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Research Article

Microwave-mediated green synthesis of silver nanoparticles using glycosmis pentaphylla leaf extract and exploration of their antimicrobial properties

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Abstract

Over the past few decades, nanotechnology emerged as one of the most significant area of research in material science. Due to the remarkable physical, chemical, optical, and biological features, noble metal nanoparticles like gold, silver, palladium, and platinum are broadly used in various fields. In this study, a quick microwave-assisted, inexpensive, and environmentally friendly method was used to synthesize silver nanoparticles (AgNPs). Without using any harmful chemical substances, AgNPs were generated using Glycosmis pentaphylla leaf extract as a reducing and stabilizing agent. UV-visible spectrophotometry, X-ray diffraction, and Scanning electron microscopy were used for the evaluation of successful synthesis. The presence of AgNPs confirmed by the appearance of peak at 417 nm utilizing UV-Vis method and also established by X-ray diffraction patterns. The synthesised AgNPs were also examined for antimicrobial activity using the agar well diffusion method and found that synthesized AgNPs efficiently inhibited the growth of various gram-positive and gram-negative bacteria.

Keywords: **Green synthesis, microwave, silver nanoparticles, *Glycosmis pentaphylla.***

1. Introduction

Nanotechnology refers to the process of synthesizing particles in the nano range, 1-100 nm. It displays large surface area to volume ratio hence they show excellent properties like optical, physical, chemical, electronic etc. Exhaustive research is being done on silver nanoparticles due to their extensive range of applications in therapeutic field 1, pharmaceutical industry [2], water purification [3], food industry [4], catalysis [5], sensors [6], etc.

There are diverse techniques are used for the preparation of metal nanoparticles. Some of them are chemical reduction, photochemical reduction, microemulsions, hybrid methods and sonochemical method, radiation method, etc [7, 8]. Chemical reduction is the most commonly used method in which as reducing agents like hydrazine [9]. sodium borohydride [10], trisodium citrate [11], and ascorbate [12] are used. However, most of these physiochemical methods are time consuming and are significantly limited in extensive application owing to the presence of injurious chemicals, high expenditure, excessive energy and time consuming, and

complication in waste refinement [13]. Hence, there is an increasing requirement to use low-cost, clean and environmentally friendly methods that utilize harmless chemicals in the synthesis of nanoparticles. The green synthesis of metal nanoparticles by means of numerous microorganisms, plants, and algae are reported [14-16].

Amongst the noble metal nanoparticles, the silver nanoparticles have been generally studied because of its lower cost and noxious nature than Pt, Au, Pd [17]. In this study we have used *Glycosmis pentaphylla* leaf extract as reducing and stabilizing agent for the formation of silver nanoparticles. It is a species of flowering plant belongs to the family *Rutaceae*, and is commonly known as Bannimbu, pannal orange berry and gin berry. The plant is used in native medicine for the treatment of fever, liver disorders, cough, reheumatism, and anaemia and also exhibits anti-cancer, anti-arthritic, anti-inflammatory, anti-oxidant and antibacterial activities [18, 19]. The synthesized nanoparticles were analysed by using UV-Visible spectroscopy, XRD and SEM analysis. The antimicrobial properties of synthesized silver nanoparticles were tested against both Gram positive and Gram negative bacteria by Agar well diffusion method.

2. Experimental part

2.1 Characterization

UV-Vis spectra were recorded in the range of 200–800 nm by using Shimadzu UV-vis. Spectrometer. For XRD analysis, PANalytic X'PERT-PERO X-ray spectrometer was used. Morphological analysis was done by the help of Scanning Electron Microscopy (SEM), JEOL (model-JSM-6390) after sputtering with platinum.

Preparation of *Glycosmis pentaphylla* leaf extract

The *Glycosmis pentaphylla* leaves were collected and taxonomically identified. Then the leaves are washed several times with distilled water and. The 10 g of leaves was chopped into small parts and transferred into a round bottom flask equipped with condenser. Then 100 ml distilled water was added and boiled for 30 minutes. It was then cooled, strained and stocked in a refrigerator for further studies.

Synthesis of Silver nanoparticles

For the preparation of silver nanoparticles 1 molar silver nitrate solution was used. Firstly, 24 ml silver nitrate solution was taken in a 100 ml beaker and is then mixed well with 6 ml *Glycosmis pentaphylla* leaf extract. Then this mixture subjected to microwave irradiation by placing it in a domestic microwave oven of operating power 800 W and frequency 2450 MHz. The solution was then irradiated with microwave radiation for one minute.

Antimicrobial Study

Agar well diffusion method was used for the analysis antimicrobial properties synthesized silver nanoparticles. Here, the clinical isolates of human pathogenic bacterial strains of bacterium separated from contaminated food were used. Firstly, the bacterial strains

were sub cultured in liquid nutrient agar and fungal strains were sub cultured in potato dextrose agar (PDA). Then Mueller Hinton agar (MHA) medium was prepared and after its solidification, using sterile cotton swabs, the culture of test bacteria was homogeneously spread over its surface. The wells with 8 mm diameter were produced on the MHA plates with sterile gel puncture. Then using a micropipette, solution of *Glycosmis pentaphylla* leaf extract, silver nitrate and AgNp-*Glycosmis pentaphylla* were poured in to the wells. All the MHA plates were incubated for 24 hours at 37 ˚C and the zone of inhibition formed around the wells was measured in mm.

3. Results and Discussions

The formation of silver nanoparticles was confirmed by using UV-Visible spectrometric analysis. Here the nanoparticles were formed by the reduction of Ag^{+} ions into Ag^{0}. The photograph of *Glycosmis pentaphylla* plant and synthesized silver nanoparticles are shown in Fig. 1. Initially the leaf extract does not show any typical absorption peak in UV- Visible spectra and also there was any appreciable changes were not observed after the addition of *Glycosmis pentaphylla* extract to silver nitrate solution. But upon microwave irradiation, the color of the reaction mixture slowly altered to brown from colorless state. After one minute of irradiation, a surface plasmon resonance (SPR) of silver nanoparticles were appeared at 417 nm (Figure 1). This SPR peak was originated from the collective oscillation of conduction electrons in nanoparticles by visible light. It is depends on the shape and size of nanoparticles. The main attraction of microwave synthesis is that it has lesser reaction time and gives uniform sized particles. The quick consumption of starting materials in the microwave aided reactions decreases the possibility of the formation of agglomerates and gives nanoparticles with narrow size distribution. Extract of *Glycosmis pentaphylla* contain carbohydrates and flavonoids. When *Glycosmis pentaphylla* extract is mixed with the $AgNO_3$ solution, these phytochemicals reduce Ag+ ions to Ag atoms and stable nanoparticles were formed.

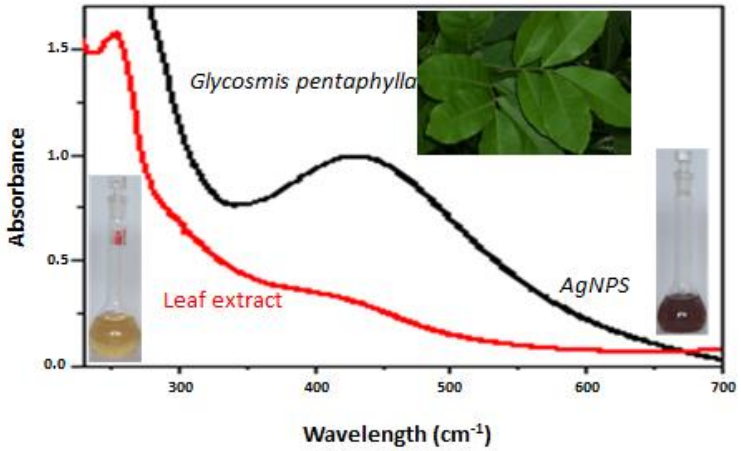

Figure 1: UV-Vis. absorption spectrum of AgNP

In order to study the crystalline nature of formed nanoparticles, the X-ray diffraction studies were carried out. In the XRD image of nanoparticles showed four peaks corresponding to the 2Θ values of 38.36, 44.52, 64.66, and 77.66 respectively. By comparing these values

with JDPDS file number 04-0783, it was concluded that the above peaks are originated from the reflections (111), (200), (220), and (311) planes of a face centered cubic silver nanoparticles. Hence the formed silver nanoparticles were purely crystalline in nature. The SEM studies also confirmed the formation of silver particles in the nanoscale range. The nanoparticles predominately adopt a spherical morphology and quite uniform in size (Figure 2). This shows that microwave method using *Glycosmis pentaphylla* extract was a good pathway for preventing aggregation and synthesizing nanoparticles.

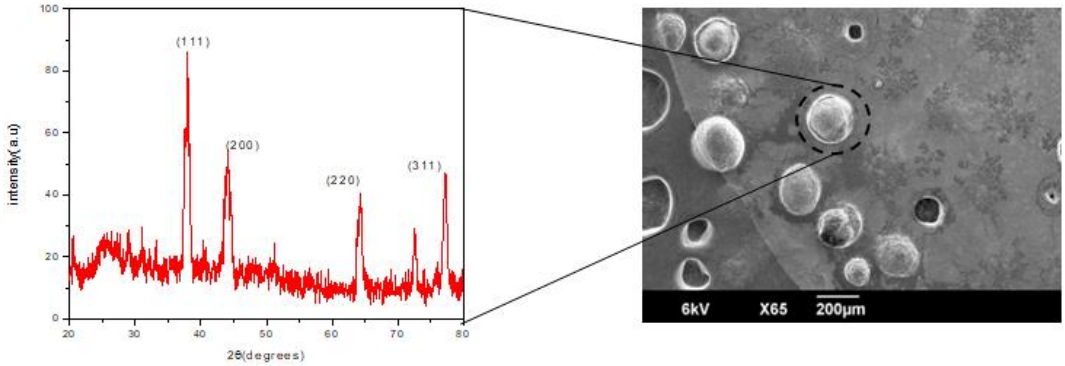

Figure 2: XRD and SEM of silver nanoparticles

The antimicrobial properties of the prepared silver nanoparticles were tested against both the Gram positive (*S. aureus, B.cereus*) and Gram negative bacteria (*E.coli, P.aeruginisa*) by well diffusion method. The photographs of antimicrobial activity are shown in Figure 3 and the details about the zone of inhibition are shown in table 1. From the photographs it is clear that the nanoparticles exhibit more antimicrobial properties than that of leaf extract and also it is found that it is more effective against Gram negative than Gram positive bacteria. The thickness and molecular composition of the membrane of gram-positive and gram-negative bacteria are different and hence resulted in the difference in their sensitivity towards the silver nanoparticles. [20, 21].

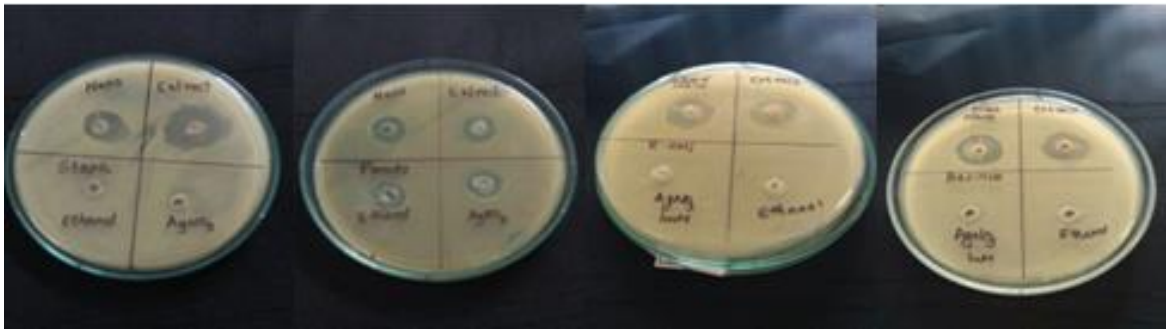

Figure .3: The antimicrobial activity against (a) *E.coli*, (b) *S. aureus*, (c) *P. aeruginosa*, (d) *B. cereus* and the values of zone of inhibition obtained by well diffusion method

Table 1: Antimicrobial study

Sl No	Organism	Zone of clearance (mm)			
		Extract	AgNPs	$AgNO_3$	Ethanol
1	*E. coli*	12	16	0	0
2	*S. aureus*	16	19	0	0
3	*P.aeruginosa*	10	15	12	5
4	*B. cereus*	12	18	0	0

The interaction of silver nanoparticles with bacterial cell wall causes changes in its structure and leading to increased membrane permeability and subsequently death. AgNPs can interact with sulfur and phosphorus rich biomaterials and extracellular components, and the silver ions can enter into cells, leading to the accumulation of damaged DNA and effect on protein synthesis [22, 23].

3. Conclusions

In this study we synthesized silver nanoparticles with the help of *Glycosmis pentaphylla* leaf extract by a microwave supported technique. The phytochemicals in the leaf extract were performed the role of both reducing and capping agents in the formation of nanoparticles. The formation of nanoparticles were confirmed by using UV-visible spectroscopy, XRD and SEM investigations. The antimicrobial actions of formed nanoparticles were analyzed for both gram positive and gram negative bacteria by agar well diffusion method. The nanoparticles exhibited excellent antimicrobial properties.

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