Bio-mediated Synthesis of Silver Nanoparticles Using Fruit Extract of Ananas Comosus L. Merrill (Pineapple)

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ABSTRACT

Silver is an agent used for different wounds and ulcer treatment as it is nontoxic. However, silver in an ionic or nanoparticles form is highly toxic to microorganisms. Hence, silver nanoparticles has wide range of applications than silver ion. Over the physical and chemical methods green synthesis is Eco-friendly and cost effective. The present study reveals the formation of silver nanoparticles by using the fruit extract (Ananas comosus) by observing the colour change. The produced nanoparticles are characterized by the physicochemical techniques, X-ray diffraction, UV-Visible and antimicrobial activity. The diffraction peaks attributed to 2θ values of 38.11° and 44.27° (111, 200) reveals the formation of silver nanoparticles. UV-Vis spectrophotometer shows surface plasmon resonance (SPR) at 459nm. The antibacterial studies promise the formation of silver nanoparticle with the ability to inhibit growth of Escherichia coli.

Keywords: Antimicrobial activity, Silver nanoparticles, Bio-synthesis, Fruit extract, Ananas comosus, Polyphenols.

INTRODUCTION

Nanotechnology is the interesting and important field of research. It has various range of application in optics, bio-labeling, medicine, biology1, agriculture, cosmetic, defense, catalysis1-2, sensors, environmental safety, food, drug delivery3, health, biomedical4, pharmaceuticals, therapeutic agents5. It is witnessed that AgNPs were used for color and dyes removal as methyl orange and methylene blue from aqueous solutions6. AgNPs are used in the treatment of wounds7. The physical and chemical methods involved for the synthesis. Whereas, the methods were reported the production of toxic byproduct and are expensive. Among the various metal nanoparticles, AgNPs is been paying more attention from last few years owing to the potential and variety of exclusive properties like high electrical conductivity, chemical stability, antimicrobial activity8-9. Preparation of controlled size
nanoparticles is important task. In many techniques like reduction of metal with some stabilizing agents are necessary. Recently, advanced and novel methods which are also called as green biological route or biosynthesis has been developed for the synthesis of metal nanoparticles from various plant extract. Particularly Murraya koenigii, Petroselinum crispum, Coriandum sativum (coriander), Ocimum10, Moringa oleifera, Brassca juncae (mustard greens)11 and S. virginianum fruit, Dioscorea batatas rhzome extract12. The synthesis of Nanoparticles by chemical method requires capping agent these are very hazardous13-14 as it generates various gases, energy (exothermic), consumes various type of energy, tedious, time consuming, and requires many synthetic steps15-18. A green synthesis method is more advantageous over to chemical method, as it consumes less energy, less time, single step, cost effective, simple and easily available precursors19-21. Furthermore, amongst many biological methods of synthesis such as by using microorganism, single cell, plant tissues, the fruits or plant leaf, flowers, seed extract is very simple and cost-effective approach of the synthesis. Plant extract play dual role as reducing agent and stabilizing agent22-24 as well.

In the present paper we report the use of Ananas comosus L. Merrill of Bromeliaceae family, which is a large and diverse. This family consists of two thousand species and it is subtropical fruit. Pineapple has most important property as antioxidant. The content of Ananas comosus includes mixture of protease which acts as a nutritional supplement to promote digestive health and is used as an anti-inflammatory agent. Main content of fruits and vegetables is phenolics which is important bioactive compound for the benefit to health24. Polyphenols presents in fruits possess antioxidant and free radical scavenging properties, which play vital protective role in human25-26. Specifically contains thiamine, riboflavin, niacin, oxalic acid, sucrose, glucose, fructose and several amino acids. Here these are reducing agent.

In this paper we propose the synthesis of silver nanoparticles by using Ananas comosus fruit extract. The synthesized silver nanoparticles were characterized by X-ray diffraction27, spectroscopic and anti-microbial activity27-31. This might be scaled up for large-scale production possible. The scope of paper was only silver nanoparticles by bio-mediated synthesis.

MATERIALS AND METHODS

A fresh Ananas comosus fruit was collected from local market to obtain the fruit extract. The ripened 200 g of Ananas comosus fruit was washed with distilled water and then filtered through Whatman No. 42. A. R. grade silver nitrate (AgNO3) was used as source of silver.

PREPARATION OF FRUIT EXTRACT

Fresh and clean pieces of Ananas Comosus fruit were grinded in mixer grinder and filtered through Whatman No. 42. The filtered fruit juice was further centrifuged in hand centrifugal machine for one hour and again filtered through Whatman No. 42 to obtain the clear extract. This extract was preserved in refrigerator.

Preparation of silver nitrate solution

The weighed amount of silver nitrate was transferred in a beaker and dissolved in minimum amount of water. It was transferred to 50 mL volumetric flask and made the volume 50 mL by using distilled water. The resultant solution was stirred and temperature of the solution was maintained between 50-60ºC. The addition of fruit extract to silver solution continued as shown in Table 1. The silver nanoparticles were formed in the suspended form in the solution. These are dried in the same test tube at room temperature.

| Sr. no. | Volume of stock solution | Volume of distilled H2O | Volume of fruit extract | Final conc. of AgNO3 |
|---------|--------------------------|------------------------|-------------------------|---------------------|
| 1       | 0.4 mL                   | 1.6 mL                 | 2 mL                    | 5mM                 |
| 2       | 0.8 mL                   | 1.2 mL                 | 2 mL                    | 10mM                |
| 3       | 1.6 mL                   | 0.4 mL                 | 2 mL                    | 20mM                |
| 4       | 2.0 mL                   | 0.0 mL                 | 2 mL                    | 25mM                |
RESULT AND DISCUSSION

The addition of *Ananas comosus* broth to aqueous solution of AgNO₃ the reaction mixture becomes colorless (Fig. 1). Subsequently significant change in color was observed after 45 min (Fig. 1 and 2) and remarkable changes were observed after 24 h (Fig. 3). Silver nanoparticles were successfully synthesized through a simple, rapid and green route using *Ananas comosus*. The method is very cheap, hence AgNPs may be used in the production of soaps, cloths, plastics, medical bandages, water purification. This was advantageous over the other route also.

**Fig. 1. Starting color of the reaction mixture**

**Fig. 2. Colour of reaction mixture after 45 minutes**

**Fig. 3. Colour of reaction mixture after 24 hours**

**X-ray diffraction**

The X-ray diffraction pattern shows formation of AgNPs (Fig. 4). The two intense peaks are attributed to the formation of AgNPs in the spectrum of 2θ values ranging from 20° to 80°. A comparison of our XRD spectrum obtained with the standard data confirmed for formation of silver particles as evidenced by the peaks at 2θ values of 38.11°, and 44.27° (111, 200). These peaks are matching with the face centered cubic structure (fcc) of silver nanoparticles (JCPDS card no. 04-0783). Furthermore, it is observed that there is formation of nanoparticles only if the concentration of silver nitrate solution is 20mM and 25 mM. It is evident that higher concentration of silver nitrate stock solution leads the formation of silver nanoparticles.

**Fig. 4. XRD spectrum of 1) 5mM, 2) 10mM, 3) 20mM, 4) 25mM concentration of AgNO₃**

**SEM**

The measurements of SEM were used to determine the structural of biosynthesized AgNPs. The images of SEM of silver nanoparticles were depicted in Fig. 5. It depicts the formation cubic silver nanoparticles. In sight of Fig. 5-a, b, c, shows the cubic silver nanoparticles, whereas the Fig. 5-d demonstrates the cauliflower like structure which was milled to smaller size.

**Fig. 5. SEM images of 5mM (a), 10mM (b), 20mM (c), 25mM, (d) concentration of AgNO₃**

**Antibacterial activity**

**Observation of zone of inhibition**

The silver nanoparticles have antimicrobial activity properties in medicine. The biologically synthesized silver nanoparticles by using fruit extract were found highly inhibitive against different pathogenic bacteria. It exhibits antifungal action against different fungi and bacteria.

**Fig. 6. Inhibition Zone of silver nanoparticle 5mM (a), 10mM(b), 20mM(c), 25mM(d) concentration of AgNO₃**
The antibacterial activities of *Ananas comosus* stabilized silver nanoparticles against *Escherichia coli* were evaluated. The image (Fig. 6) shows the zone of inhibition and it was effective against bacterial strain for this study. The diameter of inhibition zone was recorded by ruler as 3.2 cm and compared with standard AgNPs. Due to presence of silver nanoparticles as a drug that impedes their growth revealed in Fig. 4. From these results, silver nanoparticles found to be more active against the bacteria. The biosynthesized AgNPs may be potential antimicrobial agent for future.

**UV-Vis Spectroscopy**

The representative optical absorbance was recorded on UV-Vis spectrophotometer in 200-800nm wavelength range (Fig. 7). After addition of *Ananas comosus* fruit extract to the aqueous solution of AgNO₃ of different concentrations, the mixture showed a gradual change in color at room temperature with time from yellowish to brown and then color intensified after 24 h to dark brown. The color is characteristic property of the Surface plasmon resonance (SPR) of silver nanoparticles. The reduction of silver ion to silver nanoparticles was reflected in spectral data obtained by using a UV-Vis spectrophotometer. Absorbance peak around 459 nm was shown for representative sample 5mM, which in turn confirms silver nanoparticles.

**CONCLUSION**

Silver nanoparticles are prepared by using simple, single step, cost effective, reliable and environment friendly green synthesis method. In chemical methods different reducing chemicals used are associated with toxicity and environmental hazards; therefore plant mediated synthesis of AgNPs is advantageous over other traditional methods. The present AgNPs have X-ray diffraction peaks at 38.11°, and 44.27° confirms the formation of silver nanoparticle having antimicrobial activity. The intensity of SPR band at 459nm indicates the formation of silver nanoparticles. It also concludes that higher concentration stock solution of silver nitrate is favorable for the formation of silver nanoparticle.

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**Conflict of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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