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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED USING *CINNAMOMUM ZEYLANICUM*

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Abstract:

Silver is a proven antimicrobial compound and silver nanoparticles are gaining their importance due to their biological activities. The aim of the current study was to use cinnamon bark extract for the biosynthesis of silver nanoparticles and to evaluate their antibacterial and anti- oxidant activity in vitro. The physical characterization of these silver nanoparticles was verified using UV - visible spectroscopy. The reaction mixture of silver nitrate with bark extract was monitored from 350 to 600 nm for 70 min. The surface plasmon resonance band was formed at 450nm for silver nanoparticles. The synthesized silver nanoparticles containing cinnamon extract showed a higher antioxidant and antimicrobial activity compared to bark extract alone or silver nitrate. Free radical scavenging assay showed 22.41 % activity for silver nanoparticles derived from cinnamon bark extract. It could be concluded that C. zeylanicum bark extract can be used effectively in the production of potential antioxidant and antimicrobial nanoparticles for commercial application.

Keywords: *Cinnamon, Nanoparticles, Silver, Surface plasmon resonance*

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INTRODUCTION:

The synthesis of noble metal nanoparticles attracts an increasing interest due to their new and different characteristics as compared with those of macroscopic phase, that allow attractive applications in various fields such as antimicrobials, medicine, biotechnology, optics, microelectronics, catalysis, information storage and energy conversion [1]. Nanoscience is a new interdisciplinary subject that depends on the fundamental properties of nanosize objects [2,3]. Nanoparticles possess wondrous optical, electronic, magnetic, and catalytic properties than the bulk material owing to their high surface area to volume ratio [4,5]. Metal nanoparticles like silver and gold show different colors due to their Surface Plasmon Resonance (SPR) phenomenon. It is a collective oscillation of free electrons of the metal nanoparticles in resonance with the frequency of the light wave interactions causing the SPR band to appear in the visible and infrared region [6].

Fig.1 Cinnamon bark

Silver nanoparticles are prepared by chemical reduction method. Typically, silver nitrate ($AgNO_3$) is the metal precursor which provides the Ag ions in solution. The redox reaction of Ag+ requires a reducing agent. Silver nanoparticles have received attention due to their physical, chemical, and biological properties that attributed to the catalytic activity and bactericidal effects and found applications in nanobiotechnological research [7,8]. They are used as antimicrobial agents in wound dressings, as topical creams to prevent wound infections [9], and as anticancer agents [10].

A plant species *Gliricidia sepium* used for the synthesis of silver nanoparticles, showed an absorption maximum at 440 nm [11]. Green synthesis of AgNPs using *Argemone mexicana* leaves broth generated particles of 20 nm and was found to be effective against many bacterial and fungal pathogens [12]. *Cycas* leaf extract was used to prepare silver nanoparticles of 2 to 6 nm [13]. A plant species *Solanum torvum* produced AgNPs of 14 nm dimension and showed the absorbance peak at 434 nm. The antimicrobial activity of synthesized nanoparticles was tested against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus flavus* and *Aspergillus niger*, showing a zone of inhibition [14]. *Emblica officinalis* fruit extract was used for fabrication of gold and silver nanoparticles of 10 nm, showed a maximum absorption of light at 430 nm [15]. Bio-reduction of silver using various plant extracts such as *Helianthus annus*, *Basella alba*, *Oryza sativa*, *Saccharum officinarum, Sorghum bicolor* and *Zea mays* have been studied by Ankawar et al. [16]. Thus the aim of the study was to investigate the potential of nanoparticles synthesis through Cinnamon, its stability, antioxidant and antimicrobial activity.

MATERIALS AND METHODS:

Plant material and preparation of sample extract

The bark of *C. zeylanicum* (Family: Lauraceae) was collected from the local market, Thanjavur. About 5g bark powder was weighed and boiled with 100ml of distilled water at 60°C for 15min. The mixture was filtered through Whatman No. 1 filter paper and stored at 4°C for nanoparticle synthesis process [17].

Biosynthesis of silver nanoparticles

Fifty ml of 0.1 N aqueous solution of silver nitrate was prepared in a Stoppard Erlenmeyer flask and 9 ml of the bark extract was added at room temperature in the dark until the brownish color was developed which indicated the formation of silver nanoparticles [18]. It attained maximum brown colour after 1 hour. Meanwhile, the nanoparticles settled down and the colour of the solution turns pale brown. Then it was stored at 4°C.

Characterization of silver nanoparticles

The bio-reduction of silver nitrate ($AgNO_3$) to silver nanoparticles was monitored by UV–Vis spectroscopy (Shimadzu UV-1800). A UV–Vis spectrograph of the silver and nanoparticles was recorded using a quartz cuvette with water as reference. The UV–Vis spectrometric readings were recorded at a scanning speed of 350–600 nm [19].

Total Antioxidant Assay of silver nanoparticles

To assess the scavenging ability on DPPH, each extract (5–20 mg/ml) in water and ethanol was mixed with 1 ml of methanolic solution containing DPPH radicals (0.2 mM). The mixture was shaken vigorously and left to stand for 30 min in the dark before measuring the absorbance at 517 nm against a blank [20]. Then the scavenging ability was calculated using the following equation:

I (%) = 100 X (A blank – A sample/ A blank)

Where I (%) is the inhibition percent, A blank is the absorbance of the control reaction (containing

all reagents except the test compound) and A sample is the absorbance of the test compound.

Total antibacterial activity of silver nanoparticles

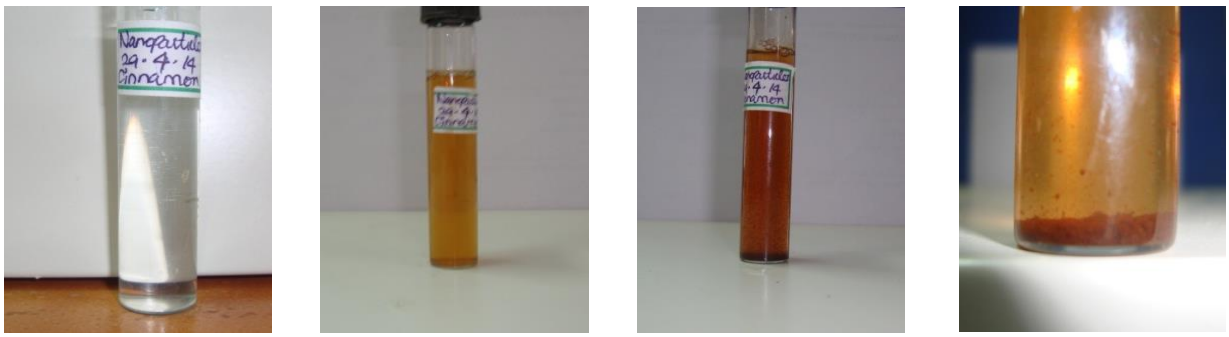
The agar diffusion method (well and disc) described by Srinivasan et al. [21] was adopted to assess the antibacterial activity of the cinnamon nanosilver particles. One ml of standardized *Escherichia coli* bacterial stock suspensions (10^8 – 10^9) Colony Forming Units (cfu) per ml was thoroughly mixed with 250 ml of sterile nutrient agar. Twenty ml of the inoculated nutrient agar was distributed into sterile Petri dishes. The agar was left to set, 10 mm disk in diameter was cut using a sterile cork borer No.4 and the agar disks were removed. The wells were filled with 0.1 ml of cinnamon silver nitrate solution; silver nitrate solution and cinnamon water extract were allowed to diffuse at room temperature for 2 h. The plates were then incubated in the upright position at 37° C for 18 h. After incubation the diameters of the growth inhibition zones were measured averaged and the mean values were recorded.

Stabilization of Silver Nanoparticles using Carboxy Methyl Cellulose (CMC)

CMC which is an ester derivative of cellulose was used as a stabilizer of the synthesized silver nanoparticles. 0.5% solution of CMC was prepared. 9 ml of 0.1N Silver Nitrate +1ml of 5% Cinnamon extract + 5ml of 0.5% CMC were taken in a boiling test tube and heated at 40°-45°C for 15 minutes. The formation of brown colour indicated the formation of silver nanoparticles.

RESULTS AND DISCUSSION:

In the present study, we adopted a simple procedure to synthesize silver nanoparticles from cinnamon bark extract. The photographs of the silver nitrate solution, silver nitrate blended with cinnamon solution after 10 minutes, 1 hour and 4 hours of incubation were shown in Fig 2. The UV–Vis spectrometric readings were recorded and shown in Fig 3. The surface plasmon resonance band was formed at 450 nm for silver nanoparticles. The absorbance intensity was increased while increasing the time of reaction and the synthesis was maximum attained at 70 min. This has been observed by change in colour of the solution from brown to yellow after 2 hrs and it became dark brown over the time.



0.1 N $AgNO_3$ Solution

$AgNO_3$ Solution + Cinnamon after 10min of incubation

$AgNO_3$ Solution + Cinnamon after 1 hour of incubation

Silver Nanoparticles precipitation after 4hr of incubation

Fig. 2: Photographs of silver nanoparticles synthesis using cinnamon bark extract

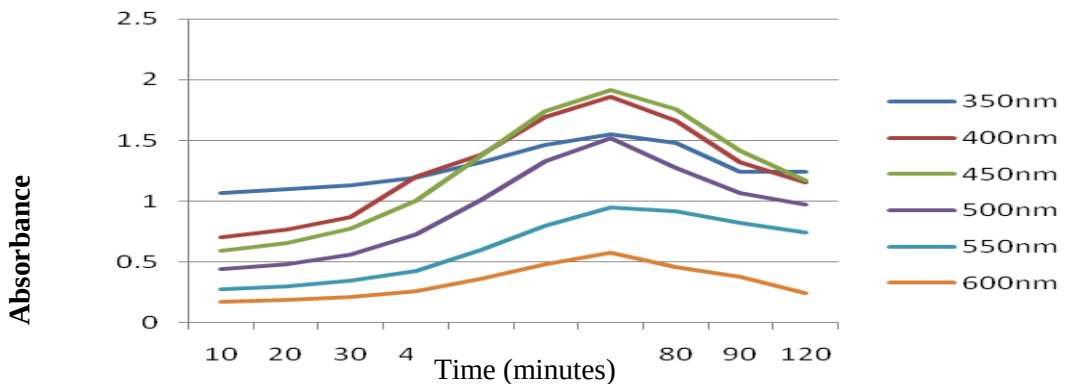


Fig. 3: UV-Vis spectrum of cinnamon extract added with 0.1 N $AgNO_3$ shows distinct peak at 450 nm

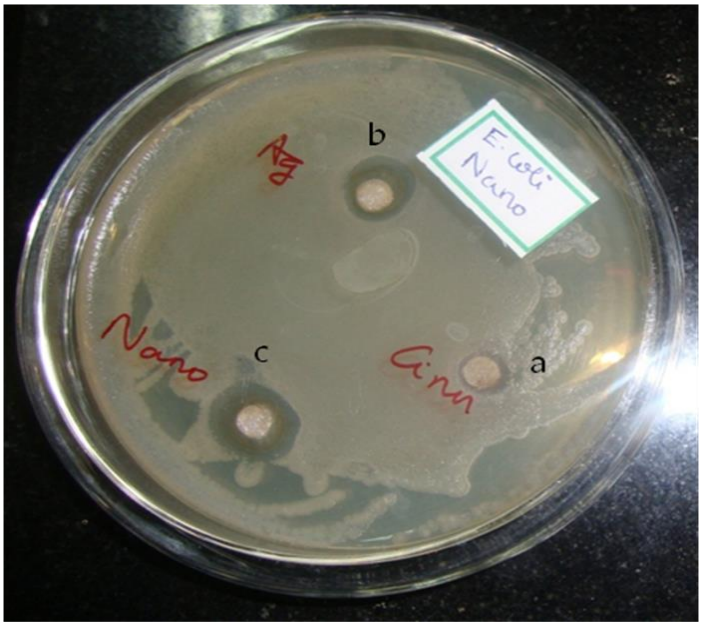


Fig. 4: The antibacterial effect of Cinnamon bark extracts (a), silver nitrate (b) and the biosynthesized silver nanoparticles (c) using the test bacterium *Escherichia coli*.

In the stabilization study of the cinnamon silver nanoparticles, CMC was used as a stabilizer. The solution was observed for any settlement of nanoparticles. The solution retained in brown colour and it was stored at 4°C. This indicated that the produced nanoparticles were stabilized by CMC. It has proven that the polysaccharide CMC is a natural reservoir of silver nanoparticles synthesized from Cinnamon extract [22]. The solution was stable even after 8 days of incubation. The antioxidant activity of the synthesized Cinnamon silver Nanoparticle was compared with the activity of cinnamon extract by DPPH Method. The percentage of inhibition was 11.34 ± 0.56 % by Cinnamon extract and 22.41 ± 0.25 % by Silver nanoparticles synthesized from cinnamon. Thus the silver Nanoparticle showed antioxidant activity 2 times that of cinnamon extract. Hence it can be concluded that the synthesized silver nanoparticles is better than cinnamon extract in its antioxidant activity.

The antibacterial activity of the synthesized Cinnamon silver nanoparticles was compared with the activity of cinnamon extract and silver nitrate. They showed activity against the selected bacteria *E. coli*. The zone of inhibition was measured. It was 1.7 mm with synthesized Cinnamon silver nanoparticles, 1.1 mm with Silver nitrate and 0.7 mm with Cinnamon extract. The agar diffusion assay distinguished the antimicrobial activity of the different preparations and explained the increment of the clear zone using the same concentration before (AgNO3) and after silver nanoparticles formation (Fig. 4).

CONCLUSION:

The current study revealed that silver nanoparticles can be synthesized in a simple method using *C. zeylanicum* bark extract. The aqueous silver ions were reduced to silver nano particles after mixing with bark extract followed by incubation for 70 minutes in the dark condition. The color turned to reddish brown and this change in color. The color change appeared due to the surface plasmon resonance of deposited silver nanoparticles. The in vitro antioxidant and antibacterial properties of the biosynthesized silver nanoparticles have been evaluated and showed a higher antioxidant and antimicrobial activity compared to *C. zeylanicum* bark extract alone or silver nitrate.

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