DESIGN, SYNTHESIS AND *IN-VITRO* ANTI-CANCER ACTIVITY OF BIOGENIC SILVER NANOPARTICLES FROM AQUEOUS LEAF EXTRACT OF *OCIMUM BASILICUM*

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***Abstract*: Nanotechnology is becoming a new area of increasing research and industrial interest.Silver nanoparticles are one of the promising products in the nanotechnology industry. Silver nanoparticles can be synthesized by several physical, chemical and biological methods. One of such promising process is green synthesis. However for the past few years, various rapid chemical methods have been replaced by green synthesis because of avoiding toxicity of the process and increased quality. In this present study green synthesis of silver nanoparticles from aqueous silver nitrate (1mM) through a simple and eco-friendly route using leaves of *ocimum basilicum* . The aqueous silver ions when exposed to leaf broth were reduced and resulted in the green synthesis of silver nanoparticle. The bioreduced silver nanoparticle were characterized by scanning electron microscope (SEM) and Fourier transform infra-red (FTIR) spectroscopy. Size of silver nanoparticles range 0.1µm-0.5µ m observed by SEM. The FTIR measurement was carried out to identify the possible biomolecules responsible for efficient stabilization of silver nanoparticles. The synthesis of silver nanoparticles using aqueous extract of leaves of *ocimum basilicum* would be helpful for the preparation of potent cytotoxic agent against DLA and EAC cells to destroy cancer cells.**

***Keywords*: *Oscimum basilicum*, Silver nano particles, Green synthesis, *in vitro* anticancer activity.**

INTRODUCTION:

Due to swift industrialization and urbanization, our environment is undergo huge smash up and a large amount of perilous and superfluous chemical, gases or substances are released, and so now it is our need to learn about the secrets that are present in the Nature and its products which leads to the growth of advancements in the synthesis processes of nanoparticles. Nanotechnology applications are highly suitable for biological molecules, because of their exclusive properties. Nanotechnology is the fastest growing area of manufacturing in the world today and there is an increasingly frantic search for new nanomaterials and methods to make them. It has been well known that living cells are the best examples of machines that operate at the nanolevel and perform a number of jobs ranging from generation of energy to extraction of targeted materials at very high efficiency[1]. Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process [2, 3]. The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burn and open wounds [4]. Further these biologically synthesized nanoparticles were found highly toxic against different multi-drug resistant human pathogens. In most items, chemical synthesis of methods leads to the production of some toxic chemicals that are absorbed at the surface and can be prevented their use in medical applications[5]. Therefore, an environmentally and economically compatible synthetic of method is required for preparation of nanoparticles. The search for a method led to the bio-like production of silver nanoparticles [6]. There are three main sources for the synthesis of silver nanoparticles by biological methods such as bacteria, fungi and plants extract [7-9]. The main advantage of using plant extracts to synthesize silver nanoparticles is easy accessibility, Safe and in most cases non-toxic plants which can help to reduce silver ions. They have different types of metabolites that can help in the reduction of silver ions and be synthesizing faster than microbes. The herbal of chemicals which are including terpenoids, flavones, ketones, aldehydes, amides and carboxylic acids, they are directly involved to the reduction of ion and the formation of silver nanoparticles [10]. Silver nanoparticles are one of the promising products in the nanotechnology industry. The development of consistent processes for the synthesis of silver nanoparticles is an important aspect of current nanotechnology research. Silver nanoparticles can be synthesized by several physical, chemical and biological methods. One of such promising process is green synthesis. However for the past few years, various rapid chemical methods have been replaced by green synthesis because of avoiding toxicity of the process and increased quality [11-14]. The aim of the present wok is phytochemical investigation, Design, synthesis and characterization of biogenic silver nanoparticles from the aqueous leaf extract of *ocimum basilicum* and exploration of it's in-vitro anti-cancer activity.

MATERIALS AND METHODS

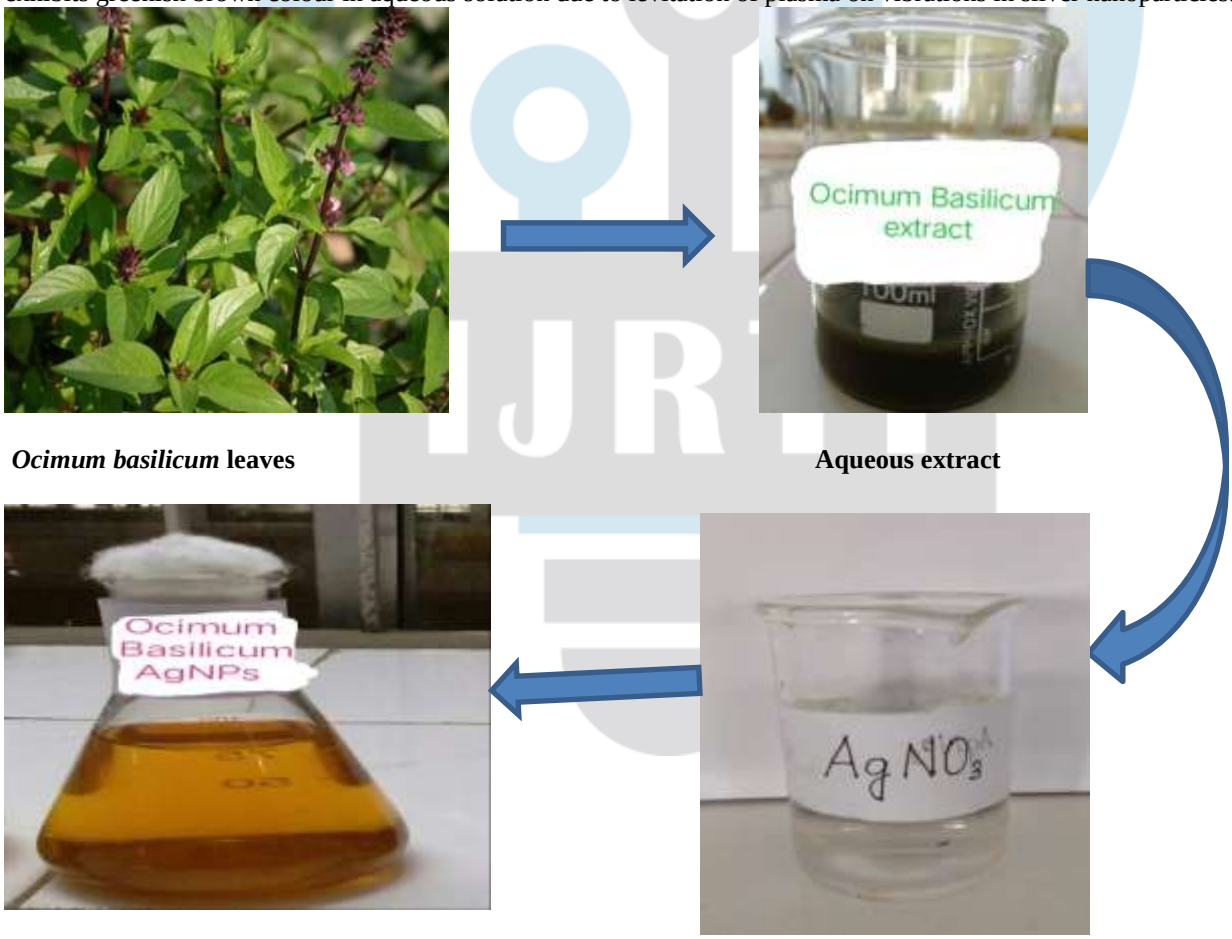
PREPARATION OF LEAF EXTRACT:

The plant material (*Ocimum basilicum*) was collected fresh from Watrap is a small village located in Coimbatore district, Tamil Nadu, India. 5gms of the leaves were surface cleaned under running tap water, followed by distilled water, air dried. The dried and powdered plant materials (100g) were extracted successively with 600ml of ethanol by using soxhlet extraction, for 48hrs at a temperature not exceeding at boiling point of the solvent. The extracts were filtered using Whatman No.1 filter paper and then concentrated in vaccum at 400°C using a Rotary evaporation. The extract was transferred to glass vials at 40°C before use.

Figure 1:***Oscimum basilicum*** **Plant**

SYNTHESIS OF SILVER NANOPARTICLES USING PLANT EXTRACT :

0.1M of aqueous solution of Silver nitrate was prepared and used for the synthesis for silver nanoparticles. 10ml of ethanolic leaf extract *oscimum basilicum* was added to vigorously stirred 90ml of aqueous solution of 0.1M silver nitrate and kept at room temperature. Reduction takes place rapidly and is completed in 10min as shown by tube light greenish-brown colour of the solution indicating the formation of silver nanoparticle. The green synthesis of Silver nanoparticles through plant extracts were carried out and confirmed by visual observation. The colour was changed greenish brown colour due to reduction of silver ions. The leaf extracts were mixed with the aqueous solution of the silver ion complex, it was changed into reddish brown color due to excitation of surface plasmon vibrations, which indicated that the formation of Ag nanoparticles. It was well known that Silver nanoparticle exhibits greenish brown colour in aqueous solution due to levitation of plasma on vibrations in silver nanoparticles.



Ocimum basilicum **leaves** **Aqueous extract**

Colour change of the solution **AgNO3 solution**

Fig 2: Formation of silver nanoparticles of *Oscimum basilicum* leaf extract

RESULTS AND DISCUSSION:

SCANNING ELECTRON MICROSCOPE (SEM):

Scanning electron microscope (SEM) SEM is a type of electron microscope that images a sample by scanning it with a high-energy beam of electrons in a raster scan patterns. In this experiment after the synthesis of nanoparticles using the plants and then lyophilisation was done using VIRTIS BENCHTOP machine. SEM analysis was done using JEOL-MODEL 6390 machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the films on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min..

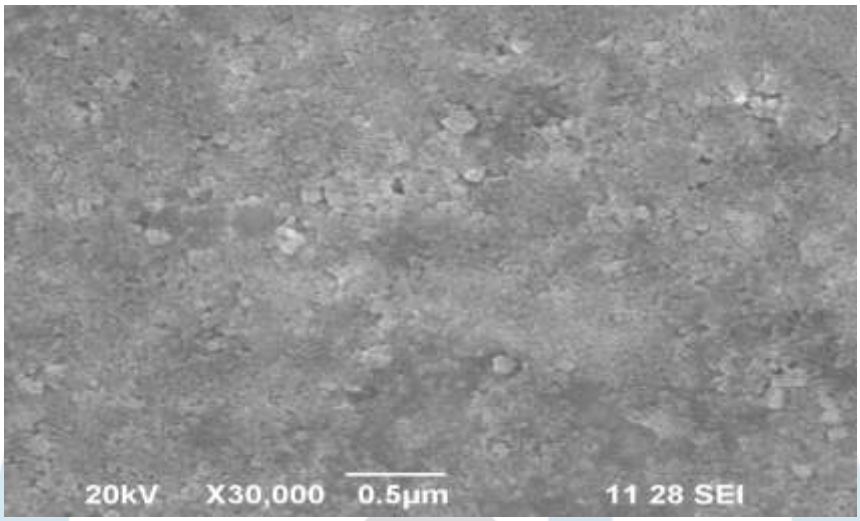


Fig.3: SEM image of silver nanoparticles synthesized by *Oscimum basilicum*

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR):

FTIR is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid, or gas. An FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer, which measures intensity over a narrow range of wavelength at a time. Fig.2: shows that the FTIR image of *O. Bsilicum* medicated synthesized silver nanoparticles indicates presence of biomolecules involved in the reduction process. FTIR spectrum silver nanoparticles shows the characteristic peaks in the region of 4000-500 cm-1. *Ocimum Basilicum* AgNPs had the absorption at 3414 cm-1 is notably enhanced indicating -NH stretching. Peak at 1618 cm-1 represents C=O stretching and peak at 617 cm-1 indicates C-H stretching.

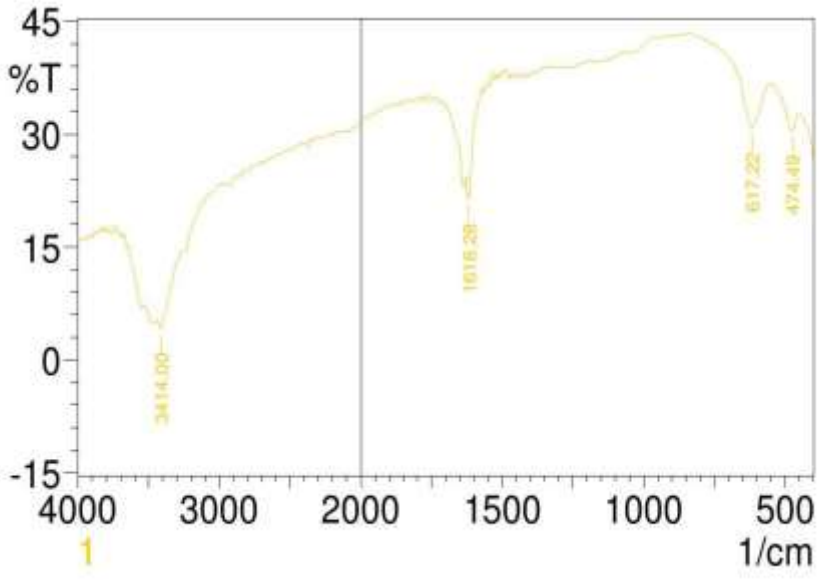


Fig 4: FTIR spectrum of silver nanoparticles synthesized by *Oscimum basilicum*.

***IN VITRO* ANTI – CANCER STUDIES**

The test compound was studied for short term in vitro cytotoxicity using Dalton's lymphoma ascites cell (DLA) and Ehrlich ascites carcinoma cell (EAC).The tumor cells aspirated from the peritoneal cavity of tumor bearing mice were washed thrice with PBS or normal saline. Cell viability was determined by trypan blue exclusion method. Viable cell suspension (1×10^6 cells in 0.1 ml) was added to tubes containing various concentration of the test compounds and the volume was made up to 1 ml using phosphate buffered saline (PBS). Control tube contained only cell suspension. These assay mixture were incubated for 3 hour at 37 C. Further cell suspension was mixed with 0.1 ml 1% trypan blue

and kept for 2-3 minutes and loaded on a haemocytometer. Dead cells take up the blue colour of trypan blue while live cell do not take up the dye. The number of stained and unstained cells were counted separately.

$$\% \text{ cytotoxicity} = \frac{\text{No. of dead cells}}{\text{No. of live cells + No. of dead cells}} \times 100$$

S.NO	Drug concentration ug/ml	Percentage of cell death inDalton's lymphoma ascites cells (DLA)%	Percentage of cell death in Ehrlich Ascites Carcinoma(EAC) cells%
1.	200	84.1	88.6
2.	100	80.8	80.3
3.	50	77.5	78.2
4.	20	55.2	59.1
5.	10	41.7	42.8

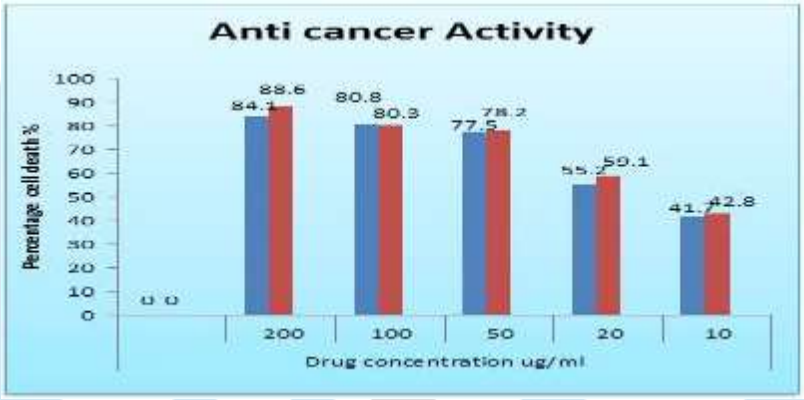


Fig 5:*In vitro* anticancer activity of silver nanoparticles synthesized by *Oscimum basilicum*

CONCLUSION:

Reduction of silver ion into particles during exposure to the plant extracts could be followed by color changes. Silver nanoparticles exhibit dark yellowish – brown color in aqueous solution due to surface plasmon resonance phenomenon. The synthesis of nanoparticles is in lime light of modern nanotechnology. Biosynthesis of nanoparticles by extracts is currently under exploitation. The development of biologically inspired experimental processes for the synthesis of nanoparticles is evolved into an important branch of nanotechnology. The present study emphasizes the use of plants medicinal for the synthesis of silver nanoparticles with potent anti cancer effect. The silver nanoparticles formed were predominantly cubical with uniform shape .It is known that the shape of metal nanoparticle can considerably change their optical and electronic property. The SEM imaged showed relatively spherical shape nanoparticles formed with diameter in the range of 1 – 100nm.The stability of silver nanoparticles were detected by Fourier Transform Infra Red Spectroscopy (FT-IR) . FT-IR spectra of silver nanoparticles showed the peaks for the functional groups , -NH, C=O and C-H which indicate the stability of synthesized silver nanoparticles. Anti cancer activity of silver nanoparticle tested against Daltons Lymphoma Ascites [DLA] and Ehrlich Ascites carcinoma[EAC] cells. The synthesis of silver nanoparticles using aqueous extract of leaves of *ocimum basilicum* would be helpful for the preparation of potent cytotoxic agent against DLA and EAC cells to destroy cancer cells

References

[1] D. S. Goodsell, *Bionanotechnology: Lessons from Nature*, Wiley-Liss, Hoboken, NJ, USA, 2004.
[2] J. R. Morones, J.L.Elechiguerra, A. Camacho, K. Holt, J.B. Kouri, J.T. Ramírez, et al. et al. "The bactericidal effect of silver nanoparticles. Nanotechnology", Vol. 16, pp. 2346-2353, 2005.
[3] C. Lok, C. Ho, R. Chen, Q. He, W. Yu, H. Sun, et al. et al. "Silver nanoparticles: partial oxidation and antibacterial activities", J Biol Inorg Chem. Vol. 12, pp.527-534, 2007.
[4] M. Ip, S.L. Lui, V.K.M. Poon, I. Lung, A. Burd, "Antimicrobial activities of silver dressings: an *in vitro* comparison", J Med Microbiol. Vol. 55, pp. 59-63, 2006.
[5] V.K. Sharma, R.A. Yngard, Y. Lin, "Silver nanoparticles: green synthesis and their antimicrobial activities", Adv. Colloid Interf. Sci., Vol.145, pp. 83-96, 2009.
[6] S. Prabhu, E.K. Poulose, "Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects", Int. Nano Lett., Vol.2, pp.32, 2012.
[7] U.K. Parashar, P.S. Saxena, A. Srivastava, "Bioinspired synthesis of silver nanoparticles", Dig. J. Nanomater. Biostruct., Vol.4, pp.159-166, 2009.

[8] K. Kalishwaralal, V. Deepak, S. Ramkumarpandian, H. Nellaiah, G. Sangiliyandi, "Extracellular biosynthesis of silver nanoparticles by the culture supernatant of *Bacillus licheniformis*", Mater. Lett., Vol. 62, pp. 4411-4413, 2008.
[9] A. Ahmad, P. Mukherjee, S. Senapati, D. Mandal, M.I. Khan, R. Kumar, M. Sastry, "Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*", Colloids Surf. B: Biointerfaces, Vol. 28, pp. 313-318, 2003.
[10] A.R. Shahverdi, S. Minaeian, H.R. Shahverdi, H. Jamalifar, A.-A. Nohi, "Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria: a novel biological approach Process", Biochem., Vol. 42, pp. 919-923, 2007.
[11] K. Vijayaraghavan, S.K. Nalini, N.U. Prakash, D. Madhankumar, "Biomimetic synthesis of silver nanoparticles by aqueous extract of *Syzygium aromaticum*", Mater. Lett., Vol. 75, pp. 33-35, 2012.
[12] A.K. Jha, K. Prasad, K. Prasad, A. Kulkarni, "Plant system: nature's nanofactory Colloids Surf. B:", Biointerfaces, Vol. 73,pp. 219-223, 2009.
[13] X. Li, H. Xu, Z.-S. Chen, G. Chen, "Biosynthesis of nanoparticles by microorganisms and their applications", J.Nanomater., https://doi.org/10.1155/2011/270974, 2011.
[14] A.D. Dwivedi, K. Gopal, "Biosynthesis of silver and gold nanoparticles using *Chenopodium album* leaf extract", Colloids Surf. A Physicochem. Eng. Asp., Vol. 369, pp. 27-33, 2010.
[15] F.K. Alsammarraie, W. Wang, P. Zhou, A. Mustapha, M. Lin, "Green synthesis of silver nanoparticles using turmeric extracts and investigation of their antibacterial activities", Colloids Surf. B: Biointerfaces, Vol. 171, pp. 398-405, 2018.
[16] S. Das, J. Das, A. Samadder, S.S. Bhattacharyya, D. Das, A.R. Khuda-Bukhsh, "Biosynthesized silver nanoparticles by ethanolic extracts of Phytolacca decandra, Gelsemium sempervirens, Hydrastis canadensis and Thuja occidentalis induce differential cytotoxicity through G2/M arrest in A375 cells", Colloids Surf. B: Biointerfaces, Vol. 101, pp. 325-336, 2013.
[17] C. Dipankar, S. Murugan, "The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from Iresine herbstii leaf aqueous extracts", Colloids Surf. B: Biointerfaces, Vol. 98, pp. 112-119, 2012.
[18] D. Philip, C. Unni, S.A. Aromal, V. Vidhu, "*Murraya koenigii* leaf-assisted rapid green synthesis of silver and gold nanoparticles", Spectrochim. Acta A Mol. Biomol. Spectrosc., Vol. 78, pp. 899-904, 2011.
[19] O.A. Ojo, B.E. Oyinloye, A.B. Ojo, O.B. Afolabi, O.A. Peters, O. Olaiya, A. Fadaka, J. Jonathan, O. Osunlana, "Green synthesis of silver nanoparticles (AgNPs) using *Talinum triangulare* (Jacq.) Willd. Leaf extract and monitoring their antimicrobial activity", J. Bionanoscience, Vol. 11, pp. 292-296, 2017.
[20] B. Kumar, K. Smita, L. Cumbal, A. Debut, "Green synthesis of silver nanoparticles using Andean blackberry fruit extract",Saudi J. Biol. Sci., Vol. 24, pp. 45-50, 2017.