Facile preparation of Silver nanoparticles from *Vitex negundo* leaf extract with multiple applications

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Abstract. Synthesis of silver nano particles following green protocols is an emerging area of interest throughout the world, as it has multiple applications in different fields. Moreover the methods are non-toxic, sustainable, eco-friendly and economically viable ones. In the current work, that we have presented is an approach towards the development of zero cost and eco-friendly approach for the preparation of nanoparticles following the green route, using the leaf extract of *Vitex negundo*, a common medicinal plant in South India. The specific phenomenon of Surface Plasmon Resonance (SPR) has been used for the depiction of the silver nano particles prepared. The silver nanoparticles created was characterized by using UV-Visible Spectroscopy and its performance towards the naked eye detection of several heavy metal ions was done. The larvicidal activity of silver nano particles were assessed as per the standards prescribed by WHO.

1. Introduction

Nanotechnology and Nano science is one of the most promising fields of the present times because of its multiple applications in optical, industry, drug delivery, catalysis, environment, medical, space, energy, textile and consumer goods with applications in our day-today-life. The conventional route of synthesis of nanoparticles by physical and chemical methods are highly expensive, technically difficult, need high energy requirements and needs hazardous chemicals which may pose serious threat to environment.[1-5] Many researchers have explored several approaches for the preparation of silver nanoparticles in environmentally benign techniques. Plant mediated synthesis of nanoparticles has made significant attention owing to their unique properties like, biodegradability, non-toxicity, cost effective and environmentally sustainable. The syntheses of the nanoparticles also possess several advantages over the other conventional approaches, as they are cost-effective, environment friendly, very modest and reasonably reproducible and results in more stable constituents [2].

Heavy metal pollution is a serious issue that we are facing nowadays. Due to its widespread distribution in water, air and soil and it is persistent and highly toxic to living organisms. Mercury is considered as one of the most hazardous heavy metal which has much distribution in the biota worldwide. The major sources of mercury include mining activities, paper and pulp industries, emissions from coal fired power plants, medical and municipal waste combustion etc. Mercury can exists in different forms like, metallic, inorganic and organic forms. It can cause several harmful health effects in humans from behavioural and neurological disorders, damage to brain, kidneys, endocrine system and central nervous system. Presently there are several methods for the detection of
mercury but most of them needs sophisticated instruments and are highly expensive and time consuming. Therefore, it is important to detect mercury in both biological and environmental samples. There are several studies reported regarding the identification and detection of mercury using silver nanoparticles [3-5] only a few efforts are there towards the naked eye detection of metals. Here we have attempted to make silver nanoparticles from fresh leaf extract of Vitex negundo, a medicinal plant in South India. The optical sensor that we have developed is highly significant as it is ecofriendly, highly selective, less time consuming and cost effective. It has been well known that silver nanoparticles exhibits excellent antimicrobial properties for different types of microorganisms like, fungi, virus and bacteria [6-9]. This work is an attempt for the facile synthesis of silver nanoparticles and exploring the possibility of developing chemosensors for mercury detection and its anti larvicidal potential against mosquito larvae.

2. Materials and Methods

2.1 Details of materials used
The plant used for the present work was Vitex negundo, is an aromatic shrub, commonly known as five leaved chaste tree, widely used in folk medicine and a native of South East Asia. This species belongs to the Vitex genus and the Lamiaceae family. The genus consists of 250 species of which about 15 species are found in different parts of India. (Figure.1). All the reagents and chemicals used was purchased from Sigma–Aldrich, India were employed for the analysis.

2.2 Preparation of the extract
Fresh unmodified leaf extract from Vitex negundo was used for the preparation of the silver nanoparticles. Fresh leaves of the medicinal plant were collected from the premises of the University Campus at Kollam district, Kerala. The leaves were washed thoroughly with running tap water for the removal of adhering debris and followed by washing with distilled water. Five gm of fresh leaf samples were taken and boiled with 25mL double distilled water for 10 minutes. The extract was cooled and filtered through with Whatman no: 1 filter paper and the extract was stored at room temperature for further detailed studies [10-11].

2.3 Preparation of Silver Nanoparticles
The plant extract prepared from the V. negundo was mixed with 25mL of 1millimolar silver nitrate solution. The reduction of Ag⁺ to Ag⁰ was observed initially by the colour change of solution from colourless to brown colour, which was further confirmed by UV -Visible spectroscopy.

2.4 Heavy metal detection studies
Known amount of solutions with different metal ions like $K_2Cr_2O_7$, $NiSO_4\cdot6H_2O$, $Pb(NO_3)_2$, $HgCl_2$ are made in 100ml de ionized water. Silver nanoparticles prepared following green route was taken for studying the interaction with different metal ions. The colour change obtained was monitored visually and the photographs were taken using digital camera at equal time intervals [12-13].

2.5 Anti-larvicidal Analysis

The larvae of *Aedes aegypti* were obtained manually from the adjoining water bodies and were raised in plastic containers filled with tap water and was accustomed to the lab conditions at STP. The mosquito larvae were fed with 15% sucrose solution. The bioassays were executed with the fourth instar of mosquito larvae as per the standard guidelines prescribed by WHO [14]. Different concentrations of *Vitex negundo* leaf extracts (1, 2, 3, 4, 5 mg/L of extracts) were taken in beakers containing 100 mL of double-distilled water. For performing the larvicidal studies of the mosquito larvae, about 20 larvae were introduced in to the container. For each concentration tried, five trials were constituted and each trial entails of five different replicates and a control (with aqueous *Vitex negundo* leaf extract) were analysed for its anti-larvicidal activity.

The *A. aegypti* larval mortality rates were calculated to find out the acute toxicity levels on the mosquito larvae at different intervals of 1, 3, 6, 12, 16 and 24 hours of contact. The mortality rate of the larvae was assessed from the initial hour of exposure to the silver nanoparticles prepared and the death percentage was reported as the average of five duplicates performed in the study. The death rate of larvae were calculated as per by the formula prescribed by Abbott [15].

3. Results and Discussion

The silver nanoparticles were synthesised from *Vitex negundo* leaf extract following green pathway. The leaf extract prepared was used as the reducing, oxidising and stabilizing agent. The reduction of silver nitrate into zero valent metallic silver nanoparticles using the plant extract was examined by UV-visible spectrometer and the Surface Plasmon Resonance (SPR), the distinguishing phenomenon exhibited by silver nanoparticles were used to confirm the occurrence of nano silver in the extract. The UV spectrum of nano particles synthesized by the *Vitex negundo* leaf extract is as presented in Figure 2. The colour change in the solution as a result of silver nanoparticle formation is given in Figure 3.

For the development of biosensor, the nanoparticles were prepared from the *V. negundo* leaf extract and millimolar solution of silver nitrate.

Fresh unmodified silver nanoparticles synthesised from *V. negundo* leaf extract was mixed with some heavy metal solutions like $HgCl_2$, $Pb\ (NO_3)_2$, $NiSO_4$, $CdSO_4$ and which exhibits instant colour change was as given in the figure 4. The change in the colour can be directly linked with the spectral studies, but an instant change that can be noticed for an insidious metal mercury is highly noteworthy. This result is an important phase towards the development of biosensors coupled with the naked eye detection of the highly toxic element mercury in the liquid phase.

The percentage death rate of *A. aegypti* larvae with different concentrations of silver nanoparticles prepared using the *Vitex negundo* leaf extract is as given in the figure 4. It is obvious that at minor concentrations, the mosquito larvae needs more exposure period for death but as the concentration seems on increasing it is evident that more death rate is observed at lower time of exposure. The better results were attained with medium to higher concentrations of nano silver and instantaneous death was witnessed as compared to the control. The silver nanoparticles prepared using *Vitex negundo* leaf extract seems very effective to be used as eco-friendly and cost effective mosquito destructive substance. This property can be credited to the characteristic capacity of silver nanoparticles to pierce easily through the cell wall of the *A. aegypti* larvae and finally results in the death of the cell.
Figure 2 UV spectrum of silver nanoparticles prepared using *Vitex negundo* leaf extract.

Figure 3 Silver nano particle formation from *Vitex Negundo* leaf extract.

Figure 4 The Colour change observed when *Vitex negundo* leaf extract facilitated synthesis of nanoparticles interaction with selected metal salt solutions.
Figure 5 Percentage mortality of mosquito larvae after exposure to different concentrations of nano silver prepared using Vitex negundo leaf extract

4. Conclusion

The present study is an exceptional method towards the synthesis of silver nanoparticles from Vitex negundo leaf extract following green pathway. The work attempts naked eye recognition of an insidious pollutant mercury in a very cost effective and environmentally sustainable manner. Moreover the A. aegypti mosquito larvicidal toxicity produced by the nanoparticles was also estimated following standard methods. The silver nanoparticles prepared using Vitex negundo leaf extract seems very effective to be used as eco-friendly and cost effective mosquito destructive material.

5. References

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