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Evaluation of Antibacterial Efficacy of Phyto Fabricated Gold Nanoparticles using *Bacopa Monniera* Plant Extract

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ABSTRACT
The biosynthesized metal nanoparticles provide an expanding research area due to the potential application for the development of novel technologies. We have reported a fast, extracellular and convenient method for the synthesis of metallic gold nanoparticles by reducing the aqueous HAuCl4 with the help of Bacopa monniera plant extract. The synthesized nanoparticles were characterized by using UV-Vis. spectrophotometer, Transmission electron microscope and Fourier transform infrared spectrophotometer. The bactericidal property of nanoparticles was evaluated. The biologically synthesized nanoparticles were found to be highly effective against different multidrug resistant human pathogens such as E.Coli, Staphylococcus aureus, Bacillus subtilis and Enterococcus.

Key words: *Bacopa monniera, gold nanoparticles, UV-visible spectroscopy, FT-IR*

1. INTRODUCTION

Nanotechnology offers unique approaches to control a wide variety of biological and biomedical processes due to their nanometer length i.e, 1 nm-100 nm in size [1]. Nanoparticles have received considerable aid because of their structure and properties which differ significantly from those of bulk materials [2]. Moreover, ultra transition metal nanoparticles have attracted great interest due to their unique physical, chemical and thermodyanamic properties that have made them useful in such diverse fields as catalysis, [3] electronics, [4] optics [5] and even in biological and medical sciences [6]. Nanoparticles of noble metals such as gold, silver, palladium and platinum are widely applied in products that directly come in contact with the human body such as shampoos, soaps, detergents, shoes, cosmetic products and toothpaste, besides medical and pharmaceutical applications. Among the above four, gold nanoparticles being inert and less cytotoxic are extensively used in the field of drug and gene delivery [7, 8, 9]. Gold has a long history of use, red colloidal gold has been used as medicine for revitalization in China and India [10].

Nano size gold, an emerging nanomedicine is renowed for its promising therapeutic possibility high surface reactivity, resistance to oxidation and plasmon resonance [11]. The role of gold nanoparticles are invading the treatment for various inflammatory diseases and other relative disorders evidences towards the anti-oxidative effect of traditional gold in treatment of several diseases [12]. The interaction of several biomolecules with gold nanoparticles has been studied with a view to understand the binding chemistry, improved biological action and possibility of acheiving drug delivery [13]. Biosynthesis of nanoparticles has received considerable attention due to the growing need to develop environmentally begign technologies in nanoparticles synthesis. Plant extracts have been found to be ecofriendly and cost effective for the large scale synthesis of nanoparticles [14].

The use of environmentally begign materials like plant extracts offers numerous benifts of ecofriendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. *Bacopa monniera* belongs to Scrophulariacae family commonly known as Brahmi found throughout the Indian subcontinent in wet and marshyplaces. In Ayurvedic medicine, it is used as a nerve tonic to mend intelligence, memory and functioning of sense organs [15]. *Bacopa monniera* have been used in the treatment of various nervous systems ailments such as insomnia, anxiety, epilepsy, hysteria etc [16]. Its ethanolic extract contains a mixture of triterpenoids, steroids, saponins designated as Bacosides A and B [17,18]. The present study was aimed to rapid synthesis of gold nanoparticles

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using ethanol extract of *Bacopa monneria* and evaluates its anti bacterial activity.

2. MATERIALS AND METHODS

2.1. Preparation of Bacopa monneria extract

The whole plant of *Bacopa monniera* was thoroughly washed with double distilled water and dried in shade, in dust free condition for one week at room temperature before being ground to a fine powder. Finely powdered plant material (10 g) was extracted with ethanol (100 ml). The mixture solution was left on constant magnetic stirring at room temperature for 24 hrs. The extract was filtered and stored at 4^oC for further experiments.

2.2. Synthesis of gold nanoparticles

0.001M aqueous solution of $HAuCl_4$ was prepared and used for the synthesis of AuNPs.

5 ml of plant extract was added to 120 ml of aqueous solution of 0.001M $HAuCl_4$ for reduction into Au particles and kept at room temperature for one hour.

2.3. UV-Vis spectral analysis

The reduction of pure Au^{+3} ions was monitored by measuring the UV-Vis spectrum of the reaction medium after diluting a 0.5 ml aliquots of the sample into 5 ml distilled water. UV-Vis spectral analysis was done by using Genesis 10S UV-Vis spectrophotometer (Thermo Scientifics).

2.4. Transmission Electron Microscopy (TEM) measurements

The sample was first sonicated for 10 minutes. A drop of this solution was loaded on carbon coated copper grid and solvent was allowed to evaporate under Infrared light for 30 minutes. TEM measurements were performed on Philips Model CM 200 instrument operated at an accelerating voltage at 200KV.

2.5. FTIR analysis of dried residue after bioreduction

To remove any free biomass residue or compound that is not capping ligand of the nanoparticles, the residual solution of 100 ml, after reaction was centrifuged at 9000 rpm for 30 min. The obtained pellet was washed with ethanol for three times. There after the purified suspension was air dried to obtain dried powder. Finally the dried nanoparticles were analyzed by Bruker Alpha-T Model 109974 FTIR.

2.6. Antimicrobial activity:

The antimicrobial activity of gold nanoparticles was evaluated against staphylococcus aureus, Escherichia coli, Enterocoocus and Bacillus subtilis by disc method. The 24 hrs old cultures were prepared in nutrient broth (composition (gm/L) peptone 15.0; yeast extract 3.0; sodium chloride 6.0; D (+) glucose (1.0). Two replicas of respective microorganisms were prepared by spreading 100µl of revived culture on the nutrient agar plate (composition (gm/litre) peptone 15.0; yeast extract 3.0; sodium chloride 6.0; D (+) glucose 1.0; agar-agar 12.0) with the help of spreader. Discs were prepared by using Whatmann No.1 filter paper and placed on agar plates. The sample of synthesized gold nanoparticles is placed on the disc with the help of micropipette. The plates were incubated at 37°C overnight. Gentamycin disc was used as reference drug.

3. RESULTS AND DISCUSSION

Gold nanoparticles were fabricated using *Bacopa monniera* extract at room temperature. Reduction of the gold nanoparticles during exposure to the plant extract could be detected by the colour change. The appearance of ruby red colour was observed with in 15 min after the adding of extract to the $AuCl_4$ solution and the solution changed from ruby red to steel grey in about 1 h (Figure1).

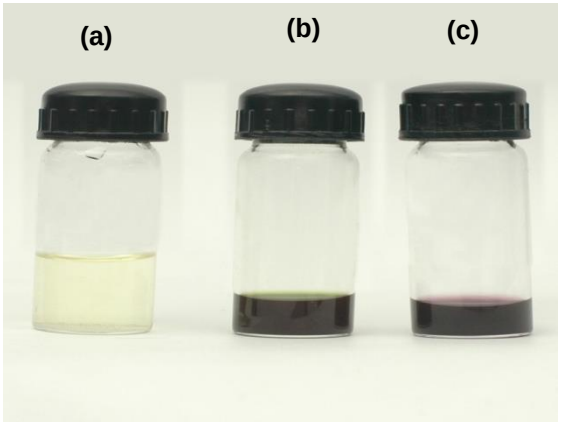

Figure 1. Aqueous solution of 0.001M Aqueous solution of $HAuCl_4$ (a) with *Bacopa monniera* extract (b) before adding the extract and after addition of extract at 1hr (c).

Formation of nanoparticles was confirmed by using UV-Visible spectrophotometer [19]. Figure 2 shows the strong surface Plasmon resonance absorption peak at 557 nm shows the formation of gold nanoparticles at room temperature. It is thought that phenols, alcohols and proteins present in the ethanolic extract of *Bacopa monniera* are responsible for the reduction of gold ions, and also for the stabilization of nanoparticles throughout by electrostatic attraction [20]. Especially in the case of gold nanoparticles bioencapsulation would be one of the methods for stabilizing these nanoparticles [21].

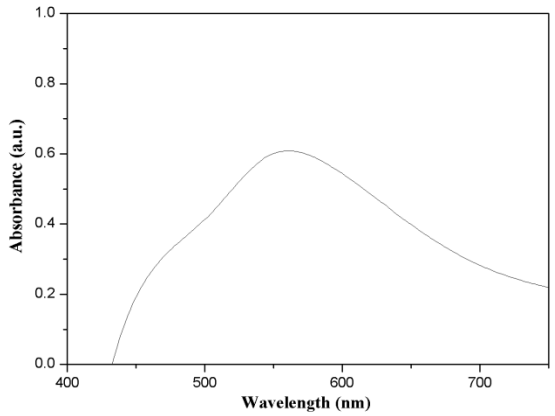

Figure 2. UV-Vis absorption spectra of gold nanoparticles synthesized by exposure of *Bacopa monniera* with 0.001M aqueous solution of $HAuCl_4$

TEM visualization provides an extraordinary opportunity for the morphological evaluation and particle size of nanoparticles. The typical TEM micrograph of the synthesized gold nanoparticles is presented in Figure 3. It is observed that most of the gold nanoparticles were spherical in shape. There is a variation in particle sizes and the average size estimated was 21 nm, most of the particles are ranged from 15 to 35 nm in size.

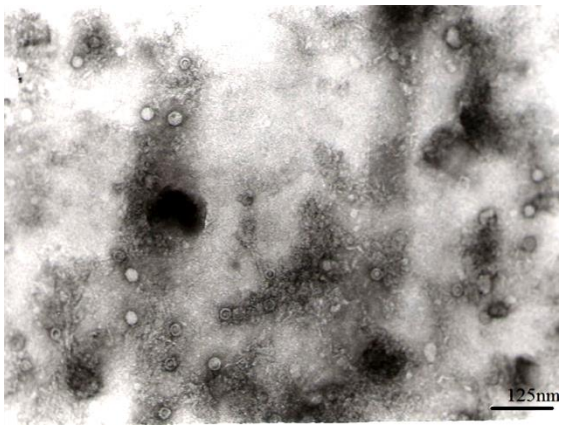

Figure 3. TEM image of the gold nanoparticles formed by the reaction of 0.001M $HAuCl_4$ and *Bacopa monniera* extract at room temperature.

FTIR analysis was used for the characterization of the synthesized nanoparticles and the spectrum provides the information about the chemical change of the functional groups involved in bioreduction and mode of interaction between gold nanoparticles and *Bacopa monniera*. Figure 4 shows the FT-IR absorption spectrum of gold nanoparticles. The intense broad absorbance at 3400 cm^{-1} is the characteristic of the hydroxyl functional group in alcohols and phenolic compounds. The band at 2148 cm^{-1} correspond to C=C stretch vibration of alkynes. The band at 1647 cm^{-1} corresponds to N-H bend of primary amines and the bands observed at 1447, 1166, 1049 and 728 cm^{-1} indicates C-C stretch of aromatic carboxylic acids, esters, ethers and primary and secondary amines respectively. This indicates the synthesized gold nanoparticles using the *Bacopa monniera* extract are surrounded by some proteins and metabolites having functional groups of amines, alcohols, aldehydes and carboxylic acids.

Further the biosynthesized gold nanoparticles were assayed for antibacterial activity against Escherichia coli, Bacillus subtilis, Staphylococcus aureus and Enterococci by using disc diffusion method. The gold nanoparticles showed zone of inhibition against all the studied bacteria. Maximum zone of inhibition was found in Bacillus subtilis. Where as intermediated activity was revealed against Escherichia coli, Staphylococcus aureus and Enterococci (Figure 5). The inhibitory activities in culture media of the gold nanoparticles reported in Table 1. Gentamycin was taken as reference drug.

Metal nanoparticles are harmful to bacteria and fungi [22]. Gold nanoparticles possess well developed surface chemistry, chemical stability and appropriate smaller size, which make them easier to interact with the micro organisms [23]. The enhanced activity might be due to the nano size of gold nanoparticles, large surface area and high penetrating power hence such nanoparticles could effectively bind to the substrates on the outer membrane and cell membrane of organisms.

4. CONCLUSION:

Gold nanoparticles have been successfully synthesized by using a simple and effective green synthesis methodology. Using plant and plant extract for nanoparticle synthesis can be advantageous over other biological methods. In this study *Bacopa monniera* extract have been used as a reducing agent for synthesis of gold nanoparticles. It is used as an ayurvedic herb to treat neurological disorders. These biologically synthesized gold nanoparticles shows antibacterial activity against four strains of bacteria. We are currently pursuing the drug delivery nature of *Bacopa monniera* stabilized gold nanoparticles in mammalian models.

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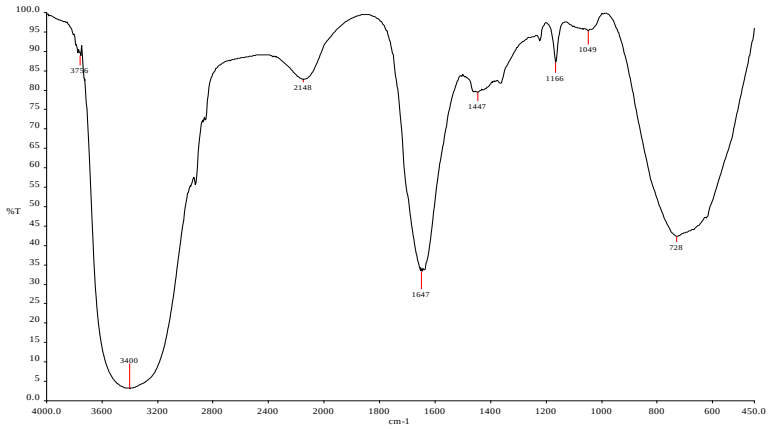

Figure 4. FTIR spectra of the gold nanoparticles synthesized by the reduction of Auric chloride with the *Bacopa monniera* extract.

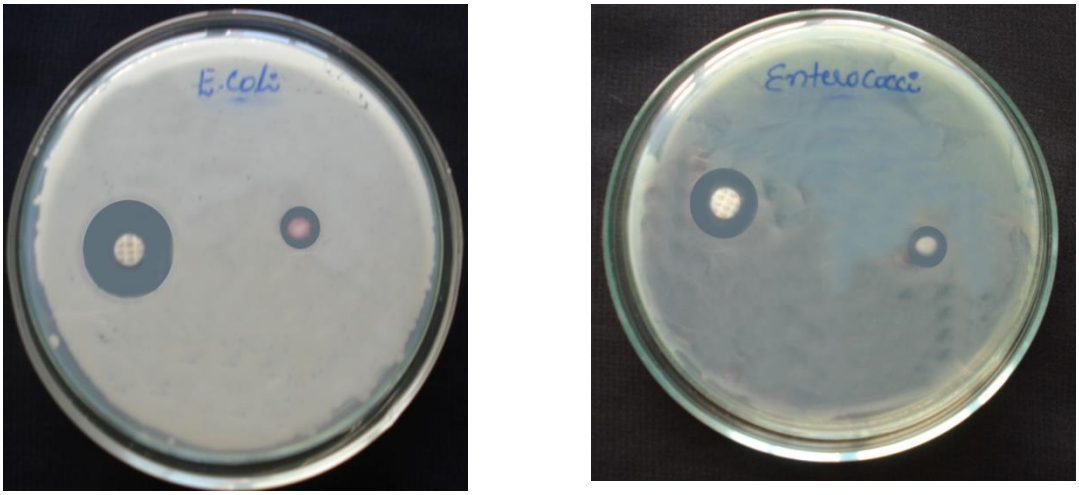

Figure 5. Antimicrobial activities of gold nanoparticles by using *Bacopa monniera* extract and 0.001M aqueous solution of $HAuCl_4$. Gentamycin as standard.

Table 1. Inhibitory activity of gold nanoparticles on bacteria

S. No.	Organism	Zone of inhibition(cm) Ref. drug Gentamycin	Nanoparticle
1	Staphylococcus aureas	0.9	0.5
2	Bacillus subtilis	0.9	0.6
3	Escherichia coli	0.9	0.35
4	Enterococci	0.6	0.3

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***Biographical Sketch**

Dr.N. John Sushma *obtained her Ph.D in 1999. Her research areas are Toxicology and Nanobiotechnology. She has published 27 research papers in peer reviewed National and International journals. She has presented her research work in several National and International conferences. She has received "National Environmentalist Award" in 2006 by National Environmental Science Academy.*