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The Cytotoxic Effect of Silver Nanoparticles Derived from Amla Fruit Seed Extract

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ABSTRACT

INTRODUCTION : Silver metal has a variety of applications in nanoparticles. AgNPs are most frequently used for their medical, food-packaging, antimicrobial, anti-cancer, and wound-healing capabilities. Although spherical nanoparticles are typically utilised, thin sheets, diamonds, and octagons are other frequent geometries of AgNPs.

AIM : The study's objective was to investigate the cytotoxic effects of amla fruit seed-derived silver nanoparticles (AgNPs).

MATERIALS AND METHODS : To gather and synthesise amla fruit seed extract - *To produce Silver Nanoparticles using amla fruit seed extract *Brine shrimp lethality assay was used to test the cytotoxic effects of produced AgNPs.

RESULTS : The green hue of the plant extract combines with the silver nitrate solution to produce brown colour, which denotes the formation of AgNPs. The results of the present investigation show that the graph peaked at a wavelength of 460 nm. As a result, it can be inferred from the present study that the amount of cytotoxicity was reduced by half as we used less concentration. Therefore, if the concentration is greater than 50%, it can be applied to biomedical purposes.

CONCLUSION : As a result, it can be inferred from the present research that biosynthesized AgNPs from amla seed fruit yields promising outcomes for biological applications. The synthesis of AgNPs from amla fruit seed extract was demonstrated by an absorption peak at 460 nm in the UV-vis spectrum.

KEY WORDS : Amla fruit ; Amla seed extract ; Silver nanoparticles ; cytotoxicity ; nauplii

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INTRODUCTION

Silver is considered a precious metal and has a variety of applications in its nanoparticle form. The most common applications of silver nanoparticles (AgNPs) are for medicinal, food packing, plasters, antibacterial purposes, anti-cancer, and wound healing properties[1]. Awe-inspiring improvements have been made in the field of nanoparticles since the time its under the limelight. It has a unique optical, electrical, and thermal property. In general, nanoparticles are used in a spherical shape, but diamond, octagonal, and thin sheets are also popular in common shapes of AgNPs[2]. The size and shape of metal nanoparticles are typically measured by analytical techniques such as transmission electron microscopy, scanning electron microscopy or atomic force microscopy[3].

Humans have also experimented with AgNPs in which the main routes to penetrate into the human body are gastrointestinal tract, respiratory tract as well as skin[4]. However, AgNPs use in human cells are still under investigation. Nanoparticles are applied in numerous technologies such as diagnostic, antibacterial, conductive, and optical[5]. Cytotoxicity in AgNPs plays a major role against cancer cell lines. Cytotoxicity roughly translates to a property which means being harmful to a cell. Compounds that have cytotoxic effects often compromise cell membrane integrity[6]. Most cytotoxic T cells express receptors that can recognize a specific antigen. An antigen is a molecule capable of stimulating an immune response and is often produced by cancer cells or viruses[7]. In the treatment of cancer, chemotherapy is undergone to kill or damage the cells which are reproducing [8].In this present analysis, we have used amla fruit seed for the grain synthesis of AgNPs and the synthesised nanoparticles were characterised using ultraviolet. Preparation and application, experience in other research field related to many studies were done previously.[9–17]

MATERIALS AND METHODS

Preparation of Plant Extract

Amla fruits were collected from Chennai. The collected fruits were washed 3–4 times using distilled water and then dried in the shade for 7–14 days. The well-dried fruits and seeds were made into powder using mortar and pestle. The powder was collected and stored in a sealed container. One gram of amla powder was dissolved in distilled water and boiled for 5–10 min at 60–70°C. The solution was filtered using Whatman No. 1 filter paper. The filtered extract was collected and stored at 4°C for further use.

Synthesis of Nanoparticles

1 Mm of silver nitrate dissolved in 90 mL of double distilled water. The fruit extract of amla [Figure 1] was added with the metal solution and was made into 100 mLsolution. The colour transformation was seen visually, and pictures were taken to document it. The solution is kept in magnetic stirrers for nanoparticle synthesis(Menon et al. 2018; Mueez et al. 2022). Characterization of Nanoparticles The synthesised nanoparticle’s solution is preliminarily characterised using UV-vis-spectroscopy. 3 mL of the solution is taken in curettes and scanned in double beam UV-vis-spectrometer from 300 nm to 700 nm wavelength. The outcome was recorded. Graphs and tables were formatted for analysis.

Preparation of Nanoparticles Powder

The nanoparticle’s solution is centrifuged using a lark refrigerated centrifuge. The solution in AgNPs is centrifuged at 800 rpm for 10 m and the pellet is collected and washed with distilled water twice. The final purified pellet is collected and dried at 60°C for AgNPs for 24 hours.

The powdered nanoparticles are then collected and placed in a sealed Eppendorf tube for storage. Eggs for the BSLA brine shrimp were procured from Chennai's Aquatic Remedies. For the purpose of hatching the shrimp eggs, filtered, artificial saltwater was created by combining 36 g of sea salt with 1 l of distilled water. The seawater was placed in a little plastic container with a barrier separating the light and dark (covered) sections (the hatching chamber). The chamber's dark side was added with shrimp eggs, and the light from the bulb above the chamber's other side will draw the shrimp hatchlings. The shrimp were given two days to hatch and develop into nauplii (larva). When the shrimp larvae were ready after two days, 5 mL of the synthetic seawater and 5 mL of the nanoparticles solution were injected to each test tube, followed by 10 brine shrimps [Figure 2]. There were thus 30 shrimps altogether per dilution. Up to 10 mL of synthetic saltwater each test tube serves as the control. Under the illumination, the test tubes were left unattended. After 24 hours, the number of shrimps that had survived were counted and noted. The 95 percent confidence intervals for the lethality concentration (LC50) were calculated using probit analysis. [Figure 3]. LC50 of <100 ppm was considered as potent (active). As mentioned by Meyer and others, LC50 value of <1000 μg/mL is toxic while LC50 value of >1000 μg/mL is non-toxic. The percentage mortality (%M) was also calculated by dividing the number of dead nauplii by the total number and then multiplied by 100%. This is to ensure that the death (mortality) of the nauplii is attributed to the compounds present in the nanoparticles.

RESULTS AND DISCUSSION

Visual Observation The plant extract color is green and it reacts with silver nitrate solution and forms brown color and indicates the synthesis of AgNPs [Figure 4]. When the current study compared with the study of Kumar et al. in 2017, 2018, it was noted that the results of the current study correlate with the study of his article[1819]. UV-Vis Spectroscopy UV-spectra of AgNPs synthesized with and without amla fruit extract at room temperature. It is generally recognized that UV-Vis spectra could be used to examine the size and shape-controlled nanoparticles in aqueous solution with 200–800 nm wavelength range.

The AgNPs have a characteristic band in the UV-Vis region due to their surface plasmon resonance(Elechiguerra et al. 2005). From the current study, it is seen that the graph reached its peak at a wavelength of 460 nm]. When the recent study was compared with the study done by Karthick et al., using Garcinia mangostana, it was noted that his results were at a wavelength of 420 nm(S. Rajeshkumar and Malarkodi 2014). Hence, from the comparison, it is recorded that the cytotoxic effect of AgNPs can be synthesized using amla fruit seed extract. Cytotoxic Effect From the brine shrimp lethality test done it is noted that on the 1st day five of nauplius survived, while on day 2 it got decreased to three nauplius, and on day 3 only one nauplii remained to survive [Figure 5]. As far, the concentration of the nanoparticles increased the toxicity decreased and nauplius survived(Kim et al. 2006). When the concentration of nanoparticles decreased, the toxicity increased, and nauplius died(Kim et al. 2006; Arulvasu et al. 2014). As a result, it can be seen from the current study that the toxicity was reduced by half since we utilized a lower concentration. Hence, if the concentration is above 50%, it can be used for biomedical applications.

CONCLUSION

Hence, from the current study, it is noted that biosynthesization of AgNPs from AgNPs show promising results for biomedical applications. An absorption peak at 460 nm in the UV-vis spectrum proved the formation of AgNPs from amla fruit seed extract. The benefits of nanoparticles to medicine are numerous. Therefore, in recent trends, the combination of green chemistry with nanotechnology is one of the most challenging researches at the nanoscale. Hence, with low cost and more benefits, this study can be useful in the field of nanomedicine for the upcoming generation.

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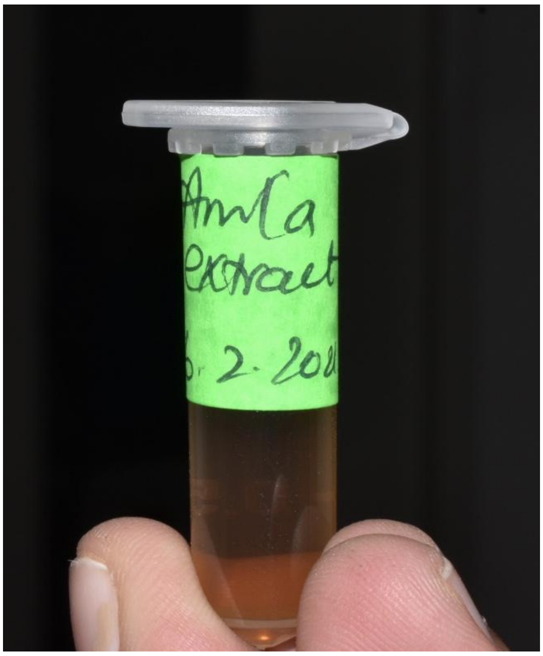

Figure 1 : Amla fruit seed extract

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Figure 2 : Brine shrimp

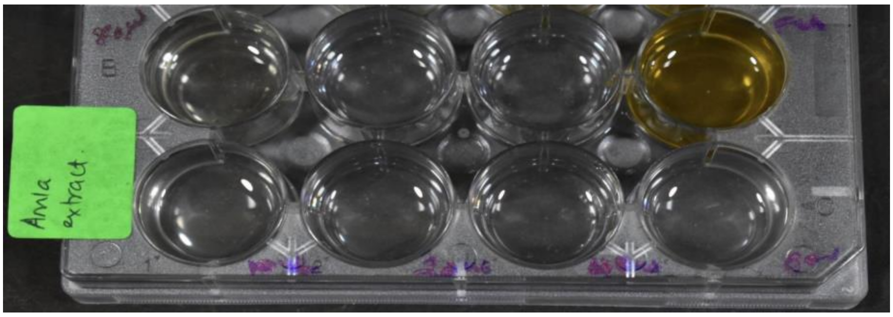

Figure 3 : Cytotoxicity analysis of brine shrimp

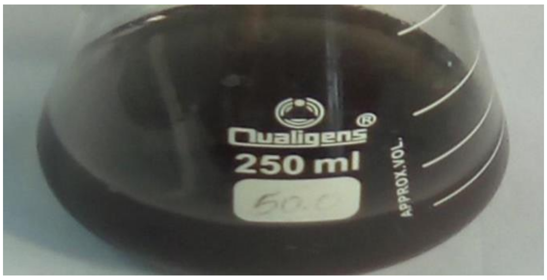

Figure 4 : visual observation of AgNPs synthesis

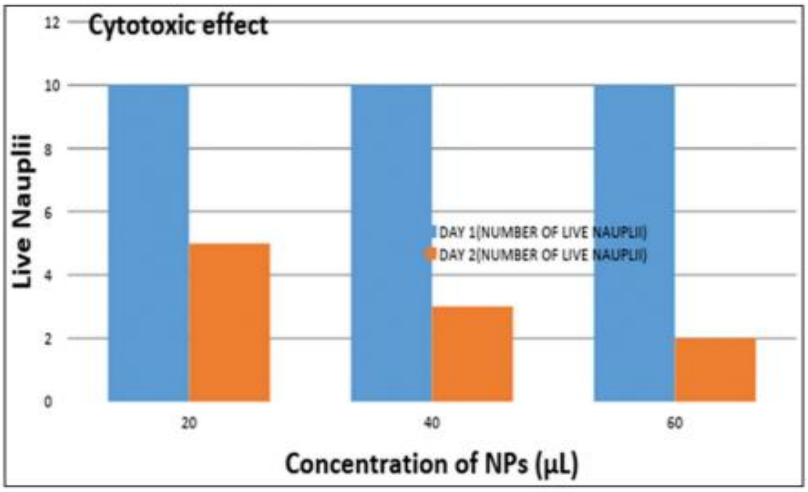

Figure 5 : cytotoxic effect of amla fruit extract derived silver nanoparticles