Section A-Research paper

Comparative study between the green mediated synthesised Silver Oxide nanoparticles from NegundoVitex leaf and Eucalyptus leaf extract: Synthesis, Characterization, Antibacterial studies and Cytotoxicity studies.

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Abstract

Developing a low cost, high-efficiency and environmentally friendly approach to synthesize Silver Oxide nanoparticles for biomedical applications has become a highly research focus in the current scenario. In the present investigation, the AgO NPs were synthesized via a green process using different plant extracts such as Vitex Negundo leaf extract and Eucalyptus leaf extract. X-ray diffraction (XRD), Scanning electron microscopy (SEM), Energy dispersive x-rayanalysis (EDX), and Fourier Transform Infrared Spectroscopy (FTIR) were used to investigate the evolution of the size, morphology, chemical composition and functional group of synthesized AgO NPs. Antibacterial efficiency and Cytotoxicity studies were carried out for the green synthesized AgO NPs using both extract and compared their efficiency.

Keywords:*Biogenic synthesis, Nickel Oxide nanoparticles, Eucalyptus leaf, Negundo Vitex, Cytotoxicity, Antibacterial activity*

1. Introduction

Nanoparticles have a special place in current technology not only because of their special properties resulting from their reduced dimension, but also because of they are promising building blocks for more complex structures.'Nano' is a prefix used to describe one billionth or 10^{-9} of something [1].Nanotechnology is an important field of modern research dealing with synthesis, strategy and

manipulation.Nanotechnology is invented to describe extra high precision and ultra-fine dimension. Particles with size in the range of 1-100nm considered in any dimension is called nanoparticles.Nanoparticles are now being widely used in various fields such as biomedical, electronic, optical, agriculture etc. [2,3].Silver oxide nanoparticles is an important transition metal oxide material which is used in numerous applications such as battery electrodes, photoelectron devices, magnetic materials, catalysts, anticancer properties, cytotoxic activityetc.[13]. Synthesis of nanoparticles involves physical, chemical, biological method.Physical and chemical methods may produce well defined particles but they have low productivity, high cytotoxicity, and low antimicrobial activity and are not eco-friendly.Biological synthesis provides an eco-friendly, simple inexpensive, produce low cytotoxicity compared to chemically synthesized nanoparticles.In addition the plant secondary metabolites act as stabilizing agent as well as capping agent [6].

Eucalyptus is an evergreen tree that's widely used in many areas for its medicinal properties.Although native to Australia, this popular tree now grows in many areas of the world.It has a gum-infused bark, long stems, and circular leaves that are hard to digest if eaten whole. However, eucalyptus leaves can be made into a tea that's safe for consumption.Additionally, the leaves can be made into essential oil for topical use or inhalation. It is also rich Anti-oxidant. [9]

Negundo Vitex belongs to the family of verbanaceae, which is commonly known as chase tree and also called as Karu Nochi in Tamil, Nirgundi in Hindi. It is a large shrub grown in waste lands throughout India. It is one of the common plants used in traditional medicine and reported to have variety of biological and pharmacological applications [10].The leaves avonoids, alkaloids, monoterpenes, eurostoside, saturated steroidal and triterpinoidal saponins, amino acids, and aromatic amines.This herb is traditionally utilized to cure and treat many deficiencies, such as amenorrhea, corpus luteum insufficiency, dysmenorrhea, hyperprolactinemia, and breastfeeding disorders [11].

In this current study we have used the green mediated synthesis of Silver Oxide nanoparticles from Vitex Negundo leaf extract and Eucalyptus leaf extract. The synthesized nano particles were subjected to characterization techniques like FTIR, SEM,EDAX and XRD. Their efficiency as Anti-bacterial and Cytotoxicity studies were also studies for the synthesized nanoparticles from both leaf extract.

2. Materials and Methods

2.1 Materials:

Fresh and healthy leaves of Negundo Vitex leaf were collected from the nearby areas of Coimbatore and Eucalyptus leaf was collected from the nearby areas of Coimbatore. All the Chemicals used in this research work are of A.R grade and water used is double distilled water.

2.2 Methods:

2.2.1Preparation of the aqueous extract:

The collected Vitex Negundo leaves and Eucalyptus leafs were thoroughly cleaned with running tap water to remove debris and other contaminants, followed by distilled water and air dried at room temperature for a 2-3 days separately. The aqueous extract of the leaves was prepared by boiling the leaves with distilled water at about 60 ℃ for 50 minutes until the color of the aqueous solution changes to brown red. The extract was cooled to room temperature and filtered using Whatmann No. 1 filter paper. Finally, the extract was stored in a refrigerator at 4 °C in order to be used for further experiments[16].

2.2.2 Preparation of Silver Nitrate solution for Vitex Negundo leaf extract:

About 0.001M of Silver nitrate solution was prepared by dissolving 0.169g of silver nitrate in distilled water in 1 litre beaker and placed in magnetic stirrer.

2.2.3Preparation of Silver Nitrate solution for Eucalyptus leaf extract:

About 0.001M of Silver nitrate solution was prepared by dissolving 0.169g of silver nitrate in distilled water in 1 litre beaker and placed in magnetic stirrer.

2.2.3 Synthesize of AgO nanoparticles:

To synthesize AgO nanoparticles, 500 ml of the precursor solution was mixed with 500 ml of Vitex Negundo aqueous extract in a 1:1 ratio.To synthesize AgO nanoparticles, 500 ml of the precursor solution was mixed with 500 ml of Eucalyptus aqueous extract in a 1:1 ratio.

Both the aqueous extract was slowly poured into the precursor solution separately until one can observe significant color change. The final mixture was stirred using magnetic stirrer for an hour under ambient conditions. After cooling down to room temperature, the resulting solution was washed three times, centrifuging each time at 3500 rpm for 10 minutes with distilled water to remove unwanted impurities.

The 2 different combination of resulting precipitate was collected and oven dried at 120 ℃. Finally, the powder was calcinatedseparately using Muffle furnace for 1 hour at 600 $°C$ to collect AgO nanopowder.

3. Characterization:

3.1 FTIR Studies:

The green synthesized AgO nanoparticles were analyzed for the different functional groups by the Fourier Transfer Infra-Red (FTIR) spectrophotometer (Shimadzu, Japan).

3.2 SEM Analysis

The green synthesized AgO nanoparticles were subjected for Scanning electron microscope studies with 30kV scanning electron microscope JEOL (Japan) Model JSM-6360.

3.3 EDAX Analysis:

The elemental composition of materials present in green synthesized AgO nanoparticles are determined using Energy Dispersive X-Ray analysis (Oxford Instruments, UK).

3.4 XRD Analysis:

The unknown crystalline materials present in formic acid treated and dyed modal fabrics were determined using XRD technique (Shimadzu XRD6000).

3.5 Antibacterial Activity:

Preparation of the bacterial inoculum:

Stock cultures were maintained at 4° C on slopes of nutrient agar and potato dextrose agar. Active culture for experiments were prepared by transferring a loop full of cells from stock cultures to test tubes of 50ml nutrient broth bacterial cultures were incubated with agitation for 24hours and at 37°c on shaking incubator and fungal cultures were incubated at 27°c for 3-5 days. Each suspension of test organism was subsequently stroke out on nutrient agar media and potato dextrose agar. Bacterial cultures then incubated at 37°c for 24 hours and fungal incubated at 27°c for 3-5 days. A single colony was transferred to nutrient agar media slants were incubated at 37°c for 24 hours and potato dextrose slant were incubated at 27°c for 3-5 days. These stock cultures were kept at 4°c. For use in experiments, a loop of each test organism was transferred into 50ml nutrient broth and incubated separately at 37°c for 18-20 hours for bacterial culture.

Well Diffusion method

The antibacterial activity of crude extracts was determined by Well Diffusion method. The 2-20 µl of Nanoparticle extract was poured into the wells. After that, the plates were incubated at 37°C for 24 hours. Assay was carried into triplicates and control plates were also maintained. Zone of inhibition was measured from the edge of the well to the zone in mm. The tested cell suspension was spread on MullerHinton agar plate and potato dextrose agar. Well were put into the agar medium using sterile forceps. Plant extract were poured on to wells. Then plates were incubated at 37°c for about 24 hours and control was also maintained. Zone of inhibition was measured from the clear zone in mm.

Antibacterial activity was performed by agar diffusion method. Van der Watt *et al.*, 2001. The stock culture of bacteria *(E.Coli and Streptococcus)* were received by inoculating in nutrient broth media and grown at 37 % for 18 hours. The agar plates of the above media were prepared. Each plates was inoculated with 18 hours old cultures the bacteria were swab in the sterile plates. Cut the 5 wells Pour the extract in ratio 25 µl, 50 µl 75 µl 100 µl. All the plates were incubated at 37^{o}C for 24 hours and the diameter of inhibition zone was noted in Cm.

Agar well diffusion method has been used to determine the antimicrobial activities and minimum inhibitory concentrations or plant extracts against Gram positive, Gram negative bacteria. The extracts exhibited antibacterial activities against tested microorganisms.

3.6 In-vitro Anticancer Activity:

Cell line

The human cancer cell line (Cervical cancer –HELA) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^{0}C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^{5}$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^{0}C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat

dimethylsulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore,the amount of formazan produced is directly proportional to the number of viable cells.

After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader.

The percentage cell viability was then calculated with respect to control as follows

$$\% \text{ Cell viability} = [A] \text{ Test} / [A]\text{control} \times 100$$

The % cell inhibition was determined using the following formula.

$$\% \text{ Cell Inhibition} = 100 - \text{Abs (sample)}/\text{Abs (control)} \times 100.$$

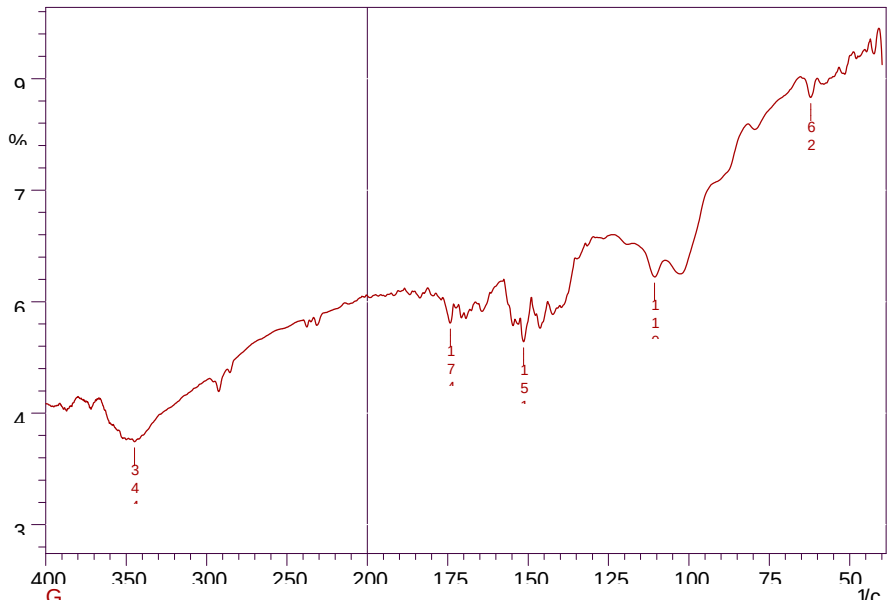
Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC50 was determined using GraphPad Prism software.

4. Results and Discussion

4.1 FTIR Analysis

4.1.1 The FTIR spectra of synthesized silver oxide nanoparticles from Negundo Vitex leaf extract for functional group identification are shown in the figure. It can be observed that there are some strong peaks in the region of 1,000 to 1,800 cm^{-1} .These peaks correspond to stretching vibration of –OH group.

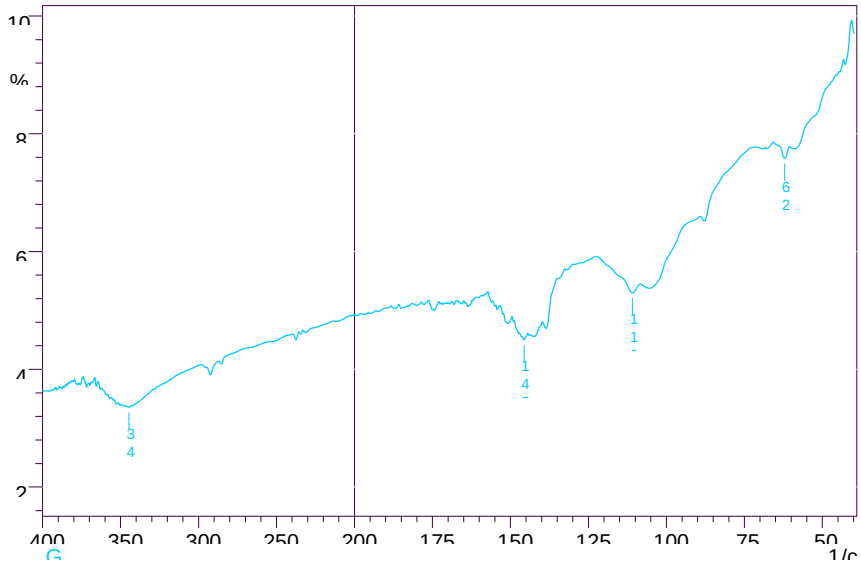
The peak at 1,741 cm^{-1} was attributed to lattice water molecule. The appeared weak bands between 1,100-1,200cm^{-1} were due to silver oxide bond.The bands at 621cm^{-1},1,107 cm^{-1}, and 1514 cm^{-1} were ascribed to asymmetric stretching , symmetric stretching and bending vibration of silver oxide bond respectively.



4.1.1.a FTIR studies of AgO nanoparticles from Negundo Vitex leaf extract

4.1.2 The FTIR spectra of synthesized silver oxide nanoparticles for functional group identification are presented in figure.It can be observed that there are some strong peaks in the region of 1,000 to 1,500 cm^{-1} .These peaks correspond to stretching vibration of –OH

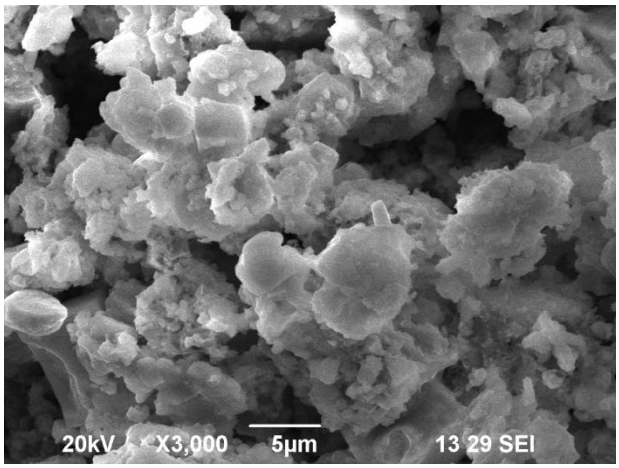
group.The peak at 1,456 cm^{-1} was attributed to lattice water molecule. The appeared weak bands between1,100-1,200cm^{-1} were due to silver oxide bond.The bands at 621cm^{-1},1,109 cm^{-1}, and 1456 cm^{-1} were ascribed to asymmetric stretching, symmetric stretching and bending vibration of silver oxide bond respectively.



4.1.2.aFTIR studies of AgO nanoparticlesfrom Eucalyptus extract

4.2 SCANNING ELECTRON MICROSCOPY

4.2.1 Scanning electron microscopy(SEM) was used to examine the morphological properties of the biogenically synthesized silver nanoparticles.The high magnification SEM images of the Silver nanoparticles are shown in figure.The Silver nanoparticles which was prepared from Negundo Vitex leaf extract have a consistent size distribution, as illustrated in the images.Individual particles were discovered in the crystals, which is in the flakes like structure.



4.2.1.a SEM images of AgO nanoparticles from Negundo Vitex leaf extract

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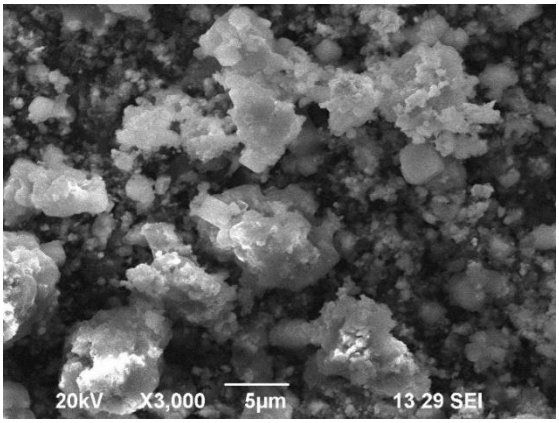
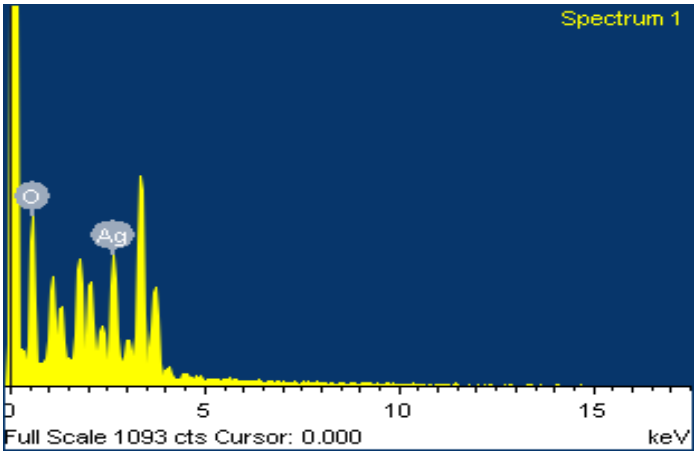


4.2.2.a SEM images of AgO nanoparticlesfrom Eucalyptus extract

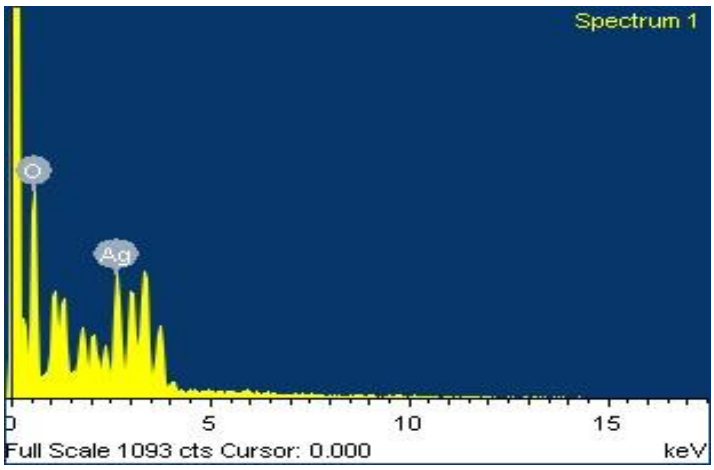
4.3 EDAX

4.3.1 The elemental analysis of EDAX spectrum using Negundo Vitex leaf extract conforms the formation of 83.80% of silver and 16.2% of oxide.



4.3.1.a EDAX images of AgO nanoparticles from Negundo Vitex leaf extract

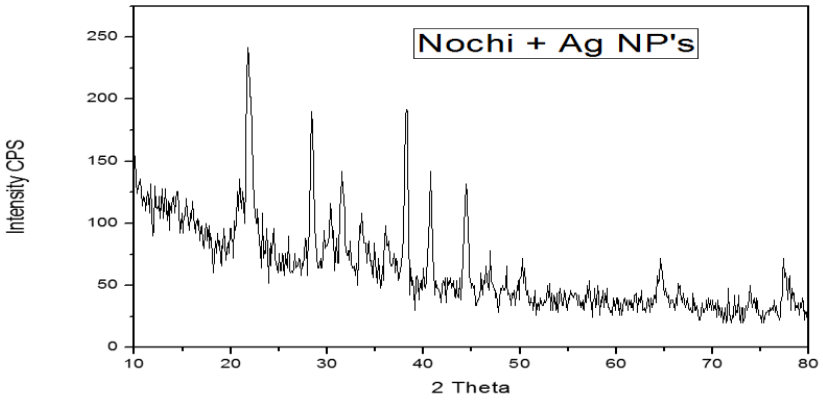
4.3.2 The elemental analysis of EDAX spectrum using Eucalyptus leaf extract conforms the formation of 95.8% of oxide and 4.2% ofSilver.



4.3.2.a EDAX images of AgO nanoparticlesfrom Eucalyptus extract

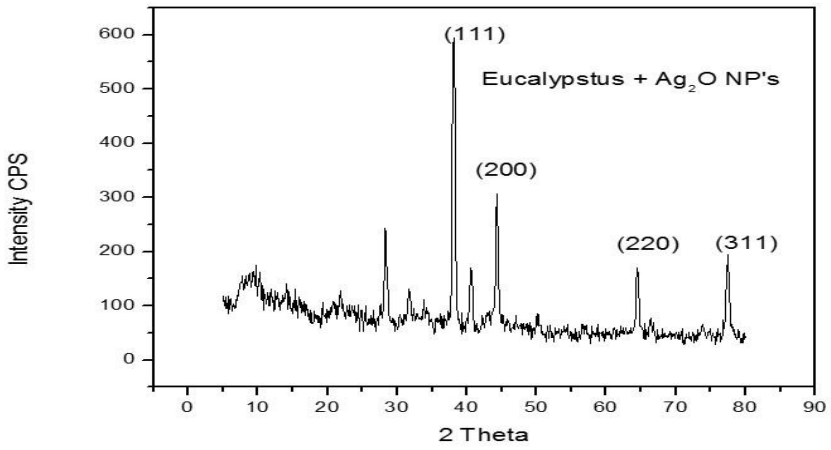
4.4 X-Ray Diffraction Studies:

4.4.1 The XRD technique was used to determine the crystal structure of green synthesized nanoparticles. Figure displays the XRD pattern of silver nanoparticles synthesized in the present work.A number of brag reflection with 2 theta values of 38.01, 64.86 and 77.33 corresponding to the (111),(200) & (220) sets of lattice planes, respectively are observed. (JCPDS card no: 04-0783)It is indexed as the band for face centred cubic (fcc) structure of Silver nanoparticles[4].



4.4.1.a XRD studies of AgO nanoparticles from Negundo Vitex leaf extract

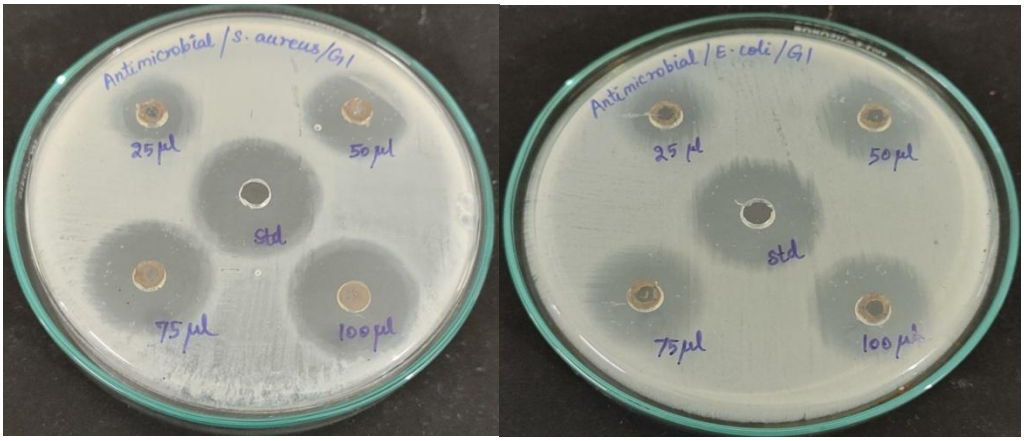
4.4.2 The XRD technique was used to determine the crystal structure of green synthesized Silver nanoparticles. Figure displays the XRD pattern of silver nanoparticles synthesized in the present work.A number of brag reflection with 2 theta values of 38.66,44.00,64.26corresponding to the (111), (200),(220)sets of lattice planes, respectively are observed.(JCPDS card no: 04-0783).It is indexed as the band for face centered cubic(fcc) structure of silver nanoparticles.



4.4.2.a XRD studies of AgO nanoparticlesfrom Eucalyptus extract

4.5 ANTIBACTERIAL ACTIVITY

4.5.1 Antibacterial activity of the sample was identified by using well diffusion method against E.Coli and S. Aureus. Muller Hinton agar(39gm in 1000ml) was prepared and swabbed 70µl of the bacterial culture (E.Coli, S.Aureus) using cotton swab and well were made with cork borer followed by the sample in different concentrations (25µl,50µl,75µl,100µl) was added. Antibiotic disc (ciprofloxacin 5mcg) was placed as a positive control DMSO was used as negative control, the plate was incubated at 37^{o}c for 24 hrs (Figure 4.5.1.a). After incubation anti-bacterial activity of the sample was confirmed based on the zone of inhibition in mm[4,5,12,15,19].



4.5.1.a Anti-Bacterial Activity of AgO nanoparticles from Negundo Vitex leaf extract

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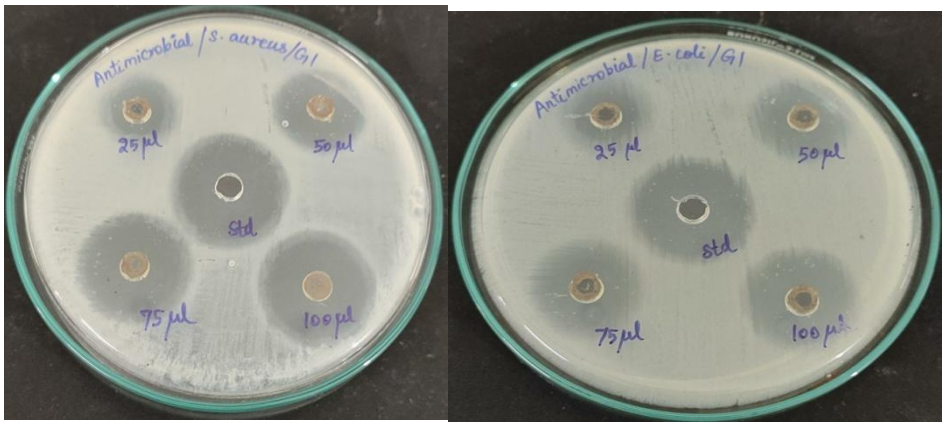
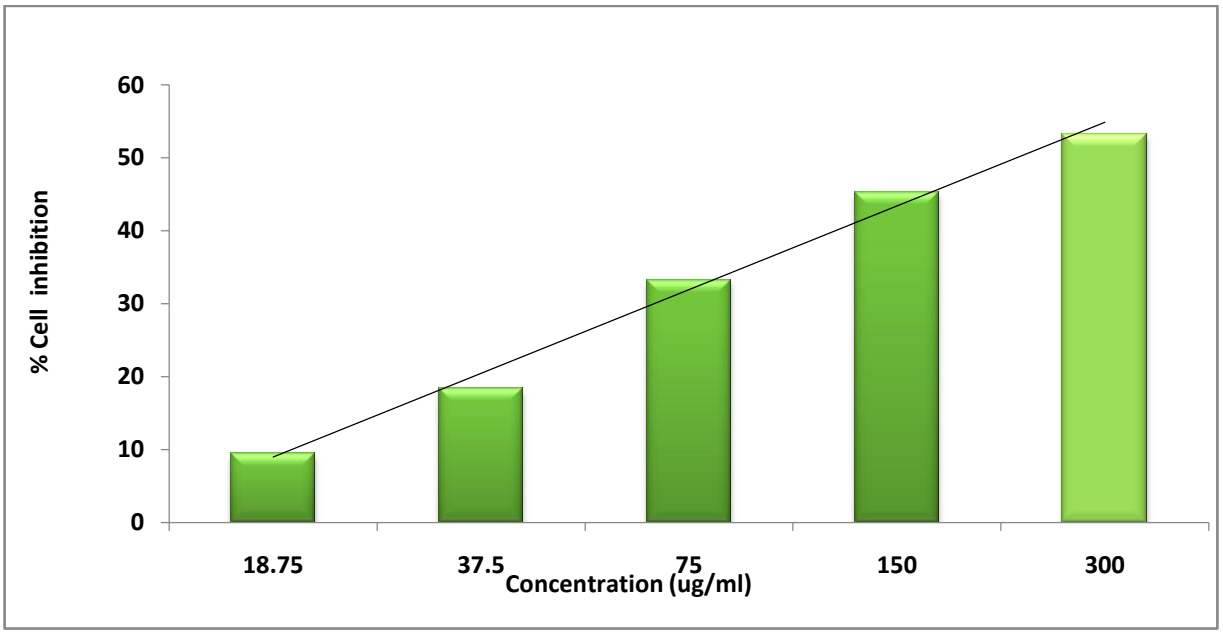


4.5.2.a Antibacterial Activity of AgO nanoparticlesfrom Eucalyptus extract

4.6 ANTI-CANCER ACTIVITY

4.6.1 In this present study Silver nanoparticles synthesized using different Negundo Vitex leaf extract were further evaluated for the cytotoxic activity using HT-29 colon cancer cell lines .The % of viability of the cells in each dose was measured by using MTT assay method. The absorbance is directly proportional to the number of living cells in the culture. The observed result revealed, the cells treated with AgO nanoparticles showed significant cytotoxicity in cell lines at the concentration above 75 µg/ml (Figure 4.6.1.a). Morphological observation of HT29 cells, showed significant changes in the cell morphology (Swelling of cells, Cell breakage) when treated with the synthesized AgO nanoparticles. From the above finding, we suggest further cytotoxic study to determine the anticancermechanism by the synthesized AgO NPs[7,8,14,18].



4.6.1.a Cytotoxicity Studies of AgO nanoparticlesfrom Negundo Vitex extract

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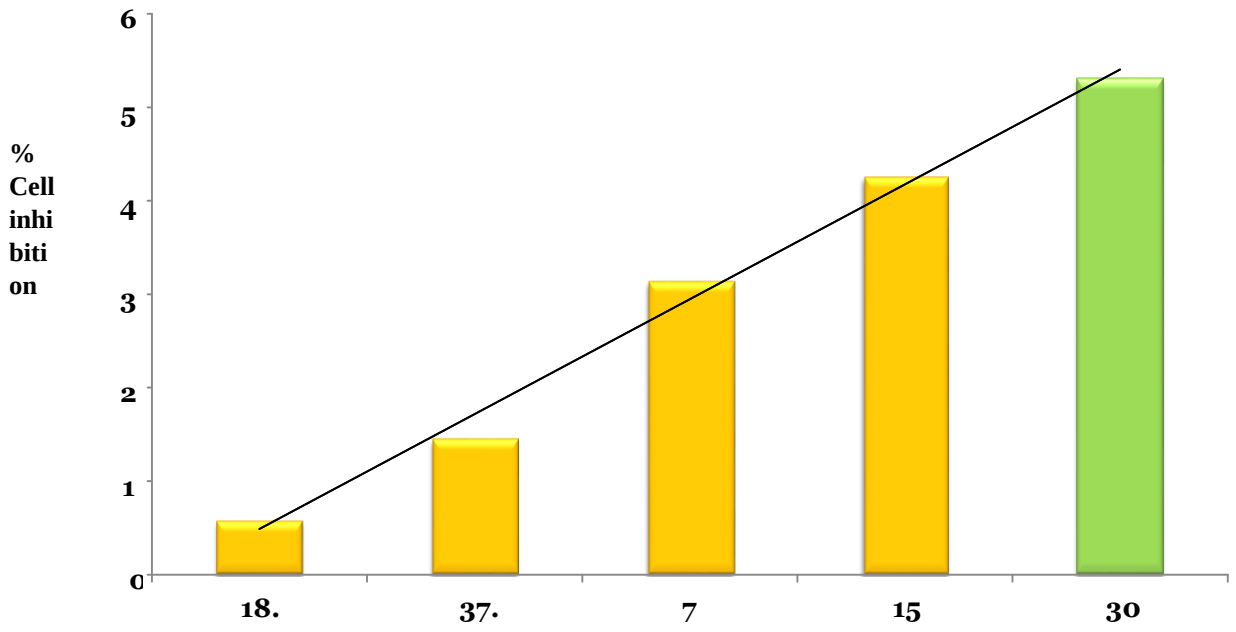

4.6.2.a Cytotoxicity Studies of AgO nanoparticlesfrom Eucalyptus leaf extract

CONCLUSION

The results indicate that eucalyptus leaf extract and Negundo Vitex leaf extract which is grown as an medicinal plant in many gardens in India and other parts of the world can be beneficially used in the nano biotechnology based industries for bio inspired rapid synthesis of silver nanoparticles.

A simple, cost effective and green approach was developed for synthesis of silver nanoparticles.Eucalyptus leaf extract and Negundo Vitex leaf extract added acted as both reducing and stabilizing agent.

The formation and structure of silver nanoparticles were confirmed based on EDAX, XRD and SEM analysis.The silver nanoparticles that are formed from both extract are highly stable and had moderate antibacterial action on E.Coli and Staphylococcus bacteria.It also act as a good Anti-cancer agent against HELA cells.

Acknowledgement

The authors feel thankful to the Management, Principal and Secretary Dr B.L Shivakumar, Sri Ramakrishna College of Arts & Science, Coimbatore for their constant support and funding to complete this project.

Statements and Declarations

Disclosure of potential conflicts of interest

Funding

The authors feel thankful to the Management, Principal and Secretary Dr B.L Shivakumar, Sri Ramakrishna College of Arts & Science, Coimbatore for their constant support and funding to complete this project.

Conflict of interest

The authors have no relevant financial or nonfinancial interests to disclose.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

REFERENCE

1. A.F.Abd El-Rahman and Tahany.G.M (2013) Mohammad. Green synthesis of silver nanoparticle using Eucalyptus globulus leaf extract and its antibacterial activity, 2013 December; 9(10): pages 6437-6440.
2. AfrahEltayebMohammed (2015) Green synthesis, antimicrobial and cytotoxic effects of silver nanoparticles mediated by Eucalyptus camaldulensis leaf extract,Asian Pacific Journal of Tropical BiomedicineVolume 5, Issue 5, May 2015, Pages 382-386, https://doi.org/10.1016/S2221-1691(15)30373-7
3. Anal K. Jha &K Prasad (2019) Green Synthesis of Silver Nanoparticles Using *Cycas* Leaf, International Journal of Green Nanotechnology: Physics and Chemistry 1(2):P110-P117, DOI: 10.1080/19430871003684572.
4. Akl M. Awwad, Nidà M. Salem, (2012) Green Synthesis of Silver Nanoparticles byMulberryLeavesExtract, Nanoscience and Nanotechnology2012; 2(4): 125-128 doi: 10.5923/j.nn.20120204.06
5. Xi-Feng Zhang& etal (2016),Silver Nanoparticle:Synthesis, Characterization, Therapeutic approach,Int J Mol Sci Sep 13;17(9):1534.,doi: 10.3390/ijms17091534.

6. Sudip Mukherjee & etal (2014), Potential theranostics application of bio-synthesized silver nanoparticles (4-in-1 system) Thernostics,2014 Jan 29;4(3):316-35. doi: 10.7150/thno.7819**.**
7. Ramar Manikandan & etal (2015), Biosynthesis of silver nanoparticles using ethanolic petals extract of Rosa indica and characterization of its antibacterial, anticancer and anti-inflammatory activities**,** Spectrochim Acta A Mol Biomol Spectrosc Mar 5;138:120-9. doi: 10.1016/j.saa.2014.10.043.
8. Sang Hung Lee & etal (2019),Silver Nanoparticles: Synthesis and Application for Nanomedicine**,**Int J Mol Sci . 2019 Feb 17;20(4):865, doi: 10.3390/ijms20040865.
9. Palanivel Sathishkumar& etal (2016), Anti-acne, anti-dandruff and anti-breast cancer efficacy of green synthesised silver nanoparticles using Coriandrum sativum leaf extract**,**J Photochem Photobiol B, 2016 Oct;163:69-76, doi: 10.1016/j.jphotobiol.2016.08.005.
10. Zia-ur-Rehman Mashwani & etal (2016),Applications of plant terpenoids in the synthesis of colloidal silver nanoparticles,Advances in Colloid and Interface Science,Volume 234, August 2016, Pages 132-141, https://doi.org/10.1016/j.cis.2016.04.008.
11. Li.Xu & etal, (2020), Silver nanoparticles: Synthesis, medical applications and biosafety, *Theranostics* 2020; 10(20):8996-9031. doi:10.7150/thno.45413.
12. Wen-Ru Li& eta (2009) Antibacterial activity and mechanism of silver nanoparticles on Escherichia coli,Applied Microbiology and Biotechnology volume 85, pages1115–1122.
13. Ivan Sondi & etal (2004), Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria, Journal of Colloid and Interface Science Volume 275, Issue 1, 1 July 2004, Pages 177-182, https://doi.org/10.1016/j.jcis.2004.02.012.

14. Anthyalam Parambil Ajaykumar& etal (2023), Green Synthesis of Silver Nanoparticles Using the Leaf Extract of the Medicinal Plant, *Uvaria narum* and Its Antibacterial, Antiangiogenic, Anticancer and Catalytic Properties,*Antibiotics* 2023, *12*(3), 564; https://doi.org/10.3390/antibiotics12030564**.**
15. Virender K. Sharma& etal (2009), Silver nanoparticles: Green synthesis and their antimicrobial activities, Advances in Colloid and Interface Science, Volume 145, Issues 1–2, 30 January 2009, Pages 83-96.
16. Aleš Panáček &etal (2009), Antifungal activity of silver nanoparticles against *Candida* spp, Biomaterials, Volume 30, Issue 31, October 2009, Pages 6333-6340, https://doi.org/10.1016/j.biomaterials.2009.07.065
17. Kholoud M.M. Abou El-Nour & etal (2010), Synthesis and applications of silver Nanoparticles, Arabian Journal of Chemistry, Volume 3, Issue 3, July 2010, Pages 135-140, https://doi.org/10.1016/j.arabjc.2010.04.008.
18. Shazina Jabeen & etal (2021), Application of green synthesized silver nanoparticles in cancer studies – A critical review, Materials Research Express, 8(21).
19. Anandalakshmi K1 and Venugobal J2* Green Synthesis and Characterization of Silver Nanoparticles Using Vitex negundo (Karu Nochchi) Leaf Extract and its Antibacterial Activity Department of Physics, Periyar Government Arts and Science College, Cuddalore, Tamil Nadu, India 2 Department of Physics, Sir Issac Newton Engineering College and Technology, Nagapattinam, Tamil Nadu, IndiaDOI: 10.4172/2161-0444.1000460.