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Babar Vishal Bharat
Research Scholar, PG Studies, Pharmacy Department, PAHER University, Udaipur, Rajasthan, India

Dr. Karajgi Santoshkumar R
Associate Professor, Department of Quality, Assurance BLDEA's Shri SanganbasavaMahaswaniji, College of Pharmacy and Research Cente, Vijayapur, Rajasthan, India

Sudarshan Narayan Nagarale
Department of Pharmaceutical Chemistry, Dattakala College of Pharmacy, Swami-Chincholi, Daund, Pune, Maharashtra, India

Corresponding Author:
Babar Vishal Bharat
Research Scholar, PG Studies, Pharmacy Department, PAHER University, Udaipur, Rajasthan, India

Green synthesis for antimicrobial and anticancer assessments of isolated bioactive compound from Calycopteris floribunda leaves

Babar Vishal Bharat, Dr. Karajgi Santoshkumar R and Sudarshan Nagarale

Abstract
The present study aimed with "Green Synthesis for Antimicrobial and Anticancer Assessments of Isolated Bioactive Compound from *Calycopteris Floribunda* Leaves". The potential application of silver nanoparticles synthesized by green method is considered to be cost effective and less toxic and biocompatible one. Hence these nanoparticles exhibit a variety of therapeutic applications. On the basis of these objectives the *Calycopteris floribunda* Lam. plant was collected, identified and authenticated by botanist. The preliminary phytochemical investigation revealed that the ethyl acetate extract of leaves of *Calycopteris floribunda* Lam. was potent extract. Which was taken for further investigation to isolate, purifies, identify the bioactive compound i.e. Gallic acid and characterize confirmation done with analytical methods TLC, column, and HPLC. Synthesize and characterization of the nanoparticles was confirmed by FTIR, UV-VIS, and TEM. The screening of photosynthesized AgNPs selected nano formulations of leaves extract of *Calycopteris floribunda* for anticancer, antibacterial, antifungal, antioxidant and cytotoxicity. The isolated bioactive compound Gallic acid and its AgNPs was shows good effect against Neoplastic cell line (HT29 Adenocarcinoma) and *Pseudomonas fluorescens*, *Bacillus subtilis*, *Proteusmirabilis* and *Staphylococcus aureus* microorganisms. The synthesized nanoparticles were checked with different parameters (Concentration, pH, temperature and location) and stability. Stability check of bio-inspired AgNPs exploitation completely different incubation temperature was taken by exploitation UV-Visible spectrometry that revealed an honest stability at 25 ^{0}C.

Keywords: Green Synthesis, Nanoparticles, Antimicrobial, Anticancer Assessments, Calycopteris Floribunda Leaves.

Introduction
Now a day there's worldwide analysis on the utilization of Silver nanoparticles to fight against pathogenic microorganisms. In between varied metals, Silver has been acknowledged since times of years as active antimicrobial drug on therapy as interface. Nanotechnology is raising field of science that involves preparation and development of varied nanomaterials. Nanotechnology is a vital field of recent analysis handling coming up with, preparing and manipulation of structure of the particles starting from 1-100 nm. The prefix ''nano" in term of Nanotechnology is outlined from a Greek word "Nanos" which suggests two "dwarf" it refers to any designed matter that's one billionth (10-9 m) in size and expressed as milimicron (nm). Nanotechnology has currently started going the scope of laboratories; and gaining control new applications to vary our lives [1-3].

With the recent achievements within the area of Nanotechnology, Silver nanoparticles are wide utilized as a completely unique therapeutic drug extending their use as medication, antifungal, antiviral, medicine, and anti-cancerous drug. Useful preparation of Silver nanoparticles involves variety of physical and chemical ways as well as chemical reduction in liquid or non-aqueous liquid, template, small emulsion and microwave-assisted ways. Silver nanoparticles are more and more utilized in completely different applications. Due to their distinctive properties Silver nanoparticles are receiving lots of considers for potential utilization in medical specialty, technology, Therapeutic agent's discovery, Antimicrobial agents etc. [4-6].

The present study aimed with "Green Synthesis for Antimicrobial and Anticancer Assessments of Isolated Bioactive Compound from *Calycopteris Floribunda* Leaves". The potential application of silver nanoparticles synthesized by green method is considered to be cost effective and less toxic and biocompatible one by using isolated bioactive compound i.e. Gallic acid from potent ethyl acetate extract of leaves of *Calycopteris Floribunda*..

The biosynthesized nanoparticles were characterized by using UV-Visible Spectroscopy, FTIR, and TEM analysis to confirm their particle size, shape, distribution and stability.
The synthesized AgNPs were further evaluated for their antibacterial and anticancer activities. The antibacterial activity was performed using disc diffusion method against selected bacterial pathogens followed by studies on the synergistic effect with standard Gallic acid. Evaluation of anticancer activity was carried out by HT 29 Adenocarcinoma cell line by MTT assay.

Material and Methods

Plant Sample Collection

The herbal plant *Calycopteris floribunda* Lam. family: *Combretaceae* was collected from the local areas of Baramati, Dist: Pune, State-Maharashtra. Plant parts means leaves of *Calycopteris floribunda* plant were identified and authenticated by botanist [7-10]. For further procedure extraction and fractionation of the leaves of plant by using different solvents hexane, chloroform, ethyl acetate, methanol and aqueous. The Pharmacognostical, Physico-chemical, and Identification of potent extract analysis of leaves extract of plant was done and confirmed by macroscopic, microscopic, phytochemical observations were created utilizing microscope, various preliminary phytochemical tests and UV-Visible spectroscopy [11-13].

Isolation and Characterization of Bioactive Compound

Extraction of the bioactive molecule from the ethyl acetate potent leaves extract of plant by using Kupchan-cold extraction method. Potent Ethyl acetate layer dehydrated with anhydrous sodium sulfate and evaporated below low pressure on rotary evaporator to get leftover and isolation done by Thin Layer Chromatography method. The end batch was characterized, structural description was done out and confirmed as Gallic acid by UV-Visible photometer, Red spectrometry KBr press pellet method [13-16].

Biosynthesis of Silver Nanoparticles (AgNPs)

The purely isolated Gallic acid was used to synthesis the AgNPs by extracellular biosynthesis.One ml molar solution of AgNO3 (AgNO3) was ready and used for the preparation of silver nanoparticles. Five ml of extract from every sample was additional with 100ml of AgNO3 liquid. The amendment of color from pale inexperienced to highly dark brown was checked of times. They were kept in incubator for incubation at temperature for twenty-four hours. The amendment of color indicated the synthesis of silver nanoparticles [17].

Characterization of AgNPs

The colour of the solution becomes more darken and cloudy after 72 h of incubation on the rotary shaker 160 rpm at 25 °C indicated the formation of silver nanoparticles. These AgNPs were further investigated by using UV–Visible Spectrophotometer, and analysis was carried out from the wave length 300–600 nm to check the maximum absorbance (λmax). FTIR analysis was used to reveal the proteins, and functional group contained in the AgNPs which responsible for the stability of the nanoparticles and FTIR powder form of the sample was mixed with potassium bromide and observed the spectra by FTIR spectroscopy. The particle size, shape and electrostatic charge of the AgNPs are characterized by using TEM analysis. For TEM the nanoparticles solution was diluted, and one drop of diluted nanoparticles was put on the carbon-coated grid and subjected to TEM analysis [18-20].

Antimicrobial Activity of AgNPs

The eco-friendly synthesized silver nanoparticles were evaluated for its antibacterial properties against *Staphylococcus aureus, Pseudomonas fluorescens, Aspergillus niger, and Aspergillus flavus*by disc diffusion method. From the chosen tube (showing MIC i.e., 62.5 and 125µg/ml) was taken and Sterile paper discs (6 millimeters in diameter) were inseminated with their concentration 62.5µg/ml, 125µg/ml every of the EA leaves extract, refined compound (Gallic acid), silver nanoparticles and management (EA & AgNO3) were used and left to dry to get rid of the residual solvent, which could interfere with the determination. The extract discs were then kept on the Luria and potato grape sugar agar plates and incubated at 37 ^{0}C for twenty-four hours for bacterium forty-eight hours for fungi and examined for microorganism and plant growth. The MBC and MFC, all-time low concentration of the plant extract giving 99.9% reduction of the microorganism growth of varied plants components against the microorganism pathogens, was reported. The zone of inhibition was calculated [18].

Anticancer Activity of AgNPs

Neoplastic cell line (HT29 Adenocarcinoma) was maintained in MEM in carbonic acid gas incubators at 37 ^{0}C in a very cleaned and sterile flask. From this the cancer cells were detached by reacting with 0.25% enzyme in sterile saline. Then the cells were gentile washed with sterile phosphate solution a minimum of thrice and also the cell density was adjusted to a hundred and five cells/ ml by creating use of a white blood cell investigating chamber, letter was wont to alter the cell density by adding needed volume. The cells were transferred to a ninety-six well microtiter tissue culture plate, in triplicate. The EA leaves extract, refined compound (Gallic acid), silver nanoparticles were conjointly transferred to induce the ultimate concentration of ten, twenty and thirty µg/ml in every well. The expansion management and drug management with periwinkle plant derivative and Cisplatin (50µl/ were conjointly founded. The plate was unbroken in carbonic acid gas apparatus for seventy-two unit of time (72min). Once the treatment of cells with the taken a look at compounds, the cell monolayer is washed with the nice and cozy PBS exploitation micropipette. 100µl of culture containing MTT assay was additional to every well and incubated for any four unit of time (4min) [21].

Stability of AgNPs at Different Incubation Temperature

Stability check of biosynthesized silver nanoparticles exploitation completely different (4, 25, 35, 45 ^{0}C) temperature incubation was done. One ml of leaves extract was mixed with hundred ml of AgNO3, then the amendment of color was determined and monitored by ultraviolet illumination Visible spectrometry ranges from 200-800nm [22].

Result and Discussion

The synthesis of silver nanoparticles was first confirmed by the amendment of color of the liquid.

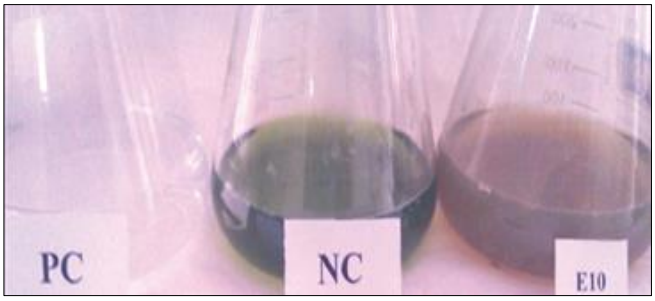

Fig 1: Biosynthesis of silver nanoparticles.

The transformation of color from inexperienced to dark brown color of the EA leaves extract of *Calycopteris floribunda* was because of the excitation of the SPR and SPR band each of that play a crucial role within the confirmation of silver nanoparticles synthesis "Fig. 1". It conjointly showed that the reduction of silver particle occurred by the EA leaves extract of *Calycopteris floribunda*.

UV- Visible spectral data obtained from the catalytically reaction of reduction of silver ions that poly dispersed nanoparticles has given broadening peak within the absorbance band at the wavelength of 424 nm "Fig. 2".

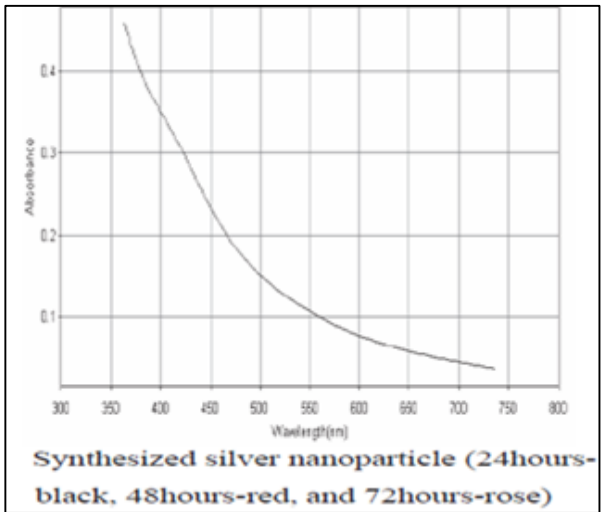

Fig 2: Synthesized silver nanoparticles under UV-VIS spectroscopy.

Straight directed analysis of variance comparison the Ultraviolet-Visible spectroscopic readings, discovered the variations in Surface Plasmon Resonance for 24, 48 and 72 hours.

FTIR analysis discovered the purposeful team's given within the silver nanoparticles synthesized exploitation the leaves extract of *Calycopteris floribunda*. Moreover, capping was proven by FTIR scaling of the identical sample and refined conductor nanoparticles "Fig. 3".

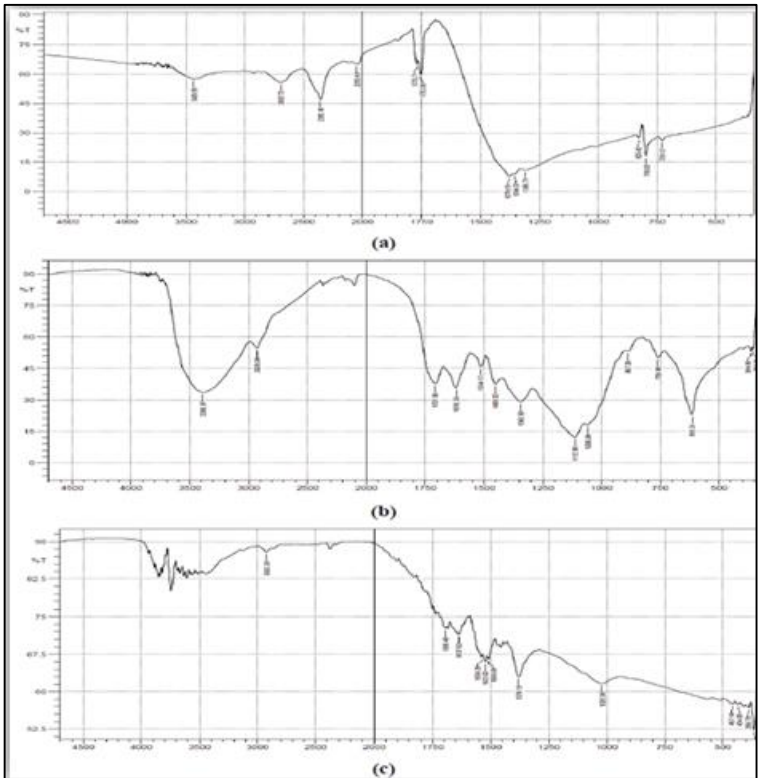

Fig 3: Fourier Transform Infra-Red (FTIR) spectroscopy Analysis

FTIR analysis reports infrared widths at 1022.59 cm-1, 1510.57 cm-1, 1527.63 cm-1, 1541.37 cm-1, 1648.71 cm-1, and 1694.98 cm-1. The width at 1022.59 cm-1 distanced to -C-O- bonding; the height at 1508.38 cm-1 distanced to aromatic paraffin –C-N- bonding. The bandwidths came at 1527.63 cm-1 and 1541.37 cm-1, were because of Carboxyl Ate particle –COO- and -C-O- bonding severally. The bandwidths came at 1648.71 cm-1 and 1694.98 cm-1 may well be assigned to Carboxyl Ate particle -COO- and -C=C-, -C=O-stretching severally.

Morphology and particle size of AgNPs were characterized exploitation TEM. Typical TEM micrographs at completely different ranges (5, 10, 20, 50nm) were given in "Fig. 4" that the silver nanoparticles produced were dispersed well with no agglomeration.

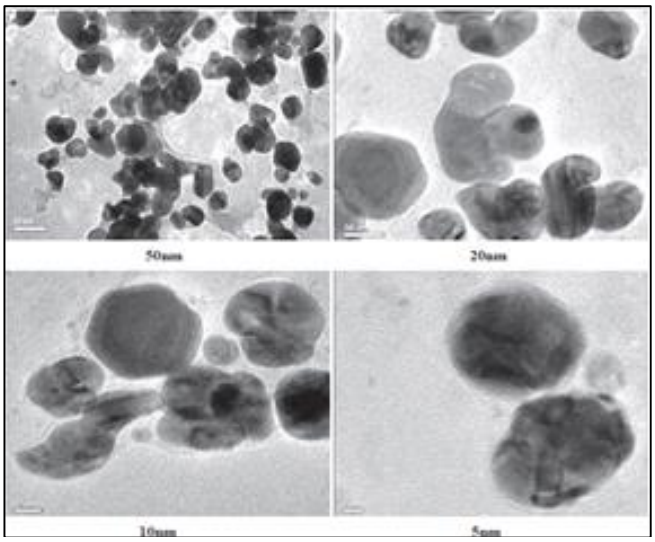

Fig 4: TEM image of Bioinspired AgNPs

It had been clear from the TEM pictures the formation of biosynthesized nanoparticles of pentagons, spherical and triangular formed nanoparticles with size 5.5 to 15.3 nanometre.

Table 1: Comparative antimicrobial activity of ea leaves extract, gallic acid (partially pure), biosynthesized silver nanoparticles of*calycopteris floribunda*

Microbes	ZI in mm at Concentration in µg/ml					
	EA leaves extract		Gallic acid pure		Silver Nanoparticles	
	62.5	125	62.5	125	62.5	125
Staphylococcus aureus	10±1.6	13±1.1	18±1.4	21±0.8	28±1.4	36±0.9
Pseudomonas fluorescence	12±1.6	14±1.8	15±1.1	20±1.1	27±0.9	26±1.3
Aspergillus niger	06±1.7	08±0.7	09±1.1	11±0.9	13±1.4	14±1.1
Aspergillus flavus	07±1.3	10±1.5	16±1.5	17±0.8	18±1.3	20±0.8

AgNPs revealed most antibacterial acting drug activity is best against *Staphylococcus aurous* with 28mm, 36mm diameter of area of inactivation at 62.5µg/ml and 125µg/ml concentrations than bacteria genus *Pseudomonas fluorescens*. With fungus genus *Aspergillus flavus* it revealed 20mm diameter at 125µg/ml area of inactivation than *Aspergillus* niger "TABLE. I". The higher than results it may well be terminated that AgNPs had bigger antibacterial drug activity and antifungal action than EA leaves extract, acid refined Gallic acid compound "Fig. 5".

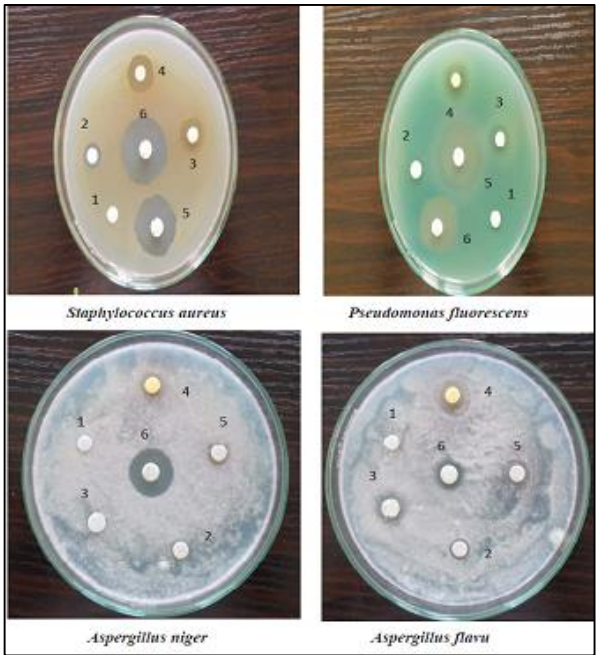

Fig 5: Antimicrobial activity

Malignant neoplasm activity exploitation neoplastic cell lines (HT twenty-nine Adenocarcinoma) were analyzed by MTT Assay methodology "Fig. 6".

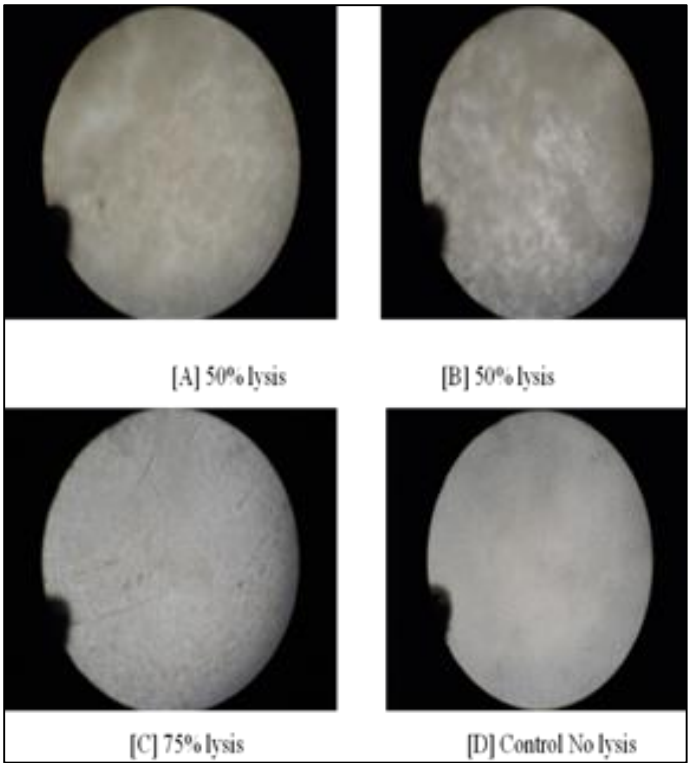

Fig 6: Anticancer activity

The 20µg/ml and 30µg/ml AgNPs revealed 50% cell deaths and 75% cell deaths indicating malignant neoplasm activity and these 2 concentrations showed that it had been accountable in complete killing of neoplastic cell lines (HT twenty-nine Adenocarcinoma) cell. AgNPs revealed that good malignant neoplasm effect than refined compound and EA leaves extract.

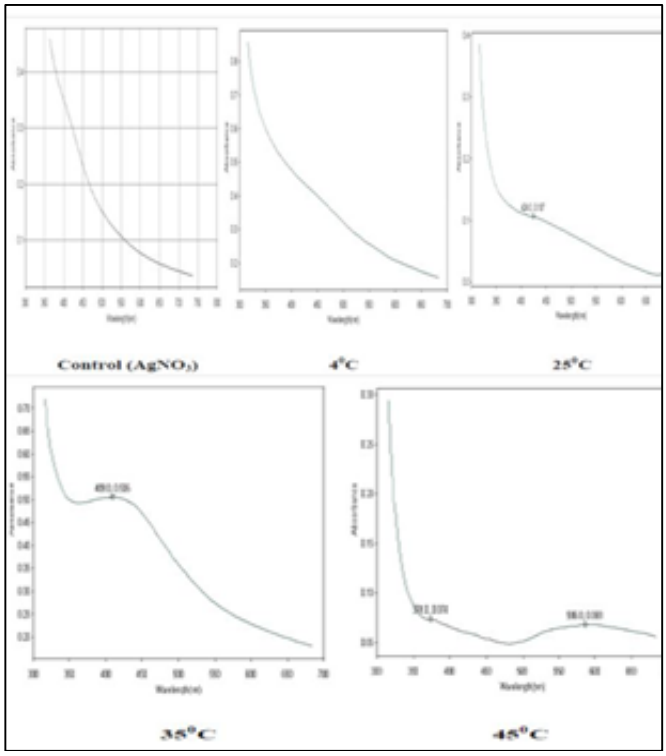

Fig 7: Stability of Bioinspired AgNPs by UV-Visible spectroscopy

Stability evaluation of biosynthesized silver nanoparticles exploitation completely different incubation temperature was done by exploitation UV-Visible spectrometry that reported an honest stability at 25^0C "Fig. 7".

Conclusion

Silver nanoparticles biosynthesized from leaves extract of *Calycopteris floribunda* showed good antimicrobial activity and anticancer activity against the neoplastic cell line (HT29 Adenocarcinoma). Hence these silver nanoparticles could become good anticancer and antimicrobial agent but needs further cytotoxic and animal model before brought into the market.

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