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Antibacterial properties of biosynthesized silver nano particles

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ABSTRACT

Nanoparticles have their demand in various fields of science and technology and their applications extend even in medical and pharmaceutical arena. They have been used as preservatives, diagnosing aids and potent antibacterial agents. But their production is a serious matter of concern when it comes to cost, efficacy and toxicity issues. Overcoming these limitations green synthesis has taken its advantage for their commercial and large scale synthesis. This research will focus on the preparation of nano particles of silver with the help of purified leaf extract from *Lannea coromandelica* and evaluation of the same using UV-Vis Spectrophotometry. The nanoparticles exhibited surface plasmon resonance at 420nm in UV spectroscopy. Futhermore, nanoparticles have been evaluated for their antibacterial activity on *Putida vulgaris, Staphylococcus aureus,* and *Bacillus subtillis*. The results proved the eco friendly synthesized silver nanoparticles have a good antibacterial and can be used effectively in therapies targeting infections and infectious wounds.

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INTRODUCTION

Nanoparticles, now days have gained their importance in various fields of medicine and technology. Concerning their advantages over other forms they are widely and exclusively used as diagnostic and medical aids. They can be synthesized using various methods with their own advantages and disadvantages. But most significantly green synthesis of metallic nanoparticles is found to be an emerging trend in nano biotechnology when the health safety issues are concerned. These processes are eco-friendly, less toxic and most efficient approaches for the preparation of nano particles [1].

Silver is used as an antimicrobial agent as evident from the history since 500 AD. Since ages silver is used to store water, wine, vinegar and milk based on an assertion that silver prevents them from spoilingthough without scientific evidence. Silver nano particles have been used extensively used for health and household purposes even though the mechanism and toxicity were not completely investigated. But still the harmful effects of silver nano particles is now a matter to consider and is a topic on which probing research work has to be done. The high potency and efficacy of silver against any kind of bacteria makes it is the best antimicrobial agent known to and prescribed by physicians. Even though scientists developed many derivatives which overcome bacterial resistance to synthetic antibiotics, such drugs come under cause unwanted side-effects or costly to afford. So, in view to the above disadvantages of the antibacterial drugs nano silver took its way to establish itself as a potent and

safer antibacterial agent from histories. Not only as a pharmaceutical product but also silver nano particles have a varied ranges of usage in electronics & related technologies [2].

Many investigations reveal the synthesis of silver nanoparticles through plants like Neem leaf (A.indica), graveolens leaves (*P.graveolens*), Soap nuts (*Sapindus trifoliandus*), Cinnamon (*Cinnamomum zeylanicum*), Citrus (*Citrus limon*), Tea, Coffee, Tannic acid and many micro organisms. So this research concentrates on the biogenesis of silver nanoparticles using the *Lannea coromandelica* leaves. Silver nano particles synthesized by reducing the ions to metallic silver nanoparticles. Lannea extract contains rich polyphenols which can be utilized for the reduction of ions to form silver nano particles [3].

MATERIALS & METHODS

Collection and extraction of herbal material

Leaves of *Lannea coromandelica* were obtained from S.V.University, Tirupathi and duly authentified by Prof. P. Jayaraman, PARC, Chennai. The leaves were air dried; finely powdered and 50gm of the powder was macerated with 250 ml double distilled water for 24hrs with constant stirring. The macerate was filtered under vacuum. The obtained filtrate was filtered using a whattman filter paper twice to get a clear solution and was directly used for further experiments [4–7].

Cultures

Micro-organisms used in the experiment were procured from pure mother cultures, Microbiology lab, Rao's College of Pharmacy. All these cultures were one day cultures freshly prepared from pure mother cultures obtained from the lab.

Preparation of silver nanoparticles

50ml of 1mM Silver nitrate was mixed with different volumes of plant extract like 1ml, 5ml, 10ml separately and final solution of volume is made to 200ml and centrifugation is done at 18000rpm for 25min to separate any precipitates. The supernatant was heated at 50ºC to 60ºC. A change in the colour of the solution was observed during the heating process. The resultant solutions were named as SNP 1, SNP 5, SNP 10 respectively.

Evaluation

The reducing of pure silver ions was analysed by measuring the UV spectrum of the reaction solution at 30 min, 1, 2 and 3 hrs after dilution of a small sample in to distilled water. UV spectral analysis was performed by using UV Spectrophotometer UV2450.

Antibacterial activity

The Anti-microbial efficacy of different formulations of silver nano particles and were performed on various micro organisms by using Dip well method as per standard procedure. Three sterile petri plates were taken for testing the antimicrobial activity against three different micro-organisms i.e., *Putida vulgaris, Staphylococcus aureus*, and *Bacillus subtillis*.

Nutrient broth medium was prepared and was poured into test tubes and micro organisms were inoculated and kept in incubator at 37^0C for 24 hours for the growth of cultures. To the Solidified Nutrient Agar in the petri plates, cultured micro organisms from nutrient broth were inoculated. Four cavitites were made in the inoculated nutrient agar media and filled with 25 μl different concentrations of Silver Nanoparticles in three cavities along with standard clindamycin gel in fourth cavity. Care was taken that the samples should be placed at the level of cavity and incubated at 37^0 C for 24 hrs. Petri plates were seen for anti microbial activity after 24 hrs. The extent of zone of inhibition was calculated and the values were compared with each other which show the antibacterial activity of silver nano particles [8].

Statistical Analysis

The results are represented as mean SEM with n=3 and subjected to One way ANOVA followed by Dunnett's analysis. The values of $p<0.01$ were marked significance.

RESULTS AND DISCUSSION

The formation of silver nano particles was clear with the variation in colour of the *Lannea coromandelica* extract containing silver nitrate after heating. The solution was pale yellow before heating and changed to dark brown in Figure 1, suggests the formation of silver nano particles.

UV spectral analysis revealed surface plasmon resonanace by metallic nanoparticles at 420 nm. This is due to excitation of surface plasmon vibrations of the formed silver nano particles. Peaks were observed at time intervals of 30 min, 1hr, 2hrs, 3hrs respectively and are shown in Figure 2. The spectra obtained at 3hr showed the absorption maximum at 420nm. The height of the peaks increased indicating a formation of nanoparticles as proportional to the time. The peak tends to become sharp and more intense at 3^{rd} hr suggesting the formed nanoparticles are even in size compared to those formed at 1^{st} and 2^{nd} hrs. The absorption at shorter wavelengths infers that the silver nanoparticles were formed due to the intervention of some organic molecules like

Table 1: antibacterialactivity of silver nanoparticles

Putida vulgaris		Bacillus subtilis		Staphylococcus aureus	
Group	**zone of inhibiton**	**Group**	**zone of inhibiton**	**Group**	**zone of inhibiton**
STD	4.5±0.54mm	**STD**	4.9±0.34mm	**STD**	2.1±0.12mm
SNP-1	2.7±0.23mm	**SNP-1**	1.4±0.5mm	**SNP-1**	1.8±0.8mm
SNP-5	5.1±0.1mm*	**SNP-5**	5±0.21mm*	**SNP-5**	3.2±0.32mm**
SNP-10	5.8±0.22mm**	**SNP-10**	6.3±0.17mm**	**SNP-10**	4.9±0.28mm**

Results were presented as mean±SEM, n=3; *P<0.01significant compared to std; **more significant.

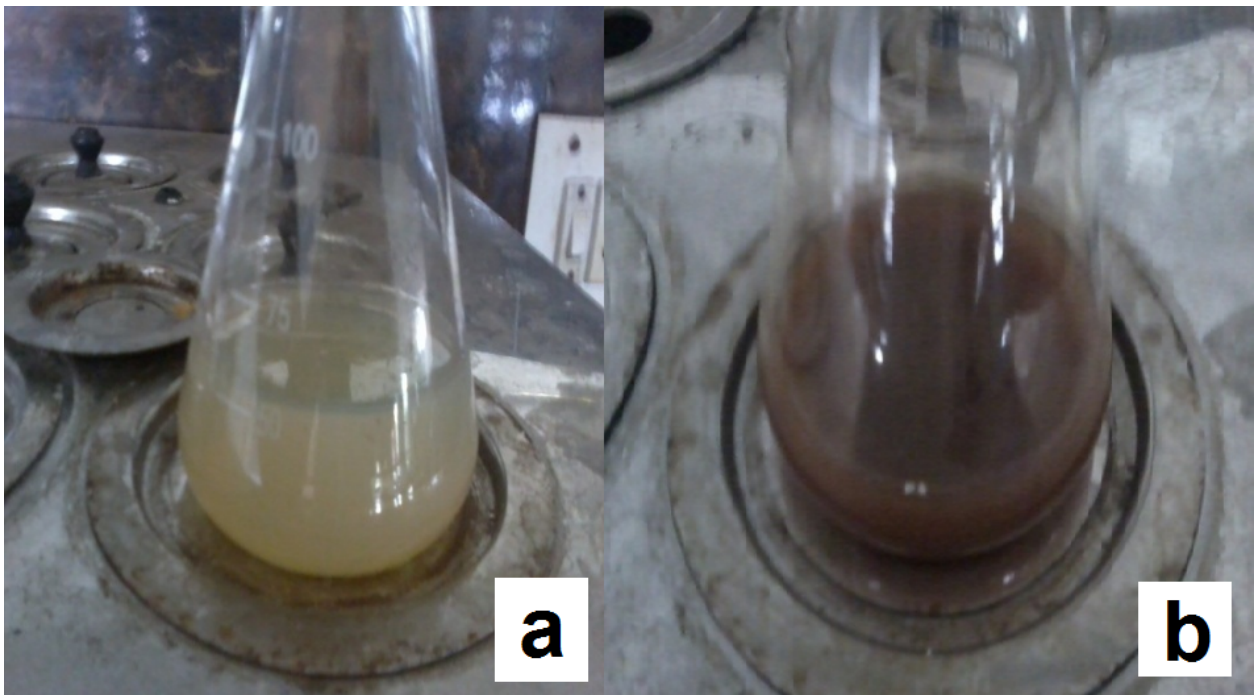

Figure 1: Color change in extract solution; a. before heating b. after heating

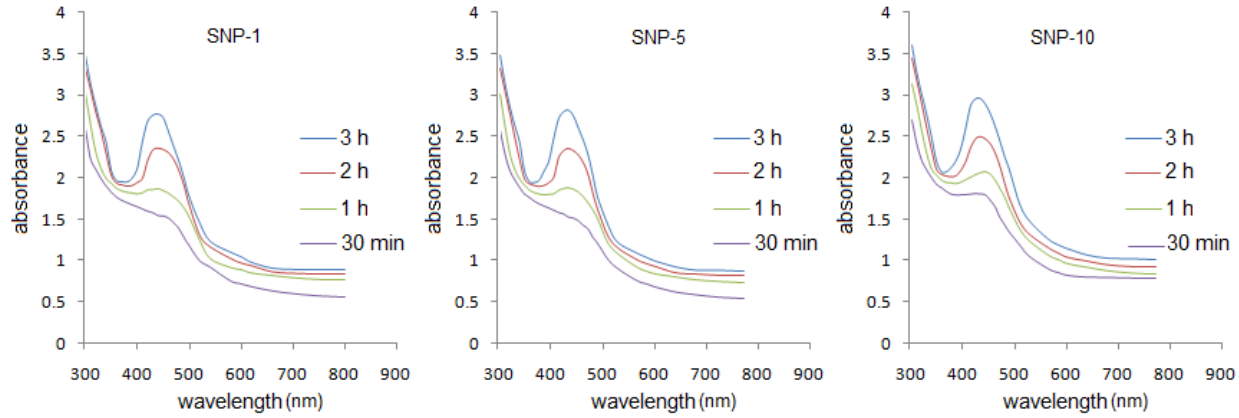

Figure 2: UV spectrum of silver nano particles

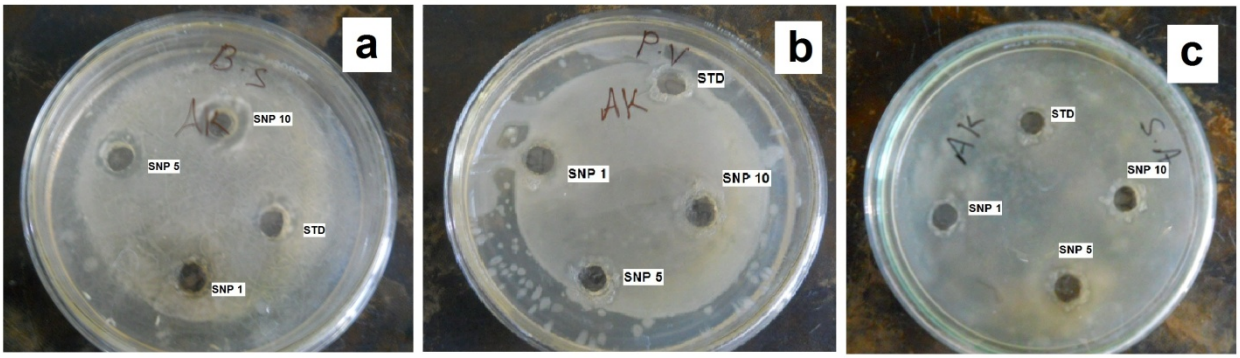

Figure 3: Anti-bacterial potential of silver nano-particles against, a. B. subtilis, b. P. vulgaris and c. S. aureus

secondary metabolites from plant extract. So, it can be reported that reducing agents and antioxidants like polyphenols and flavonols present in the plant might be responsible for the reduction of silver ions into metallic silver nanoparticles.

Metallic silver possesses antimicrobial activity in a very less concentrations and it is relatively less toxic to animal cells. The anti-bacterial activity for silver nano particles was investigated on various organisms like Putida vulgaris, Bacillus subtilis, Staphylococcus aureus. Silver nanoparticles (SNP 10) showed highest zone of inhibition compared to SNP-5, SNP-1, and control as shown in Figure 3. The zone of inhibition of SNP-10 on Putida vulgaris, Bacillus subtilis and Staphylococcus aureus is 5.8±0.22mm, 6.3±0.17mm and 4.9±0.28mm respectively which are significantly ($p<0.01$) higher compared to remaining groups as in Table 1. This high efficacy might be due to the low particle size of the prepared silver nanoparticles produced with 10 ml of extract. Smaller nanoparticles particles have relatively larger effective surface area than larger particles for interaction with cells and show more bactericidal effect. The bacterial cell walls, composed of negatively charged phospholipids and lipoproteins attract positive charged silver nanoparticles thereby disturbing the permeability and cellular respiration. They also penetrate into the bacterial cell and interact with phosphorus and sulfur containing cell components such as DNA and RNA. Thus the normal cell metabolism and cell division gets disturbed leading to bactericidal and bacteriostatic action.

CONCLUSION

Silver have been used as medicinal and diagnosing agent and it is evident from the literature, nano particles are potent and effective compared to normal drugs. The silver nanoparticles were found to have potential advantages over many available drugs. But their synthesis became a serious concern towards the cost and toxicity. So the bio-mimetic synthesis of silver nanoparticles was carried out using leaf extract of Lannea coromandelica and investigations revealed the prepared nanoparticles are potent antibacterial agents. The bio-mimetic synthesis of metallic nanoparticles was proven safer and cost effective. They are relatively safe and effective compared to available antibacterial drugs and chemically produced silver nanoparticles either. The potency and effect of silver nanoparticles was proven yet the toxicity was to be considered for establishing it as a therapeutic agent.

CONFLICT OF INTEREST

Authors declared no conflict of interest.

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