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Synthesis, Characterization and Antimicrobial activity of Silver nanoparticles using *Santalum album* aqueous seeds extract

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Abstract : Silver nanoparticles play a significant role in the field of biology and exploited in medicine for treatment burn, skin diseases and also for making dental materials. The present work investigated the synthesis of silver nanoparticles using *Santalum album* aqueous seeds extract. The formation of silver nanoparticles was confirmed by visual observation, UV-Vis spectroscopy, FTIR and TEM analysis. Appearance of dark brown colour indicated the synthesis of silver in the reaction mixture. The silver nanoparticles were found to be circular, spherical and triangular in shape with variable size, as evident by TEM studies. The nanoparticles appeared to be associated with some chemical compounds which possess hydroxyl and carbonyl groups, confirmed by FTIR. The synthesized nanoparticles were tested for antibacterial and antifungal activities using agar well diffusion method. The AgNPs at 40µl showed potent antifungal activity against *C. albicans* (16.0±0.0), *T. rubrum* (14.83±0.28) and *E. coli* (21.0±0.0). AgNPs inhibit the growth of human pathogens which are responsible for causing superficial fungal infections. This is the first and novel report of silver nanoparticles synthesised from *S. album* aqueous seeds extract and their antimicrobial activity. **Keywords:** *Santalum album*, medicinal plant, silver nanoparticle, antimicrobial activity.

Introduction

In recent years, the nanoparticle materials have received an increasing attention for their exclusive physical and chemical properties, which are significantly different from their conventional counterparts (1). There is an increasing commercial demand for nanoparticles due to their wide applicability in various areas such as electronics, catalysis, chemistry, energy, and medicine. Different types of nano materials like copper, zinc, titanium (2), magnesium, gold (3), alginate (4) and silver have been developed but in the past few years, advances in research on metal nanoparticles such as silver nanoparticles has received special attention and have known to proved that silver and its compounds are most effective and has been an increasing interest as they exhibit potent antimicrobial properties and are even being projected as future generation antimicrobial agents (5-13). Silver nanoparticles play a significant role in the field of biology and exploited in medicine for treatment burn, skin diseases, for making dental materials, used for coating stainless steel materials, textile fabrics, water treatment, and also as ingredients of sunscreen lotions, etc. and posses low toxicity to human cells, have high thermal stability and low volatility (14). Therefore, the preparation in terms of size, shape, and physical and chemical properties of uniform nanosized silver particles with specific requirements is of great interest in the formulation of new pharmaceutical products (15,16,35-37).

Santalum album Linn. a small evergreen tree, medicinally useful in biliousness, fever, thirst, skin eruption, hemicranias and skin diseases. It is commonly used in cosmetic and hair oil. *S. album* is bitter, cooling, sedative diuretic, expectorant, stimulant and astringent (17,18). The seed oil yield dark red viscid fixed oil containing stearolic acid and santalbic acid (19). Many studies have shown the biological effects of silver nanoparticles, however, its effects against pathogens responsible for skin disease have not yet been fully studied. So far, there have been no reports on the synthesis of nanoparticles by using seed extract. Therefore, the present report on the synthesis of AgNPs using the aqueous seed extract of *S. album* and antimicrobial properties against human pathogenic fungal strains *C. albicans* and *T. rubrum* as well as *E.coli* bacteria.

Experimental

Plant material and synthesis of silver nanoparticle

Mature seeds of *S. album* were collected from botanical garden of Gulbarga University campus and local area of Kalaburagi districts, Karnataka, India. 25 g of seeds were washed repeatedly with distilled water, so as to remove impurities present on it and pulverized into fine powder. The powder are then taken separately into 1000 ml Erlenmeyer flask containing 500 ml double distilled water and exposed to microwave for 180° C for 30 minutes to suppress the enzymes present in the solution. Then, extract was filtered twice by Whatman filter paper No. 42. The aqueous solution of 1mM silver nitrate (AgNO₃) was prepared and used for the synthesis of silver nanoparticles (AgNPs). Seed extract was added to silver nitrate solution in the ratio 1:10 and kept for incubation for 20 min at room temperature to synthesize the nanoparticles.

Characterization

The S. album seed extract containing silver nanoparticles were characterized by the following methods:

Visual observation

A change in colour of the solution was observed during a process and colour changes indicate the formation of silver nanoparticles (AgNPs).

UV- Visible spectra analysis

The formation of AgNPs was confirmed by the spectral analysis. The UV spectra of synthesized plant mediated AgNPs were recorded using UV-visible 5704SS Elico spectrophotometer operated at with 1 nm resolution with optical length of 10 mm. UV-visible analysis of the reaction mixture was observed at a wavelength range of 400–800 nm.

TEM analysis

TEM analysis was performed on a Technai-20 Philips instrument operated at 190 keV. Sample for TEM analysis were prepared by placing a drops of AgNPs solution on carbon coated copper grids. The film of TEM grid is exposed to IR light for drying for 5 min and extra solution was removed using blotting paper.

FTIR analysis

The powder sample of AgNPs was prepared by centrifuging the synthesized AgNPs solution at 10,000 rpm for 20 min. The solid residue formed is then washed with deionised water. The resultant residue was then dried completely and the powder obtained was used for FTIR measurements carried out on a Nicolet iS5 FTIR with diamond ATR (Attenuated Reflectance Technique).

Antimicrobial studies

Microorganisms

Fungi such as, *Trichophyton rubrum*, and *Candida albicans* and bacterial strains such as, *Escherichia coli* were used in the present study. The tested strains *Trichophyton rubrum* and *Escherichia coli* were procured from Mahadevappa Rampure Medical College, Kalaburagi, Karnataka, India and *Candida albicans* MTCC

3017 from Institute of Microbial Technology, Chandigarh, India. These test cultures were grown in SDB, nutrient broth (Himedia, M002) at 37°C and maintained on nutrient and potato agar slants at 4°C.

Antimicrobial activity

The antimicrobial activities of synthesized AgNPs were carried out by agar well diffusion method. About 15 ml of potato dextrose agar medium was poured in the sterilized petri dishes and allowed to solidify. 1 ml of 10^8 CFU/ml of test strain was spread over the medium using a sterilized glass spreader. Using flamed sterile borer, wells of 4 mm diameter were punctured and required concentrations (40µl and 20 µl) of AgNPs solution were added to the wells. The plates thus prepared were then incubated at 37^{0} C for 48h. After incubation, the plates were observed for zones of inhibition. The diameter of zone of inhibition was measured and expressed in millimetres. AgNo3 solution and plant aqueous extract was used as negative control. Ketoconazole and streptomycin sulphate used as positive control. The experiments were conducted in triplicates. The same method was followed for testing antibacterial activity using nutrient agar medium and incubated at 37^{0} C for 18h.

Statistical Analysis

All the analyses were performed in triplicate and the results were statistically analyzed and expressed as mean $(n=3) \pm$ standard deviation (SD).

Results and Discussion

AgNPs were synthesized using *S. album* seed extract and AgNO₃ solution. After 20 minutes at room temperature the colour of the reaction mixture changes from yellow to dark brown in colour. The reduction of silver ions into silver particles during exposure to the plant extract in aqueous solution exhibit yellowish brown color due to excitation of surface plasmon resonance in AgNPs. Surface plasmon resonance arises due to the assembling of free conduction electrons which is induced by an interacting electromagnetic field (20). As the plant extract mixed in the aqueous solution of the silver ion complex, it started to change the colour from yellowish to brown, which is the indication of formation of AgNPs (21) (Fig 1.).



Fig-1: Synthesis of AgNPs using *Santalum album* seed extract treating with AgNO3 solution at room temperature. a. AgNO3 solution, b. *Santalum album* seed extract, c. formation of AgNPs.

The formation and stability of the reduced AgNPs in the colloidal solution was examined by using UV–visible spectrophotometer. The UV-vis spectroscopy is a technique to confirm the formation of silver nanoparticles in aqueous solution. The synthesized AgNPs were evaluated through Elico double beam spectrophotometer at a wavelength range of 400–800 nm; The UV-vis spectrum recorded from reaction mixture was plotted. The strong surface plasmon resonance of the synthesized AgNPs band appears at the range of 421nm. The spectrum is shown in fig 2. The broadening of peak indicated that the particles are mono dispersed. The typical optical spectra for AgNPs appear at the range from 350 nm–550 nm in visible light region (22), confirming the formation of silver nanoparticles. The present wave length of the silver nanoparticles peaks were similar and supported by the recent report from *Elettaria cardamomum* seed extract at the range of 440–480 nm (23), and also from *Argemone mexicana* leaf extract at the range of 440nm (24) and 423nm from *Santalum*

album leaf extract (25). The synthesis of silver nanoparticles was also confirmed from the UV spectra of *Syzygium cumini* seed extract reported previously where the maximum absorbance was found 450nm after 24 hours of incubation. After the reduction of silver ions by *S. album* seed extract, the solution was centrifuged at 10,000rpm for 15minutes (26). This process of centrifugation was repeated to separate the AgNPs free from other organic compounds present in solution. After centrifugation AgNPs pellet was obtained and redispersed in distilled water and washed for several 2-3 times. The synthesized AgNPs were characterized by transmission electron microscope (TEM). TEM procedure was employed to visualize the size and shape of AgNPs formed. A typical TEM image of synthesized AgNPs suggests that the particles are uneven in shape. Some are circular, spherical and triangular shaped particles with a varying size (Fig 3).



Fig-2: UV-vis spectrum of synthesized AgNPs showing surface plasmon peak at 421 nm



Fig-3: TEM image of bio functionalized AgNPs.



Fig-4: FTIR spectrum of bio functionalized AgNPs

To identify the possible biomolecules responsible for capping and efficient stabilization of AgNPs synthesized in *S. album* seed extract, FTIR measurement was carried out. The spectrum as shown in fig. 4 lot of absorption bands indicates the presence of active functional groups in the synthesized AgNPs. FTIR spectroscopic study confirm the carbonyl group of amino acid residues and peptides of proteins has a stronger ability to bind metal, so that the proteins might probably form a capping of metal nanoparticles, to prevent the agglomeration of the particles, and thus, the nanoparticles are stabilized in the medium. The FTIR spectrum of synthesized AgNPs using *S. album* seed extract indicates a strong band at 3789 cm⁻¹ indicates the presence of OH group stretch. In addition to this, other peaks obtained at 1740 to 1410 cm⁻¹ indicate to C=O aromatic and carboxyl stretching. C-O stretch of ether group at 1121 cm⁻¹ stretch, which play a major role in the synthesis of seed powder mediate AgNPs (27). The peak at 979 to 1209 cm⁻¹ corresponds to aromatic C-H in plane bending. The presence of active functional groups in seed extract results in the swift reduction of silver ions to silver nanoparticle. AgNPs exhibited a potent antimicrobial activity against tested strains. The AgNPs at 40µl and 20µl showed potent antifungal activity against *C. albicans* (16.0±0.0). *T. rubrum* (14.83±0.28) and *E. coli*

20µl showed potent antifungal activity against *C. albicans* (16.0±0.0), *T. rubrum* (14.83±0.28) and *E. coli* (21.0±0.0). The activity was directly proportional to the concentration of AgNPs. Plant aqueous extract and AgNO3 solution was taken as negative control. Plant aqueous extract showed less activity against tested strains whereas AgNO3 solution did not showed any activity. Streptomycin sulphate and ketoconazole showed the inhibition zones 35.0 ± 0.0 and 24.0 ± 0.0 , respectively (Table 1).

SI. No.	Organism	Concentration of AgNPs from <i>S. album</i> seed extract		Control 1.	Control 2.	Standard
		40µl	20µl			
1.	E.coli	21.0±0.0	15.33±0.57	11.16±0.28	NA	35.0±0.0
2.	T. rubrum	14.83 ± 0.28	11.16±0.28	8.0±0.0	NA	24.0±0.0
3.	C. albicans	16.0±0.0	13.33±0.57	10.83 ± 0.28	NA	24.0±0.0

Table-1: Antimicrobial activity of AgNPs synthesized from Santalum album seeds extract

Note: NA- no activity, Control 1- Seed extract, Control 2- AgNO3, Standard- Streptomycin sulphate(bacteria) and Ketoconazole(fungi)

Many of the researchers reported that silver and its compounds have a broad spectrum of antimicrobial activities against bacteria, fungi, and virus (9, 28-30) and non-toxic to human (31). Hence, AgNPs have been applied to a wide range of healthcare products, such as for purification of water, preparing medical apparatus, burn dressings and many other products (16, 32, 34). Therefore, for the formulation of new pharmaceutical products, an immense interest is developed in the researchers for the preparation of consistent nanosized silver particles with specific requirements in requisites size, shape, physical and chemical properties (15, 34). The results of this study elucidate that the effect of AgNPs shown effective effect on tested strains comparing with the standard. This is found to be with both the concentration 40μ l and 20μ l, because when the concentration is low, the drugs have more lethal characteristics. Therefore, the vital significance of this finding is the indication that AgNPs inhibit the growth of human pathogens which are responsible for causing superficial fungal infections.

Conclusion

This is the first report on synthesis of AgNPs using *S. album* seed extract. The synthesized AgNPs have shown significant antimicrobial activity against human pathogens responsible for causing skin disease. Hence can used in formulation of new pharmaceutical products

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