Green Synthesis of Silver Nanoparticles from Phytochemicals of Medicinal Plants and their Cytotoxicity against Human Umbilical Vein Endothelial Cells

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Abstract

In the past few decades, green synthesized nanoparticles are being extensively studied for their biological potential and in treatment of variety of diseases. The current study was designed to evaluate the cytotoxic potential of silver nanoparticles (AgNPs) green synthesized with medicinal plants Cissus quadrangularis, Catharanthus roseus, Acalypha indica and Coleus aromaticus on immortalised Human Umbilical Vein Endothelial Cells (HUVEC) cell line. The aqueous extracts of the medicinal plants were used as reducing agents during the synthesis of AgNPs. The qualitative phytochemical analysis of the aqueous extracts denoted the presence of several bioactive compounds, including saponins, tannins, phenols, flavonoids and steroids in all four species. Phytochemicals such as flavonoid are the major constituents. Flavonoids are phytochemicals known for their anti-cancer, antioxidant, anti-inflammatory and cell-signalling regulatory properties.

It is found that flavonoid content in C. quadrangularis AgNPs was greater than other three plants at a value of 968.31mg/g (QCE). The synthesised nanoparticles were then characterised by Scanning electron microscope (SEM), X-ray diffraction (XRD), UV–vis spectrophotometer, Energy-dispersive X-ray spectroscopy EDAX and Fourier-transformed infrared spectroscopy (FT-IR). The synthesised nanoparticles were then cultured with the HUVEC cell line to assess their cytotoxic potential. The AgNPs produced considered cytotoxicity in the HUVEC cell line, however it was found that AgNPs synthesised with aqueous extract of Cissus quadrangularis produced the greatest cytotoxicity, with an IC50 value of 28.689 µg/ml. The findings of the study suggest the anti-cancer potential of AgNPs green synthesized with Cissus quadrangularis.

Keyword: Green synthesis, *Cissus quadrangularis*, Nanoparticles, Anti-cancer, Cytotoxicity, HUVEC.

Introduction

All plants including fruits, vegetables, grains and legumes, produce phytochemicals. Phytochemicals such as anthocyanidins, beta-carotene, catechins, carotenoids, flavonoids, isoflavones etc. are utilized to treat a variety of illnesses and have anti-inflammatory, anti-cancer and antioxidant properties. They also act as reducing agents. They assist in scavenging free radicals, which may harm DNA[23]. Nanoscience is a valuable advancement that has the potential to bring unexpected transformations across various research fields and practical applications.

The past few decades have also seen the application of nanotechnology in a variety of industries including food, agriculture, healthcare and medicine[18]. Since the early 1900s, it has been recognised that plant extracts can reduce metal ions. In the past decades, there has been growing interest in using plants and their extracts to reduce metal salts to nanoparticles[17]. The use of plant-based systems for nanoparticle synthesis introduces an eco-friendly and sustainable alternative to conventional chemical methods[17]. Phytochemical-rich plant extracts provide intrinsic anti-cancer and anti-angiogenic properties, which, when integrated with nanoparticles, offer synergistic therapeutic potential[16].

Unlike synthetic agents, these biogenic nanoparticles are biocompatible, reduce adverse effects and enhance drug delivery efficiency. By leveraging phytochemicals as stabilizing agents and reducing agents, use of phytochemicals established possibilities for the synthesis of non-toxic, environmentally conscious and sustainable phytochemical treated nanoparticles[2]. Nanoparticles improve the pharmacokinetics and biodistribution of chemotherapeutics, enabling targeted delivery to tumour sites while minimizing systemic toxicity[11]. Anti-cancer therapies have emerged as a pivotal component in oncological interventions, aiming to disrupt the vascular support system of tumours[15]. While conventional anti-cancer and anti-angiogenic drugs have shown promise, they are often limited by systemic toxicity, resistance and incomplete tumour suppression. These challenges necessitate the exploration of innovative, biocompatible and efficient alternatives[21].

Doxorubicin, a widely used chemotherapeutic agent, demonstrates significant anti-cancer efficacy but is hindered by dose dependent cardiotoxicity and limited tumour specificity[14]. The current research explores at the phytochemical analysis of medicinal plants notably *Acalypha indica, Cissus quadrangularis, Coleus aromaticus*

and Catharanthus roseus. Silver nanoparticles are created via phytochemical extraction as a reducing agent which also has cytotoxic effects on HUVEC. Finally, a promising margin in the cytotoxic activity of cancer cells is provided by the photosynthetic AgNPs. The incorporation of plant extracts in nanoparticle synthesis not only enhances their therapeutic potential but also aligns with the growing demand for sustainable and non-toxic biomedical solutions. By targeting angiogenesis, this hybrid system aims to disrupt the tumour microenvironment, thereby inhibiting cancer growth and progression[32].

In conclusion, the convergence of nanotechnology and plant-derived therapeutics offers a promising margin in anti-cancer research. This work contributes to the evolving paradigm of targeted, sustainable and effective cancer therapies, emphasizing the critical role in combating malignancies[8].

Material and Methods

Collection and identification of plants: Four plants *Cissus quadrangularis*, *Acalypha indica*, *Catharanthus roseus*, *Coleus aromaticus* were collected in and around Tamil Nadu, India. The plant was identified and authenticated by Dr. M. Job Gopinath, Assistant Professor, Department of Zoology, Voorhees College (Affiliated to Thiruvalluvar University), Vellore 632001. Fresh plant material was washed 5 with water, air dried under shade and then blended to fine powder. The powder was stored in airtight containers for further use.

Extraction of plant for its phytochemicals: About 100 g dried samples were extracted in a Soxhlet extraction system (BUCHI Extraction System Model B-811) using water as solvent. The crude extract solutions obtained were then concentrated using a vacuum rotary evaporator or hot air oven (BUCHI Rotavapor Model R-144) at a temperature of 60°C or lower to remove the solvents. All extracts were then stored at room temperature before weighing gravimetrically to determine the yields.

The extraction yield was calculated using:

$$Y = Wf/Wi \times 100$$

where Y = Extraction yield, Wi = Initial weight of sample and Wf = Final weight after extraction.

Qualitative analysis: Qualitative analysis was done for analysing the presence of flavonoids, tannin, phenol, glycoside, saponin, terpenoids, alkaloids and steroids in the extract[10].

Quantification of total flavonoid in plant extract: The plant extract was quantitatively estimated for the detection of total flavonoid[24] and phenol content[19].

Synthesis silver nanoparticle using flavonoid rich fraction: For synthesis of silver nanoparticles, about 47.5 ml of 10−3 M aqueous $AgNO_3$ (HiMedia, India) solution was taken in 100 mL beaker. 20 ml of aqueous plant extract(10mg/ml) was transferred dropwise to $AgNO_3$ solution. The mixture was then continuously stirred for 3 hours at 25 °C.

Fig. 1.1

Fig. 1.2

Fig. 1.3

Fig. 1.4

Figure 1: Plants used for NPs Synthesis
(1.1) *Cissus quadrangularis*, (1.2) *Acalypha indica*, (1.3) *Catharanthus roseus*, (1.4) *Coleus aromaticus*

During the reaction progress, visual colour changes were monitored. The obtained solution was subjected to centrifugation (Remi R12C plus) at 4000 rpm for 20 min. The pellet present at the bottom was washed with 90% ethanol to remove the organic components. Then it is lyophilized to get powdered form of nanoparticle and then kept at room temperature for further studies[3].

Characterization of silver nanoparticles

UV spectrum analysis: UV-visible spectro photometric analysis was conducted for the synthesised nanoparticles using UV-Visible double beam spectrometer, Kinglab, India with a slit width of 2 nm, using a 10 mm cell at room temperature. The sample was examined under visible and UV light in the wavelength ranging from 200 to 800 nm for proximate analysis[1].

SEM- EDX analysis: The AgNPs were subjected to SEM analysis using a Scanning electron microscopy (VEGA3, TESCAN (Czech Republic)). The energy of the electron beam was kept at 15 keV for both imaging and EDX analysis. The characterisation of elements or chemicals present in the synthesised biogenic AgNPs was determined using energy-dispersive X-ray spectroscopy EDAX (BRUKER, D-12489, Germany)[6].

XRD: The crystal structure, phase and texture of the synthesised AgNPs were analysed using an PANalytical X'pert3 powder X-ray diffractometer operating at a voltage of 40 kV, a running current of 30 mA with Cu Kα radiation[29].

FT-IR: Fourier-transformed infrared spectroscopy (JASCO FT/IR-6300, Japan) was used to identify the functional groups present in plants responsible for the reduction of silver iron and stabilisation of synthesised AgNPs. FTIR was measured in the range from 4,400 to 400 cm^{-1}. The spectra (16 scans per spectrum) were collected with a spectral resolution of 16.0 cm−1 with an interval of 2.0 cm^{-1} [20].

Cell culture: Primary cultures of immortalised human umbilical vein endothelial cells (HUVEC) were purchased from NCCS, Pune and cultured at 37 °C under 5% CO_2 for 2–3 days in flasks in EGM endothelial cell growth medium (supplemented with 2% Fetal bovine serum, 1µg/ml hydrocortisone, 10ng/ml human epidermal growth factor, 3 ng/ml basic fibroblast growth factor, 10 ng/ml VEGF and 10 µg/ml heparin or 1% endothelial cell growth supplement). Medium without growth factors and 2.5% serum supplement served as endothelial basal medium (EBM). Upon reaching 80% confluence, cells were routinely passaged at a ratio of 1:2. HUVECs between passages 2 and 6 were used in the experiments[13].

Anti-cancer activity: The assay was carried out by using (3-(4, 5-dimethyl thiazol-2yl) - 2, 5- diphenyl tetrazolium bromide (MTT). Briefly, HUVEC cells trypsinized were seeded in 96 well plate and incubated with medium to allow for the formation of monolayers at 37°C for 48 h. After 48 hours, medium was replaced with low serum medium containing samples (with and without green synthesized AgNPs) in different concentrations for 24 - 48 hours. After treatment, media were removed and wells were added with MTT (5 mg/ml prepared in phosphate-buffered saline) and left for 4 hours at room temperature. Thus, the formazan crystals formed were dissolved in 100 µl DMSO and absorbance was read in a microplate reader at 570 nm[26].

Results and Discussion

(1) Plant extraction and yield percentage: The dried plant powder of *C. quadrangularis, A. indica, C. roseus and C. aromaticus* were subjected to Soxhlet extraction with water as solvent to extract their bioactive compounds. After Soxhlet extraction, plant extract was dried and yield percentage was calculated. The percentages of extraction yield obtained were 79.97, 79.63, 84.23 and 84.06% in *Cissus quadrangularis, Acalypha indica, Catharanthus roseus and Coleus aromaticus.*

(2) Qualitative analysis: The phytochemical analysis of the plant extracts from *C. quadrangularis, A. indica, C. roseus and C. aromaticus* demonstrated the presence of several bioactive compounds including saponins, tannins, phenols, flavonoids and steroids in all four species A as given in table 2. The qualitative findings of *C. quadrangularis* were consistent with the findings of Kaur et al[12] except that phenol and saponin were found to be absent. However, in findings of Dharshini[4], phenol and saponins were detected in aqueous extract of *C. quadrangularis*.

The phytochemical analysis of *A. indica* is like the results obtained by Sahukari et al[25] in which ethanolic extract of the plant root were qualitatively estimated. From the phytochemicals detected in *C. roseus*, similar results were observed in study conducted by Jan et al[7] in which the plant extract before and after exposure to silver nanoparticles was qualitatively estimated.

Table 1
Plant Extraction and Yield Percentage

S.N.	Plant Sample	Initial Wt. (gm)	Final Wt. (gm)	Ext. Yield (%)
1	*Acalypha indica*	50.345	40.09	79.63
2	*Catharanthus roseus*	51.84	43.67	84.23
3	*Cissus quadrangularis*	51.678	41.33	79.97
4	*Coleus aromaticus*	51.945	43.67	84.06

Table 2
Phytochemical Analysis of the Extracts

S.N.	Phyto-chemical	*A. indica*	*C. roseus*	*C. quad.*	*C. arom.*
1	Flavanoid	-	-	-	-
2	Alkaloid	-	-	+	-
3	Saponin	++	++	++	++
4	Tannin	++	++	++	++
5	Phenol	++	++	++	++
6	Terpenoid	-	+	+	+
7	Glycoside	-	-	-	-
8	Steroid	+	+	+	+

Table 3
Quantification of Total Phenol and Total Flavonoid contents of the aqueous extracts

Plant Name	Phenol Quant.		Flav. Quant.	
	Abs @ 650nm	GAE (mg/g)	Abs @ 420nm	QCE
A. indica	0.344 ± 0.004	84.11 ± 1.09	1.083 ± 0.003	82.79 ± 1.71
C. roseus	0.696 ± 0.003	170.99 ± 1.69	2.234 ± 0.003	721.83 ± 1.67
C. quad.	0.675 ± 0.003	159.94 ± 1.90	2.677 ± 0.002	968.31 ± 1.39
C. arom.	0.546 ± 0.003	92.26 ± 0.18	2.090 ± 0.003	642.01 ± 1.67

From the phytochemicals obtained in extract of *C. aromaticus*, similar results were observed in study conducted by Sehrawat et al[27]. These compounds are known for their significant pharmacological properties such as antioxidant, antimicrobial and anti-inflammatory activities. Glycoside were absent in all the plant extract and alkaloids were present in only *C. quadrangularis.*

(3) Quantitative analysis: The total phenol and flavonoid contents of aqueous extracts of *C. quadrangularis, A. indica, C. roseus and C. aromaticus* were quantitatively estimated by measuring their absorbance at different concentrations. Total phenolic contents in the aqueous extracts of *C. quadrangularis, A. indica, C. roseus and C. aromaticus* were evaluated by plotting a standard curve using different concentrations of GAE (Gallic acid equivalent) with their respective absorbance at 650 nm.

The linear regression equation is:

$y = 0.0019x + 0.3711$

It was used to evaluate the total amount of phenolic contents present in each extract which was expressed as mg of GAE/g.

Aqueous extract of *C. roseus* showed higher phenolic content 170.99 ± 1.69 mg GAE/g of dry plant extract. Then *A. indica, C. aromaticus* and *C. quadrangularis* have a phenolic content in the range of 84.11 ± 1.09, 92.26 ± 0.18 and 159.94 ± 1.90mg GAE/g of dry plant extract. The aluminium chloride method was used to determine the total flavonoid content in the aqueous plant extract. The standard curve of different concentration of quercetin (QCE) was plotted to obtain calibration curve to evaluate the total amount of flavonoid content present in *C. quadrangularis, A. indica, C. roseus and C. aromaticus* plant extract which was expressed as mg of QCE/g dry plant extract (Table 3). The amounts of flavonoid content in *C. quadrangularis, A. indica, C. roseus and C. aromaticus* were 968.31±1.39, 82.79±1.71, 721.83±1.67 and 642.01±1.67 mg QCE/g dry plant extract.

UV-visbile spectrum analysis: The UV-visible spectrum of the nanoparticles synthesized from *C. quadrangularis, A. indica, C. roseus and C. aromaticus* has major characteristic peaks at 455, 480, 480 and 470nm respectively. The presence of major peaks between 450 and 480 nm confirms the formation of silver nanoparticles synthesized by aqueous extract in the figure 3.

The above has clearly indicated that reducing agents are present in the plant extracts. These results can be verified by comparing UV results of synthesised AgNP from Duraisamy et al[5] and Vishwajeet et al[31].

EDAX: The elemental composition of synthesised nanoparticles was analysed by EDAX, the detected elements with their relative weight and atomic percentage were given in table 4. According to the EDX result, the green synthesized AgNPs also produce a strong signal at 3 keV as shown in figure 4 confirming the existence of silver (Ag) and the organic components which are present along with the NPs.

SEM: Surface morphology and topography of the AgNPs were observed by using SEM. Figure 5 depicts that the morphology of the synthesised AgNPs is near to be spherical in shape and in agglomerated condition. The spherical shape of the synthesised nanoparticles coincides with the SEM

analysis of green synthesised AgNPs by Valli et al[30] and Sujitha et al[28].

XRD: Silver nanoparticles typically exhibit characteristic diffraction peaks corresponding to the face-centred cubic (FCC) structure of metallic silver (Ag), often appearing at 2θ ≈ 38°, 44°, 64° and 77°, which correspond to the (111), (200), (220) and (311) planes. The plane values and nanoparticles size for nanoparticles synthesized were analysed by XRD.

Figure 4 shows the graphical representation of XRD results obtained. The XRD data confirms the presence of crystalline AgNPs in green synthesised AgNPs and the size of *C. quadrangularis, C. aromaticus, A. indica* and *C. roseus* synthesised AgNPs were 12.41, 11.28, 18.79 and 11.22nm respectively.

FTIR: FTIR analysis was carried out for the identification of the possible functional groups in bio molecules, present in the plant extract responsible for the reduction of the silver ion into AgNPs. FTIR spectra of synthesized AgNPs with plant extracts are present in figure 6. FTIR spectrum of AgNPs synthesized with *Cissus quadrangularis*, showed different peaks at 1795, 1918, 2030 and 3583; *A. indica* at 1747, 1996, 2185 and 3610; *C. roseus* at 1799, 1834, 3749 and 3942; *C. aromaticus* at 1800, 2567, 3622 and 3640, these peak values denote the presence of groups C=O, N=C, O=H. These results were like the findings of Duraisamy et al[5] in which *C. quadrangularis* AgNPs were analysed.

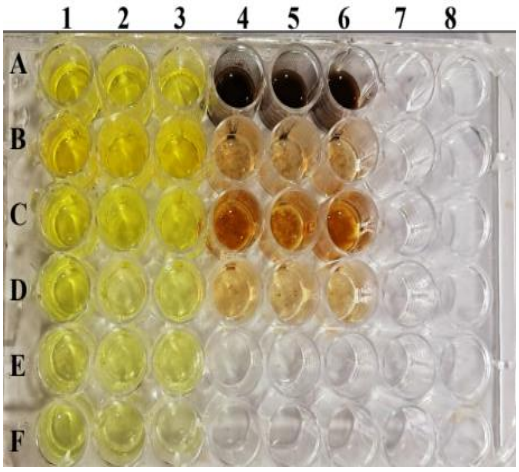

Figure 2.1: Total phenolic contents of the aqueous extracts

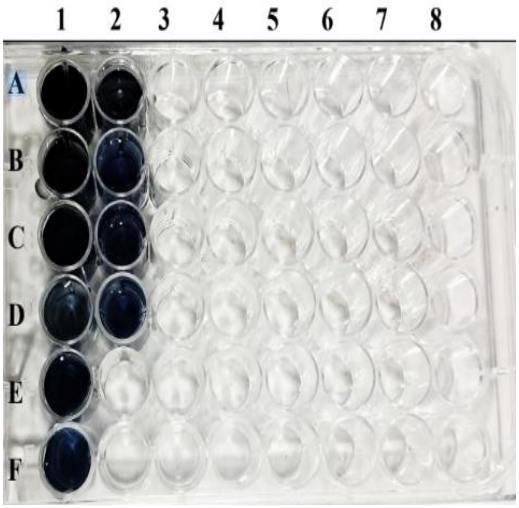

Figure 2.2: Total Flavonoid contents of the aqueous extracts

Table 4
IC50 Value against HUVEC Cell lines

Sample	Concentration (µg/mL) IC_{50}
Coleus aromaticus	41.535
Catharanthus roseus	41.383
Acalypha indica	47.776
Cissus quadrangularis	28.689

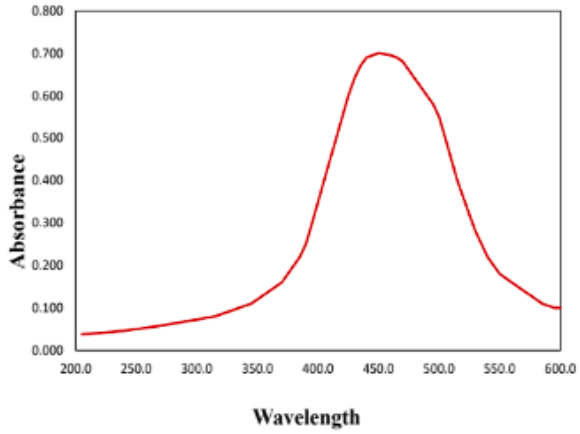

Fig. 3.1: ***Cissus quadrangularis Ag-Uv spectrum***

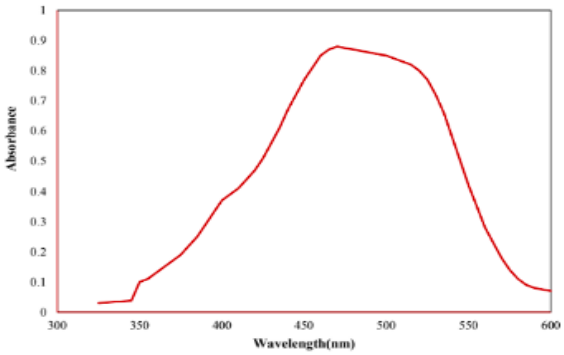

Fig. 3.2: ***Acalypha indica Ag-Uv spectrum***

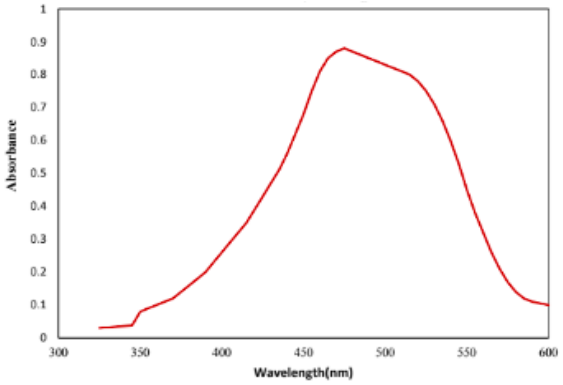

Fig. 3.3: ***Catharanthus roseus Ag-Uv spectrum***

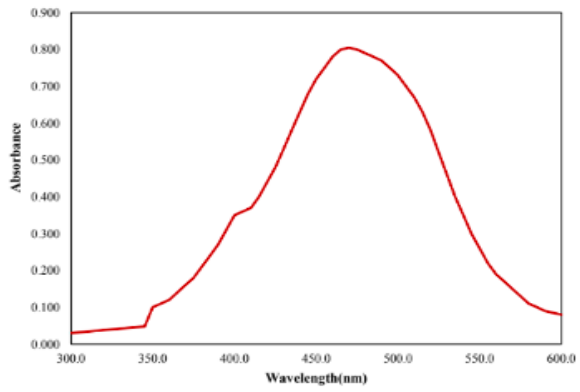

Fig. 3.4: ***Coleus aromaticus Ag-Uv spectrum***

Figure 3: UV visible spectrum of the nanoparticles using the extracts as follows:
(3.1) ***Cissus quadrangularis,*** **(3.2)** ***Acalypha indica,*** **(3.3)** ***Catharanthus roseus*** **and (3.4)** ***Coleus aromaticus***

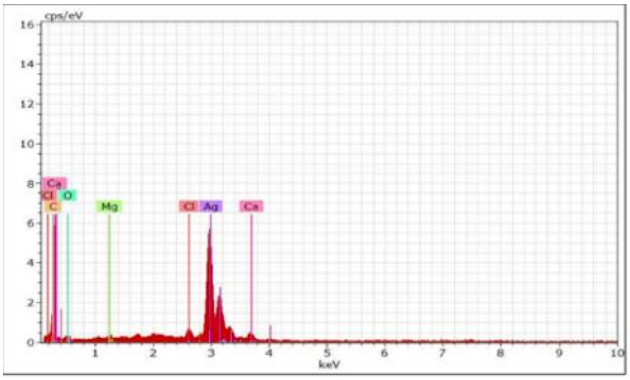

Fig. 4.1: ***Cissus quadrangularis***

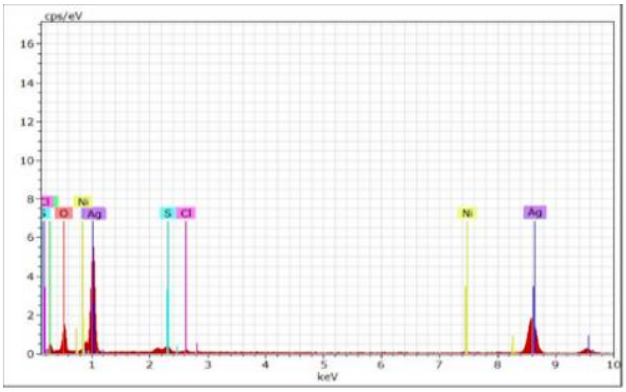

Fig. 4.2: ***Acalypha indica***

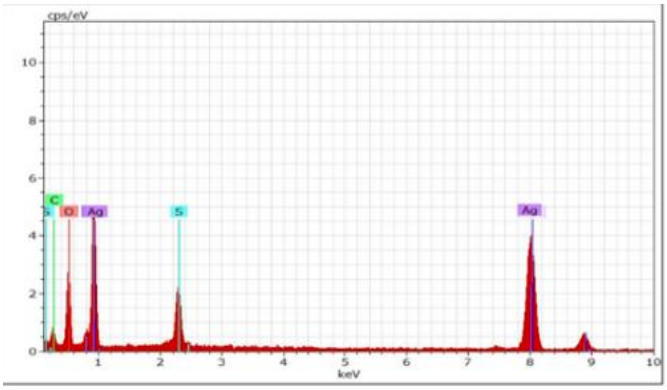

Fig. 4.3: ***Catharanthus roseus***

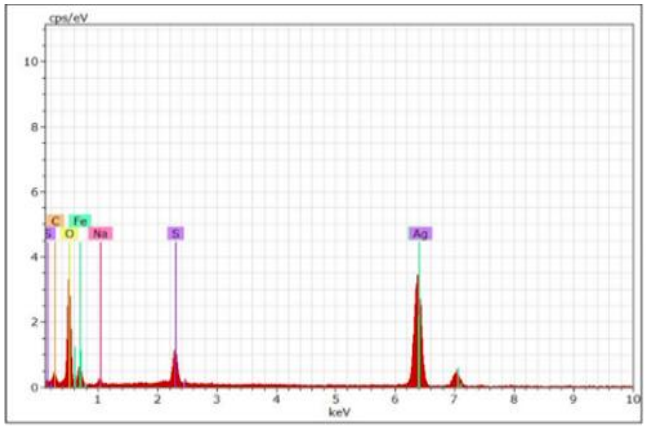

Fig. 4.4: ***Coleus aromaticus***

Figure 4: Analysis of Elemental Composition of the synthesised nanoparticles by EDAX of the extracts as follows: (4.1) ***Cissus quadrangularis,*** **(4.2)** ***Acalypha indica,*** **(4.3)** ***Catharanthus roseus*** **and (4.4)** ***Coleus aromaticus***

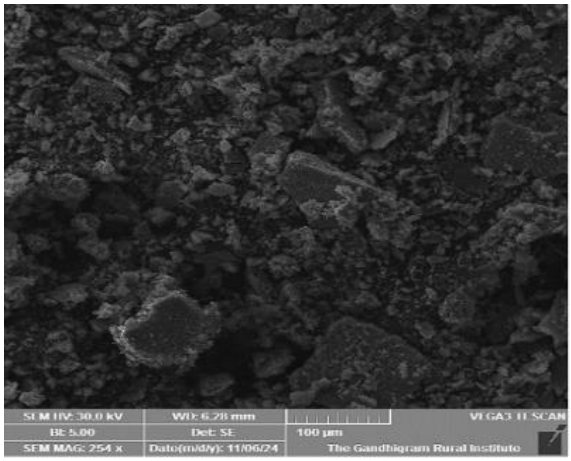

Fig. 5.1: *Cissus quadrangularis* – SEM analysis

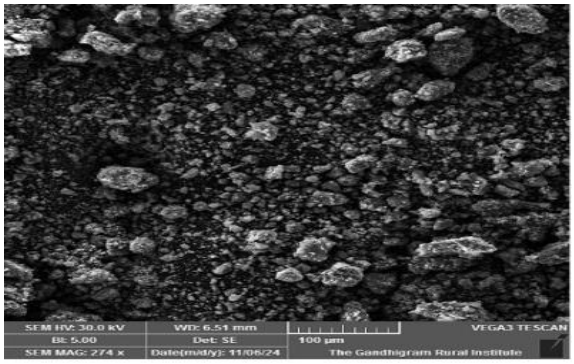

Fig. 5.2: *Acalypha indica* – SEM analysis

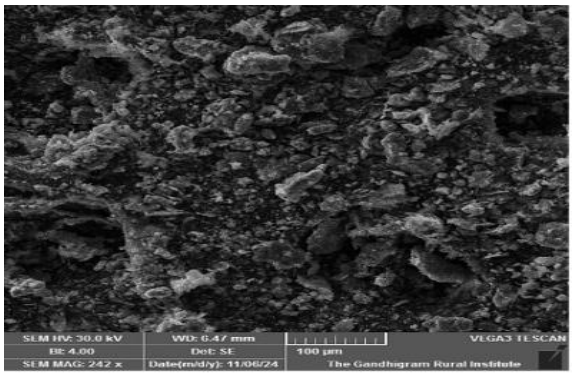

Fig. 5.3: *Catharanthus roseus* – SEM analysis

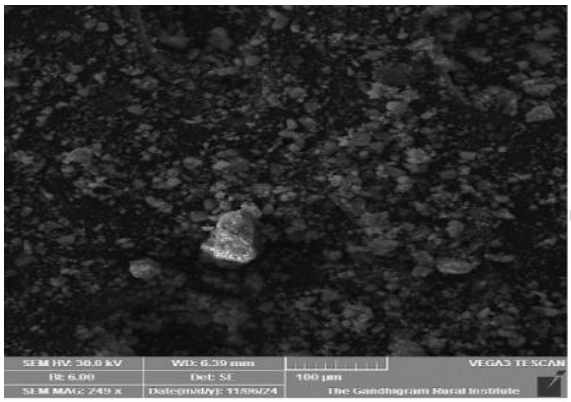

Fig. 5.4: *Cissus quadrangularis* – SEM analysis

Figure 5: Surface Morphology and Topography of the AgNPs using SEM of the extracts as follows: (5.1) *Cissus quadrangularis,* (5.2) *Acalypha indica,* (5.3) *Catharanthus roseus* and (5.4) *Coleus aromaticus*

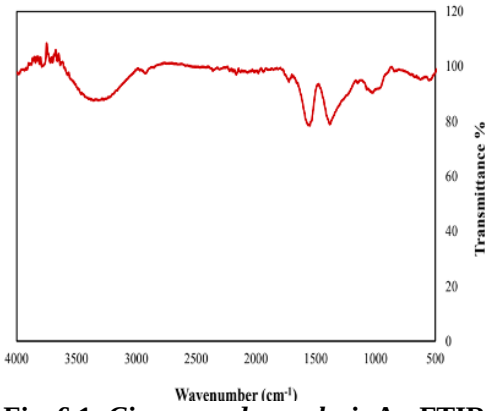

Fig. 6.1: *Cissus quadrangularis* Ag-FTIR

Fig. 6.2: *Acalypha indica* Ag-FTIR

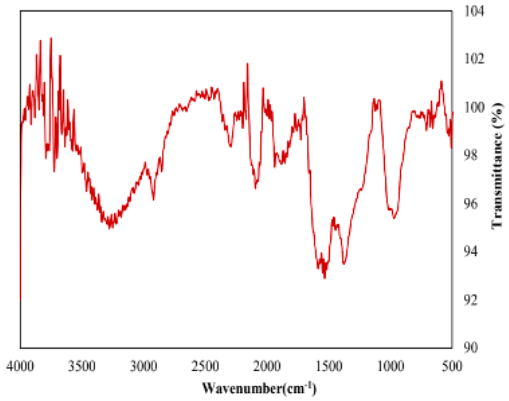

Fig. 6.3: *Catharanthus roseus* Ag-FTIR

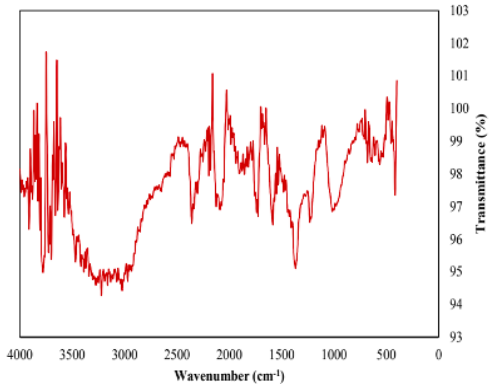

Fig. 6.4: *Coleus aromaticus* Ag-FTIR

Figure 6: FTIR Analysis of the synthesised AgNPs of the extracts as follows:
(6.1) *Cissus quadrangularis*, (6.2) *Acalypha indica* (6.3) *Catharanthus roseus* and (6.4) *Coleus aromaticus*

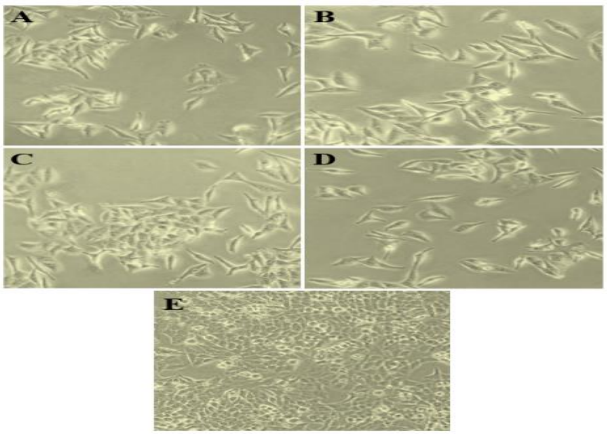

Figure 7: HUVEC Cells treated with the nanoparticles using the plant extract A. *Coleus aromaticus*, B. *Acalypha indica*, C. *Catharanthus roseus*, D. *Cissus quadrangularis* and E. *Control-untreated cells*

Anti-cancer activity: The cytotoxicity test results of nanoparticles green synthesised with the extracts of plants using MTT assay as IC50 scores are presented in table 4. Figure 7 represents HUVEC cell treated with nanoparticles. The cytotoxicity test results using MTT assay were measured with IC50 scores. The IC50 score of 47.776 µg/mL was observed in *Acalypha indica* and 28.689 µg/mL IC50 value was observed in *Cissus quadrangularis*. *Catharanthus roseus* and *Coleus aromaticus* showed IC50 of 41.383 and 41.535 µg/ml respectively. This clearly shows that *Cissus quadrangularis* AgNPs have better cytotoxic activity against immortalised HUVEC cells proving its anticancer effect. Since anti-angiogenesis is inhibition of angiogenesis of endothelial cells, the cytotoxic effect by AgNP synthesised from extract of *Cissus quadrangularis* can prove it to be of anti-angiogenic property[9].

Conclusion

Green synthesized silver nanoparticles are considered safer and have excellent cytotoxic effects against cancerous cell lines. The green synthesized AgNPs were tested for their stability, size and possible functional groups, which resulted in consistent findings in the results. AgNPs synthesized with *Cissus quadrangularis*, *Catharanthus roseus*, *Acalypha indica* and *Coleus aromaticus*, all showed cytotoxic activity on immortalised HUVEC cell line. However, the *Cissus quadrangularis* AgNPs showed greater cytotoxicity against the cell line.

Therefore, it is concluded that silver nanoparticles synthesized with *Cissus quadrangularis* possessed excellent anti-cancer activity. The future direction of the study will be assessing the potential of green synthesized AgNPs as drug carriers and their anti-angiogenic activity.

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