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ANTI-CANCER ACTIVITY IN HELA CELL LINES OF PHYTOSYNTHESIZED GOLD NANOCOMPOUNDS USING *ANDROGRAPHIS PANICULATA* (NILAVEMBU) - CHARACTERISATION, OPTIMIZATION, PHYTOCHEMICAL ANALYSIS & ANTI MICROBIAL STUDY

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ABSTRACT ***Andrographis paniculata*** commonly called as Nilavembu is one of the well known medicinal plant having potential anti viral properties. This plant is used for the synthesis of gold nanoparticles predominantly aiming at spherical morphology and upon synthesis screening the nanoparticles for *in vitro* anti cancer activity. The synthesis was carried out in room temperature with precursor solution, Chloroauric acid and the green extract. The distinctive color change of the precursor solution, Chloroauric acid, from pale yellow to wine red indicated the initial confirmation of the nanoparticles synthesis. The preliminary confirmation of the nanoparticles was done using UV visible spectrophotometer, which showed the characteristic peak for the synthesized nanoparticles. Further, the imaging of the nanoparticles was carried out under SEM (Scanning Electron Microscope) which revealed the size and shape of the synthesized nanoparticles. The nanoparticles were tested for the Anti-Bacterial efficiency by well diffusion assay which showed defined Zones of inhibition against the tested strains of Bacteria. Further, the anti cancer assay gave an IC50 value for the nanocompound at 150 ng/ml.

KEYWORDS : AuNP's- Gold Nanoparticles,

INTRODUCTION

Nanomaterials is currently an area of intense research, due to wide variety of potential applications in biomedical, optical, and electronic fields. A nanometre is 1 x 10^{-9} m or one millionth of a millimetre. These exhibit size and shape-dependent properties that are of great interest for applications

ranging from catalysts and sensing to optics, antibacterial activity and data storage. Reducing gold to nano-sized particles helps to make the element highly effective, for several uses in the industry of medicine and technology.

Materials And Methods:

Preparation Of The Plant Extracts

5g of the leaves was washed well and taken in a conical flask, and boiled for 5-10 minutes. The extract was allowed to cool, filtered and collected through Whatman filter paper. This extract was stored at 4^{0}C and used for further synthesis.

Synthesis Of The Gold Nanoparticles:

For the synthesis of gold nanoparticles (AuNP's), the pH of the chloroauric acid was checked and adjusted to alkaline ranging from pH 7 to pH 8. To 10ml of chloroauric acid solution about 1ml of plant extract was added, thereby the ratio of 10:1 is followed as the constant ratio for production of larger quantities . The plant extract is used as the reducing agent for the synthesis. The pale yellow color of the chloroauric acid changes into a wine red, pink or purple color confirming the formation of nanoparticle.

Characterization Of Nanoparticles Using Uv Visible Spectrophotometer

The bioreduction of gold chloride in aqueous solution was monitored by periodic sampling of the aliquots of the suspension. 1 ml of the sample was diluted with 30 ml of double distilled water and analyzed for the presence of nanoparticles. The UV visible spectra of the gold nanoparticles were recorded using a Systronic smart DBS 2303 UV Visible spectrophotometer operating in the wavelength of 400 to 700 nm.

Sem Analysis

The nanoparticles were diluted with Millipore water in the ratio of 1:20 such that the concentration of the nanoparticles becomes much less, which enables better imaging of the nanoparticles. About 10 µl of sample was placed on the aluminum foil and allowed to air dry. Then the corresponding images were obtained by scanning electron microscope through German model-S-3400, Department of NCNSNT, University of Madras, Guindy.

Fourier Transform Infrared Spectroscopy Analysis (ftir)

Fourier Transform Infrared Spectroscopy, also known as FTIR Analysis or FTIR Spectroscopy, is an analytical technique used to identify organic, polymeric, and inorganic materials. The FTIR analysis method uses infrared light to scan test samples and observe chemical properties. The synthesized nanoparticles were analysed for the presence of functional groups of plants that aided in the process of nano formation.

Phytochemical Analysis Of The Green Extracts Of The Samples Used:

Test for terpenoids (Salkowski method)

Reagents: conc. sulphuric acid, chloroform

Procedure: To 0.5 ml of each of the extract, 2ml chloroform was added. 3ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown color at the interface indicates the presence of terpenoids.

Test for flavonoids:

Reagents: 10 % ammonium solution, conc. sulphuric acid and 1% aluminum solution

Procedure: Two methods were used to test for flavonoids. First, 10% of dilute ammonia (5ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid 1 ml was added. A yellow coloration that disappears on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminum solution were added to a portion of the filterate. A yellow coloration indicates the presence of flavonoids.

Test for saponins:

Reagents: water

Procedure: To 2 ml of the extract, 8 ml of distilled water was added and shaken vigorously if the foam persists after 5 to 6 minutes, the test is positive for the presence of saponins.

Test for tannins:

Reagents: 10% ferric chloride

Procedure: About 0.5 g of the extract was boiled in 10 ml of water and then filtered. A few drops of 10% ferric chloride was added and observed for brownish green or a blue black coloration.

Test for phenols:

Reagents: 1% ferric chloride in chloroform

Procedure: Take 1ml of the extract in the test tube and add 1 ml of the reagent and note the appearance of the color.

Test for Coumarins:
Reagents: 10% sodium hydroxide

Procedure: 3 ml of 10% sodium hydroxide was added to 2 ml of the extract. The formation of yellow colour confirms the presence of coumarins.

Test for Triterpenoids:
Reagents: conc. sulphuric acid, chloroform

Procedure: 5 ml of extract was added to 5 ml of chloroform with few drops of conc. sulphuric acid, shaken well and stand for some time. The formation of yellow color lower layer indicates the presence of triterpenoids.

Test for Proteins:
Reagents: conc. sulphuric acid and HNO_3

Procedure: To little extract 2 ml of water was added followed by 0.5 ml addition of nitric acid. The formation of yellow color indicates the presence of proteins.

Antibacterial Efficiency By Well Diffusion Method
Materials:
- Nutrient agar medium
- Bacterial culture
- Petri plates
- cotton swab

Procedure:
1. The NB agar medium was poured into petri plates.
2. Allow the medium to solidify.
3. The inoculum was mixed in saline.
4. The cotton swab was dipped in the saline and was streaked onto the plate.
5. 4 wells around 10 mm were cut out aseptically with the help of a cork borer.
6. The wells were filled with 100 µl of the synthesized nanoparticles.
7. The plates were incubated at 37°for 24 hrs.

***In Vitro* Cytotoxicity/anti Cancer Activity Of The Nanoparticles**
Materials:
- Vero cell lines
- HeLa cell lines
- Standard cancer drug - Doxorubicin
- DMEM medium
- MTT
- DMSO
- Trypsin
- Short 96 well assay plate
- Elisa reader

Procedure:
Short 96 well assay: Each condition should be done in triplicate or more

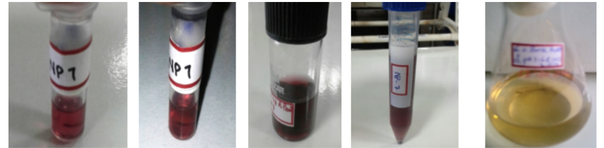
DAY ONE: Trypsinize 1 T-25 flask and add 5 ml of complete media to trypsinized cells. Centrifuge in a sterile 15ml centrifuge tube at 5000 rpm.
- Remove the media and resuspend the cells to 1.0 ml with complete media.
- Count and record the cells per ml.
- Remember to remove the cells aseptically while counting.
- Dilute the cells to 1,00,000 cells per ml. use complete media to dilute the cells.
- Add 100 µl of cells (10,000 total cells) into each well and incubate overnight.

DAY TWO: Treat the cells on day two with agonist, inhibitor or drug.
- If removing media, do it carefully.
- Final volume should be 100 µl per well.
- To this different concentration of gold nanoparticles were added to each well. All should be done aseptically.
- Incubate for 24 hrs at 37°C in the CO_2 incubator.

DAY THREE:
- Carefully remove the media. Do not disturb the cells and do not rinse with PBS.
- Add 10 µl of 5 mg/ml of MTT to each well. Include one set of wells with MTT but no cells.
- Incubate the cells for about 3 hours for the formation of formazan crystals.
- Add 100 µl of DMSO solvent to dissolve the formazan crystals.
- Incubate the plate for about 1 hour.
- Cover with tinfoil and agitate the cells on orbital shaker for 15 min.
- Read absorbance at 540 nm with a reference filter of 620 nm.
- Percentage of cell viability was calculated.
- Formula for cell inhibition = (1- O.D of treated cells/O.D of control) x 100
- Formula to check cell viability = (100 – cell inhibition)

RESULTS AND DISCUSSION
The leaves of the plant *Andrographis paniculata* used for the synthesis of nanocompounds.

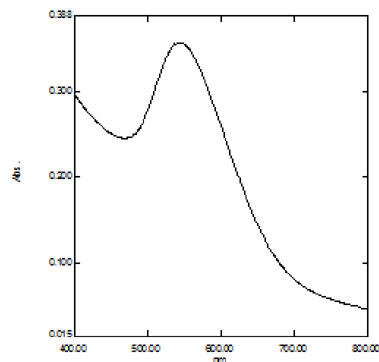
Andrographis paniculata ***A. paniculata leaves***



Synthesized gold Nanoparticles using Andrographis paniculata ***Stock Solution – Chloroauric acid***

The results of the nanoparticles synthesized were in varying colors depending upon the concentration of the precursor solution. It was ranging from deep wine red, pale pink to pink in color. Similar results were illustrated by Sumit.S.Lal and P.L.Nayak (2012).

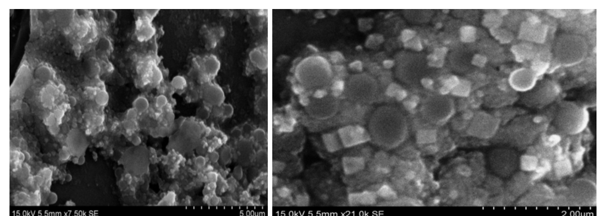
Characterization Of Nano particles Using Uv Visible Spectrophotometer



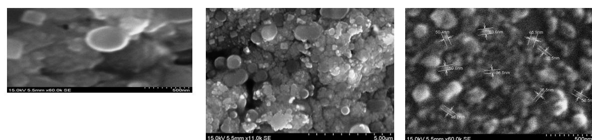
UV absorbance spectrum of gold nanparticles synthesized by A.paniculata (λmax -543 nm)

The UV absorption peak corresponds to the wavelength at which the optical absorption spectra of metal nanoparticles are dominated by surface plasmon resonances (SPR), which shifts to longer wavelengths with increasing particle size. The position and shape of plasmon absorption of gold nanoparticles are strongly dependent on the particle size, dielectric medium and surface-adsorbed species. The number of SPR peaks increases as the symmetry of the nanoparticles decreases. Thus, spherical nanoparticles, disks, and triangular nanoparticles of gold show one, two and more peaks respectively. Similar results have been reported by Sunil Pandey et al., (2012).

Sem And Edax Analysis



The SEM analysis was carried out and the corresponding images were obtained by Scanning Electron Microscope SEM along with EDAX (German model-S-3400, National centre for Nanoscience and Nano technology, NCNSNT, University of Madras, Guindy) The synthesized nanomaterials were Spherical, cubical nanoparticles in morphology, Homogenenous, evenly dispersed.

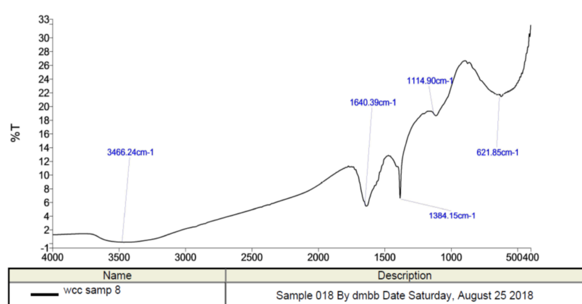


From the SEM analysis, it has been inferred that the average size of the nanoparticles are around 30 nm, which is a commendable nanodimension that has been so far achieved through a plant mediated green route of synthesis in comparison to the various studies reported by Annamalai .A et al., (2011), Sumit S Lal et al., (2012), Huang et al. (2007).

FT – IR ANALYSIS (Fourier-transform infrared spectroscopy)

The FR-IR spectra of organic compounds have two general areas, the Functional group region (4000-1500 cm-1) and the Finger print region (1500-.400 cm-1). The Peaks present in the Functional group region corresponds to the particular types of bonds

pertaining to the compound and hence can be used to identify the specific Functional groups of the compound in study. The Peaks present in the Fingerprint region are a result of complex deformations of the molecule. In particular, they may be a characteristic of molecular symmetry or combination of bonds deforming simultaneously.



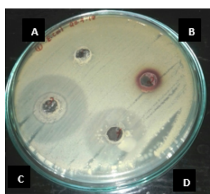
S.No.	Wave number (absorptions) (cm-1)	Functional groups
1.	621.85	=(-C-H) alkane
2.	1114.90	(C-O) alcohol, (C-O) Ester
3.	1384.15	(-C-H) alkane
4.	1640.39	(C=C) Alkene
5.	3466.24	(N-H) Amine, (O-H) alcohol

Similar results have been reported by **Jolly Mariam Johny *et al.*, (2011)**

Phytochemical Analysis Of The Green Extracts Of The Samples Used:

S.No.	Compounds	Results
1.	Coumarins	+ ++
2.	Saponins	+ ++
3.	Flavanoids	+ +
4.	Terpenoids	+ ++
5.	Triterpenoids	+ +
6.	Carbohydrates	+ ++
7.	Phenols	Greenish Black color
8.	Tannins	+++
9.	Alkaloids	+++

*+ = presence of the compound, the number of signs indicates the intensity of results seen



Zone of inhibition of the synthesized nanomaterials Vs Bacterial strain - Escherichia coli

A: NP Conc. 10-1 – No zone of inhibition

B: NP Conc. 10-2 - No zone of inhibition

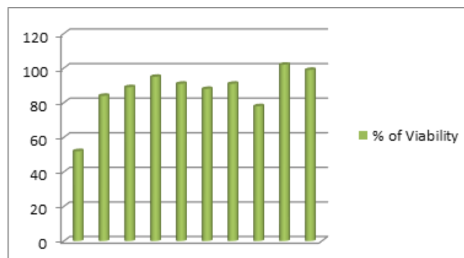
C: NP Conc. 10-3 - 35mm

D: NP Conc. 10-4 - 30mm

The results show that the gold nanoparticles possess good antimicrobial activity against the tested strain of bacteria, Escherichia coli and implies that these nanoparticles can be further exploited on their antibacterial efficacy against wide spectrum of microorganisms. It has been stated that silver has good antimicrobial activity by various studies including Sukdeb et al., (2007) and so it has been tested for gold nanoparticles.

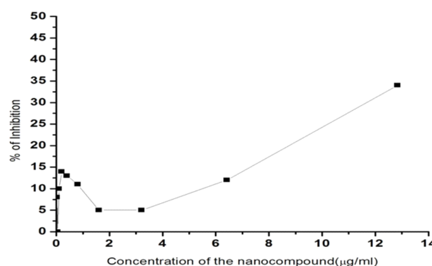
In Vitro Cytotoxicity Of The Nanoparticles

The results of the in vitro cytotoxicity through MTT assay was carried out in vero cell lines, shows that the Nanoparticles tested has higher cell viability at all the tested concentrations.



Anti Cancer Activity Of The Nanoparticles – In Hela Cell Lines Cervical Cancer

The results obtained from the studies of anticancer activity on the cervical cancer HeLa cell lines showed that the gold nanoparticles have good anti proliferative activity, in par with the standard drugs against cancer. The AuNP's proved to be efficient in most of the concentrations tested and the highest of the inhibition percentage is 34%, and as the concentration increased the anticancer efficacy as well increased.



Conclusion:

The research on Nanomaterials is of great interest because of its applications in numerous fields. In this study, the synthesis of gold nanomaterials using *Andrographis paniculata (nilavembu)* has been successfully carried out. On contrary to chemical synthesis, the green synthesis is inexpensive and eco safe. The initial characterization of the nanomaterials were performed using UV Visible Spectrophotometer and the structural elucidation from SEM analysis.

The functional group present was detected using FR-IR analysis. The various compounds of the plant extract were determined using phytochemical analysis. The anti microbial efficiency was tested against *E.coli* and anti cancer activity was done in HeLa cell lines which proved that the synthesized nanoparticles showed anti proliferative effects against cervical cancer. Further expanded research will help us to get deeper insights on the nanomaterials for *in vivo* applications in biomedicine.

Acknowledgments:

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