Facile And Green Synthesis of Silver nanoparticles Using Leaf Extract of *Blepharis maderaspatensis (L) Heyne ex Roth*: A Prospective Cytotoxic Negotiator Against A431 Epidermoid Carcinoma Cells

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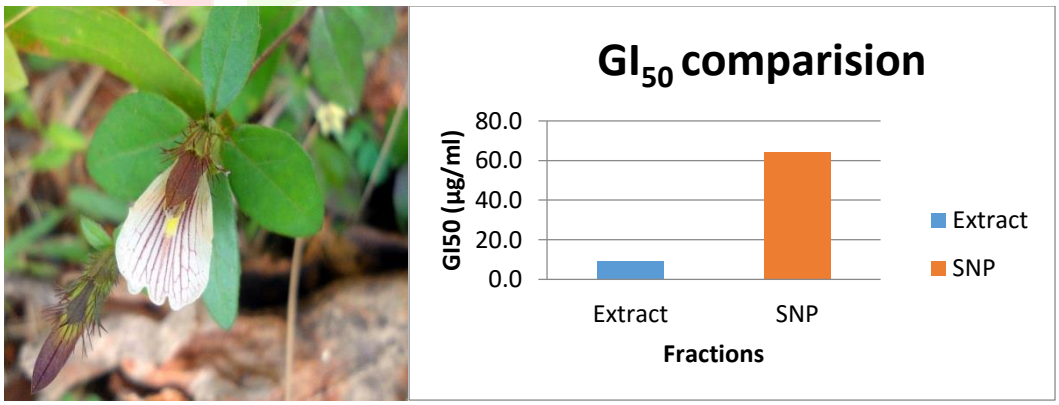
Abstract

The current study was intended to categorize the cytotoxic effect of bio-synthesized silver nanoparticles of *Blepharis maderaspatensis* (L) *Heyne* ex Roth *leaf* extract against human epidermoid carcinoma A431 cell lines. The examination of the cytotoxic consequence of the bio-synthesized silver nanoparticles of *Blepharis maderaspatensis (L) Hyne ex. Roth* leaf extracts on human epidermoid cancer cell lines by means of MTT assay. Silver nanoparticles from shade dried leaf extract of *Blepharis maderaspatensis (L) Hyne ex. Roth* and then characterized by various techniques. The eminence of the particle was examined by XRD and morphology by SEM. The silver nanoparticles (SNP) synthesis was resolute by UV-Vis spectroscopy then followed by FTIR and ZETA potential. Bio –synthesized silver nanoparticles were predominantly sphere-shaped in shape and size ranging >100nm Cytotoxicity activity of biosynthesized silver nanoparticles against in vitro human epidermoid carcinoma A431 cell lines showed a dose –dependent activity with GI50 value of 64.4 and 9.2µg/ml.

Graphical abstract



Key words: *Blepharis maderaspatensis (L) Heyne ex Roth* leaf extract, cytotoxic effect, A431 cell lines.

1. Introduction

Green synthesis is the proposal of chemical, physical and biological yield that exterminate the make use of or spawn risky substances in use for human vigor and for flora and fauna. Currently, the advancement of green separation of metallic nanoparticles and their utilization is one of the most vital areas of research. For synthesizing silver nanoparticles (AgNPs) numerous methods such as chemical creation, electrochemical synthesis, radiation synthesis photochemical synthesis, and biological synthesis have been used. Nanoparticles research is currently a zone of passionate scientific concern because of its ample range of pertinence in biomedical, optical and electrical fields. Nanomedicine is one of the most fascinating applications of nanotechnology uses nanomaterials, which are introduced into human body to achieve cellular repairs in the molecular level. For synthesizing of silver nanoparticles (AgNPs), biological synthesis has been used.. Newly, speedy, energy-valuable, lush, and cost-effective quantitative room temperature methods for separation of constant AgNPs via the tannic acid (a polyphenolic composite resulting from plant extract) were urbanized. In modern times, the silver nanoparticles are growing as optimistic agents for drug delivery in cancer therapy. Nevertheless, no studies were reported on anticancer activities of AgNPs in this plant. This is the first report of Blepharis *maderaspatensis* leaves extract containing AgNPs which acts against cancer. *Blepharis maderaspatensis (L.) Heyne ex Roth* (BM), a member of Acanthaceae family, is a scrambling or procumbent perennial or rarely twelve-monthly herb by stems up to 2.5 m elongated. Habitually it is used to treat swellings, edema and gout. The leaves paste is mixed with black gram powder, compressed onion and white, egg yolk and the combination is applied to fractured bones in Nigeria. *Blepharis maderaspatensis* (BM) leaves grasp prominent levels of polyphenolic compound it can be contributed to the reduction of silver ions. In this exertion, blend of AgNPs using aqueous *Blepharis maderaspatensis* (BM) *leaves* extract was probed and revamped. The cytotoxicity belongings of extracts containing AgNPs on cell feasibility were intentional using human epidermoid cancer cell lines (A431).

2. Results and discussion

The major parameters distressing the creation of nanoparticles, as well as pH of extract, temperature and concentrations of the extract were examined and enhanced. The silver nitrate reduction into silver ions was estimated by calibrating the UV-Vis spectrum. The captivating was documented at a decision of 1nm at 400-600 nm by means of UV-Vis spectrophotometer. (Fig .1). The structures as well as the dimension of nano particles were analyzed with a scanning electron microscope (SEM). The sample was arranged as a 1µg thin film and covered in a copper grid by plummeting method and the excess amount of solution in the grid is wiped out by means of blotting paper and it is acceptable to dehydrate under a Mercury lamp for five minutes to get tremendous outcome. The imagery of nanoparticles (NPs) was calculated by means of SEM (JPEG, model JFC -1600). (Fig 2). Perkin-Elmer spectrometer FTIR Spectrum in the series 4000-400 cm−1 at a declaration of 4 cm−1 was worn for the investigation. The trial was assorted with KBr crystals. The thin sample disc was arranged by burning with the disc preparing apparatus and positioned in Fourier Transform Infrared [FTIR] (Fig 3) for the investigation of the nanoparticles as well as for the leaf extract. The dehydrated pellet was purified and the silver nanoparticles were further analyzed with X-ray diffractrometer. The statistics

obtained for silver is synchronized with the dual group on powder diffraction standards JCPDS. The morsel size of the silver nanoparticles produced with the method was anticipated since the debye-scherrer equation. The eminence of the dried mixture was analyzed by XRD (Fig 4) procedure (PAN analytical BV, Netherlands) negotiated at a voltage of 40kv, and a current of 30mA, with CuKa radiation in a h-2-h configuration. The zeta potential (Fig 5) was calculated with the help of the Zetasizer Nano ZS (Malvern) software.

Fig 1: UV-VIS spectral analysis of silver nanoparticles from *B. maderaspatensis*

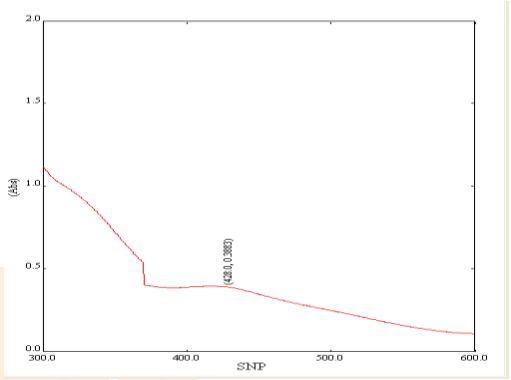


Fig 2: Scanning electron microscopy image of silver nanoparticles of *b.maderaspatensis*

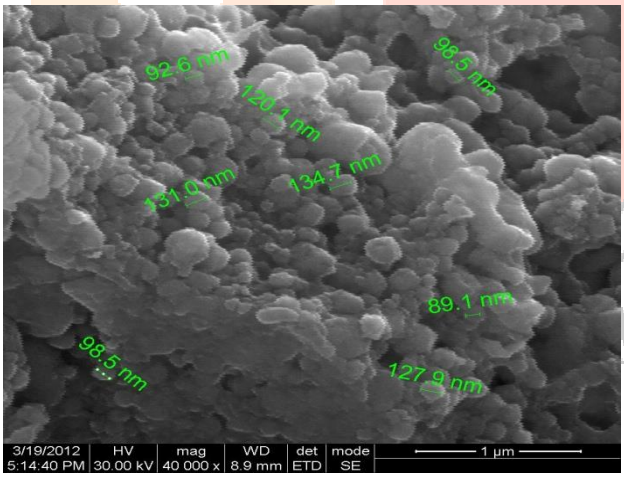


Fig 3: XRD analysis of biosynthesized silvernanoparticles of *b. maderaspatensis*

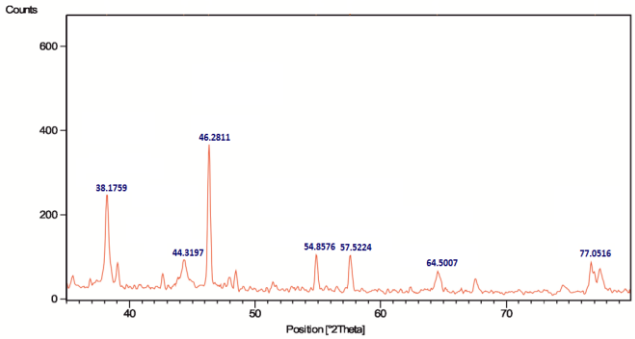


Fig 4: FTIR spectrum of biosynthesized silvernanoparticles from *B.maderaspatensis*

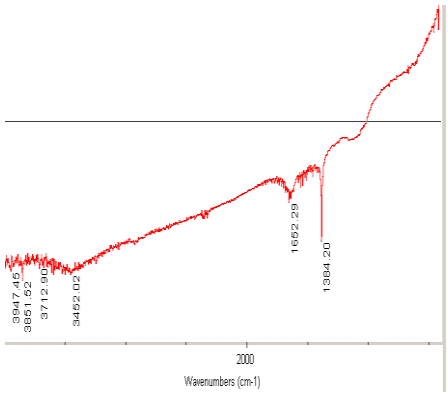
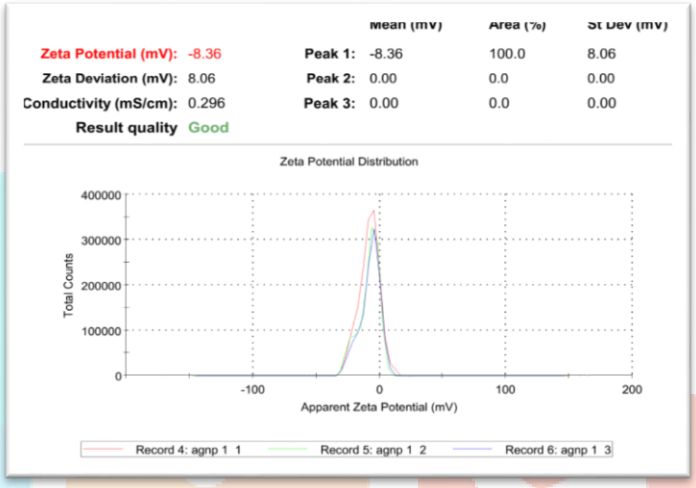


Fig 5: zeta potential results of bio-synthesized silver nanoparticles from b.maderaspatensis



In MTT assay, Test items were supplemented as 2X concentration of the cell in 100 µl volume and the deliberation range were: 1000, 100, 10, 1.0, 0.1, µg/ml. given that the compounds are soluble in the medium the compounds are thinned an suitable quantity and dissolved in DMSO and additional dilutions were in the media100 µl of stock was added to the cell. The dishes were auxiliary incubated for 48 hours in the CO_2 incubator.MTT solution was self-possessed of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) at 5 mg/ml in phosphate buffered saline (1.5 mM KH_2PO_4, 6.5 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl; pH 7.4), from this solution 50 µl was pipette out into each well to accomplish 1 mg/mL as absolute deliberation. The dish was additionally incubated for 2.30 hours in incubator and the medium was carefully decanted. The formazan crystals were air dried in gloomy place and dissolved in 100 µl DMSO and the plates be gently shaken at room temperature and the OD was calculated using Synergy HT micro plate reader at 570nm. (Table 1)

Table: 1 Raw Data Absorbance values at 570nm

Test Conc.	**SNP**				**Extract**			
0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**

Blank	**0.051**	**0.052**	**0.052**	**0.051**	**0.051**	**0.05**	**0.054**	**0.052**
1000 ug/ml	**0.598**	**0.598**	**0.513**	**0.531**	**0.448**	**0.404**	**0.483**	**0.427**
100 ug/ml	**0.709**	**0.726**	**0.716**	**0.709**	**0.621**	**0.591**	**0.587**	**0.593**
10 ug/ml	**0.806**	**0.792**	**0.803**	**0.829**	**0.747**	**0.762**	**0.737**	**0.759**
1 ug/ml	**0.892**	**0.802**	**0.815**	**0.922**	**0.886**	**0.893**	**0.872**	**0.858**
0.1 ug/ml	**0.843**	**0.893**	**0.896**	**0.803**	**0.906**	**0.856**	**0.829**	**0.811**
Control	**0.864**	**0.883**	**0.901**	**0.822**	**0.917**	**0.907**	**0.948**	**0.882**
Blank	**0.05**	**0.051**	**0.05**	**0.05**	**0.05**	**0.049**	**0.05**	**0.049**

From the optical densities the percentage growths was intended with the following formula:

Percentage growth= 100× [(T-T0)/(C-T0)]

If T is greater than or equal to T0, and if T is less than T0,

Percentage growth = 100× [(T-T0)/T0)],

Where T is optical density of test,

C is the optical density of control,

T0 is the optical density at time zero.

From the percentage growths a dose response curve was generated and GI50 values were interpolated as of the growth curves.

In cell imaging, the trial compound of each concentration was performed in quadruplicate and cumulative variation was maintained less than 20% among the data points. Three sets of the cell lines were tested in a 96 well plate as described in the below 96 well format. The test compounds exhibited superior cytotoxicity/anticancer action in both experienced cell lines. Test item Extract exhibited high cell growth inhibition exhibited good inhibition in A431cell lines. RPM showed the GI_{50} range 9.2 µg/mL concentrations in the A431 cell line. (Table 2).

Table: 2 Percentage growth compare to untreated control

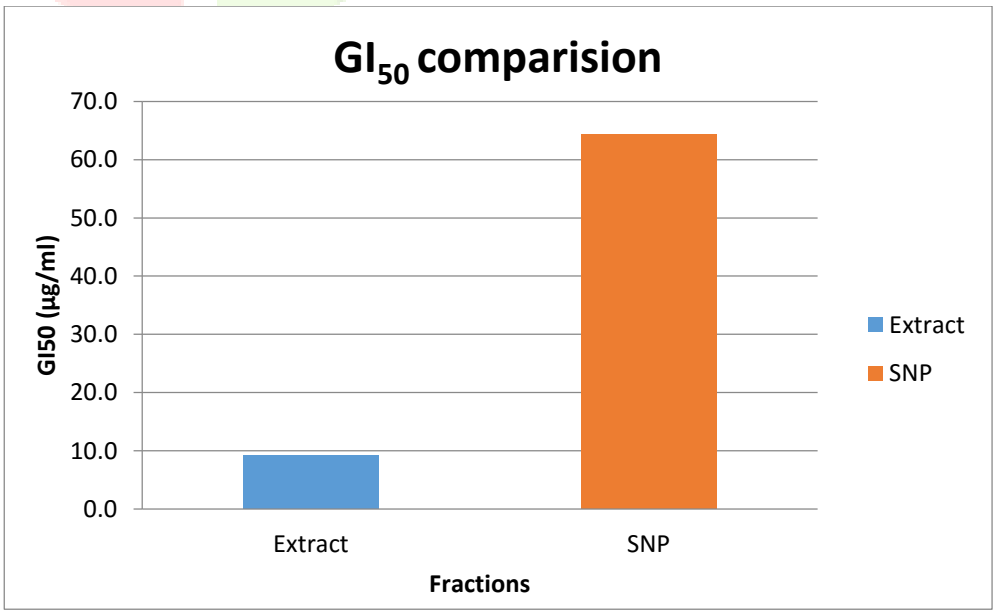
Compounds	PERCENTAGE GROWTH					GROWTH INHIBITON IN µg		
	1000µg	100µg	10µg	1µg	0.1µg	GI50	TG1	LC50
Extract	-29	0	49	89	80	9.2	>100.0	>100.0
SNP	-7	43	78	96	97	64.4	>100.0	>100.0

The outcome illustrate with the purpose of the cytotoxic effects of biosynthesized AgNPs greater than before in the presence of the extract on cancer cell line. (Table 3, 4).

Table: 3 GI_{50} value of the test items

	GI_{50} (µg)
Compound	A431
Extract	9.2
SNP	64.4

Table 4: comparison of extract and SNP of *B. maderaspatensis*



These deeds can be endorsed to the compounds in the extract that improved the action of the AgNPs. For the initial time biosynthesis of AgNPs by means of aqueous *Blepharis maderaspatensis leaf* extract was developed and enhanced. The enhancement of responsible and recyclable formula for the invention of metallic nanoparticles is of massive consequence in the field of nanotechnology. Nanomedicine is defined as a technology which uses molecular apparatus and association about human body for medical finding and healing. (Duncan 2004). The biosynthesized nanoparticles have been characterized by SEM, FT-IR, XRD, Zeta potential and UV-VIS spectroscopy. The AgNPs are crystalline in manners and measurement of silver nanoparticles is ranging from <100nm**.**more over the developmental outlay and risks of these possessions are small as compared to other drugs. (Wagner et. Al 2006)**.** To full fill the reduction of silver ions to silver nanoparticles, different concentrations of the extract were assorted with a stable quantity of silver nitrate (1 mM). The XRD patterns of synthesized AgNPs are in association with cubic Ag reference pattern, the Spectra data reveal the five diffraction lines (111), (200), (222), (311), and (222) which are attuned with the typical pattern. The SEM image shows that the synthesized AgNPs are in sphere-shaped structures. Zeta potential is an essential constraint for accepting the state of the nanoparticles surface and predicting the long-term stability of the distribution. FT-IR spectra of *Blepharis maderaspatensis* leaf aqueous extract and synthesized AgNPs shows charisma of similar peaks with small shift in both spectra reveals that the Synthesized AgNPs are containing natural compounds from extract. Firmness of AgNPs can be endorsed to endurance of these compounds in wrapping of nanoparticles. The biosynthesized AgNPs were isolated from the extract and their anticancer effects were examined. In this study, the anticancer activities of biosynthesized AgNPs were compared with extract containing AgNPs. As observed the increasing in concentration of AgNPs in *Blepharis maderaspatensis* leaves extract, cytotoxicity in A431 cells was greater than before. The IG50 value of detached AgNPs in the extract was 64.4 AND 9.2µg/mL Biosynthesized AgNPs could take part in an imperative task in civilizing their bioavailability as well as compatibility for therapeutically applications in disease like cancer. (Fig 5)

Fig 5: Percentage growth curve of cells treated with test compounds

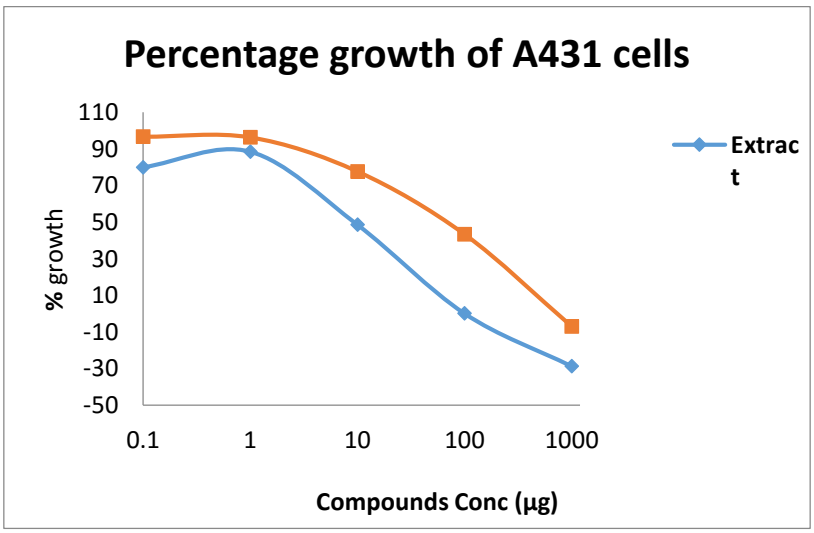


Fig 6: Microscopic analysis of A431 cells treated SNP

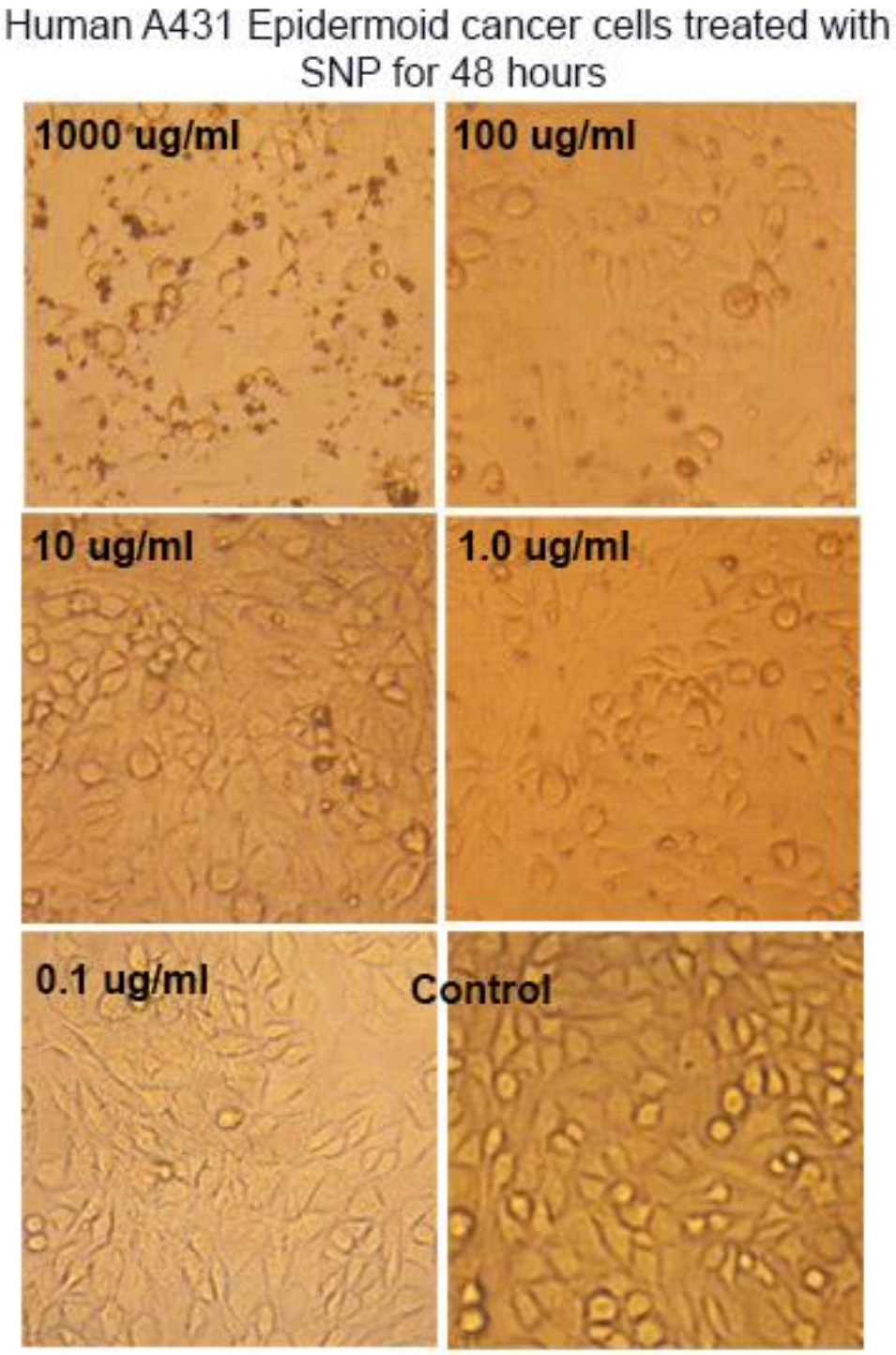
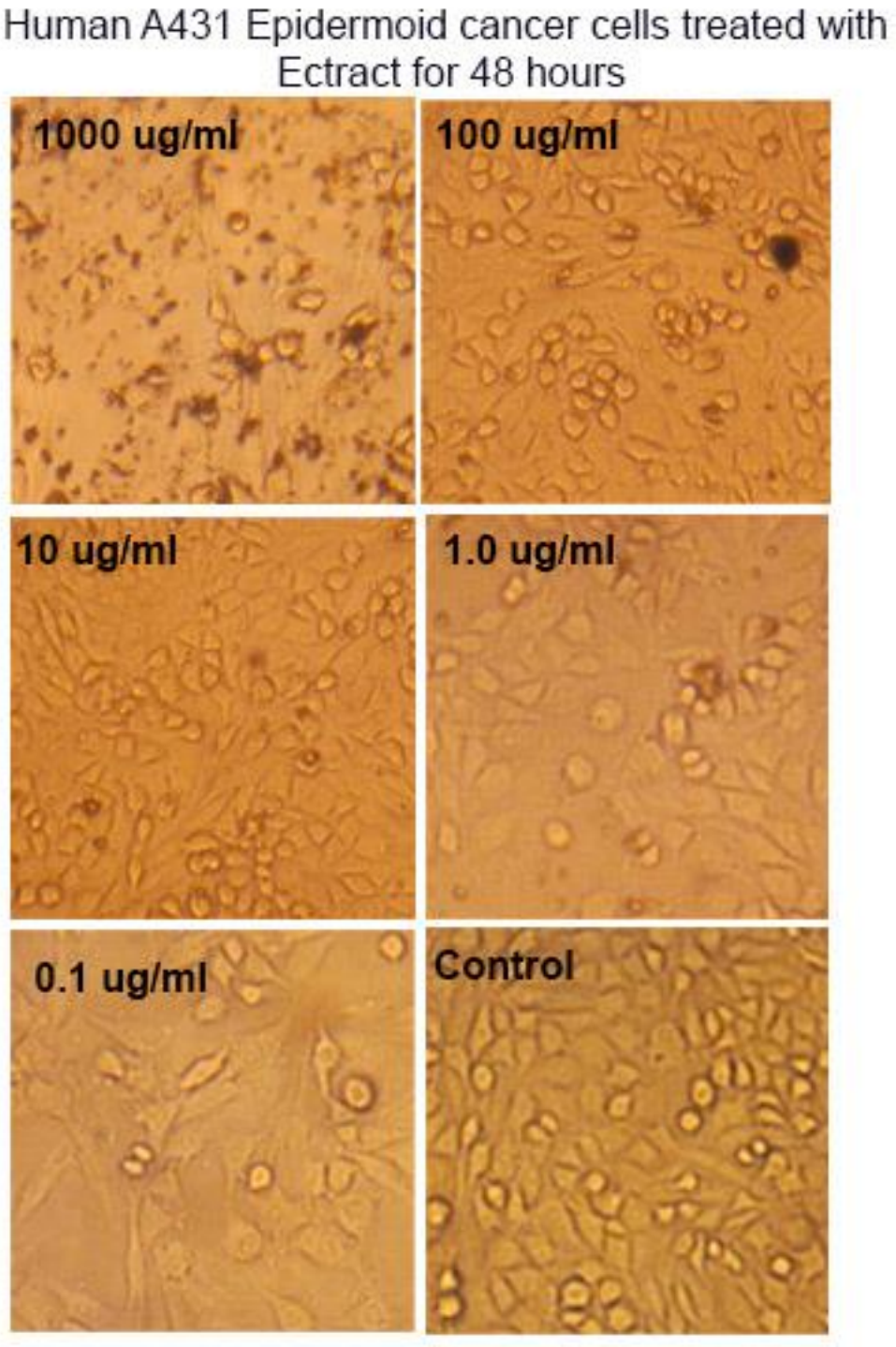


Fig 7:



This study demonstrates the opportunity of using AgNPs to slow up the growth of cancer cells and their cytotoxicity for forthcoming therapeutic treatments, and offers a new method to warfare various diseases, such as cancer, arthritis, and revascularization. The test items have been tested in the MTT assay for the cytotoxic potential in A431 cell line. (Fig 6,7). The extract exhibited excellent anticancer activity comparable to SNP. The test items SNP and extracts exhibited $G1_{50}$ of 64.4 and 9.2 µg in A431 cell line. This extract inhibition is persuasive and momentous further the test items can be taken for other anticancer studies including apoptosis and cell cycle investigation to hit upon the mechanism of cell growth inhibition/cytotoxicity.

3. Experimental

3.1 Biosynthesis of silver nanoparticles from plant material

Spanking new leaves of *Blepharis maderaspatensis* (BM) were unruffled from Megamalai Hills Theni DT Tamil Nadu India .The collected samples were cleaned, washed three times with tap water and two times with distilled water. The leaves were dried in shade for 2 to 3 weeks at room temperature and then pulverized. Momentarily, 5 g of powdered leaf was weighed and assorted in 100 ml of Millipore water. This fusion was boiled in water bath at 60°C for 10- 20 min. After cooled to room temperature, the mixture was filtered by using Whatmann no. 1 filter paper. A sum of 10ml of filtrate was treated with 90ml 1Mm silver nitrate solution and incubated at room temperature for 10minutes resulting in the formation of brownish black colour representative the synthesis of silver nanoparticles. (SNPs)

3.2 Media preparation (Sigma)

The sachet (12.0g) was dissolved in 800 ML of sterile distilled water to which 2.5g of sodium bicarbonate was added. The beaker was covered with aluminum foil and cheerful using magnetic stirrer for 10 minutes. The medium pH was adjusted to 7.2 using 0.1M NaOH. The quantity of the medium was prepared to 1000 ML and filtered in the course of sterile 0.2µ membrane filter unit. The medium quality control was checked by incubating 5 ML of filtered medium in the CO2 incubator for 2 days. The antibiotics and serum were accompanying previous to it was used for cell culture.

3.3 Human epidermoid culture

The human cancer cell line A431 was purchased from NCCS Pune. The cells were grown-up in a RPMI 1640 medium supplemented with 10% fetal bovine serum and antibiotics. Cytotoxicity (MTT) assay can be performed by the following method explained by Carmichael *et al*., (1987) and the percentage of cell feasibility was firm by spectrophotometric purpose of accumulated formazan derived in treated cells at 570 nm in evaluation with the crude ones.

3.5 MTT assay

For the MTT assay, the cells were full-fledged in 25 cm ×25 cm ×25 cm tissue culture flasks containing RPMI1640 medium as a culture medium supplemented with 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin (GIBCO) and grown at 37°C beneath a humidified ambiance of 95% air and 5% CO2. Cells were habitually passaged and maintained prior to the experiment. When the cell compactness in a culture flask attained 70-80% convergence, they were trypsinized and seeded in 96-well plates at unreliable cell number according to the size and shape of the cell Hela were seeded in the density of 3500 cells per well in 100 µl and incubated for 24 hours at CO_2 incubator.

3.6 Cell imaging

After 48 hours of incubation the cells were radical beneath the microscope for cell morphology exploration and images of each consideration was captured and recorded. The Test items were tested against human epidermoid carcinoma cell line. The test items concentration ranging from 0.1 µg to 1000µg in logarithmic range.

3.7 Characterization of silver nanoparticles

A UV–Vis spectrum was assessed using a UV–Vis spectrophotometer (Jenway, model 6505, UK). The optical absorbance of silver nanoparticles was assessed on UV-Vis spectrophotometer at 400-500nm. The way of life of biomolecules of synthesized AgNPs was examined using FT-IR (BRUKER, model TENSOR 27, Germany) analysis. The creation and excellence of silver nanoparticles of *Blepharis maderaspatensis* leaf extract was checkered by XRD. The figure of AgNPs was assessed by SEM (Hitachi model S-4160, Japan). The zeta potential study of biosynthesized AgNPs was evaluated through Malvern Zetasizer Nano range tool (Malvern Instruments Ltd., Malvern, UK).

3.7 Statistical analysis

The mean and standard deviation were anticipated for every factor. All dimensions were activated in triplicate. The One-way investigation of conflict (ANOVA) was done for expressing trial consequence of outcome. In cell feasibility tests, the p values \0.05 were measured as considerable.

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Compliance with Ethical Standards

Conflict of interest

The authors declare no conflict of interest.

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