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Green Synthesis Of Gold Nanoparticles Using Kalanchoe Pinnata and Its Free Radical Scavenging Activity

Research Article

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Abstract

The bio-synthesis of gold nanoparticles is achieved by the reduction of gold metal ions in interaction with the aqueous plant extract of Kalanchoe pinnata. An absorption peak of the biosynthesized gold nanoparticles is detected at the wavelength range of 350 – 700nm. The UV-Visible spectra of gold nanoparticles synthesized by Kalanchoe pinnata extract shows maximum absorption peak at 530nm. The morphological features of gold nanoparticles were analysed by Scanning Electron Microscope that confirms the spherical shape of gold nanoparticles. The elemental analyses of gold nanoparticles synthesized by Kalanchoe pinnata extract extract have the weight percentage of gold as 50%. The study reveals that the synthesized gold nanoparticles shows potential antioxidant activity.

Keywords: *Kalanchoe pinnata*; Green Synthesis; Gold Nanoparticle; Antioxidant Activity.

Introduction

Nanotechnology possess materials in 10-9 meter scales, including biotechnology, material sciences, computer sciences, medicines, pharmacy and engineering [1-4]. Nanoparticles (NPs) in crystalline and undefined structures (amorphous) got numerous considerations worldwide for their utilizations in numerous commercial applications, and powered many research centres to commit in creating and expanding different nano-applications [5-10]. Resulting upon their noteworthy nature, spherical and gold nanorods (Au NRs) nanoparticles attract extraordinary consideration [11-12].

The utilization of eco-accommodating materials like microorganisms, organisms, plants, and parts of plant materials and green synthesized gold nanoparticles (AuNPs) can be utilized in biomedical applications as they don't use harmful chemical compounds [13-15].

Kalanchoe pinnata is a miraculous plant that grows 3-5 feet tall. Regularly known as 'air plant,' it has tall hollow stems, dark green leaves that are particularly scalloped and trimmed in red, and bell like pendulous blossoms. It is a therapeutic plant utilized in folklore medication to treat kidney stones, gastric ulcer, respiratory disease, rheumatoid joint inflammation etc. The chemical compounds present in the plant include various classes, for example, alkaloid, diterpenoidal lactones, glycosides, steroids, phenolics, aliphatic mixes, and so forth. The remarkable pharmacological properties contain anti- diabetic, anti- neoplastic, antioxidant, immunomodulation, anti-lipidaemic, anti-allergic and many more activities [16].

In the present investigation, Kalanchoe pinnata plant extract was used to synthesize gold nanoparticles in green synthesis method. The synthesized gold nanoparticles were characterized using various spectroscopy and microscopic techniques and tested for antioxidant properties.

Materials and Methods

The chemicals used in this study such as chloroauricacid ($HAucl_4$), Mueller Hinton agar were purchased from Hi-media laboratories Pvt. Ltd, India. DPPH, ascorbic acid from Sigma Aldrich.

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Preparation of Kalanchoe pinnata Plantextract

Fresh leaves of Kalanchoe pinnatawere collected from garden in Katpadi town, Vellore. The Kalanchoe pinnataleaves were washed thoroughly and removed all the contaminants present on the leaves' skin with soap water followed by deionised water. The washed leaves were then air dried well to remove moisture from the leaves. The dried leaves were then crushed finely with the help of mortar and pestle to make it a fine powder. 1g of powdered Kalanchoe pinnata leaves were dissolved in 100mL of distilled water by slowly adding into the fine powder. The extract is heated at 80ºC for about 10 minutes using a heating mantle. The extract were filtered using No 1 Whattman filter paper and stored at cool and dry place for future usage.

Green synthesis and characterization of nanoparticles

To synthesize gold nanoparticles, 10mL of Kalanchoe pinnata extract was added into 90mL of 1mM aqueous gold chloride solution. The reaction mixture was kept in magnetic stirrer for 72 hours. The formation of gold nanoparticles was monitored periodically by measuring the UV-Vis spectra (450-600nm) of the gold chloride solution. The colour change of the gold chloride solution were observed and noted which preliminarily indicates the bio-reduction and formation of gold nanoparticles. Biosynthesized gold nanoparticles was collected by centrifugation method at 8000rpm for 10 minutes. The obtained gold nanoparticle pellet was washed with double distilled water for 3-4 times and then heated in hot air oven at 70°C for 2 hours. The powdered Kalanchoe pinnata mediated gold nanoparticles were stored in air tight vials for further studies.

The maximum absorbance of Kalanchoe pinnata mediated gold nanoparticles were measured by using double beam UV-vis spectrophotometer (uv-2450, Shimadzu) in the wavelength range of 450-600nm.The synthesized gold nanoparticles was subjected to test the elemental analysis using Energy dispersive X-ray detector (EDX) attached to the SEM machine. The Atomic force microscopy was used to analyse and provide the three dimensional image of Kalanchoe pinnata gold nanoparticles with sub-nanometer resolution.

Antioxidant activity

The DPPH (1,1-diphenyl-2-picryl-hydrazil) free radical assay of Kalanchoe pinnata mediated gold nanoparticles was performed by the technique reported in (Rajeshkumar, 2017). Different concentrations (2-10 µg/ml) of Kalanchoe pinnata extract intervened gold nanoparticles and plant extract was mixed with 1 ml of 0.1 mM DPPH in methanol and 450 µl of 50 mM TrisHCl buffer (pH 7.4) and incubated for 30 minutes. After incubation, the reduction in the amount of DPPH free radicals was evaluated based on the absorbance at 517 nm. BHT was used as control. The percentage inhibition was calculated from the following equation,

% inhibition = (Absorbance of control- Absorbance of test sample/Absorbance of control) × 100

Results and Discussion

Visual observation

The colour change during nanoparticle synthesis confirms the reducing and stabilizing ability of plant extract [17, 18]. Formation of light yellow to dark brown colour in the reaction mixture could confirm the presence of formation of gold nanoparticles which also denotes the ability of the Kalanchoe pinnata plant extract to reduce gold chloride into gold nanoparticles.

UV - Visible spectroscopy

The UV-Visible spectroscopy was used to characterize the Kalanchoe pinnata mediated gold nanoparticles to find structural properties of gold nanoparticles. The absorbance spectra range from 450-600nm and the reaction time (12hrs, 24hrs, 48hrs, 72hrs) of gold nanoparticles as shown in fig 2. The absorption peak of Kalanchoe pinnata intervened gold nanoparticles was obtained at 530nm which denotes the intense absorption in visible light

Figure 1. Visual observation Gold chloride solution and synthesized gold nanoparticles.

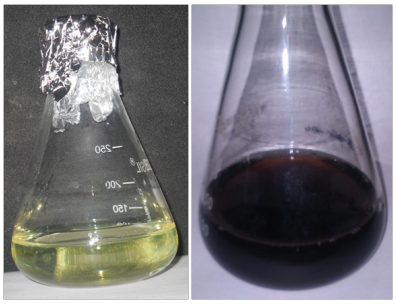


Figure 2. UV-Visible spectra image of Kalanchoe pinnata mediated gold nanoparticles.

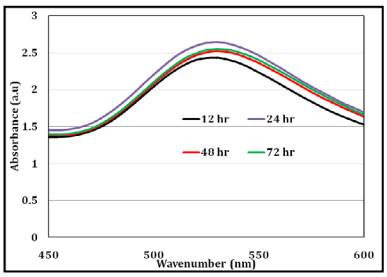


Figure 3. SEM &EDX spectrum of gold nanoparticles.

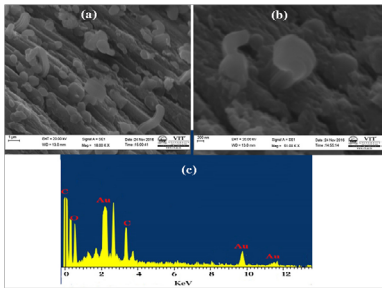
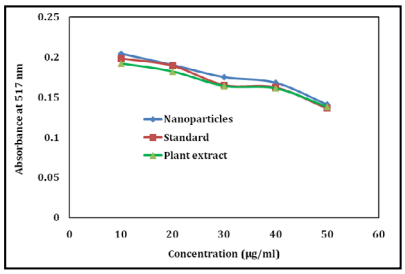


Figure 4. Antioxidant activity of gold nanoparticles by DPPH assay.



region. It was states the reduction and stabilization capability of Kalanchoe pinnata extract. The UV results of previous studies such as [19, 20] seems to be concurrent with this study.

Scanning Electron Microscope

The surface morphology of Kalanchoe pinnata mediated gold nanoparticles was analysed using SEM. Fig 3 (a&b) shows the SEM image of biosynthesized gold nanoparticles and found the shape to be spherical, polydisperse in nature. The size of the gold nanoparticles was found to be 65 nm.

The EDX measurements were done to evaluate the elemental composition of the gold nanoparticles (fig 3 C). The EDX spectra shows three strong peaks of metallic Au. It also affirms that the Kalanchoe pinnata leaf extract can be effectively used in the biosynthesis of gold nanoparticles. Early works such as [21, 22] found to be concordant with the SEM-EDX results of Kalanchoe pinnataintervened gold nanoparticles.

Antioxidant Activity

The *Kalanchoe pinnata* intervened gold nanoparticles was tested for antioxidant activity by using DPPH method. Fig 4 shows the scavenging activity of synthesized gold nanoparticles, Standard (Ascorbic acid) and Kalanchoe pinnata extract. Various concentrations 10µL, 20 µL, 30 µL, 40 µL, 50 µL of Kalanchoe pinnata mediated gold nanoparticles and kalanchoe extract was used. The results showsthe antioxidant property of synthesized gold nanoparticles increases in dose dependent manner and also remains equal to the standard antioxidant values. The plant extract also showed substantial antioxidant effect against the free radicals. However, the synthesized gold nanoparticles shows higher absorbance that implies more inhibition with 85% of scavenging activity than Kalanchoe pinnata plant extract. The antioxidant activity of gold nanoparticles were also reported in earlier studies such as [23, 24].

Conclusion

This study is an eco-friendly approach of the synthesis of gold nanoparticles using Kalanchoe pinnata plant extract. The extract exhibit the properties of both reducing and stabilizing agent owing to the presence of different compounds in the plant extract. Synthesized gold nanoparticles were initially identified by a formation of dark brown colour and UV–Visible spectrophotometer analysis exhibits the surface plasmon resonance band at 530 nm. SEM image affirms the size, shape and distribution of nanoparticles. In this study the amplified antioxidant activity was found to be at an increased concentration of gold nanoparticles. The biosynthesized gold nanoparticles were competent in the biomedical applications in treating various diseases for their high antioxidant activity.

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