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Biosynthesis, Characterization And evaluation of *In-Vitro* Cytotoxic Potential of Silver Nanoparticles of Stems of *Abutilon Hirtum* (Lamp) Sweet

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Abstract

Abutilon hirtum (Lamp) *Sweet* is one of the species of *abutilon*, commonly known as Vadathuthi. *Abutilon hirtum* has antioxidant, and hepatoprotective properties. The present work involves the biosynthesis, characterization and in-vitro cytotoxic evaluation of silver nanoparticles of stems of *Abutilon hirtum.* Silver nanoparticles was synthesised by green method and were confirmed by various spectral studies. The UV/Vis spectra absorption peak confirms their production. scanning electron microscopy, transmission electron microscopy and energy diffraction X- ray analysis revealed that the silver nanoparticles are polygonal and of different morphologies ranging from 16 to 56 nm in size. The green synthesized AgNPs were evaluated for its in-vitro cytotoxicity against HepG2 cell lines The synthesized AgNPs showed significant cytotoxicity against HepG2 cell lines in a dose dependent manner.

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

Introduction

Cancer is the world's second largest cause of death. Overall, cancer has become more common; in the United States alone, around 1,665,540 persons were diagnosed with cancer in 2014, with 585,720 of them dying as a result of the disease [1] Cancer is caused by a mutation of gene changes that cause the cell's functions to be altered. Chemical substances are well known for their function in the formation of gene mutations and cancer cells. Furthermore, Alcohol and tobacco contains a number of carcinogenic chemical components that cause hepatocellular cancer and lungs cancer [2]. Hepatocellular carcinoma (HCC), the most frequent kind of hepatic malignancy caused by a buildup of genomic and epigenomic changes in hepatocytes is one of the most lethal cancers in the world.[3,4,5]. HCC treatment options differ from patient to patient and are strongly depending on the stage of the disease [6]. Early stages of HCC can be treated with a complete surgical resection or a liver transplant. Patients with HCC in the intermediate stages may benefit from locoregional therapies such as transarterial chemoembolization, ablation, and selective internal irradiation [7]. Systemic therapies, such as chemo- and immunotherapy, are the treatment options for patients with advanced HCC [8]. Nanotechnology has emerged as one of the most significant and promising areas of research, with unique characteristics and numerous uses in a wide range of industries, including agriculture, food, and medical. A great number of studies have been conducted on the green synthesis of AgNPs utilising the leaves of plants; however, the biosynthesis of AgNPs employing wild and indigenous species that have been shown to demonstrate potential anticancer and antibacterial action has not been thoroughly investigated.

Abutilon hirtum (Lamp) *Sweet* is one of the species of *abutilon*, commonly known as Vadathuthi. It belongs to the malvaceae family. *Abutililon hirtum* is distributed in tropical and subtropical countries of America, Africa, Asia and Australia. In India, it is widely distributed in Tamilnadu and Andrapradesh. It was reported that the methanolic leaf extract of *Abutilon hirtum* contains alkaloids, flavonoids, saponins, cardiac glycoside, sterpenes and steroids, phenols, and resins, but balsam was not present. The plant has been reported for its anti-inflammatory activity, analgesic activity, antipyretic activity and anti-diabetic activity, Hepatoprotective activity, anti-oxidant and anti-cancer activity, and In-vitro cytotoxic activity [11,12]. The present study aims to synthesize and characterize the silver nanoparticles (AgNP) utilizing stems of *Abutilon hirtum* and to evaluate its in-vitro cytotoxic potential.

Methods

Plant Material

Fresh stems 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 stems 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. Different solvents and chemicals used in the present study were of and analytical grade.

Preparation of Extracts

About 10 g of dried stems 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

In order to make silver nanoparticles, 10 ml of stems aqueous stem extract of Abutilon hirtum was combined with 90 ml of silver nitrate (1 mM) solution in a shaker. The colour changes from light to a colloidal brown is visible [13]

Characterization of Silver Nanoparticles

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

MTT Assay

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 stems of *Abutilon hirtum* was synthesized by mixing 100 ml AgNO3 with 10 mL of plant extract. The reaction was confirmed by the colour change from the yellow colour to brown colour solution. This indicates formation of silver nanoparticles was shown in Fig.No.1.

UV Spectroscopy

UV–Vis spectroscopy, the most basic and indirect approach, was used to assess the bioreduction of silver ions to silver nanoparticles. In this study, UV-Vis spectroscopy ranging from 300 to 800 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* stem extract. The appearance of an absorbance peak at around 390 nm clearly reveals the synthesis of AgNPs in solution as a result of electrons from the surface plasmon resonance (SPR) of the nanoparticle (16) was shown in fig no.2.

ATR- FTIR Analysis

The functional groups of the biomolecules responsible for capping and stabilizing the nanoparticles were analysed using ATR-FTIR spectroscopy. Peaks at 3321.87, 2352.40, 1693.54 and 1284.56 cm^{-1} indicates the functionalgroups involved in capping and stabilizing of nanoparticles was shown in fig.no.3.The interaction of silver ion with hydroxyl group, which referred to the stretching of OH group was found at 3321.87 cm^{-1} suggesting the existence of polyphenols. Furthermore, the presence of a C=C stretch at roughly 1693.54 demonstrates that the produced nanoparticles include a wide range of alkene groups. Due to C-N stretching, another medium signal at 124.568 cm-1 suggests the existence of aliphatic amines. The stem of *Abutilon hirtum,* which participates in the bioreduction process for the creation of silver nanoparticles, included typical functional groups such as alcohols, aldehydes, flavonoids, phenols, and nitro compounds as phytoconstituents (17).

SEM Analysis

The topology and size of the Ag-NPs were studied using SEM, which revealed the synthesis of higher density polydispersed spherical Ag-NPs of varied sizes. SEM analysis revealed that the size of AH-AgNPs was in the range of 16–56 nm was shown in fig.no.4. similar results were reported by Malik et al(18).

EDX

Energy Dispersive X-ray Analysis determines the qualitative and quantitative status of components that may be implicated in AgNP

production. The spectrum indicated a peak in the silver region at 3KeV (54.39 percent in mass) which is typical of metallic silver nanocrystalline absorption due to surface plasmon resonance was shown in fig.no.5.

HR-TEM Analysis

Fig.no.6 showed the HR-TEM image of silver nanoparticles produced by AH-AgNPs.. The generated silver nanoparticles were near spherical and polydisperse, with an average diameter of 16.73 nm. The synthesized organic biomolecules encased the AgNPs.

Cytotoxicity Assay

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

Hepatocellular carcinoma (HCC) is the most common type of cancer and is associated with a high fatality rate. There are several therapy procedures available for the treatment of HCC, each of which has its own set of side effects. Many natural products are beneficial in the co-treatment and prevention of HCC. A number of processes are involved in the activity of these herbal medicines and their bioactive substances in the prevention and co-treatment of HCC. They can limit the formation and progression of liver cancer in a variety of ways, including shielding against liver carcinogens, boosting the effects of chemotherapeutic medications, inhibiting tumour cell proliferation and metastasis, and regulation of oxidative stress and chronic inflammation. Abutilon species are well known for its medicinal properties. *Abutilon hirtum* is a traditional medicinal plant belongs to the family of malvaceae, reported for its anti-inflammatory, demulcent, diuretic, ulcer and diarrhea activity. Hence the present study focussed on the synthesis and characterisation of silver nanoparticles of stems 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 stems of *Abutilon hirtum* by green method. The UV spectra of the synthesised silver nanoparticles showed an intense peak at 391 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 3321.87, 2352.40, 1693.54 and 1284.56 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 16-56nm was measured by SEM, EDX and HR-TEM analysis. 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	12.85
2	12.5	23.54
3	25	36.64
4	50	50.12
5	100	64.36
6	Silymarin	83

Figure

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

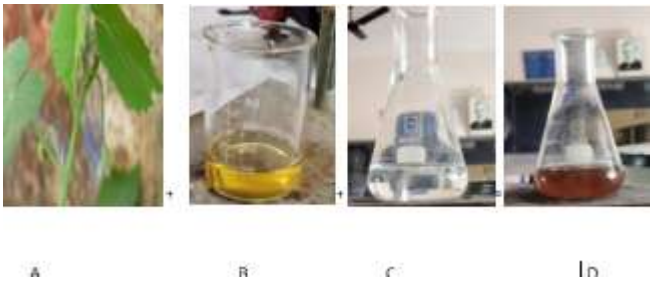

Fig. No. 2 UV- spectrum of AH-AgNPS

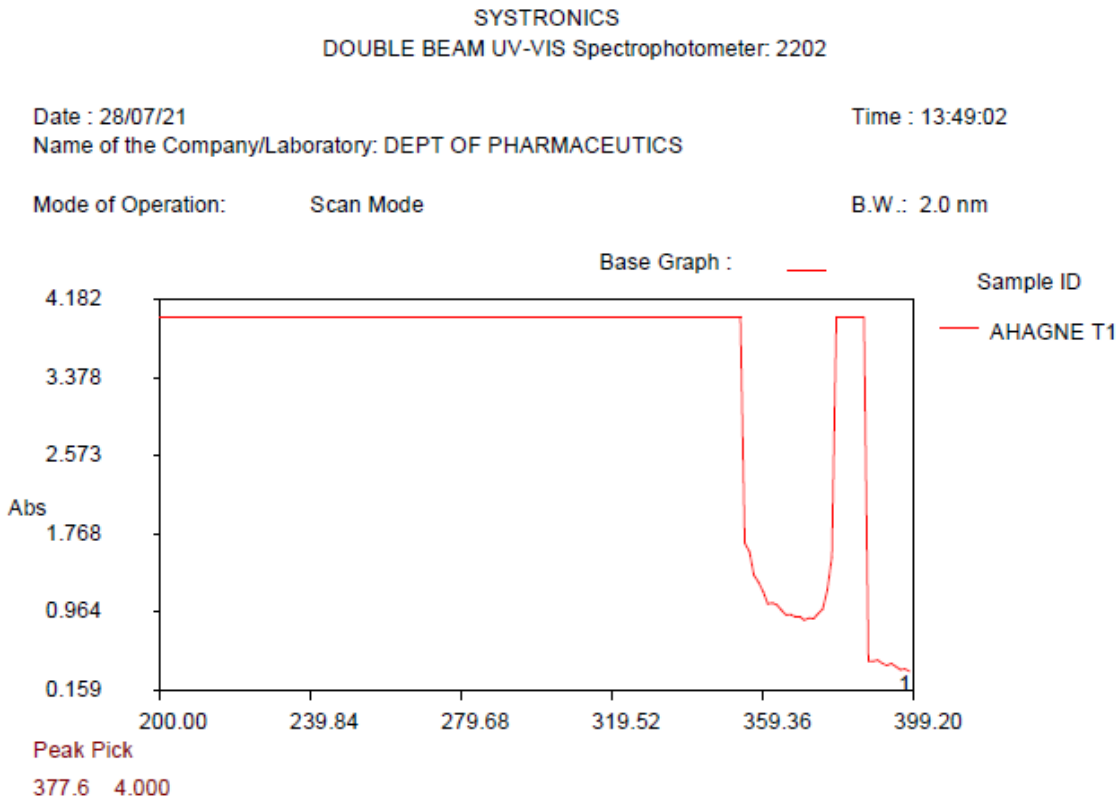

Fig.no:3. ATR-FTIR analysis of AH- AgNPs

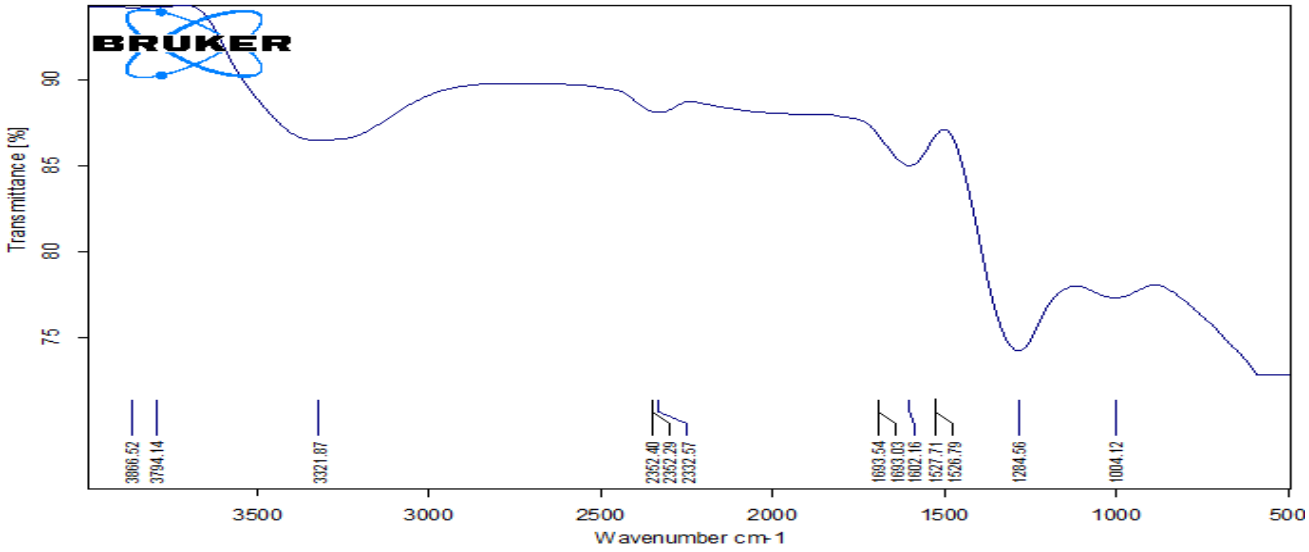

Fig.no.4 SEM analysis of AH-AgNPs

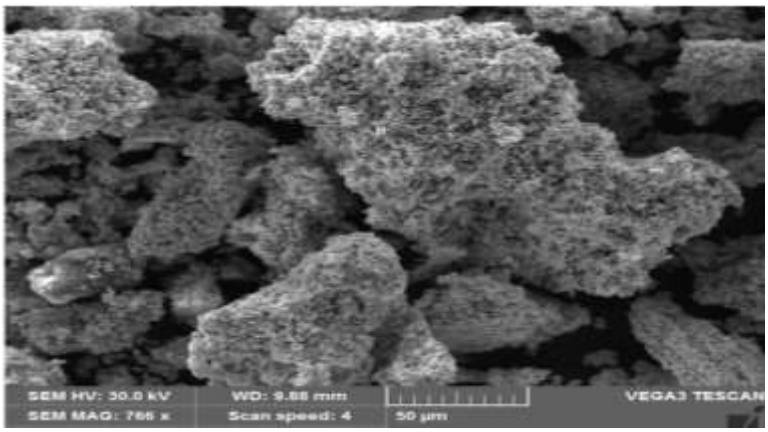

Fig.no.5 Energy dispersive x-ray analysis of AH-AgNPs

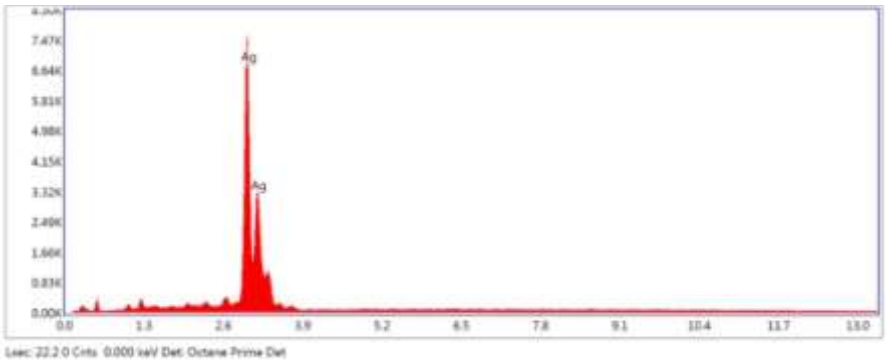

Fig.no:6. HR-TEM analysis of AH- AgNPs