Sustainable Utilization of Bio waste towards the Green Synthesis of Nanoparticles and its Utility in the Naked Eye Detection of Metals Coupled with its Larvicidal and Antimicrobial Properties

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Green synthesis of nanoparticles has become a prominent zone of attention in the field of nanotechnology, as it is a nontoxic, economically feasible and green approach. In the present work we have developed an eco-friendly and zero cost method for the synthesis of silver nanoparticles using common a bio waste banana blossom peel. The well-known characteristic phenomenon of surface Plasmon resonance (SPR) has been exploited towards the characterization of the green synthesized nanoparticles. The aforementioned nanoparticles were characterized by UV spectroscopy and the behaviour of these particles towards naked eye detection of metal ions were observed. The sensitivity of the nanoparticles towards the detection of metal ions was carefully monitored by the shift in the SPR band. Moreover the larvicidal potential of these green synthesized silver nanoparticles were evaluated as per WHO standards. The synthesized silver nanoparticles were found to be an effective antibacterial agent against Gram negative bacteria- E.coli. The method we followed for the synthesis of silver nanoparticles is economically feasible as well as environment friendly and also capable of rapid synthesis of nanoparticles at ambient conditions.
1. Introduction

Synthesis of silver nanoparticles by using biomaterials following green routes has recently attracted considerable attention due to their biodegradability, non-toxicity, and cost effectiveness. Greener syntheses of nanoparticles also provides advancement over other methods as they are simple, one step, use of minimum amount of chemicals, cost-effective, environment friendly, avoids the use of chemical reducing agents and stabilizers which are otherwise mandatory for the preparation of nanoparticles and reproducible [1].

Mercury is ubiquitously prevalent metal in the environment in metallic, organic and inorganic forms and has a high level of and the detection of it has prime importance considering human health and safety. The major source of mercury is from anthropogenic sources such as industrial emissions, e-wastes, etc. when exposed to environment causes soil, water and air pollution. The elemental mercury release is through industrial processes to air and the rest into the terrestrial environment [2-4]. Mercury can transform among the different forms depending on the environmental conditions and is potentially harmful to human health [2]. Mercury can enter through our skin, respiratory tissues and gastro-intestinal tract as well into human body easily. It can severely damage our endocrine systems and may led to serious health and environmental concerns [5]. Many approaches are reported for precise, rapid and reliable methods for sensing, monitoring and quantification of trace amount of ionic mercury in both environmental and biological samples. The techniques available include HPLC (High Performance Liquid Chromatography), X-ray fluorescence spectrometry, polarography, cold vapour atomic fluorescence spectrometry, and electron spectroscopy. Each method has its own disadvantages. Some of these methods have less selectivity, high limit for detection, complicated procedure, sophisticated synthesis of probe materials and the requirement of expensive and complex instruments [5]. The development of highly selective and sensitive analytical method for mercury ions detection is of great importance. Therefore, this colorimetric method presented, which can be observed by the naked eye, is an on-site method appropriate for real-time detection of mercury due to its very modest configuration, usage of minimum chemicals and less impact on environment.

2. Materials and Methods

2.1. Preparation of Silver Nanoparticles:

The substrate selected for this study is banana blossom peel, which is a common plant in Kerala. The fresh banana blossom peels were collected from nearby area. The extract was prepared using banana blossom peel. Silver nitrate was obtained from Merck. Bioreduction of Ag⁺ was carried out using the prepared extract and the bioreduction was carefully monitored by a UV visible spectrometer. The methodology followed is similar to that of the earlier work [4, 8].

2.2. Biosensor development: 1 molar solution of AgNO₃ solution was prepared in 250 ml double distilled water. 5gm of banana blossom peel was boiled in 25ml double distilled water at 100°C for 10 minutes. The extract is filtered and 5ml of the peel extract was added to 50ml 1M AgNO₃ solution. 1 mM solution of HgCl₂, Pb(NO₃)₂, NiSO₄, CdSO₄ were prepared. 2ml of fresh unmodified green synthesized silver nanoparticles was mixed with 2ml of each of these metal solutions and color change was observed.
2.3. Mosquito Larvicidal Assay: *Aedes aegypti* larvae were collected from surrounding water body and were reared in plastic trays in tap water and acclimatized to lab conditions. The larvae were maintained at 27.0°C. The larvae were fed with 10% sucrose solution. Bioassays were performed with the fourth instar stage of *A. aegypti* following the WHO guidelines [6]. Various concentrations of *Banana blossom peel* extracts (1, 2, 3, 4, 5 mg/L) were taken in glass beakers containing 100 mL of double-distilled water. For mortality studies, 20 larvae were introduced in the beaker. At each concentration tested, five trials were made and each trial consists of five replicates and a control (aqueous banana blossom peel extracts) were tested for its anti-larval properties. The mosquito larval deaths were assessed to decide the acute toxicities on the 4th instar larvae of *A. aegypti* at different intervals of 1, 3, 6, 12, 16 and 24 hours of exposure. The number of mortality was calculated from the 1st hour of exposure to the synthesized silver nanoparticles following green pathway, and the mortality percentage was reported from the average of five replicates studied. The larval mortality data were counted and modified by the formula developed by Abbott [7].

2.4. Antimicrobial Assay: The nanoparticles synthesized were investigated for its antibacterial activity against a gram positive and gram negative bacteria as per CLSI (Clinical Laboratory Standards Institute) Guidelines.

3. Results and Discussion

The nanoparticles were prepared using bio-waste material, *banana blossom peel*. The bioreduction of silver nitrate into zero valent metallic nanoparticles was monitored using a UV visible spectrometer and the strong surface Plasmon resonance characteristic of silver nanoparticles confirm the presence of nano silver in the medium. Figure 1 depicts the SPR band of nano silver prepared using the banana blossom peel extract. For the Biosensor development, the silver nanoparticles were prepared by mixing the banana blossom peel extract and molar solution of AgNO₃ solution. 2ml of fresh unmodified green synthesized silver nanoparticles was mixed with 2ml of each of these selected heavy metal salt solutions like HgCl₂, Pb(NO₃)₂, NiSO₄, CdSO₄ and shows immediate fading in colour when compared to the control under ambient conditions and was as presented in figure 2. These colour changes can be correlated with spectral studies but an immediate effect that is observed in the case of a very important toxic metal like mercury is definitely remarkable. This is an important step towards the development of one step naked eye detection of the heavy metal mercury in solution phase.
Figure 1  The UV spectrum of silver nanoparticles prepared using banana blossom peel extract.

Figure 2 Colour change of the silver nanoparticles prepared using Banana blossom peel with metal salt solutions.

The percentage mortality rate of the *Aedes* mosquito larvae after exposure to various concentrations of the green synthesized nanoparticles using the banana blossom peel extract is presented in figure 3. It is evident that at lower concentrations the larvae requires greater time of exposure for mortality but on increasing the concentration it is seen that higher mortality rate is observed at lower exposure times. The best results are obtained with moderate to higher concentrations of nanosilver and immediate mortality is observed as compared to control. The results reveals the effectiveness of the material synthesized using banana blossom peel to be utilized as cost effective and eco-friendly mosquito destructive
material. This can be attributed to the inherent ability of nanoparticles to penetrate through the cell membrane of the mosquito larva which eventually leads to cell death.

![Graph showing percentage mortality of mosquito larvae after exposure to different concentrations of nano silver prepared using banana blossom peel extract.](image)

Figure 3 Percentage mortality of mosquito larvae after exposure to different concentrations of nano silver prepared using *Banana Blossom peel extract*.

The synthesized nanoparticles were analysed for antibacterial activity against one gram positive and one gram negative bacteria- *Salmonella typhi* and *Escherichia Coli*. The results indicate that the nanoparticles synthesized following the green pathway shows good antibacterial activity against gram positive bacteria *Escherichia coli*. Figure 4 shows zone of inhibition of 9mm diameter in the Culture of *E.coli* diameter in the Culture of *E.coli*. 
4. Conclusion

The present work depicts a unique approach towards the synthesis of silver nanoparticles using a green synthetic pathway enabling the use of biowaste material banana blossom peel which would otherwise be discarded as waste. In addition it also aims towards the development of chemosensors for the detection of mercury, one of the most harmful heavy metal in a cost effective manner. Furthermore the mosquito larval toxicity caused by the silver nanoparticles and the antimicrobial activity of the synthesized nanoparticles was also evaluated. In this manner the approach is surely a novel method towards the effective utilization of waste materials. Moreover the conjunction of green chemistry and nanotechnology has made the entire process more eco-friendly and sustainable.

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