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Research article

Physiochemical characterization of silver nanoparticles using rhizome extract of Alpinia galanga and its antimicrobial activity

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ABSTRACT

Nanoparticles feature one-of-a-kind, size-dependent characteristics, making them useful in a variety of fields. Nanoparticles are particularly fascinating for medicine and pharmacology because of their capacity to regulate their characteristics. The design of particular nanostructures that can be utilized as innovative diagnostic and therapeutic modalities is connected with the usage of nanoparticles in medicine. High biocompatibility, durability, and the potential of large-scale manufacturing without organic solvents are all benefits of noble metal-based nanoparticles, which are significant for medical applications. PXRD, UV-Vis, FESEM, and EDX, as well as FTIR and DLS, were used to describe with silver nanoparticles attached to Alpinia galanga rhizome extract. The extract was evaluated against some pathogens like E. coli, Pseudomonas's., Staphylococcus's. and Candida species, the enormously stable Alpinia galanga silver nanoparticles extract were shown to be promising as antibacterial and antifungal agents. The antibacterial and antifungal characteristics of AgNPs produced from Alpinia galanga rhizome extract function as a significant therapeutic medication for microbial infections illness and other health-related diseases.

Keywords: Silver, Nanoparticle, Alpinia, Physiochemical, Antimicrobial

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INTRODUCTION

In our traditional medicine systems, India has a rich heritage of flora and knowledge of plants used as medicine, which have acted as a major source of medicines for centuries, used for both preventive and curative purposes. More than 88% of world's population depend on the use of plant-based drug as their primary defence for preserving their health and contending many ailments. [1,2] However, the choice as required for exploring the new and active entity from the natural resources, arise due to catastrophe in the therapy done by various synthetic or semisynthetic chemicals which actually developed some drug resistant microorganisms. Owing to their possible effectiveness and no side effects, the drugs extracted from medicinal plants expanded tremendous popularity in now a day. Plants produce secondary metabolites such as various terpene, terpenoid, alkaloid, tannin, flavonoid, coumarins polysaccharide, glycoside, gum and phenolics, are used by plants themselves providing a defence mechanism against invasion by microbes, and various small pests [3,4]. In contrast to modern synthetic drugs or medicines obtained from the plants are thought to be more appropriate to the human figure [5]. Therefore, a material property with functional flexibility is available. Because of their need to obtain full value from medicinal plants, as well as from the conventional system of medicinal products for the provision of adequate health services to rural people. The extraction of medicinal plants demonstrates very potent antimicrobial activities. By a mixture of modern scientific inputs, e.g., synthesising nanoparticles, these activities can be expanded by several folds. Like pharmacy, pharmacology, sensing instruments, microelectronics, delivery of medicines, etc.[6,7]The most vigorous and captivating nanomaterials involved in a variety of biomedical applications are known to be silver nanoparticles (AgNPs) and have been reported to possess antimicrobial, antiangiogenics and anti-inflammatory activity[8], which can be processed by various physical routes, photo-chemical routes and chemical routes which may possibly harm to the atmosphere and human race too[9,10].Nanoparticles, including fullerene in which various mineral nanoparticles which consist magnetic and new metal nanoparticles viz. gold and silver and semi-conductor nanoparticles may be narrowly grouped as organic nanoparticles (e.g. titanium-oxide and zinc-oxide).Gold nanoparticles

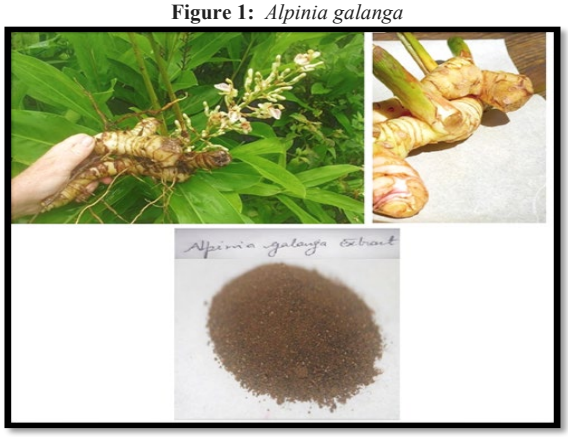
and silver nanoparticles are of growing attention because, in addition to size characteristics, these particles have been used as probable gadgets for medical imaging and for the treatment of diseases due to imaging drug agents[11]. In recent years, due to its simplicity, low cost, protection, and eco-friendly design, eco-friendly synthesis of nanoparticles using different parts/components of the plant is getting important consideration. The literature has recorded a number of plant extract-mediated eco-green synthesis of AgNPs [12].However, the role of medicinal plant extracts exerts both reducing and stabilizing mediators in synthesis of nanoparticles. In present study synthesis of AgNPs using a rhizome extract of Alpinia galanga were carried out. Alpinia galanga, belongs to family Zingiberaceae, which rhizome part is used largely as a drug in Arabic system of medicine as well as spice also. This is commonly known as "galangal", and is differentiated from the others with the common names Rasna, greater galangal, and blue ginger. The plant has a high medicinal value and has been commonly used in humans to treat a number of microbial diseases[13].This analysis illustrates existing knowledge on the different pharmacological properties of phytochemistry and, in particular, of Alpinia galanga, which may provide an opportunity for a clinical agent to test the plant.

MATERIALS AND METHODS

Plant collection and extract preparation

Alpinia galanga plant were collected and identified from Medicinal plant garden, Rishikesh, Uttarakhand. The rhizomes were isolated from the plant and was dried in hot air oven under controlled temperature 45-50^{0}C for several days. The dried mass was than size reduced into coarse powder with help of mechanical grinder and was taken for maceration using distilled water for 7 days. The whole content was filtered and filtrate was than concentrated to dryness using electronic water bath and then stored in tight closed container at room temperature.

Figure 1: *Alpinia galanga*



Preparation of drug-silver nanoparticles

5 ml of Alpinia galanga rhizome extract was taken and mixed with 120 miliL of 1Mm silver nitrate solution. A colour change in solution was observed from dark brown to pale yellow which indicate formation of silver nanoparticles. These nanoparticles were taken for centrifugation at 12,000 rpm (Remi 12c). These nanoparticles are then collected, dried, and washed twice with alcohol to remove impurities. Then these were kept for drying in oven which after drying taken for characterization.

Characterization study of AgNPs.

The given methods were applied to analyse the synthesized silver nanoparticles.

UV-Vis Spectra Analysis

The respective metal ion solution, the extract of the rhizomes, is easily identified by Ultra Violet -Visible Spectroscopy, a collection of silver nanoparticles presented decreasing. The absorption was measured using a Perkin-Elmer Lamda-45 Spectrophotometer in the limits of 200-400nm wavelength for the spectral extraction of the rhizomes and the metal concentration.

F.T.I.R. Analysis

To classify the functional group, Fourier transform infrared spectroscopy was employed. This research is very important for the identification of the molecular structure and the search for the possible number of biomolecules present in the extract of leaves responsible for the reduction of silver ions and the functional group of material sample synthesis.

D.L.S. Analysis

Dynamic light scattering was analysed to know the size distribution and average size of the synthesized Ag-nanoparticles.

P.X.R.D. Analysis

By the help of x-ray diffraction methodology, the characteristics and size of the manufactured silver nanoparticles were determined.

F.E.S.E.M. (Field emission scanning electron microscopy) Analysis

The structural features of Ag-nanoparticles which was synthesized from the Alpinia galanga rhizomes extract were rapidly calculated with a voltage of 22-25Kv.

EDAX Analysis

Energy dispersive X-ray analysis (EDX) was used for the determination of the composition by BRUKER instrument.

Antimicrobial activity

Silver nanoparticles of Alpinia galanga was used to investigate the antimicrobial potential using various microbial strains. The bacterial strains used for study were ATCC (American Type Culture Collection) and were purchased from Hi-B Chemicals, Vadodara Gujrat. The strains were Staphylococcus aureus (ATCC 2596TM), Escherichia coli (ATCC 25922TM) &Pseudomonas aeruginosa (ATCC 15442TM). The all above strains was maintained on nutrient agar media (Difco Ltd., USA) for 5^{0}C and were cultured at 37^{o}C. From National Chemical Laboratory, Pune, Maharashtra, fungal strains MDCC (Microbial Type Culture Collection) were purchased. The strains were Candida albicans (MDCC-3498) &

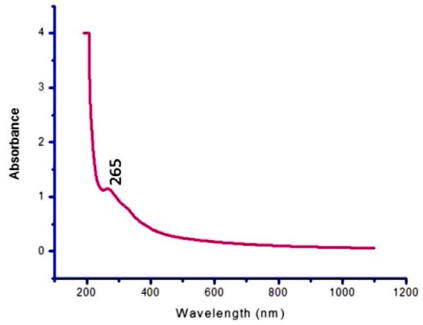
Candida vulgaris (MDCC-227). The fungal strains were maintained on Sabourrod's Dextrose Agar/broth Hi media Pvt Ltd, Mumbai, India.

RESULT AND DISCUSSION

Ultra-violet & visible spectrum analysis

Ultra-violet visible spectrum analysis was used to confirm the presence of silver nitrate. Because of the integrated trembling of electrons in silver nanoparticles with resonance to the light's wave, a specific surface plasmon resonance absorption band was produced by Ag-NPs. The spectra for AgNPs were obtained using surface plasmon resonance at 265 nm (Figure 2).

Figure 2: UV-Visible spectrum of silver-NPs using Alpinia galanga extract

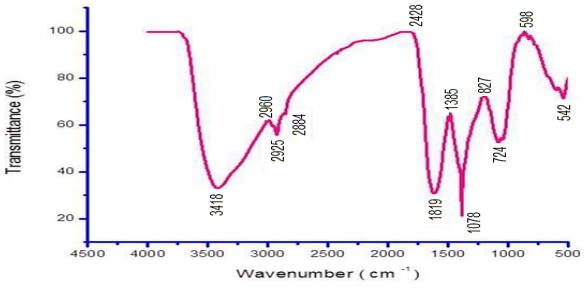


A rise that happens quickly with an improvement in reaction time, a group of nanoparticles was observed [14]. The concentration of the juice plays a role in the synthesis of symmetric nanoparticles, but the key role is responsible for it. By reducing metal ions with chemical molecules, metal nanoparticles can be formed and natural material extracts are thought to serve as agents in biosynthesis to minimize the production of metal nanoparticles.

FT-IR Analysis

FT-IR analysers are utilized to detect the presence of a variety of functional elements or groups in the molecules of AgNPs. For detection of these groups the experiential bands were compared with standard limits.

Figure 3: FT-IR spectrum of silver-NPs using Alpinia galanga extract

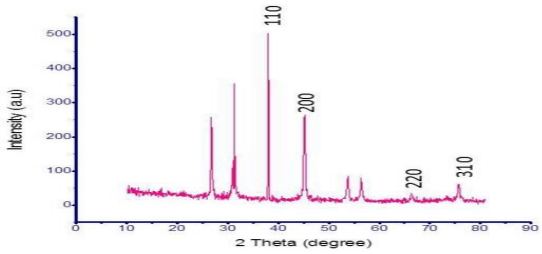


As determined that Silver-NPs shows the spectra at wave-lengths of around 3418, 2960, 2925, 2884, 2428, 1819, 1325, 1078, 827, 724, 598 and 542 cm-1 by reduction of rhizome extract of Alpinia galanga. Vibrations from the -OH groups were determined at the wavelength of 3418 cm^{-1} due to presence of saponin structure [15]. The small band at 2960 cm^{-1} was due to the extension of the -C-H alkane. A strong peak of the set amide-I group at 2925 cm^{-1} shows bending vibration. The absorption peak located at 2884 cm^{-1} is of -NH curve. The presence of bands at 2429 & 1820 cm^{-1} is associated with stretching of -CH bond. The band at 1325 and 1078 cm^{-1} caused the C-O stretching vibrations of the -COOH group. A peak of about 827 and 724 cm^{-1} shown by any aromatic class compound. The small peak observed at 598 cm^{-} possess C-O or C-N stretching may be of the either esters or amines groups found in the rhizome's extracts. C-O groups and C-C groups shows Stress oscillations at 540 cm^{-1}. However, the mechanism of formation of silver nanoparticles by FTIR analysis could be due to the silver ions reductions related to oxidation of polyphenols, due to which some bio elements bind these specific functional groups to metal salts, simultaneously interfere in reduction of their nanoparticles [16].

P-XRD Analysis

Powder Modelling in P-XRD Method of Silver NPs used for the studied plant structure which displays peaks at 39, 45, 65 and 78. These 2θ values represent 110, 200, 220 and 310 features of silver, respectively confirmed the face centred cubic crystalline (fcc) structure of metallic silver (Figure 4)[17].

Figure 4: PXRD pattern of silver-NPs using Alpinia galanga extract

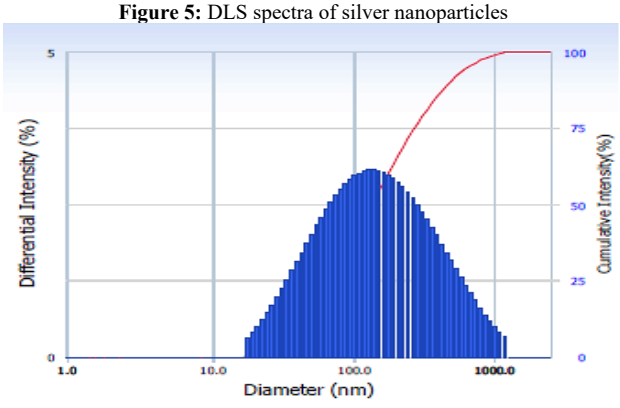


Two extra peaks with edged strength were observed due to the presence of silver, which may indicate that proteins are coated with silver nanoparticles [18].

Dynamic Light Scattering DLS Analysis

The particle size of the integrated nanoparticles was determined using the DLS calibration technique, which uses random variations in the strength of scattered light from a solution to determine particle size. The DLS diagram of the Alpinia galanga extract showed that the particle size of the AgNPs ranged from 10nm-100 nm as shown in Figure 5.

Figure 5: DLS spectra of silver nanoparticles



Field Emission Scanning Electron Micrograms (FESEM) Investigation

Figure 6: FESEM view of synthesized silver nanoparticles

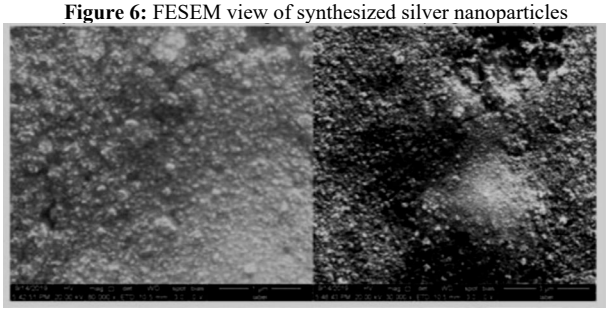
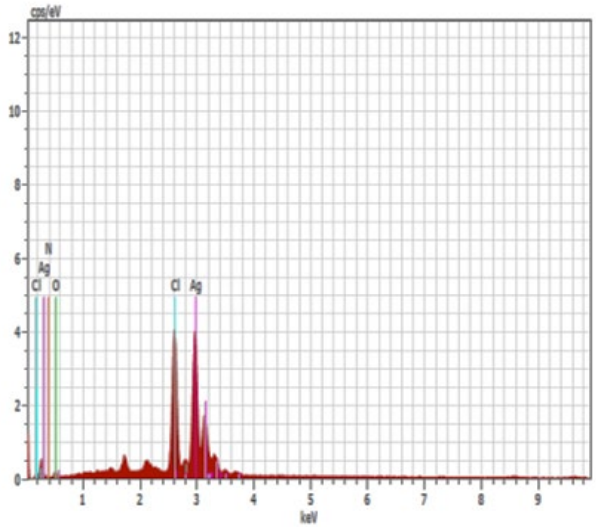


Figure 6. shows FESEM view of the synthesized Ag-nanoparticles. This investigation clearly shows that silver nanoparticles are spherical in shape and sprinkled well and non-aggregated. Larger size nanoparticles form a chain-like structure with synchronized connections [19]. The image's higher magnification reveals the nanoparticles' upper outer layer, which may be attributable to the phytochemicals and alkaloids in Alpinia galanga coating the nanoparticles and acting as a capping agent. Around the aggregation of nanoparticles, there is a decrease in the number of capping agents. Figure 6 shows the size range of nanoparticles in the 30-45 nm.

EDX Spectroscopy Analysis

Photosensitized silver NPs were mounted onto brass sample specimens instead of carbon to better understand their underlying structure [20].The sample included simple silver, carbon, oxygen, chlorine, and nitrogen, according to an EDX study. The sample-made brass includes elements such as chlorine and nitrogen. SNPs, carbon, and oxygen can be assigned to biomass compounds in the shell when the silver element is photosensitized. Interpolation is used to calculate the basic specifics of the composition and their atomic percentages. According to the EDX review, a peak ensures the formation of silver nanoparticles (Figure 7).

Figure 7: EDX spectrum of silver-NPs using Alpinia galanga extract

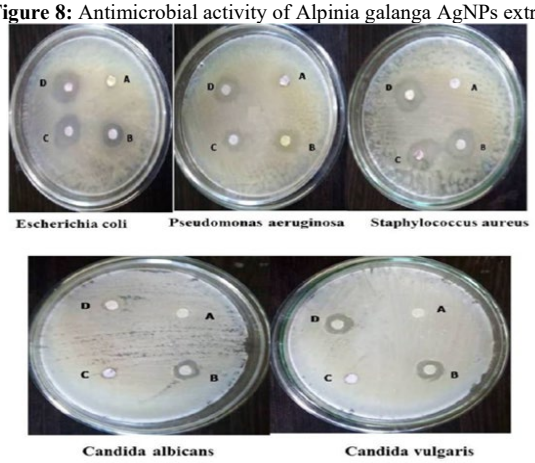


The optical absorption peak, which is typical for absorbing metallic silver nano crystallites, is about 3 keV.

Antimicrobial activity

For studying antimicrobial activity (Table 1) disk diffusion method was used.[21] Sample petri-plates were prepared with Muller Hinton's agar media and cell suspension of microorganism were spread in it. Sterile disk of 10 microliter sample were procured and disk of all concentration were prepared. Positive control was prepared using 10 µl of amoxicillin fixed antibiotic disc.

Figure 8: Antimicrobial activity of Alpinia galanga AgNPs extract



The model showed growth inhibitory activity against Escherichia coli (7 mm). All bacteria in the C sample exhibited antibacterial activity, but it was more susceptible against Pseudomonas aeruginosa (4 mm) and Staphylococcus aureus (5 mm). However, crude extract and synthesized nanoparticles showed excellent preventive action against pathogens.

Table 1: Antibacterial study of Alpinia galanga AgNPs extract

Sample	Conc. (µl/ml)	Zone of inhibition in mm		
		E. coli	P. aeruginosa	S. aureus
Amoxicillin	10	0	0	0
Zinc oxide	10	7.5	7	7.5
Plant extract	10	5	5	4
Nanoparticles	10	8	5	5

Mildew activity was observed in antifungal activity using disk diffusion method. Sample petri-plates were prepared with Sabouratin Dextrose agar media and microorganism were inoculated in it. 10 µl of 6 mm wide sterile disk was inserted into various samples.10 µl disc of fluconazole is used as standard antifungal disc.

Table 2: Antifungal study of Alpinia galanga AgNPs extract

Sample	Conc. (µl/ml)	Zone of inhibition in mm	
		C. albicans	C. vulgaris
Fluconazole	10	0	0
Zinc oxide	10	1	1.5
Plant extract	10	2.5	2.5
Nanoparticles	10	2	3.5

shows the results of the fungicide susceptibility test against different samples and test organisms. As a result, the sample no. D found most effective and show well defined activity against Candida albicans by creating a zone of 2mm.

CONCLUSION

Silver nanoparticles were biodegraded by using the extract of Alpinia galanga plant. Biological processes, as opposed to chemicals and physical systems, often provide a better solution because they are more environmentally sustainable and cost effective

[22]. By using a biochemical approach, it was able to produce the most nanoparticles in 48 hours. At pH 6.5 and 1 mM AgNO3, the Alpinia galanga plant produces the most Ag-NPs. The reduction of Ag+ ions at 265nm were verified by UV-Visible spectra. FESEM revealed that the average size of Ag NPs was 30–45 nm, and PXRD peaks revealed that the nanoparticles' structure was FCC in nature. The presence of silver nanoparticles with nitrate reductase enzymes on their surface was discovered using the FTIR process. The existence of silver nanoparticles was verified by EDAX results. The existence of an increase in the nanoparticles' stability was demonstrated in the zone of inhibition experiments.

DECLARATION

Funding None

Conflict of Interest None

Ethical approval None

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