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Green synthesis of silver nanoparticles using leaf extract of *Abutilon hirtum* and evaluation of their In-vitro cytotoxic activity

Kothai Ramalingam[1*], Karthik.A[1], Arul Balasubramanian[1]

[1]*Department of Pharmacology, Vinayaka Mission's College of Pharmacy,Vinayaka Mission's Research Foundation (Deemed to be University), Salem – 636008, Tamil Nadu, India.*
[*]*kothaiarul@yahoo.co.in*

Abstract

Silver nanoparticles are gaining popularity, because of their unique property and numerous applications. Plants play an important role in the synthesis of nanoparticles. They are cost-effective and environmentally friendly. In this study, an aqueous extract of fresh leaves of *Abutilon hirtum* was used to make silver nanoparticles. Characterization of synthesised nanoparticles was done using different methods includes UV–visible spectroscopy, ATR-Fourier transform infrared, Scanning electron microscopy, energy dispersive X-ray analysis and transmission electron microscope analysis. The absorption peak of the UV–visible spectra of silver nanoparticles in aqueous media was around 381 nm. ATR-FTIR spectrum of synthesized AgNPs reveals the biomolecules responsible for the reduction and capping of silver nanoparticles. The size and morphology was confirmed by SEM and TEM analysis. The synthesized AgNPs were evaluated for its in-vitro anticancer activities against HepG2 cell lines and exhibits significant anti- proliferative activity in a dose dependent manner.

Keywords: *Abutilon hirtum,* Hepatocellular carcinoma, Silver nanoparticles, HepG2 cell lines.

Introduction

Hepatocellular carcinoma (HCC) also known as a primary liver cancer, is a fifth leading risk to human population and responsible for cancer mortality [1]. The treatment of hepatic cancer is a serious challenge for the medical profession, and chemopreventive agents are a realistic choice to cancer prevention in certain situations. Chemopreventive agents are compounds produced from natural, synthetic, or biological sources that restrict, prevent, or block the proliferation of malignant cells. Chemopreventive agents have recently been found as innovative cancer-fighting agents that can be used against a variety of cancers. In order to discover a unique biomolecule from a natural source that can inhibit the different stages of cancer, extensive research has been conducted. Because of their widespread use as antioxidants (to decrease free radical species) and to protect cellular organelles from oxidative damage, naturally-derived chemicals have gained substantial importance in recent years. It also has the additional effect of interfering with cell signalling pathways by influencing cell proliferation, apoptotic intuition, and causing oxidative damage [2]. Hepatitis B and C, alcohol consumption, hormone exposure, haemochromatosis, aflatoxin B1, tobacco products, chemical, fried foods, cosmetics and pharmaceutical substance are the major risk factor for causing hepatocellular carcinoma [3].

Silver nanoparticles (AgNPs) are increasingly being used in a variety of fields due to their unique physical and chemical properties, which include optical, electrical, and thermal properties, as well as high electrical conductivity and biological properties [4-6]. These applications include medical, food, health care, consumer, and industrial applications. Industrial, household, and healthcare-related products, as well as consumer products such as medical device coatings, optical sensors, and cosmetics, have all been found to contain anticancer properties. Antibacterial agents have also been found to be effective in the pharmaceutical and food industries, as well as in diagnostics, orthopaedics, and drug delivery [7].

AgNPs have been employed in a variety of applications in the past, including textiles, keyboards, wound dressings, and biomedical equipment [8,9]. Since, nanosized metallic particles have a high surface-to-volume ratio, they are distinct from other materials and have the ability to significantly alter physical, chemical, and biological properties; as a result, these nanoparticles have been used in a number of applications [10,11]. The classic physical and chemical techniques, in general, tend to be both expensive and potentially harmful. For example, biologically generated AgNPs have a high yield, are highly solubilized, and are extremely stable. When compared to the multiple synthetic methods for AgNPs that exist, biological methods appear to be simple, quick, non-toxic, dependable, and environmentally friendly approaches for manufacturing well-defined size and morphology under optimal conditions for translational research.

Abutilon is a large group of flowering plants belongs to the family malvaceae with over 200 species occurring throughout the tropics and subtropics. Also, the plants from Abutilon spp. are claimed for other medicinal properties. Abutilon hirtum is an evergreen Shrub growing to 3 m. It is in leaf all year and in flower from April to September. Abutilon hirtum has been reported for the following pharmacological activity such as anti-inflammatory activity, analgesic activity, antipyretic activity and anti-diabetic activity, Hepatoprotective activity, anti-oxidant and anti-cancer activity, and In-vitro cytotoxic activity [12]. In connection with this, the present aimed to evaluate its in-vitro and in-vivo anticancer activities against DEN induced hepatocellular carcinoma in rats.

Methods

Plant Material

Fresh leaves of *Abutilon hirtum* (lamp) sweet were collected from local area of salem district, Tamilnadu, India, during November 2021. This plant was identified and authenticated by Dr. P. Radha, Siddha Medicinal Plants garden, Mettur Dam-636401, Tamilnadu, India. The leaves were kept in the shade for few days to dry at room temperature and then ground into fine powder for further use.

Chemicals

Silver nitrate and milli-Q water were procured from Sigma-Aldrich Bengaluru. The different solvents and chemicals used in the present study were of and analytical grade.

Preparation of Extracts

About 10 g of dried leaves of *Abutilon hirtum* were mixed with 100ml milli-q water and heated for 10 minutes. The aqueous solution's colour changed from green to yellow after boiling.

Synthesis of Silver Nanoparticles

Silver Nanoparticles (AgNPs) using leaves of *Abutilon hirtum* (Lamp) sweet was synthesized by a green method. The 90 mL of silver nitrate ($AgNO_3$) was mixed with 10 mL of *Abutilon hirtum* (Lamp) sweet extract. The reaction was carried out at 85 ◦C for 2 hrs [13]. The extract was filtered through whatmann filter paper no1 and stored at -15˚c finally the filtered solution can be dried to get the powdered form of AgNPs.

Characterization of Synthesized AH-AgNPs

UV-absorption spectra of synthesized AgNPs by using *Abutilon hirtum* leaf extract were measured using UV-visible spectrometer (Systronic PC Double Beam 2202). The Bruker Platinum-ATR spectrophotometer was used to identify the potential biomolecules in *Abutilon hirtum* leaf extract which is responsible for reducing and capping the bioreduced silver nanoparticles and measurements were performed at an average of 32 scans per sample in the wavenumber range 4000-400 cm-1 with a resolution of 4 cm-1. The size and morphology of the AH-AgNPs were analysed by Scanning electron microscopy (SEM) and High resolution Transmission electron microscopy (HR-TEM) electron microscopy using VEGA3 TESCAN SOC-MK University. Energy diffraction Spectroscopy (EDAX) was carried out for the elemental analysis and characterization of the chemicals in the sample [14].

In vitro cytotoxicity study

HepG2 cancer celllines

The Hep-2 cell line was purchased from National Cell Centre, Pune (India).

The MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) test was used to assess the cytotoxicity of synthesised AgNPs against HepG2 cell lines. The MTT assay is a colorimetric method for determining cell viability by detecting tetrazolium salt metabolization [15]. HepG2 cells were seeded into 96-well plates at a density of 5×10^4 cells per well. The cells were then treated with various concentrations of AH-AgNPs (0–200µL/mL) and incubated at 37°C for 24 hours in the presence of 5% CO2 and 95% humidity. MTT (5 mg/mL) was added to the cultured cells, which were subsequently incubated for an additional 4 hours. The crystals were dissolved in 200 litres of DMSO, and the absorbance was measured colorimetrically at 570 nm.

% Cell viability = ([O.D of control–O.D of test compound]/[O.D. of control]) × 100

O.D: Optical density.

Results

Synthesis of Silver Nanoparticles

In the present study, Silver nanoparticles using leaves of Abutilon hirtum was synthesized by mixing 100 ml $AgNO_3$ with 10 mL of plant extract. The reaction was confirmed by the colour change from the brown colour to black colour solution. This indicates formation of silver nanoparticles was shown in Fig.No.1.

UV- spectroscopy analysis of AH-AgNPs

In this study, UV-Vis spectroscopy ranging from 300 to 600 nm was used to observe the reduction of silver ions present in the aqueous solution of silver nitrate during the reaction with the constituents of *Abutilon hirtum* leaf extract. The appearance of an absorbance peak at around 380 nm clearly reveals the synthesis of AgNPs in solution as a result of electrons from the surface plasmon resonance (SPR) of the nanoparticle [16].

ATR-FTIR of AH-AgNPS

The FTIR spectra of AH-AgNPs revealed major absorption peaks at 3704.71, 2335.65, and 1662.24 cm–1, indicating the presence of phytoconstituents that act as capping agents was shown in Fig.no.3.

Scanning electron microscopy (SEM) analysis of AH-AgNPs

The SEM was used to analyse the morphology of the green-synthesised AH-AgNPs. SEM analysis revealed that the size of AH-AgNPs was in the range of 20–40 nm was shown in fig.no.4.

Energy diffraction Spectroscopy

The findings of the elemental analysis by EDX revealed a significant absorption peak of silver at 3 keV, which is related to the fact that silver is the main constituent (17) was shown in fig.no.5

High Resolution-Transmission Electron Microscopy (HR-TEM) analysis of AH-AgNPs

The HR-TEM was used to analyse the shape of the green-synthesised AH-AgNPs was shown in fig.no.6. TEM analysis revealed that the shape of AH-AgNPs was spherical.

Assessment of *in-vitro* cytotoxicity

The absorbance of the corresponding incubated cells in the 96-well plate was used to determine the percentage of survived cells. In HepG-2 cell lines, the effect of AH-AgNPs is strong and comparable to the conventional medication Silymarin. The extract demonstrated cell inhibit activity at a lower dosage of 6.5g/ml, but cell inhibit activity at a greater concentration of 200g/ml of AH-AgNPs and Silymarin demonstrated 66.77 and 85% respectively. As a result, the extracts exhibited dose-dependent and statistically significant cytotoxic effect. The results were shown in Table.1.

Discussion

The lowering of HCC incidence is one of the most significant global health concerns we are currently confronted with. This is due to the fact that the rate of recurrence is quite high and expanding. Botanical formulations and combinations of herbal extracts have traditionally been utilised as alternative treatments for a variety of ailments. Abutilon species shows wide spectrum of pharmacological activities. *Abutilon hirtum* is a traditional medicinal plant belongs to the family of malvaceae, which was not scientifically much explored. Hence the present study focussed on the synthesis and characterisation of silver nanoparticles of *Abutilon hirtum* and evaluated its in-vitro cytotoxic potential against HepG2 cell lines by MTT assay method. In this study, silver nanoparticles were synthesised using leaves of *Abutilon hirtum* by green method. The UV spectra of the synthesised silver nanoparticles showed an intense peak at 381 nm. The use of ultraviolet–visible spectroscopy to characterise the structure of nanoparticles is one of the most extensively utilised techniques for this purpose. The absorption peaks at 3704.71, 2335.65, and 1662.24 cm–1, indicating the functional groups of the biomolecules responsible for capping and stabilizing the nanoparticles were confirmed using FTIR spectroscopy. The synthesised silver nanoparticles were spherical in shape and in the size range of 20-40nm was measured by SEM, EDX and HR-TEM analysis. investigated the *in-vitro* cytotoxic effect of AH-AgNPs against HepG2 cell lines. The results of the MTT assay of AH-AgNPs against HepG2 cell lines showed the promising *in-vitro* cytotoxic potential in a dose-dependent manner. This may be due to the antioxidant properties of the flavonoids, tannins, and polyphenols present in it. In the future, further studies to be performed on various models for further evidence of its anticancer potential.

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Table

Table no.1. Effect of Synthesized AH-AgNPs on HePG2 cell lines by MTT-assay.

S. No	Concentration µg/ML	% Cell Inhibition
1	6.25	13.97
2	12.50	24.6
3	25	40.55
4	50	51.31
5	100	66.77
6	Silymarin	85

Figure

Fig. No.1 Green synthesis of silver nanoparticles by using leaves of *Abutilon hirtum* (Lamp) sweet. a) Leaves of *Abutilon hirtum* b) Aqueous extract of *Abutilon hirtum* c)silver nitrate solution *d)* Synthesized AH-AgNPs.

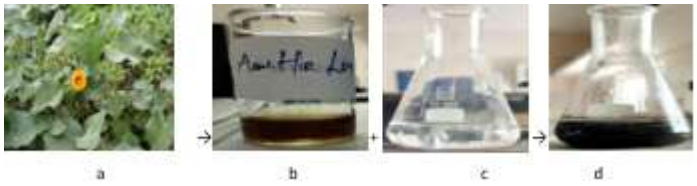

Fig. No. 2 UV- spectrum of AH-AgNPS

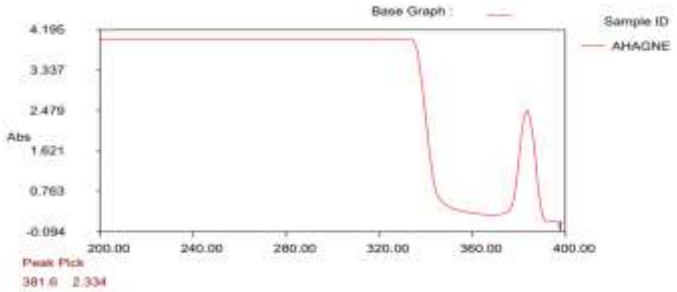

Fig.no:3. ATR-FTIR analysis of AH- AgNPs.

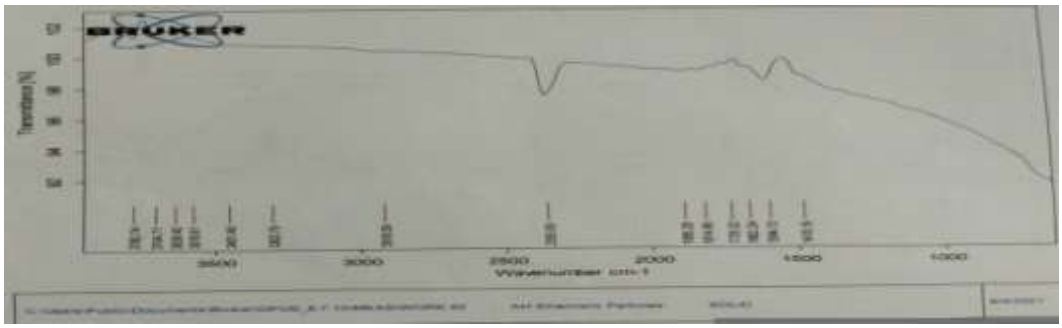

Fig.no.4 SEM analysis of AH-AgNPs

Impossibile visualizzare l'immagine. La memoria del computer potrebbe essere insufficiente per aprire l'immagine oppure l'immagine potrebbe essere danneggiata. Riavviare il computer e aprire di nuovo il file. Se viene visualizzata di nuovo la x rossa, potrebbe essere necessario eliminare l'immagine e inserirla di nuovo.

Fig.no.5. EDX analysis of AH- AgNPs.

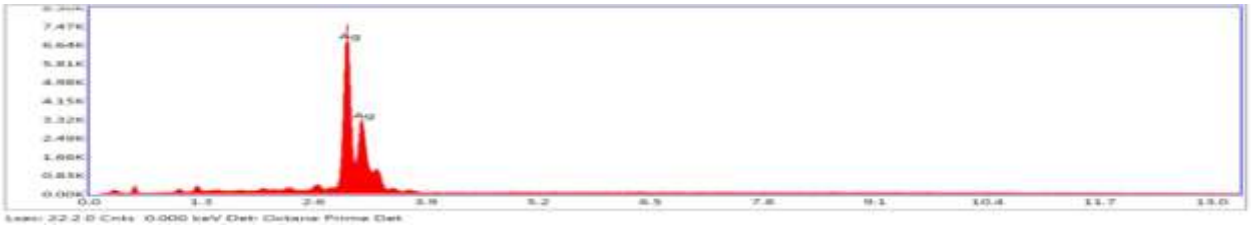

Fig.no:6. HR-TEM analysis of AH- AgNPs