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Research Article

Anti-Cancer Activity of Silver Nanoparticle Synthesized from Stem Extract of ocimum Kilimandscharicum Against Hep-G2, Liver Cancer Cell Line

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Abstract

Silver nanoparticle synthesis was performed with ethanol stem extract of Ocimum kilimandscharicum in the present investigation. It was identified as silver nanopartcle based on the characteristic excitation peak at 410nm and with different morphology and size, which was visualized clearly in Scanning Electron Microcope. EDAX analysis reveals that, the synthesized nanomaterial was corresponding to silver only. The zeta potential studies reveal that, the synthesized nanoparticle was highly stable and are positive in charge. The cytotoxicity against Hep G2, liver cancer cell line reveals an excellent IC50 value of 49µg/mL. Thus, the present study recommend the silver nanoparticle for the effective control of liver cancer cell line and further study would be initiated cell nanoparticle interaction and its associated pathways for apoptosis.

Keywords: Silver nanoparticle; Hep G2 cell line; SEM; EDAX; XRD.

Introduction

Cancer induced or mutated cells of fast growing leads to one of the major cause of death worldwide. Overall, in 2008 alone, it was estimated that 7.6 million cancer death and 12.7 million new cancer cases, with 56% of new cancer cases and 63% of the cancer deaths occurring in the less developed countries. Lung cancer remain first in cancers worldwide (1.61 million, 12.7% of the total), followed by breast and colorectal cancers with respect to 1.30 million (10.9%) and 1.23 million (9.7%). Among the cancer deaths, lung cancer accounts for 18.2% while stomach and liver cancer accounts for 9.7% and 9.2% respectively. In India, lung cancer constitutes 6.9 per cent of all new cancer cases and 9.3 per cent of all cancer related deaths in both sexes, it is the commonest cancer and cause of cancer related mortality in men, with the highest reported incidences from Mizoram in both males and females (Age adjusted rate 28.3 and 28.7 per 100,000 population in males and females, respectively) (National Cancer Registry Program 2009-11). In order to monitor, diagnose and treat such a deleterious human disease, huge sum has been invested. In this line, nanoparticles, the nucleus of nanotechnology, has been vastly being employed for the effective control of cancer cell lines as prominent therapeutic agents. There are number of synthesis procedure has been emerged so far, but use of flora based extracts for nanoparticle synthesis have been more advantageous than microbial process [1] and it's an ideal candidate for large scale production [2-7] demonstrated the anti-carcinogenic potential of caffeic acid against different cancer cell lines. Reported the anticancer properties of Ulva lactuca against human cancer cell line such as Hep2, MCF7 and HT29. Evaluated bioactivity of silver nanoparticle synthesized from Sargassum muticum. In the present investigation, anticancer activity of silver nanoparticles synthesized from stem extract of Ocimum kililmandscharicum was evaluated [8-9].

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Materials and Methods

Chemical & Reagents

Analytical grade silver nitrate and phytochemical screening chemicals were purchased from Reachem laboratory chemicals, India for the present investigation. Whatman No.1 filter paper was purchased from Hi-Media, India. Ok-AgNps characterization was performed at Karunya University, Coimbatore, Tamil Nadu, India.

Collection of Ocimum kilimandscharicum

Ocimum kilimandscharium plants were collected from vaigai

river bed, korippalayam Madurai district, Tamil Nadu, India. The plants were brought to the laboratory after proper identification.

Preparation of Ethanol Stem Extract (ETE) of Ocimum kilimandscharicum

Fresh stem sample of O. kilimandscharicum were collected and shade dried for 8 weeks. It was powdered by using a mixer (Preethi, India) and sieved. The ground plant material was subsequently used for extraction. 5gm of fine powered of stem sample was weighed and soaked with 100ml of ethanol and allowed to stand for 7 days at ambient room temperature. The soaked plant stem powder was filtered by passing through a what man No.1 filter paper (Hi-Media, India) and used as crude extract. Crude extracts of this plant were stored in a refrigerator and used as such for nanoparticle synthesis.

Synthesis of silver nanoparticles

1ml of ESE was taken from ESE stock (100mg of ESE of O. kilimandscharium dissolved in 10ml of distilled water) and added to 99ml of 1mM (0.017mg/100mL) of silver nitrate ($AgNO_3$) (Reachem Laboratory Chemicals, India). It was then allowed to constant stirring in an Orbit shaker (Neolab, Neolab instruments, Mumbai, India) and the colour change was noticed. The samples were taken at different period of time (3, 6, 9, 12 miniutes) and its absorbance was analysed by using UV-Visible Spectrophotometer (LabKit, Hongkong) at a resolution of 1 nm between 200 and 800 nm. The nanoparticle synthesized (Ok-AgNps) was characterized by using Scanning Electron Microscope (Hitachi S-4500 SEM machine), EDX (Hitachi S-340 N) and the crystalline characteristics of silver nanoparticle were determined from the width of the XRD peaks, using the Debye-Scherrer formula,

$D = 0.94\lambda / \beta \cos\theta$

Where, D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X- ray wave length and β is the full width at half maximum and θ is the diffraction angle.

Anti-Cancer Activity

Cell culture

Human cancer cell line used in this study was procured from National Centre for Cell Science, Pune. All cells were grown in Minimal essential medium (MEM, GIBCO) supplemented with 4.5 g/L glucose, 2 mM L-glutamine and 5% fetal bovine serum (FBS) (growth medium) at 37°C in 5% CO_2 incubator. The cytotoxicity study was analysed at Yaazh Xenomics, Madurai district, Tamil Nadu, India.

NRU assay

The NRU assay developed by [10] was modified and used to determine the inhibitory effects of silver nanoparticle (AgNps) synthesized from ethanol stem extract of Ocimum kilamandshcaricum on cell growth in vitro. In brief, the trypsinized cells from T-25 flask were seeded in each well of 96-well flat-bottomed tissue culture plate at a density of 2 $x10^3$ cells/well in growth medium and cultured at 37°C in 5% CO_2 to adhere. After 48hr incubation, the supernatant was discarded and the cells were pretreated with growth medium and were subsequently mixed with different concentrations of test compounds (6.81, 10, 14.7, 21.5, 31.6, 46.4, 68.1 and 100 µg/ml) in triplicates to achieve a final volume of 100 µl and then cultured for 48 hr. The AgNps was prepared as 1.0 mg/ml concentration stock solutions (Final conc. 0.5%). Each well except blank received 100 µl of freshly prepared neutral red medium (33 µg /ml in MEM) followed by incubation for 3hr at 37°C. At the end of the incubation all the solutions in the well were discarded and 200 µl of fixation solution was added to each well to fix the cells. The solution was removed and replaced with 100 µl of Extraction solution was added to each well, after which the plates were placed at room temperature for 20 min. Plates were shaken with amicroplate mixer for 30 seconds. The absorbance (OD) of the culture plate was read at a wavelength of 540 nm on an ELISA reader, Anthos 2020 spectrophotometer.

Statistical Analysis

The IC_{50} values were calculated by plotting percentage survival against concentration of extract in Excel 2007.

Results and Discussion

In the present investigation, synthesis of silver nanoparticle was performed by using ethanol leaf extract of stem of Ocimum kilimandscharicum. The study revealed that, the synthesis of silver nanoparticle was morphologically visualized in UV-Vis spectrum (Figure 1), with a potent peak observed at 410nm, which is a

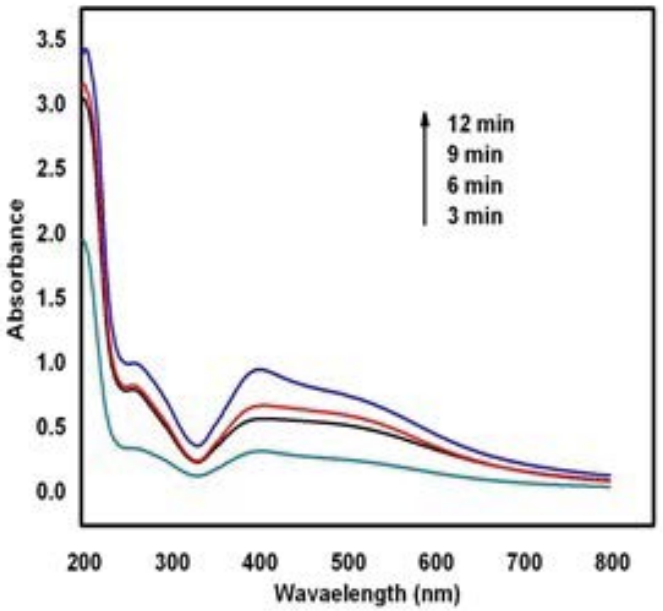

Figure 1: UV-Visible spectra of silver nanoparticl synthesized from ethanol stem extract of Ocimum kilimandscharicum.

characteristic of a Ag nanoparticle and is confirmed in SEM analysis (Figure 2) [11].

Observed excitation at 406 nm and varied size and shape in SEM by the silver nanoparticle synthesized from O. sanctum leaf. The EDAX analysis of the silver nanoparticles revealed the the peaks at 2.6 KeV, which confirmed the presence of silver nanoparticles along with Mg, Fe and Na (Figure 3).

Similarly [11] observed oxygen and aluminium besides AgNps. The XRD results revealed that, 28.01˚, 32.20˚, 46.20˚ and 76.90˚ corresponding to (111), (200), (220), (311) a set of lattice planes, which reveal the cubic structure of silver (Figure 4).

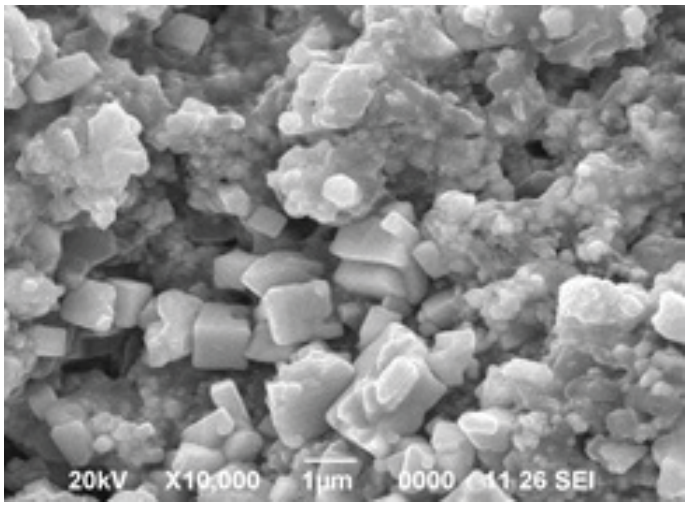

Figure 2: Scanning Electron Microscope image of Silver nanoparticles synthesized from ethanol stem extract of Ocimum kilimandscharicum

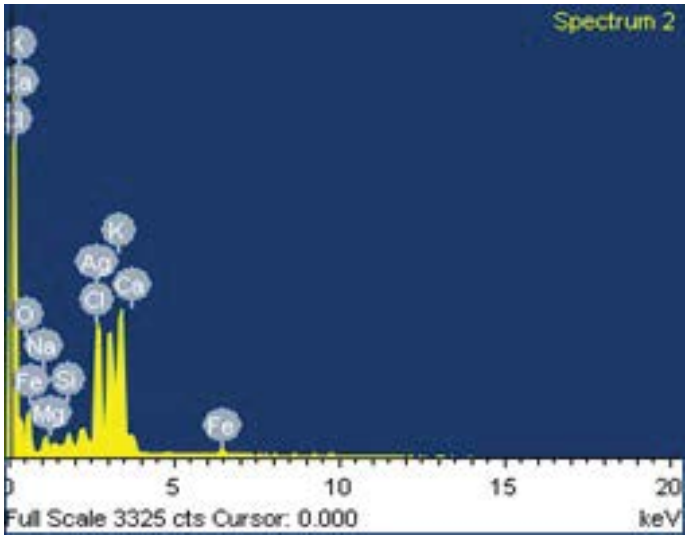

Figure 3: EDAX analysis of silver nanoparticles synthesized from ethanol stem extract of Ocimum kilimandscharicum

The surface of the nanoparticle was coated with positively charged (32.5 mV) and their stability was also high, which was clearly identified from zeta potential measurements (Figure 5).

The nanoparticle synthesized was subjected for its anticancer activity against Hep G2 (Liver cancer cell line) and found IC_{50} value of 49.5 µg/mL and almost 80% reduction in cell survival was noticed during the investigation. The anticancer activity of AgNps was highly supported by the works of [12,9] In the present study, IC_{50} value obtained was low compared to [13] who reported IC_{50} value of 82.39, 83.57 and 78.78µg/mL by AgNps synthesized from Cucubita maxima, Moringa oleifera and Acorus calamus respectively. In contrast to [13] the present study reported the potent anticancer activity of stem extract synthesized nanoparticle. [14-17] reported that, the cytotoxicity of silver nanoparticles was due to introduction of reactive oxygen species (ROS) which enunciate the apoptosis pathway and its well established mitochondrial interaction. Besides, emanation of oxidative stress pronounced

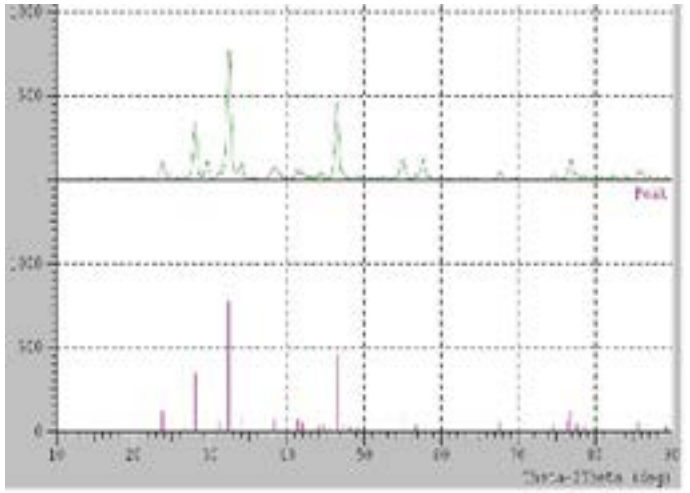

Figure 4: XRD spectra of silver nanoparticles synthesized from ethanol stem extract of Ocimum kilimandscharicum

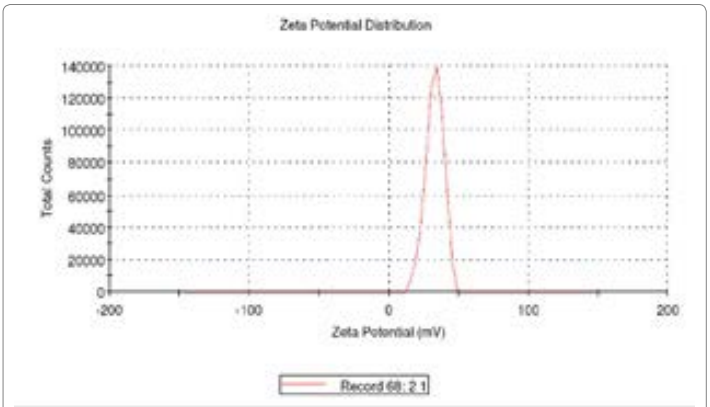

Figure 5: Zeta potential of silver nanoparticles synthesized from ethanol stem extract of Ocimum kilimandscharicum

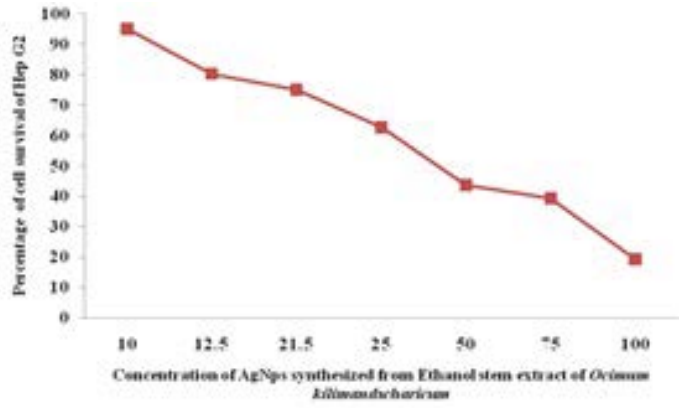

Figure 6: Anticancer activity of Silver nanoparticle synthesized from ethanol stem extract of Ocimum kilimandscharicum

the genotoxic stress as well as p53 gene upregulation [18] which initiate the apoptosis. It has greatly supported the recommendation of nanomaterials for the anticancer studies. Hence, it was observed from the present investigation that, the cytotoxic activity of silver nanoparticle synthesized from basil stem samples. It would be further studied for its mode of penetration and suppression of cancer cell line and regulation of genes of guardian of cells.

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References

1. Narayanan KB, Sakthivel N (2010) Phytosynthesis of gold nanoparticles using leaf extract of *Coleus amboinicus* Lour. Adv. Colloid. Interface Sci. 1: 156.
2. Iravani S (2011) Green synthesis of metal nanoparticles using plants. Green Chem. 13: 2638-2650.
3. Akhtar MS, Panwar J, Yun YS (2013) Biogenic synthesis of metallic nanoparticles by plant extracts. ACS Sustain. Chem. Eng. 1: 591.
4. Prasad NR, Karthikeyan A, Karthikeyan S, Reddy BV (2011) Inhibitory effect of caffeic acid on cancer cell proliferation by oxidative mechanism in human HT-1080 fibrosarcoma cell line. Mol. Cell. Biochem. 11: 349.
5. Chang WC, Hsieh CH, Hsiao MW, Lin WC, Hung YC, et al., (2010) Caffeic acid induces apoptosis in human cervical cancer cells through the mitochondrial pathway. Taiwan J. Obstet. Gyne.49: 419.
6. Chang HT, Chen IL, Chou CT, Liang WZ, Kuo DH, et al., (2013) Effect of caffeic acid on Ca2+ homeostasis and apoptosis in SCM1 human gastric cancer cells. Arch. Toxicol. 87: 21-41.
7. Guerriero E, Sorice A, Capone F, Costantini S, Palladino P, et al., (2011) Effects of lipoic acid, caffeic acid and a synthesized lipoyl-caffeic conjugate on human hepatoma cell lines. Molecules 16: 63-65.
8. Devi JS, Bhimba BV (2012) Anticancer Activity of Silver Nanoparticles Synthesized by the Seaweed Ulva lactuca Invitro. scientificreports.242
9. Vinayaga Moorthi P, Balasubramanian C, Mohan AS (2014) An improved Insecticidal activity of silver nanoparticles synthesized by using Sargassum muticum. Applied Biochemistry and Biotechnology. 175: 135-140.
10. Borenfreund E, Pruenerv JA (1985) Toxicity determination invitro by morphological alterations and Neutral Red Absorption. Toxicology Letters 24: 119-124.
11. Rao S, Kotakadi YV, Prasad S, Reddy TNVKV, Gopal AVS, et al., (2013) Green synthesis and spectral characterization of silver nanoparticles from Lakshmi tulasi (Ocimum sanctum) leaf extract. Spectrochim Acta A 103: 156–159.
12. Prabhu D, Arulvasu C, Babu G, Manikandan R, Srinivasan P (2013) Biologically synthesized green silver nanoparticles from leaf extract of Vitex negundo. L. induces growth-inhibitory effect of human colon cancer cell line HCT15. Process Biochemistry, 48: 317-324.
13. Nayak D, Pradhan S, Ashe S, Rauta PR, Nayak B (2015) Biologically synthesised silver nanoparticles from three diverse family of plant extracts and their anticancer activity against epidermoid A431 carcinoma. Journal of Colloid and Interface Science 457: 329–338
14. AshaRani PV, Mun G LK, Hande MP, Valiyaveettil S (2009) Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano 3: 279.
15. Sanpui P, Chattopadhyay A, Ghosh SS (2011) Induction of Apoptosis in Cancer Cells at Low Silver Nanoparticle Concentrations using Chitosan Nanocarrier. ACS Appl. Mater. Interfaces 3: 218
16. Setyawati MI, Tay CY, Leong DT (2014) Exploiting cancer's antioxidative weakness through p53 with nanotoxicology. Nanomedicine 9: 369-371.
17. Guo D, Zhu L, Huang Z, Zhou H, Ge Y, et al., (2013) Anti-leukemia activity of PVP-coated silver nanoparticles via generation of reactive oxygen species and release of silver ions. Biomaterials 34: 7884-7894.
18. Setyawati MI, Tay CY, Leong DT (2013) Effect of zinc oxide nanomaterials-induced oxidative stress on the p53 pathway. Biomaterials 34: 10133
19. National Cancer Registry Programme (2013) Three Year Report 2. of Population Based Cancer Registries: 2009-2011. Indian Council of Medical Research.