



Eco-friendly synthesis of silver nanoparticles using the plant *Kalanchoe gastonis-bonniieri* and its antimicrobial activity

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ABSTRACT

Ecofriendly synthesis of silver nanoparticles was achieved using the extract of the plant *Kalanchoe gastonis-bonniieri* by a simple method. The plant extract acts both as reducing agent as well as capping agent. UV –Visible spectroscopy was used to confirm the formation of silver nanoparticles. The FT-IR spectroscopy was used to identify the compounds responsible for the reduction of silver ions. The obtained nanoparticles were also characterized by XRD, SEM and EDAX analysis. The synthesized silver nanoparticles exhibit good antimicrobial activities.

Key words : Ecofriendly synthesis, Silver nanoparticles, Antimicrobial activity.

1.INTRODUCTION

Nanochemistry is the combination of chemistry and nanoscience. Nanochemistry is associated with synthesis of building blocks which are dependent on size, surface, shape and defect properties. Nanochemistry is being used in chemical, materials and physical science as well as engineering, biological and medical applications. Green chemistry is the design of chemical products and processes that reduce or eliminate the use or generation of hazardous substances. Nanoparticle research is currently an area of intense scientific research, due to its wide variety of potential applications in biomedical, optical and electronic fields. Green synthesis is an emerging area in the field of bionanotechnology and provides economic and environmental benefits as an alternative to chemical and physical methods [1]. Silver nanoparticles (Ag-NPs) are increasingly used in various fields including medical, food, health care, consumer and industrial purposes due to their unique physical and chemical properties. These include optical, electrical and thermal conductivity and biological properties [2-4]. Interestingly, biologically prepared Ag-NPs show high yield, solubility and high stability [5]. To evaluate the synthesized nano materials many analytical techniques have been used



including Ultraviolet Visible spectroscopy (UV-vis spectroscopy), X-ray Diffractometry (XRD), Fourier Transform Infrared Spectroscopy (FT-IR), X-ray Photoelectron Spectroscopy (XPS), Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Atomic Force Microscopy (AFM) and so on [6]. The biological activity of Ag-NPs depends on factors including surface chemistry, size distribution, shape, particle morphology, particle composition, coating/capping, agglomeration and dissolution rate, particle reactivity in solution, efficiency of ion release and the type of reducing agents used for the synthesis of Ag-NPs are a crucial factor for the determination of cytotoxicity [7]. Based on this approach, we have reviewed the green chemistry type of Ag nanoparticles synthesis process. We have synthesized stable Ag-NPs by the reduction of aqueous silver nitrate by the extract of the leaves of the plant *Kalanchoe gastonis-bonnieri*. The leaves have been used in traditional system of medicine for the treatment of ovarian cysts, urinary tract infections, vaginal infections and uterine fibroids [8]. There are several greener routes to synthesis of nanoparticles of zerovalent metals, metal oxides and salts emphasizing recent developments [9]. Synthesis of metallic nanoparticles using plant extracts is inexpensive, easily scaled up and environmentally benign [10].

2.Materials & Methodology

2.1.Preparation of extract:

The fresh leaves of *Kalanchoe gastonis-bonnieri* were collected and washed well under running tap water followed by sterile distilled water. The leaves were cutted by using a knife and small quantity of leaf pieces were taken in a 250mL conical flask along with 200mL of deionized water. The extract was prepared by boiling the mixture in a heating mantle for 30 minutes. Then the extract was cooled and filtered by using Whatmann No: 1 filter paper.

2.2.Green synthesis of Ag-NPs using leaf extract:

20mL of the prepared plant extract was added to 80mL of 1mM silver nitrate solution in a conical flask. Immediately the mixture turned into turbidity indicating the initiation of the reaction. The reaction was carried out at room temperature. After 15 minutes the colour of the mixture turned into brown colour which shows the formation of Ag nanoparticles. Then the mixture was put aside for 6 hours. The Ag-NPs thus formed were collected by centrifugation.

2.3.Antibacterial activity:



The Sample loaded disc was placed on the surface of Mullar-Hinton medium and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimeters.

2.4.Antifungal activity:

Antibiotic susceptibility tests were determined by agar disc diffusion (Kirby–Bauer) method. Up to 40 µl of each concentration of the extract were respectively introduced in the sterile discs using sterile pipettes. The disc was then placed on the surface of SDA (Sabouraud Dextrose Agar) medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 22°C for 48 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimeters.

3.Results and Discussions

The present investigation demonstrates the formation of the Ag nanoparticles by the reductions of the silver metal ions during exposure to the leaves extract of the plant *Kalanchoe gastonis-bonnieri*. The result discloses that the formation of Ag nanoparticles occurs within 15 minutes of the reaction time at room temperature. Initially the leaf extract was pale white in colour. After the addition of the extract in AgNO₃ the mixture becomes turbid in colour which after becomes brown in colour within 15 minutes, indicates the formation of Ag nanoparticles. This colour is primarily due to the Surface Plasmon Resonance of silver nanoparticles. In case of negative control (leaf extract alone) no change in colour was observed.

3.1.Characterization of synthesized Ag-NPs from *Kalanchoe gastonis-bonnieri* leaf extract

3.1.1.UV-Visible Spectroscopy

The formation of Ag nanoparticles was confirmed by UV-Visible spectroscopy. The respective Surface Plasmon Resonance peaks were recorded between 200-800nm. There is no evidence of absorption for the plant extract and there is a distinct absorption for the synthesized Ag nanoparticles at around 431nm (Fig:1) which confirms the formation of Ag-NPs.

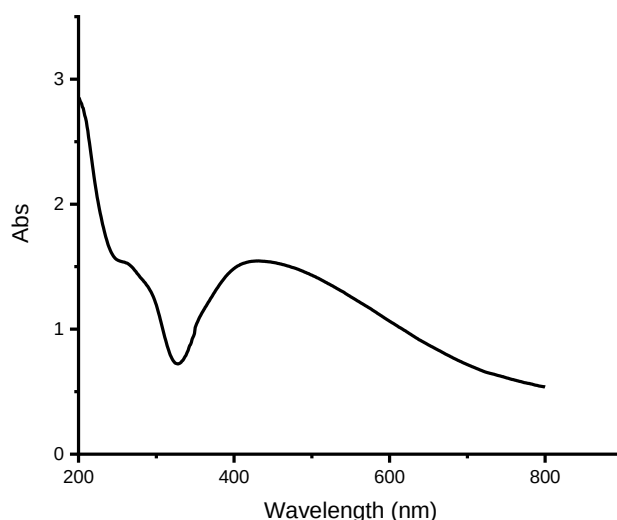


Fig:1 UV-Vis absorption spectra of Ag-NPs synthesized by *Kalanchoe gastonis-bonnieri* leaf extract

3.1.2.FT-IR Spectroscopy

The FT-IR transmittance of synthesized Ag-NPs from the plant *Kalanchoe gastonis-bonnieri* leaf extract shows the possible biomolecules responsible for capping and stabilization of nanoparticles. The FT-IR spectrum of synthesized Ag-NPs shows intense bands at 3451, 2092, 1638 and 516 cm^{-1} . The broad intense band obtained at 3451 cm^{-1} is due to the O-H stretching of an alcohol. A medium peak obtained at 2092 cm^{-1} is due to the C=C=C stretching of an allene. A sharp peak obtained at 1638 cm^{-1} is due to the strong C=C stretching of an alkene. A broad peak obtained at 516 cm^{-1} is due to the stretching frequency of an alkyl bromide.

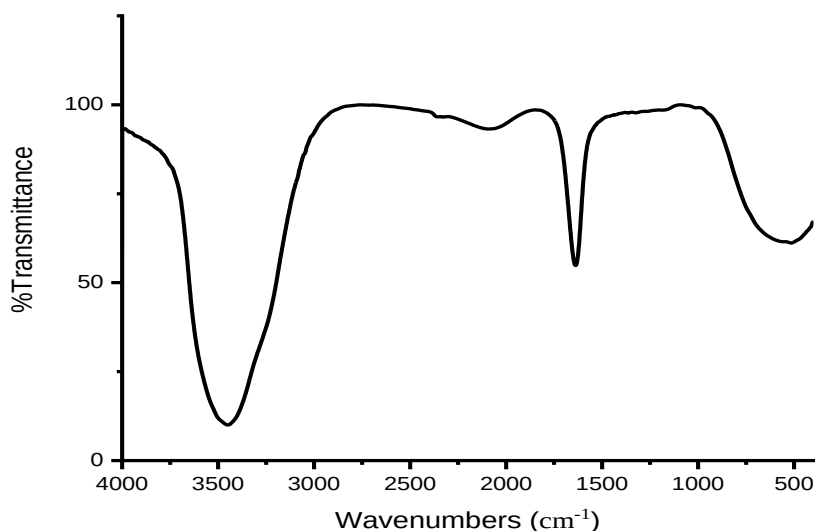


Fig:2 FTIR spectrum of Ag-NPs synthesized using Kalanchoe gastonis-bonnieri leaf extract

3.1.3.X-ray Diffraction (XRD)

The XRD pattern of synthesized Ag-NPs using the leaf (Kalanchoe gastonis-bonnieri) extract is shown in the Fig:3. Using the below given Debye-Scherrer formula the average crystallite size of the nanoparticles were calculated.

$$D = 0.9 \lambda / \beta \cos \theta$$

Where **0.9** is the shape factor,

λ is the X-ray wavelength,

β is the line broadening at half the maximum intensity (FWHM) in radians and

θ is the Bragg angle.

The average crystallite size of the Ag-NPs thus calculated were found to be 4nm in size.

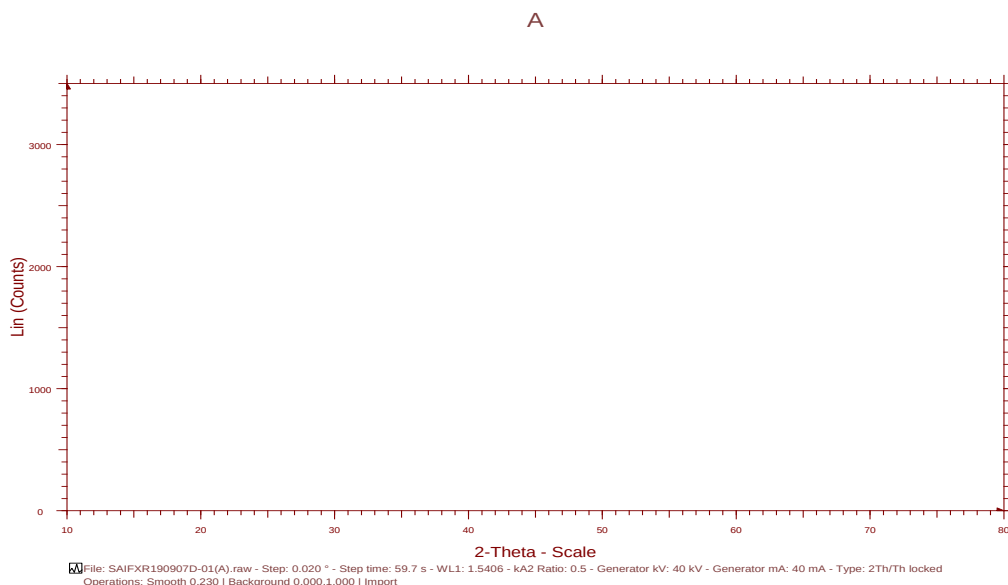


Fig:3 XRD pattern of Ag-NPs synthesized using Kalanchoe gastonis-bonnieri leaf extract

3.1.4.SEM Analysis

The morphology of the synthesized silver nanoparticles were characterized by Scanning Electron Microscope (SEM). SEM micrographs at different magnifications shows the synthesized Ag-NPs were cubical in shape (Fig:4) and they are found to be polydispersed.

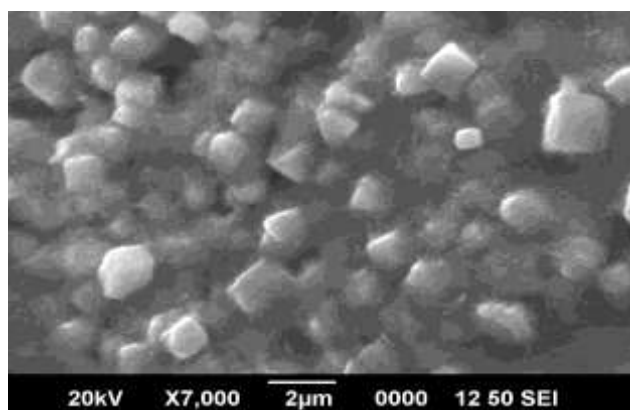


Fig:4 SEM image of Ag-NPs synthesized using Kalanchoe gastonis-bonnieri leaf extract

3.1.5.EDAX Analysis



Energy Dispersive X-ray (EDAX) spectroscopy shows strong metal signal for Ag which confirms the presence of pure Ag metal as the major constituent (Fig:5). The presence of elements such as Ag, Cl, O and C are shown in the inset of the figure which shows the quantitative information of biosynthesized Ag-NPs. In the present investigation, the synthesized silver nanoparticles shows strong absorption at around 3 keV, which is due to the Surface Plasmon Resonance.

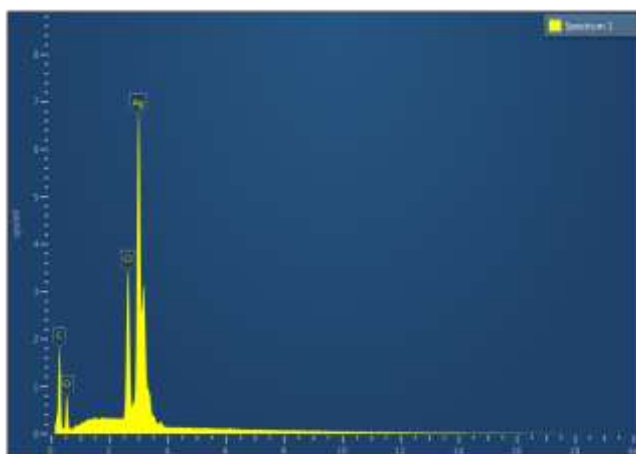


Fig:5 EDAX Spectrum of Ag-NPs synthesized using Kalanchoe gastonis-bonnieri leaf extract

Table:1 EDAX analysis of composition of Ag-NPs synthesized using Kalanchoe gastonis-bonnieri leaf extract

Element	Weight%	Atomic%
C	20.16	53.72
O	10.07	20.16
Cl	8.94	8.07
Ag	60.82	18.05
Total	100	100

3.2.Antibacterial Activity



The antibacterial activity of synthesized silver nanoparticles (Ag-NPs) were studied with respect to *Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *E.coli*. These bacterial cells were treated with Ag-NPs and observed good antibacterial activity.

Table:2 Antibacterial activity of Ag-NPs synthesized using *Kalanchoe gastonis-bonniieri* leaf extract

Bacteria Strains Name	Activity	Std.Value
<i>Staphylococcus aureus</i>	16	15
<i>Streptococcus mutans</i>	11	14
<i>Enterococcus faecalis</i>	13	15
<i>Proteus vulgaris</i>	12	18
<i>Klebsiella pneumoniae</i>	9	12
<i>E.coli</i>	12	18

3.3.Antifungal Activity

The antifungal activity of the synthesized silver nanoparticles (Ag-NPs) were tested against *Aspergillus flavus*, *Pencillium notatum* and *Rhizopus stolonifer*. It shows good antifungal activity against *Aspergillus flavus* and *Rhizopus stolonifer* where as no activity against *Pencillium notatum*.

Table:3 Antifungal activity of Ag-NPs synthesized using *Kalanchoe gastonis-bonniieri* leaf extract



Fungal Strains Name	Activity	Std.Value
Aspergillus flavus	12	18
Pencillium notatum	NIL	15
Rhizopus stolonifer	13	17

4.CONCLUSION

The present study demonstrated an eco-friendly method of synthesis of Ag-NPs using the leaf extract of the plant *Kalanchoe gastonis-bonnieri* and its antimicrobial activity was studied. Biocompatible Ag-NPs were obtained at room temperature within 15 minutes of reaction time without the use of toxic chemicals. The absorption peak observed at 431nm under UV-Vis spectra confirms the formation of Ag-NPs. The functional biomolecules responsible for the formation and stabilization of the Ag-NPs were confirmed by the FT-IR studies. The average crystallite size of the Ag-NPs were studied by using the xrd pattern. The synthesized Ag-NPs were cubical in structure was characterized by Scanning Electron Microscope and it possess good antibacterial and antifungal activity. Currently most of the applications of silver nanoparticles are in antibacterial / antifungal agents in biotechnology and bioengineering, textile engineering, water treatment and silver based consumer products. When coupled with the knowledge that Ag itself is known to be a safe substance to man, it is not surprising to learn how Ag nanoparticles can be used to prevent the spread of a wide range of diseases.

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