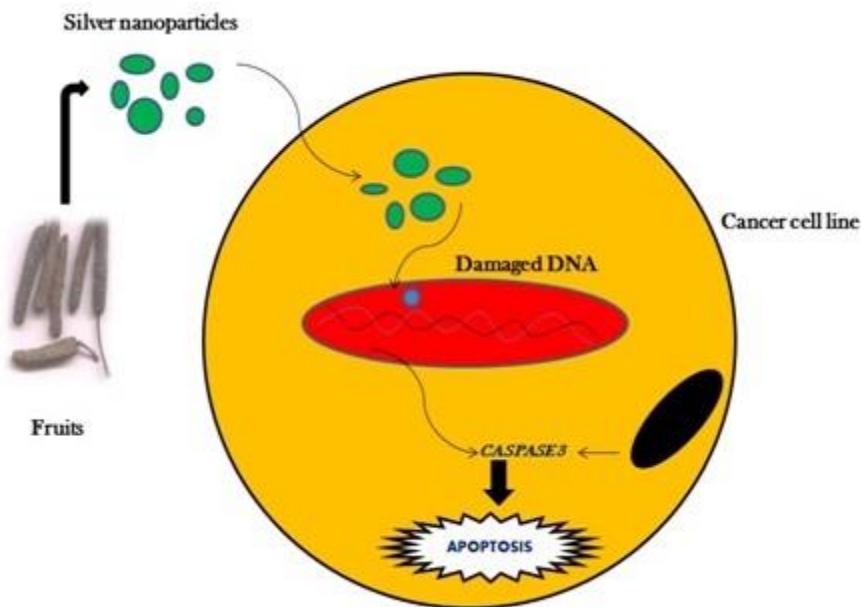


Fig.2. Mechanism involved in CASPASE mediated apoptosis

2. Methods

2.1 Collection & authentication

Fresh fruits of *Scindapsus officinalis* (Shivhare et al., 2011) were collected from Gwalior, M.P. The voucher specimen of *Scindapsus officinalis* authenticated by Botanical survey of India, Allahabad (UP)-INDIA

2.2 Chemicals

Silver nitrate was procured from Sigma-Aldrich.

2.3 Preparation of plant extract

fruits were washed, dried & grind, 10 g powdered fruits was mixed with 100 ml double distilled water & heated for 20 min. afterwards extract was get filtered.(Singh et al., 2009) Preliminary phytochemical screening was performed to know about phytoconstituents present in fruit extract.

2.4 Preparation of silver nitrate solution

Silver nitrate (1mM solution) 1.6 gm was dissolved in 1 liter double distilled water.(Medda et al., 2015)

2.5 Synthesis of Ag-nanoparticles

10 % of *Scindapsus officinalis* plant extract was mixed with silver nitrate solution in 1:9 proportions & the mixture was kept for continuous stirring at room temperature for 48 hrs. The resultant reddish brown color changes occurred in the solution due to formation of reduced silver nanoparticles.(Xia et al., 2016) Reduced nano particles were collected after centrifugation at 5000 rpm for 15 minutes.

2.6 Characterization of Silver nanoparticle

Ultraviolet–Visible spectroscopy

The reduction of Ag^+ ions in Ag was confirmed by measuring the UV–visible spectrum. (Mousavi et al., 2018) UV–visible spectral analysis was done by Perkin Elmer, Lamda 35.(Dasari and Anthony, 2017)

Fourier Transform Infrared spectroscopy

FT-IR is used to measure infrared absorption of the organic molecules found in the prepared samples. A range of 800–4000 cm^{-1} using Shimadzu.(Mukundan et al., 2017) (He et al., 2017)

Scanning Electron Microscope with Elemental Mapping

Synthesized Phytomolecules surface morphology confirmed by SEM. (Buttacavoli et al., 2018) Characterization was carried out using ZEISS instruments.

Transmission Electron Microscope

Shape & size studied by TEM & it was confirmed by using Oxford instruments. (Bagherzade et al., 2017)

Energy-dispersive X-ray spectroscopy

Elemental analysis (EDAX) confirmed by the Oxford instruments.(Khalil et al., 2014)

X-ray diffraction

X-ray diffraction of AgNPs carried out by using XPERT-PRO.(Ahmed et al., 2016)

Assessment of Cytotoxic activity on MCF-7 & HepG-2 cell lines by MTT assay method

Assay of anticancer effect of *Scindapsus officinalis* extract mediated synthesized AgNPs (Kaur and Gupta, 2017) done by the help of MTT reduction (cell viability). (Devi et al., 2012) (Bonigala et al., 2016) HepG-2 & MCF-7 cells were seeded in to separated plates & each plate had 96-wells. HepG-2 & MCF-7 cells seeded at the density of 5×10^3 cells/well. Cells were allowed to grown & attach in 96-well plate for about 24 hrs. in 200 μl of Dulbecco's Modification of basal Medium Eagle (DMEM) with 10% Fetal bovine serum (FBS) after the completion of 24 hrs. media were removed & replaced with the different conc. of AgNPs ranging from 0.97 to 250 $\mu\text{g}/\text{ml}$. HepG-2 & MCF-7 cells were incubated for 48 hrs. Cells were incubated at 37°C for another 4 hrs. after the addition of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (10 ml, 5 mg/ ml). The medium was then removed & 200 μl of Dimethyl sulfoxide (DMSO) added to each well resultant a formazan product was formed. O.D of the formazan was read at 620 nm using spectrophotometer (multi well). (Selvarani et al., 2015) Measurements were calculated & the concentration required for a 50% inhibition of viability (K et al., 2018) was determined graphically Standard Graph was plotted by taking conc. of the drug in X axis & relative cell viability in Y axis.(Devi et al., 2012)

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

3. Results

3.1 Phytochemical analysis

Glycosides, flavnoids tannins, Phenolic compounds, carbohydrate were present in fruit extract. (Table 1)

Table 1: Phytochemical test

S. No.	Chemical Test	Aqueous extract
1.	Alkaloids	-
2.	Glycosides	+
3.	Flavonoids	+
4.	Phytosterol	-
5.	Phenolic compounds and tannins	+
6.	Proteins	-
7.	Coumarins	-
8.	Saponins	-
9.	Carbohydrates	+

3.2 Characterization of Ag-nanoparticles

Physical appearance

Silver nanoparticles synthesized by using 1 mM solution of silver nitrate and aq. fruits extract of *Scindapsus officinalis*. However, after addition of silver nitrate, settled the reaction mixture for continuous stirred at room temperature for about 48 hrs, the color of solution turned light brown to dark brown in color shown in Fig.3.



Fig. 3. Biofabrication of silver nanoparticles by using *Scindapsus officinalis* aq. extract

UV–Visible spectral analysis

Light brown colored aq. extract was mixed with 1 Mm AgNO₃ solution which changed in dark brown color it's because of S.P.R (Surface Plasmon Resonance) property of silver (Supraja and Arumugam, 2015). The UV-visible observations reported that AgNPs had a maximum absorbance at 340-380 nm. Synthesis of metal nanoparticles was observed at different time intervals under UV-visible spectroscopy it shows synthesis of nanoparticles get increased with time. Fruit extract reduced AgNO₃ into Ag²⁺, the polyphenols present in extract act as a reducing & capping agent for silver nanoparticles synthesis. (Fig.4)

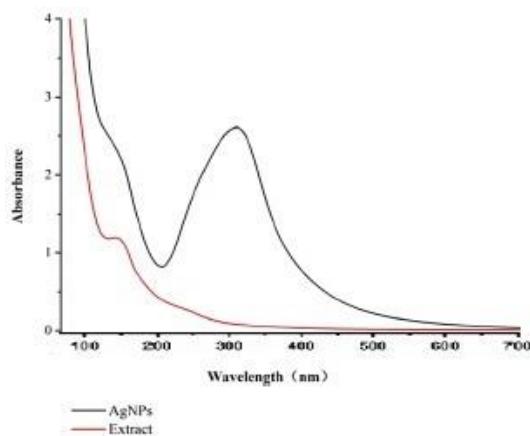


Fig.4. UV-Visible spectra of AgNPs

FTIR spectral analysis

FTIR was used to characterize the surface & functional groups of AgNPs. FTIR spectra of synthesized AgNPs showed marker absorption peaks at 504, 568, 1035, 1642, 2934 & 3476 cm⁻¹, which confirmed that the plant molecules act as capping agents that were bound on metal nanoparticle surface, peak at 3476 cm⁻¹ was confirm for -OH stretching vibration, peak at 2934 cm⁻¹ confirmed the C-H stretching, confirmation of proteins by the amine or amide at the region of 1606 cm⁻¹, peak at 1642 cm⁻¹ annex by AgNPs with the C=O functional groups, peaks at 568 cm⁻¹ represented C-H stretching of the aromatic & 504 cm⁻¹ confirm O-H group stretching of a phenolic group. FTIR spectra exhibited that phytochemicals like phenolic

compounds, amino acids might protect the AgNPs from aggregation & thereby retain them for long term stability. (Fig.5)

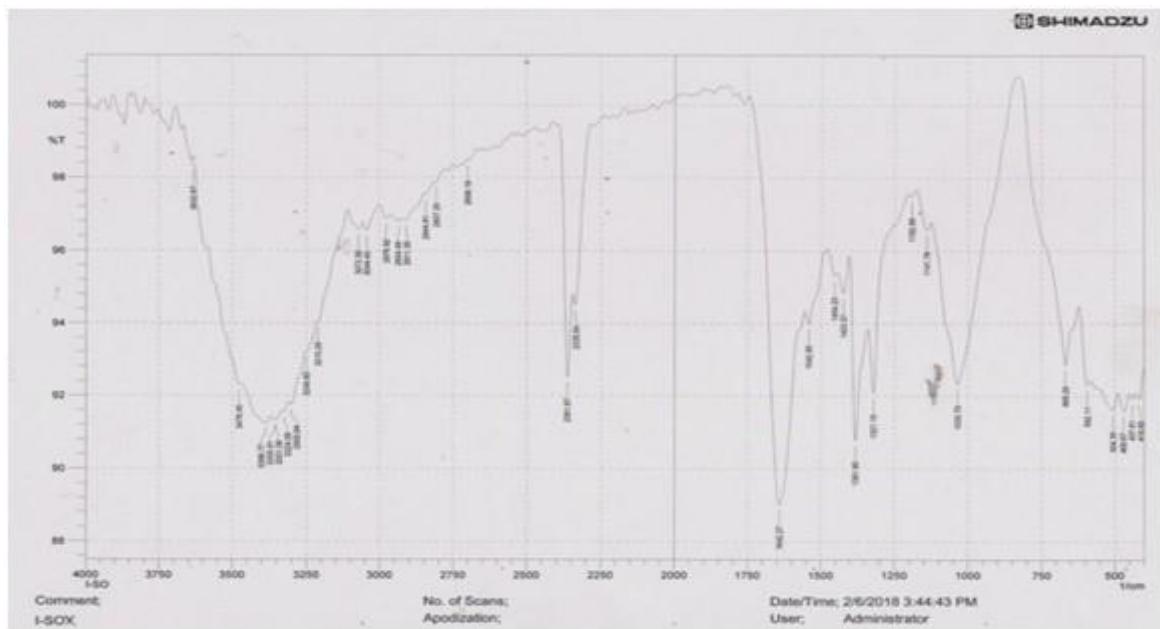


Fig.5. FTIR spectra of AgNPs

SEM imaging

Metal nanoparticles were agglomerated spheres with rough surface and with a diameter of less than 50 nm affirmed by SEM analysis. It showed a spherical shape AgNPs were enclosed by the different organic compounds. (Fig.6)

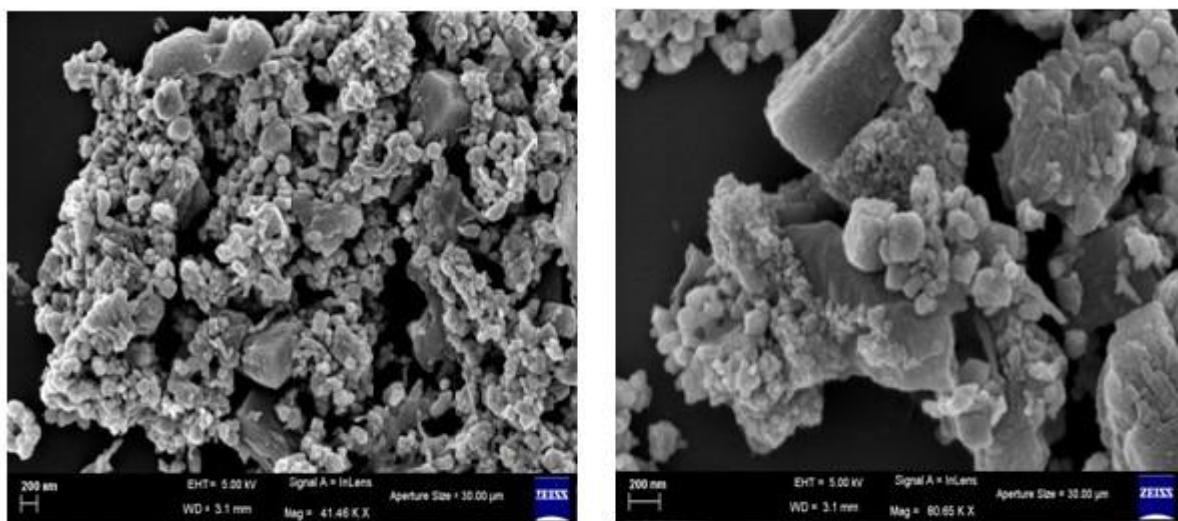


Fig.6. SEM images of AgNPs

Particle size from TEM analysis

AgNPs were spherical in shape & well dispersed while some other were irregular in shape & less than 50 nm size. This feature explained that phytoconstituents present in aq. extract of plant were effectively involved & affected the synthesis of silver nanoparticles. (Fig.7)

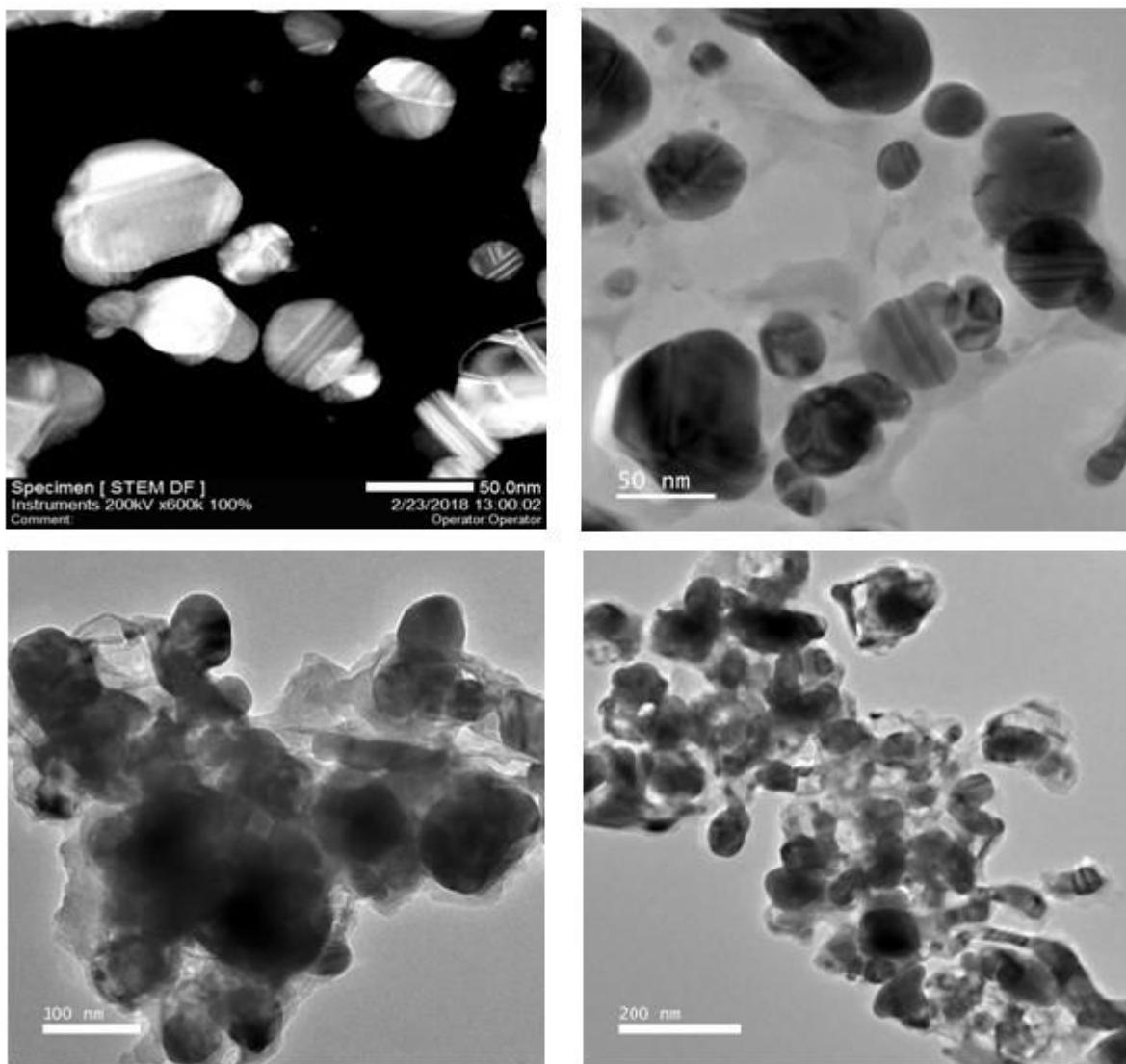


Fig.7. TEM images of AgNPs

Elemental analysis

Elemental mapping explain prepared nanoparticles exhibited maximum distribution of silver as an element which is shown in image with red color, it confirmed that silver was the main element present in sample refer to Fig.8.

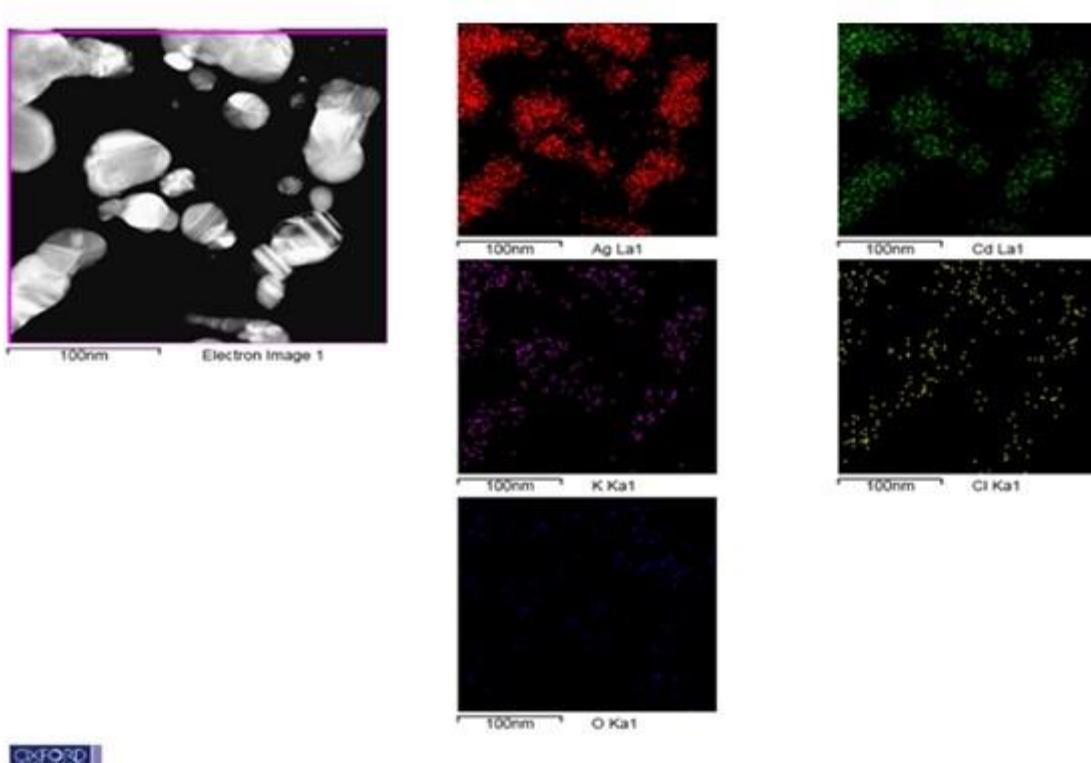


Fig.8.Elemental mapping of AgNPs

EDAX Analysis

Confirmation of the elemental silver nanoparticles of *Scindapsus officinalis* was observed in the graph obtained from EDAX analysis. Chemical composition of Ag was 54.04 wt%. This result indicates the reduction of silver ions to elemental silver. The EDAX spectra affirmed the presence of peak for elemental Ag at at 3 keV. Oxygen (O) & carbon (C) peaks might be due existence of bio-organic compounds bound on the surface. (Fig. 9)

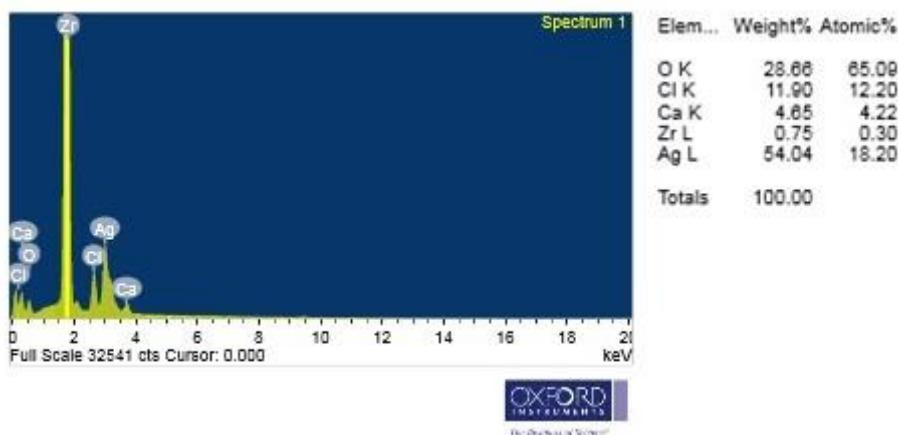


Fig.9. EDAX image

Degree of Crystallinity

XRD was carried out to identify the crystalline structure & chemical composition of a metal nanoparticles therefore, presence of Ag (silver) in nanoparticles confirmed by diffraction peaks. Synthesized silver nanoparticles crystal plane showed 2Q angles at the range of 38.68, 44.1, 64.11, 77.4 corresponding to 111, 200, 220 & 222 affirmed the formation face-centered cubic silver crystal. (Fig.10)

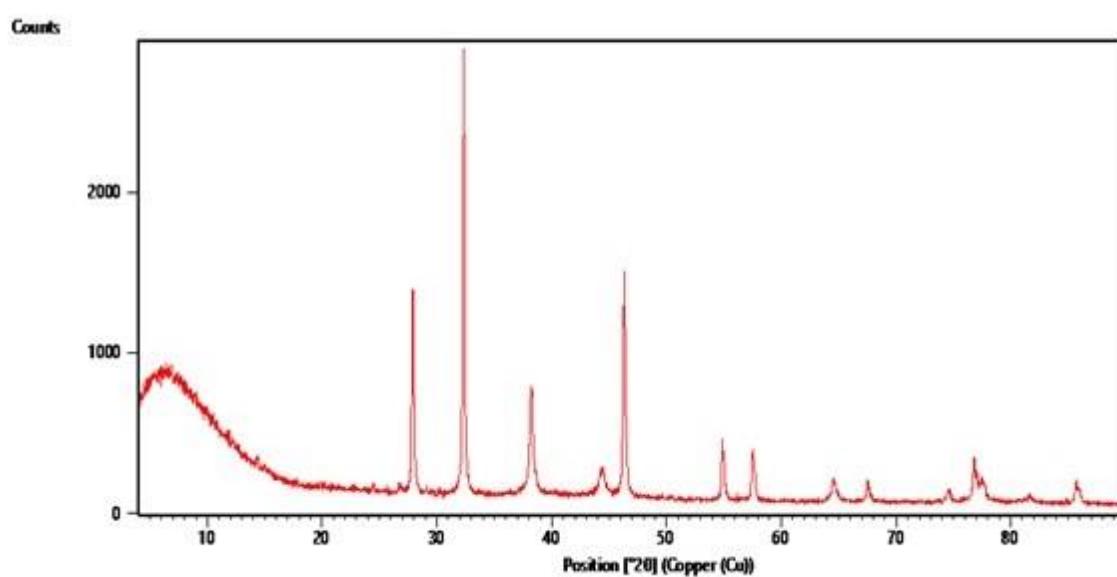


Fig.10. XRD spectrum

3.3 Cytotoxicity analysis

Cytotoxicity action of the AgNPs were studied against the HepG-2 (Table 2 &3) & MCF-7 cell line by MTT assay (Table 4 &5). Cytotoxicity effect on cancerous cell 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, 250 µg/mL) and compared with normal control. IC₅₀ of the phytoconstituted AgNPs observed at conc. of 155.81 µg/mL against HepG-2 cell line. This result shows that the minimum dose showed good cytotoxic activity. IC₅₀ value of the phytoconstituted AgNPs was confirmed at 114.74 µg/mL against MCF-7 cells. The bar diagram of efficacy of biosynthesized Silver nanoparticles against HepG-2 (Fig. 11) & MCF-

7 cells at different concentration (Fig. 12).

Table 2 Absorbance at different concentration in HepG-2 cell line.

Conc. $\mu\text{g/mL}$	Normal Control	0.97 $\mu\text{g/mL}$	1.9 $\mu\text{g/mL}$	3.9 $\mu\text{g/mL}$	7.8 $\mu\text{g/mL}$	15.6 $\mu\text{g/mL}$	31.25 $\mu\text{g/mL}$	62.5 $\mu\text{g/mL}$	125 $\mu\text{g/mL}$	250 $\mu\text{g/mL}$
Absorbance	0.922	0.893	0.872	0.712	0.758	0.699	0.721	0.573	0.506	0.42
Absorbance	0.917	0.911	0.96	0.747	0.802	0.714	0.729	0.567	0.464	0.416

Table 3 Cell Viability (%) in HepG-2 cell line at different concentration.

Conc. $\mu\text{g/mL}$	Normal Control	0.97 $\mu\text{g/mL}$	1.9 $\mu\text{g/mL}$	3.9 $\mu\text{g/mL}$	7.8 $\mu\text{g/mL}$	15.6 $\mu\text{g/mL}$	31.25 $\mu\text{g/mL}$	62.5 $\mu\text{g/mL}$	125 $\mu\text{g/mL}$	250 $\mu\text{g/mL}$
% viability	100	96.8546638	94.577007	77.223427	82.212581	75.813449	78.199566	62.147505	54.880694	45.553145
% viability	100	99.3456925	104.6892	81.461287	87.459106	77.862595	79.498364	61.832061	50.599782	45.365322

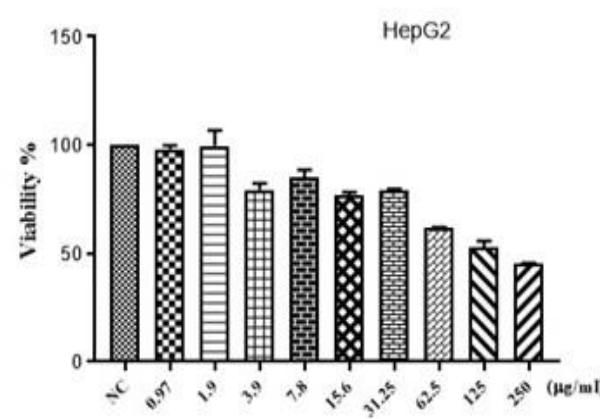
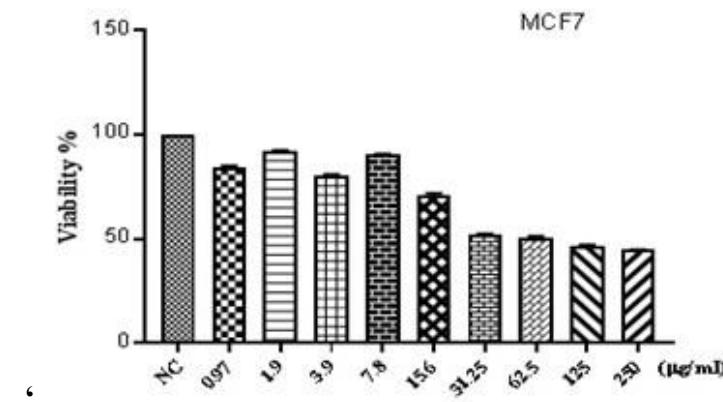
**Fig.11.** Bar diagram of efficacy of AgNPs

Table 4 Absorbance at different concentration in MCF-7 cell line.

Conc. $\mu\text{g/mL}$	Normal Control	0.97 $\mu\text{g/mL}$	1.9 $\mu\text{g/mL}$	3.9 $\mu\text{g/mL}$	7.8 $\mu\text{g/mL}$	15.6 $\mu\text{g/mL}$	31.25 $\mu\text{g/mL}$	62.5 $\mu\text{g/mL}$	125 $\mu\text{g/mL}$	250 $\mu\text{g/mL}$
Absorbance	0.977	0.823	0.9	0.789	0.885	0.687	0.501	0.492	0.46	0.435
Absorbance	0.961	0.816	0.888	0.771	0.866	0.689	0.502	0.492	0.449	0.43

Table 5 Cell viability (%) in MCF-7 cell line at different concentration.

Conc. $\mu\text{g/mL}$	Normal Control	0.97 $\mu\text{g/mL}$	1.9 $\mu\text{g/mL}$	3.9 $\mu\text{g/mL}$	7.8 $\mu\text{g/mL}$	15.6 $\mu\text{g/mL}$	31.25 $\mu\text{g/mL}$	62.5 $\mu\text{g/mL}$	125 $\mu\text{g/mL}$	250 $\mu\text{g/mL}$
% viability	100	84.2374616	92.118731	80.757421	90.583419	70.317298	51.279427	50.35824	47.082907	44.524053
% viability	100	84.9115505	92.403746	80.228928	90.114464	71.69615	52.237253	51.19667	46.722164	44.745057

**Fig.12.** Bar diagram of efficacy of AgNPs

4. Discussion

In this research plan, we have used *Scindapsus officinalis* fruits extract contains polyphenols which act as stabilizing and reducing agent for biofabrication of AgNPs. Biosynthesized AgNPs was preliminarily confirmed by color change from light brown to dark brown in mixture. (Fig.3.) Brown color arising due to the surface plasmon resonance (SPR) phenomenon, which originate due to the interaction of EMF with surface oscillated free electrons of AgNPs. SPR absorption band observed at 340-380 nm in UV-visible spectrum. (Fig.4)

AgNPs biosynthesis, shape, size, surface texture, degree of crystalline and Ag percent of presence is confirmed by FTIR, SEM, TEM, EDAX and XRD results. FTIR spectra reveled that the surface capping functionalities of the prepared AgNPs. Phytochemicals like phenolic compounds, amino acids might protect the AgNPs from aggregation & thereby retain them for long term stability. (Fig.5) AgNPs were agglomerated spheres with rough surface and a diameter of less than 50 nm reported by SEM images.(Fig.6) TEM confirmed their spherical shape with some other AgNPs found to be irregular shape. (Fig.7) Elemental analysis and EDAX reported the distribution pattern, percent (%) presence of Ag as an element in biosynthesized metal nanoparticles. (Fig.8 & 9) XRD pattern of AgNPs with distinctive peaks characteristic to the indexing planes showed the crystalline nature of the formed AgNPs. (Fig.10)

Moreover, the size of the AgNPs obtained is in good agreement confirmed with the SEM, TEM and UV absorption peak obtained at 340-380 nm, indicating small, spherical NPs formation, as the shift in the SPR band of Ag, Au NPs is associated with their size. This change in the properties of NPs with size is due to the quantum confinement effect. The formation of small and spherical nanoparticles is useful in various therapeutic conditions. Small and spherical AgNPs can easily cross the cellular membranes where as larger particles found difficulty to cross.

AgNPs showed dose dependant cytotoxicity against the hepatic (HepG-2) & breast cancer (MCF-7) cell lines. *In-vitro* cytotoxic result of biosynthesized AgNPs showed excellent IC₅₀ value of 155.81 µg/ml against HepG-2 and 114.74 µg/ml against MCF-7 cancer cell lines. (Fig.11 & 12) It is well known that the mechanism of cytotoxicity of AgNPs involves the cellular uptake of nanoparticles via macropinocytosis and clathrin-dependent endocytosis. Various studies have revealed that AgNPs works by triggering the intracellular ROS by preventing the intracellular antioxidants. The immediately formed ROS then damage the DNA which results in the cell death. It have showed that the highly reactive hydroxyl radicals produced by silver nanoparticles damage the cellular components such as DNA leading to cell death. From the earlier studies, it is concluded that the AgNPs coated with plant bioconstituents induce oxidative stress leading to the apoptosis via caspase-mediated and mitochondria-dependent pathways.

5. Conclusions

This research reports Green, facile, Simple, inexpensive synthesis of Silver nanoparticles from *Scindapsus officinalis* in aqueous medium without using any hazardous chemicals. The UV–Vis spectroscopy & FTIR affirmed the formation of silver nanoparticles. EDAX, XRD affirms presence of silver (Ag). SEM & TEM image showed spherical shape with an average particle size of less than 50 nm. The biosynthesized metal nanoparticles & *Scindapsus officinalis* extract showed promising cytotoxic activity against human hepatic cancer cell line (HepG-2) & breast cancer cell line (MCF-7). In conclusion the synthesized AgNPs using *Scindapsus officinalis* extract possess high cytotoxic action against HepG-2 & MCF-7 cell lines which suggested the potential therapeutic use of these silver nanoparticles as alternative medicine for the treatment of hepatic & breast cancer.

Abbreviations

S.O: *Scindapsus officinalis*

Ag: Silver

AgNPs: Silver nanoparticles

AgNO: Silver nitrate

MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

DMSO : Dimethyl sulfoxide

U.V: Ultra violet spectroscopy

FTIR: Fourier transform infrared spectroscopy

SEM: Scanning electron microscope

TEM: Transmission electron microscope

EDAX: Energy dispersive X-ray analysis

XRD: X-rays diffraction

HepG-2: Hepatic cancer cell line

MCF-7: Breast cancer cell line

OD: Optical density

IC₅₀: Inhibitory concentration

FBS: Fetal bovine serum

DMEM: Dulbecco's Modification of basal Medium Eagle

Aq.: Aqueous

Conc.: Concentration

N.C: Normal control

E.M.F: Electromagnetic field

R.O.S: Reactive oxygen species

% : Percent

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Conflict of interest

Authors have no conflict of interest

Author contributions

VK & PRT carried out study, data collection, analysis and interpretation. RM conducts sample characterization. PP carried out *In-vitro* testing on cell lines. AV participated in the design of the study and performed analysis. MP conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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