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Original Research Article

Green Route of Synthesis Ag NPs Using Reductant and Stabilizer Agent from Plants Extract as an Efficient Antibacterial and Antifungal Activity

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ABSTRACT

Green, fast, and low-cost synthesis of Ag NPs were utilized at ambient temperature using extracts of plants (aloe vera and hibiscus sabdarriffa L.) as an eco-friendly reductant/stabilizer agent in the preparation of Ag NPs. XRD investigated the morphology, and the Ag NPs structure to determine the crystallinity, FS-SEM to determine the morphologies, EDX to determine the elemental composition and distribution, and UV-visible absorption spectrometer to understand the optical properties. Primarily, the Preparation of Silver NPs was observed by the change in color to dark-grey, and then confirmed by SPR band at 431 nm using aloe vera leaf extract and at 410 nm using Hibiscus sabdariffa flower extract in the analysis of UV-vis spectral. The significant peaks in the XRD pattern exhibited the FCC crystal structure of Ag NPs with particles size of 16.99 nm-26.99 nm from aloe vera leaf extract (and 13.11 nm-29.50 nm from Hibiscus sabdariffa flower extract) are seen in FE-SEM images. As a result, the synthesized Ag NPs using plant extract have a powerful antibacterial activity.

GRAPHICAL ABSTRACT

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Introduction

The Ag NPs use has been increasing on a global scale particularly in the fields of textiles, technology, health, and food because of their remarkable features. Ag nanoparticles (Ag NPs) have been intensively developed for many years because of their unique properties and diverse applications. Preparation of NPs has been demonstrated through, a variety of techniques including the sol-gel method, microwave, chemical vapor deposition (CVD), and hydrothermal process [1-5]. Although chemical approaches are less efficient, due to the used chemicals' toxicity and the difficulty of removing them, these procedures may be harmful. In addition, the chemical agents utilized in these procedures are environmentally hazardous [6]. Consequently, there is a rising need for an eco-friendly nanoparticle production procedure that does not involve the use of harmful chemicals. Biological approaches for nanoparticle manufacturing including microbes, enzymes, and herbs or plant extracts have been considered environmentally eco-friendly alternatives to chemical and physical processes [7, 8].

In recent years, various studies have utilized plants or herb extracts, like henna, hibiscus sabdrriffa L. [9], aloe-vera [10], phyllanthus [11], curcumin [12], seagrasses [13], coffee, tea [14], and gum Arabic. These extracts have an effect like a reductant/stabilizer agent in the green synthesis of Ag NPs. In this work, we established the utilization of aloe-vera leaf extract and hibiscus sabdarrifa flower extract which acts as a reducing and stabilizing capping agent according to its intrinsic antibacterial action against a wide range of pathogens and antifungals.

Aloe vera (AV), belonging to the family of Liliaceae, is another medicinal plant that has been widely known for its antibacterial, anti-inflammatory, and burns healing abilities. Its gel is nutrient-rich and contain more than 200 active components, AV has a various metabolites, including as simple/complex polysaccharide, acids, proteins, enzymes, terpenes, flavonoid, minerals, vitamins, phenolic compounds, etc. which provide it with special abilities [15]. Hibiscus sabdariffa belongs to the family of Malvaceae, quite large, and colorful with many benefits in old medicine [16].

Ag NPs are considered one of the most valued precious noble metals, widely recognized for their special anti-bacterial, anti-cancer, antivirus, biosensor, and photocatalytic activity [17-20]. Ag NPs also include wound dressing, catheters, and bone cement [21]. Ag NPs are further utilized in topical ointments and creams to prevent wounds and burn infections [22]. Likewise, due to their improved antibacterial properties, they can be utilized in water filtration systems for purification [23]. Furthermore, both gram-positive and gram-negative bacteria are effectively inhibited by resultant Ag NPs. Antibacterial activities of Ag NPs are caused by the pores formation in the bacterial cell barrier, resulting in the leaking of cellular material. The Ag NPs show excellent antifungal activity against various fungus species. Ag NPs have pronounced anti-bacterial characteristics and perform better than other metal nanoparticles [24].

Materilas and Methods

Silver nitrate ($AgNO_3$) was obtained from Carlo erba chemicals; aloe vera and hibiscus sabdarriffa L. were obtained from local markets. All glasses have been rinsed with deionized water and alcohol, and then have been left to dry before being used.

Preparation of aloe vera leaf extract

Fresh leaves of aloe vera, as displayed in Figure 1a, were obtained from Local markets. First, aloe vera leaf was washed with sterile deionized water (DW) to remove the dirt particles, and then the green skin was removed. The aloe vera leaf extract was used for the reduction of (Ag^+) to (Ag^o). 100 g of finely chopped aloe vera gel were mixed with 300 milliliters of deionized water for 10 minutes. The mixture was then constantly stirred for an hour, and after that the extract centrifuging at 4000 rpm for about 10 min to completely remove the unwanted biomaterials. The extraction solution was kept at ambient

temperature so that it could be utilized in further experiments.

Preparation of hibiscus sabdarrifa L. flower extract

Hibiscus sabdarriffa L. fresh flowers, as depicted in Figure 1b, were purchased from local markets and washed thoroughly three times with D W. The leaves were sun-dried for a week, and then ground into small pieces. After that, 2 g with 100 ml of DW were mixed in a beaker, and then it was placed on a magnetic stirrer for 30 min at 60°C. In the end, the red extract was filtered by cotton wool balls twice and kept at ambient temperature for further experiments.

A

B

Figure 1: (a)Aloe vera leaves and (b) Fresh flowers of Hibiscus sabderriffa L

Green synthesis of Ag NPs via aloe vera extract

In a typical reaction procedure, using 1 g of $AgNO_3$ solution, which was slowly mixed with 10 ml of aloe vera leaf extracts at room temperature, and then put in a rotary shaker, the resulting solution becomes dark grey after 24 hours, as depicted in Figure 2a, indicating the Ag NPs production. After 24 hours, the obtained Ag NPs from aloe vera plant extract was centrifuged at 35,000 rpm for about 10 minutes, and then washed with DW using the centrifuge twice to help remove any unwanted biological components, as illustrated in Figure 2b. After that, the reaction mixture was poured in Petri plates and it was put in the incubator to dry and use as a powder.

Green synthesis of Ag NPs via hibiscus sabdarrifa L. extract

In a typical reaction procedure, using 1.67 g of $AgNO_3$ with 100 mL of DW, it was put on a magnetic stirrer for about a half hour at 60°C, and then 50 mL of hibiscus extract was added, as shown in Figure 2c. Next, 10 mL of hibiscus extract was added at every time. By adding 30 mL of hibiscus extract, the mixture color began to change, finally by adding 50 mL of hibiscus extract, the mixture color changed to dark gray, as demonstrated in Figure 2d, which indicates the complete synthesis of Ag NPs. After that, it was deposited on slides for further experiments.

Procedure for antibacterial and antifungal

Using the agar method, the inhibiting action of Ag nanoparticles produced by the green approach was demonstrated against both gram-positive (*i.e.* S. aureus) and gram-negative (i.e. Kleb. sp.), (E. coli) bacteria as well as the antifungal activities on Candida albican. It has been noticed that Ag NPS at a concentration of 2g per disc has anti-bacterial activity. 3.8 g of Mueller Hinton agar was dissolved with 100 ml of D W to form a medium for both fungi and bacteria growth. After 10 minutes of heating to fully melt the medium, it was sanitized by autoclaving at 15 lbs pressure (121 °C) for about 15 minutes, and then cooled to 45-50 °C, till it was completely combined, and then placed overnight in the refrigerator. Next, a sterile swab of cotton was used to streak agar medium on Petri-dishes that contained medium. These plates were incubated at 37 °C for 24 hr [26]. Thereafter a small amount of the prepared bacteria and fungal applied on the medium surface.

Characterization of Ag NPs

The Ag NPs structure was examined using X-ray diffraction (XRD) to determine the crystallinity The spectra were recorded via Shimadzu X-ray diffractometer employing Cu Kα radiation, and diffraction angle 2θ ranging from 20°-80°. The FT-IR spectrum was investigated for identifying the functional group using (Two Perkin Elmer). The Ag NPs morphologies were carried out by FE-scanning electron microscopy (FS-SEM), (Inspect F-50, Holland). The elemental composition of Ag NPs was obtained by energy-dispersive X-ray spectroscopy (EDX), and a UV-visible absorption spectrometer (Lambda 365 Perkin Elmer) was used to understand the optical properties.

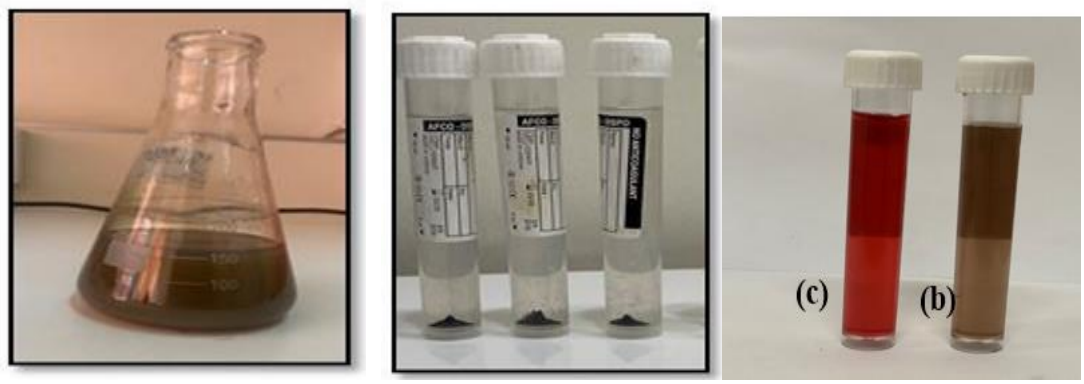


Figure 2: (a) $AgNO_3$ and aloe vera mixture after 24 hours in the rotary shaker, (b) Ag NPs after centrifuged, (c) hibiscus sabderrifa L. extract, and (d) Ag NPs via hibiscus sabdarrifa L. extract

Results and Discussion

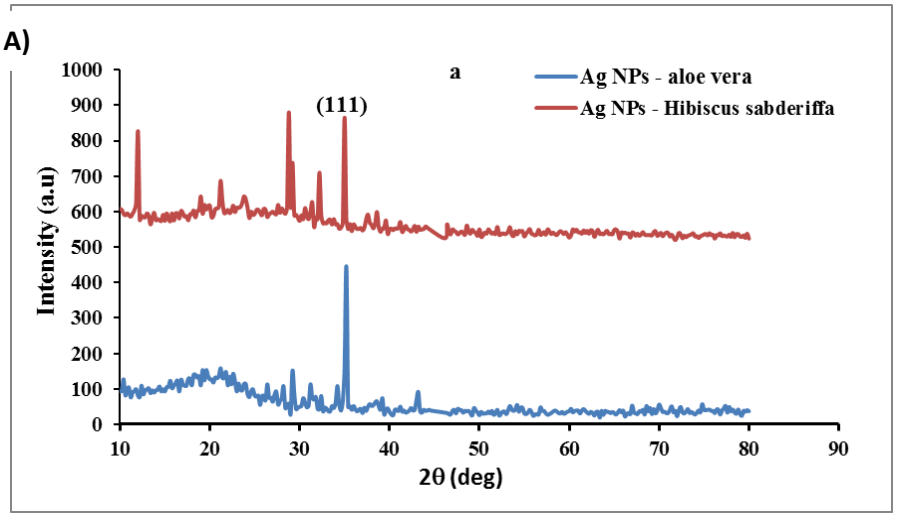
X-ray diffraction (XRD) studies

Analysis of Ag NPs from aloe vera leaf extracts using XRD pattern confirmed the crystalline of the synthesized Ag NPs classified the face center cube pattern of Ag NPs, as demonstrated in Figure 3a, where the strong diffraction peaks with 2θ = 35.2°, 43°, 67°, and 74.8° correspond to the (1 1 1), (2 0 0), (2 2 0), and (3 1 1) planes of Ag. Besides, the X-ray diffraction powder pattern had a small peak at 2θ values of 29.2° & 31.2°. This could be formed from the leaves extracted from organic ingredients. It is obvious that the peak was related to the (111) plane is the most intense of the peaks. Accordingly, Ag NPs obtained in the current synthesis are naturally crystalline and have an FCC structure.

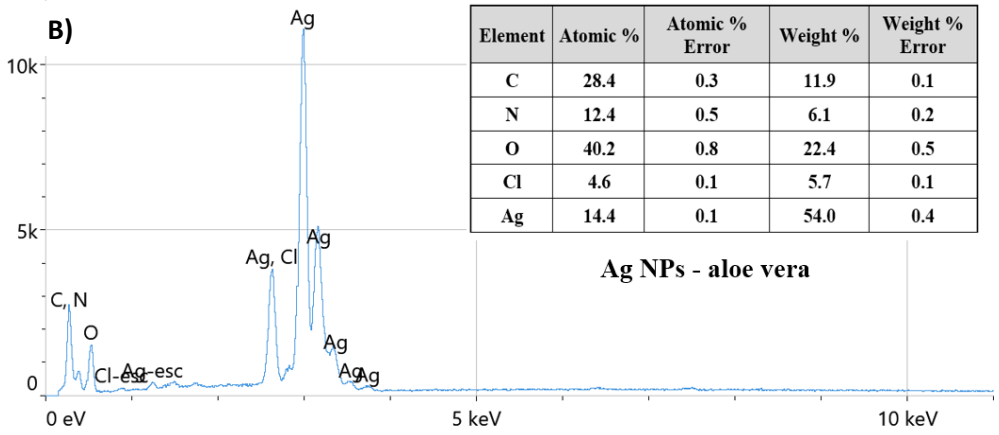
Ag NPs analysis from hibiscus sabdariffa flower extracts using an XRD pattern confirmed the face center cube pattern of the synthesized Ag NPs, as exhibited in Figure 3a, where the diffraction peaks with 2θ = 35°, 46.4°, 65°, and 72.2° correspond to the (1 1 1), (2 0 0), (2 2 0), and (3 1 1) planes of Ag. Besides, the X-ray diffraction pattern had other peaks at 2θ values. This could be formed from the flower extract organic ingredients and due to $AgNO_3$ which is not reduced and remains in a small quantity in the sample. It is obvious that the peak was related to the (111) plane is the most intense of the peaks. According to this, Ag NPs prepared in the current synthesis are naturally crystalline and have an FCC structure. Figure 3b and 3c demonstrated the EDX, the Ag NPs with both aloe vera and Hibiscus sabdariffa extracts used in this synthesis displayed an optical distinctive absorption band peak at approximately 3 keV, which is typical of the absorption of metallic Ag NPs due to surface plasmon resonance. The presence of C, O, N, and Cl were confirmed by EDS spectroscopy and are related to phenolic components of extracts adsorbing on the nano-surface of the synthesized sample that was left.

FE-SEM Analysis is used to establish the structural characteristics of the Ag NPs (Figure 3d and 3e). The green synthesized Ag NPs were mostly spherical in shape for Ag NPs synthesis using aloe vera with particle sizes ranging from 16.99 nm to 26.99 nm, also the spherical shape of the particles size 13.11 nm-29.50 nm for Ag NPs synthesis using hibiscus sabdariffa. However, some other particles with irregular shapes (or ringworm shapes) were observed.

A)



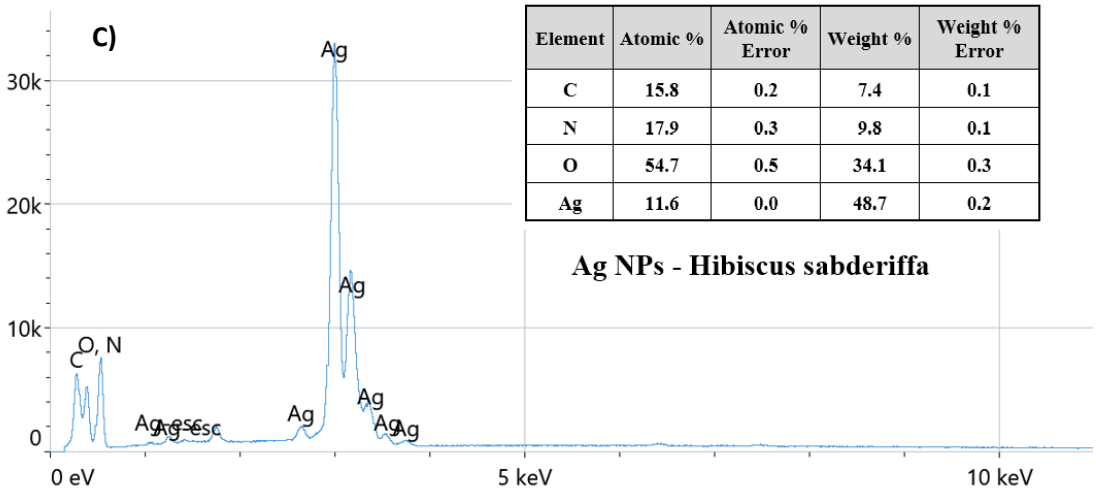
B)



Element	Atomic %	Atomic % Error	Weight %	Weight % Error
C	28.4	0.3	11.9	0.1
N	12.4	0.5	6.1	0.2
O	40.2	0.8	22.4	0.5
Cl	4.6	0.1	5.7	0.1
Ag	14.4	0.1	54.0	0.4

Ag NPs - aloe vera

C)



Element	Atomic %	Atomic % Error	Weight %	Weight % Error
C	15.8	0.2	7.4	0.1
N	17.9	0.3	9.8	0.1
O	54.7	0.5	34.1	0.3
Ag	11.6	0.0	48.7	0.2

Ag NPs - Hibiscus sabderiffa

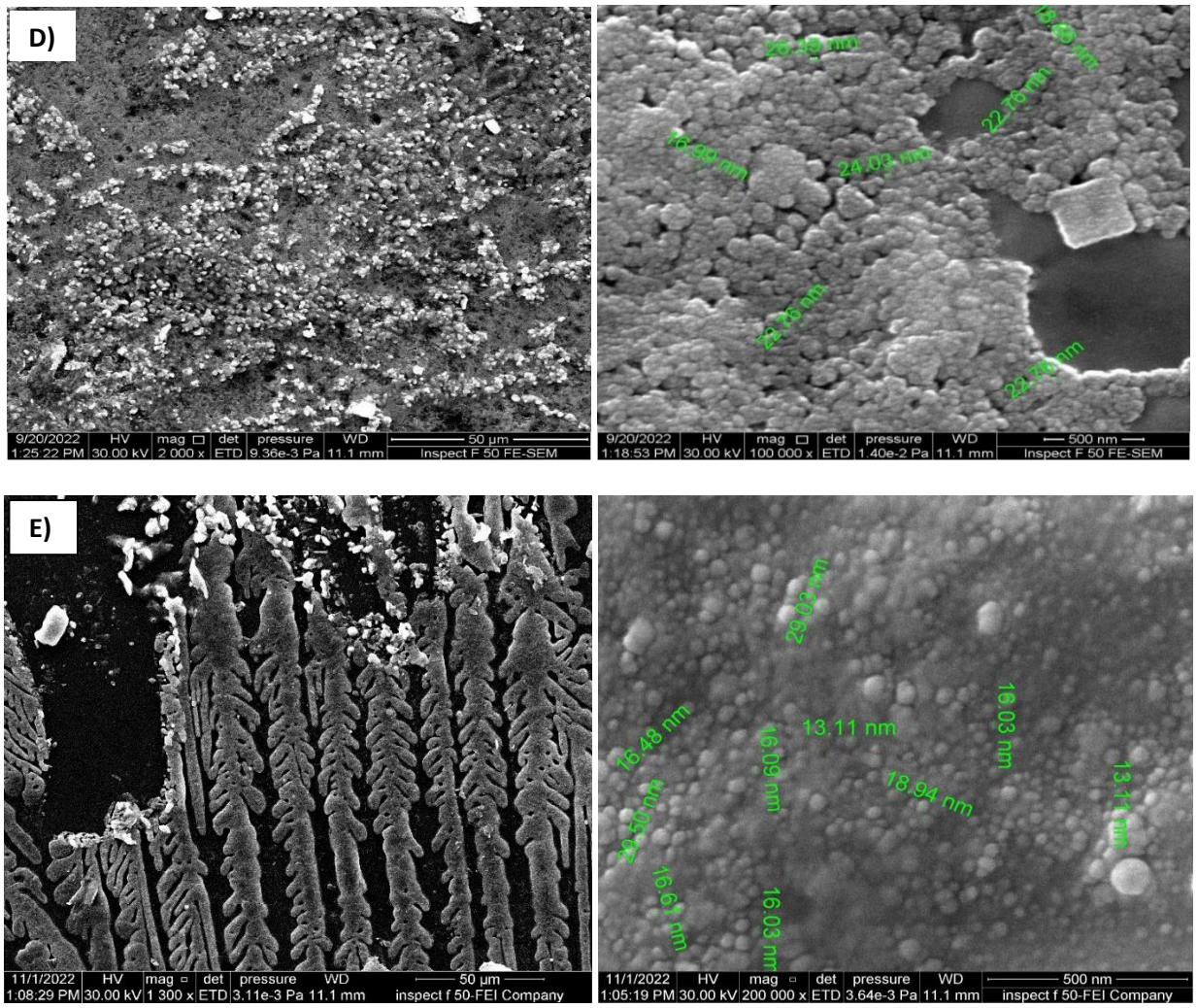


Figure 3: (a) XRD of Ag NPs, (b) EDX spectra of Ag NPs formed by aloe vera, (c) EDX spectra of Ag NPs formed by hibiscus sabdariffa, (d) FE-SEM of Ag NPs formed by aloe vera, and (e) FE-SEM of Ag NPs formed by hibiscus sabdariffa

FT-IR Spectrum

As seen in Figure 4a, the FTIR spectrum for Ag NPs has been prepared with an aloe vera plant extract. It has been found that aloe vera plant extract contains a high concentration of flavanones and terpenoids, as evidenced by the presence of the detected peaks [26]. Similarly in Figure 4b, the FTIR spectrum for Ag NPs was prepared with hibiscus sebderiffa flower extract analysis. It has been found that hibiscus sabderiffa flower extract is rich in secondary metabolites like flavanones, phenolic compounds, and tannins. Both of them analysis a few characteristic peaks with a small difference in peak positions. The peaks demonstrated with Ag NP at 3429.07 cm^{-1} and 3413.9 cm^{-1} were broad and more intense than others corresponding to (O-H) stretching due to phenol and alcohol groups in plant extracts [27]. 1637.63 cm^{-1}, peaks at 1637.28 cm^{-1} are (C=O) stretching group present in Ag nanoparticles. Peaks located at 2078.08 cm^{-1}, 2075.38 cm^{-1} assigned to (C-H) stretch aldehydes, the peaks at 2328.48 cm^{-1}, 2362.87 cm^{-1}, 2326 cm^{-1}, and 1369.69 cm^{-1}, 1381.81 cm^{-1} correspondingly to (C-O) stretching [28], 681.46 cm^{-1}, and 673.2 cm^{-1} are (C-Cl) alkyl halide groups, N-H band at 3850.74 cm^{-1} and 3798.23 cm^{-1}.

UV-Vis study

The Ag NPs were distinguished using a UV-Visible spectrophotometer, which is considered as one of the most essential techniques to confirm the NPs fabrication through green synthesis using plant

extracts responsible for the Ag NPs formation from Ag ions. The maximum absorbance of synthesized Ag NPs was at 431 nm using aloe vera leaf extract and at 410 nm using hibiscus sabdariffa flower extract, as shown in Figure 5, also the change in color of solution to gray that indicates the presence of surface plasmon resonance (SPR) of Ag NPs.

Activity as Antibacterial and antifungal of Ag NPs

After 24 hrs, it was observed that the inhibition zone, which was measured on a scale, appeared as shown in Figure 6 and Table 1. From Table 1, it can be concluded that Ag NPs via aloe vera is more effective than Ag NPs via hibiscus sabdariffa on the bacteria and fungus.

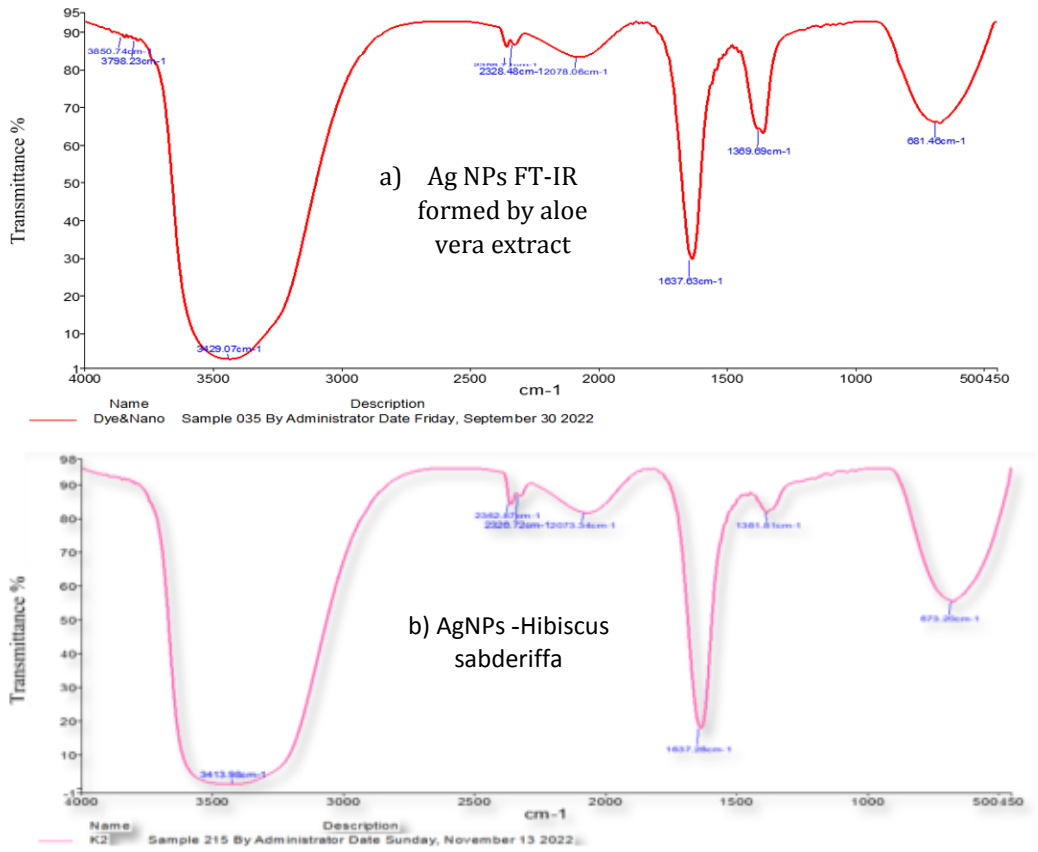


Figure 4: (a) Ag NPs FT-IR formed by aloe vera extract, (b) Ag NPs FT-IR formed by hibiscus sabderiffa flower extract

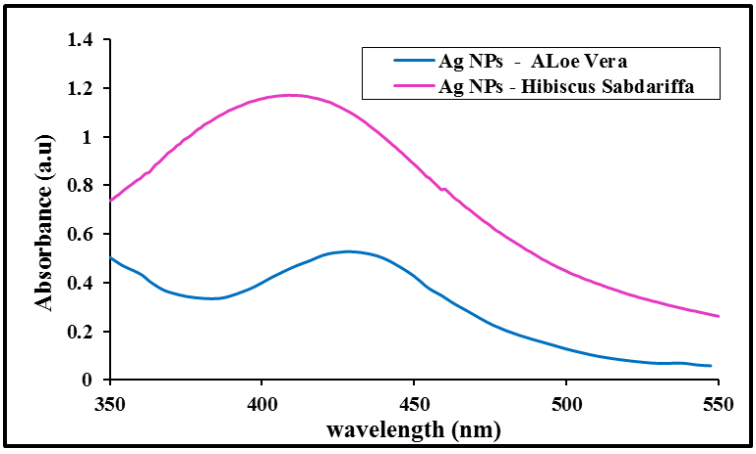


Figure 5: UV-Visible spectra of Ag NPs

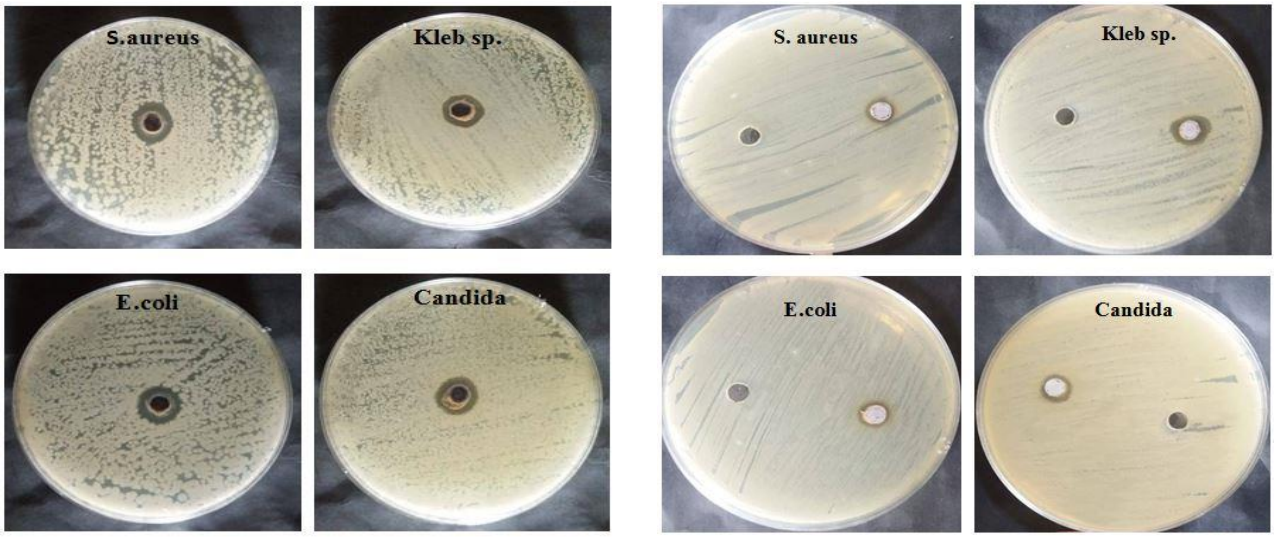


Figure 6: Effect of Ag NPs on both bacteria and fungi, (a) using aloe vera extrac and (b) using hibiscus sabdariffa extract

Table 1: Ranges of inhibition zones

Bactria and fungal	Aloe vera	Hibiscus sabdariffa
S. aureus	17	12
Kleb sp.	13	12
E.coli	13	12
Candida	14	13

Conclusion

The novelty of this study is the prepared powerful, eco-friendly, low-cost, and green route synthesis of Ag PNs using plant extracts. The results confirm that plants extract acts as a reductant /stabilizer agent in the eco-friendly green route. The UV-Vis spectroscopy of Ag PNs showed a strong absorbance band with SPR. The eco-friendly, green-synthesized Ag PNs exhibit the powerful antibacterial activity against *S. aureus, Kleb sp*., and *E.coli*. Furthermore, Ag PNs exhibit powerful antifungal activity against *Candida*.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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