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Biosynthesis of Silver Based Nanoparticles Using Plant Leaves Extracts and Their Antibacterial Activities

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ABSTRACT
The emergence of antibiotic-resistant bacteria is a major global public health concern. Antibacterial agents are one of the most exciting areas for the use of nanoparticles and have shown great potential as alternative antibacterial agents due to their outstanding physical and chemical properties. The biosynthesis and antibacterial activities of Silver Nanoparticles (AgNPs) could contribute to the development of innovative antimicrobial drugs with potential for a variety of medical applications.
The X Ray Diffraction (XRD) results showed that the formed particles were pure silver nanoparticles with a cubic crystal structure and granular size. Fourier-transform infrared (FTIR) also showed that functional aggregates formed, and the thumb region confirmed the presence of silver nanoparticles. In addition, we investigated the Uv-Visible and biological activity of AgNPs. A study of the biological activity of gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia* coli, and *Klebsiella pneumonia*) bacteria showed the importance of nano-silver particle biosynthesis.

CONTENTS

1. Introduction:

Nanoscience and nanotechnology are rapidly growing fields that have revolutionized the way we consider materials and their properties. At the nanoscale, materials exhibit unique physical, chemical, and biological properties that differ from those of the bulk materials. These properties arise from the high surface-area-to-volume ratio of nanoparticles, which allows for increased reactivity and interaction with other molecules [1].

Nanoparticles are defined as particles with at least one dimension less than 100 nanometers (nm). They can be synthesized from a variety of materials including metals, metal oxides, polymers, and carbon-based materials. The synthesis of nanoparticles has become increasingly important because of their potential applications in various fields, such as medicine, electronics, energy production, and environmental remediation [1-3].

One of the most promising applications of nanoparticles is in the field of antibacterial agents. The emergence of antibiotic-resistant bacteria is a major public health concern worldwide. Nanoparticles have shown great potential as alternative antibacterial agents, owing to their unique physical and chemical properties. They can interact with bacterial cells in multiple ways, such as by disrupting cell membranes or inhibiting enzyme activity [4-6].

AgNPs have been shown to exhibit broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria [7-8].

In this study, we aimed to synthesize AgNPs using a green chemistry approach that utilizes plant extracts as reducing agents. We investigated the antibacterial activities of these nanoparticles against various bacterial strains using standard microbiological techniques. The results obtained from this study provide valuable insights into the potential use of AgNPs as alternative antibacterial agents in various applications, including medicine and environmental remediation.

Silver nanoparticles have gained significant attention in the field of medicine owing to their unique properties such as high surface area-to-volume ratio, enhanced reactivity, and biocompatibility. These nanoparticles have been used in various medical applications such as drug delivery, imaging, and antimicrobial agents.

In particular, AgNPs have been found to possess excellent antimicrobial properties against a wide range of microorganisms, including bacteria, fungi, and viruses [9]. They have been used in wound dressings, surgical instruments, and coatings on medical devices to prevent infection. Additionally, AgNPs have shown potential in cancer therapy by inducing apoptosis in cancer cells and inhibiting tumor growth [10]. The use of AgNPs in medicine has the potential to revolutionize this field by providing effective and safe solutions for various medical applications.

1.2 Guava leaves:

Guava leaves, scientifically known as Psidium guajava, are the foliage of guava tree (figure1) and have gained significant attention for their numerous health benefits. These leaves are rich in various bioactive compounds, including flavonoids, phenolic compounds, and essential oils, which contribute to their therapeutic properties [11].

Guava leaf extract is obtained by steeping or boiling the leaves in water, and has been used traditionally for centuries in different cultures for its medicinal properties.

One of the most well-known benefits of guava leaf extract is its ability to regulate blood sugar levels. Studies have shown that certain compounds present in the extract can inhibit glucose absorption, leading to lower blood sugar levels. This makes it a promising natural remedy for managing diabetes and preventing its onset [12-13].

Furthermore, guava leaf extract possesses potent antioxidant properties. Antioxidants help protect the body against oxidative stress caused by harmful free radicals, which can lead to chronic diseases, such as cancer and heart disease. Regular consumption of guava leaf extract may help reduce oxidative damage and support overall health [14].

Moreover, guava leaf extracts have demonstrated antimicrobial activities against various bacteria and fungi. Traditionally, it has been used to treat infections and promote wound healing [15]. However, it is important to note that further research is still needed to fully understand their mechanisms of action and potential side effects.

Figure. 1: Guava tree

1.3 Sidr leaves:

Sidr leaves, scientifically known as Ziziphus spina-christi, are the foliage of the Sidr tree (figure2) and have been used for centuries in traditional medicine for their therapeutic properties. These leaves are rich in bioactive compounds such as flavonoids, tannins, and saponins [16]., which contributes to their health benefits. Sidr leaf extract is obtained by steeping or boiling leaves in water, and has been is traditionally used for its medicinal properties. One of the notable benefits of Sidr leaf extract is its potential antimicrobial activity [17].

Furthermore, Sidr leaf extract demonstrated anti-inflammatory properties. It can help reduce inflammation in the body, which is beneficial for conditions, such as arthritis and inflammatory bowel disease. The extract may also alleviate symptoms associated with inflammation, such as pain and swelling [18].

Additionally, Sidr leaf extract has shown potential in promoting digestive health. However, it is important to note that further research is needed to fully understand the mechanisms of action and potential side effects of Sidr leaf extract [19-21]

Figure. 2: Sidr tree

Guava cultivation processes in coastal areas, the Tihama plains, and Sidr trees are found in the valleys of Shabwa, as well as in some governorates such as Saada and Hajjah.

Extracts of these leaf plants (*psdium guagava and ziziphus spina Christi*) were prepared to synthesize Silver-Nanoparticles ($AgNP_s$) that were characterized by Fourier-transform infrared (FTIR), X Ray Diffraction (XRD), UV-Visible, and screened for antibacterial activity.

2. Materials and methods

2.1 Materials:

2.1.1 The Chemical Materials:

The following materials were used in this study: silver nitrate ($AgNO_3$-99%), Mueller-Hinton Agar (Sigma–Aldrich), Whatman No.1filter paper, watch glasses, measuring cylinders, standard flasks, mortar and pestle, refrigerator, beakers, and distilled water (DW or dH_2O).

2.1.2 Plant Materials:

Fresh guava leaves and Sidr leaves.

2.1.2.1 Collection of Plant:

Guava leaves were obtained from the city of Dhamar – Yemen.

Healthy leaves of Sidr (*Ziziphus spina-Christi*) were collected from Sidr trees distributed in Yareem City, Yemen.

2.2 Preparation Method:

2.2.1 Preparation of guava and sider leaves extract:

The leaves were washed several times with tap water and then with distilled water. They were then dried and ground into a powder using an electric blender. The powder (20 g) was mixed with 250 ml of distilled water and stirred using a magnetic stirrer for two hours at room temperature [22]. The solution was filtered twice using filter paper to obtain guava and sider leaf extracts, which were immediately used for sample preparation.

2.2.2 Preparation of silver nanoparticles using guava and sider leaves extract:

To prepare AgNPs, 20 ml of the extract was adding three grams of silver nitrate and mixing for two hours. A color change from brown to black was observed for the two extracts, and centrifugation was used to separate the AgNPs. The precipitate was washed with distilled water, dried, and prepared for required measurements [23].

2.3 Characterization Techniques:

In this section, the characterization methods and instruments used in this project are described.

2.3.1 XRD analysis:

The crystallinity of the synthesized Ag NPs was analyzed by XRD (XD-2 X-ray diffractometer using Cu Kα (λ = 1.54 Å) at 36 kV and 20 mA, China) in the Yemeni Geological Survey and Minerals Resources Board (YGSMRB)).

2.3.2 FT-IR analysis:

IR spectra were recorded in the (400 – 4000) cm-1 frequency range using a Varian IR Prestige-2000, FT-IR Spectrophotometer, Global Company for Medicaments, Sana'a, Yemen. The discs were made by mixing the plant leaf extracts and AgNPs with potassium bromide and pressing firmly into the disc.

2.3.3 UV-Vis analysis:

Electronic spectra were recorded using a UV-Vis spectrophotometer (Cary -50 Conc -UV-Visible Spectrophotometer Made in Australia, Varian Australia) over a wavelength range of 200–900 nm at room temperature. Global Company for Medicaments, Sana'a, Yemen. Electronic spectra of the plant leaf extracts were recorded at 10^{-3}M using solution absolute ethanol as the solvent.

3. Results and discussion

3.1 AgNPs prepared using ziziphus and guava leaves extracts:

Many researchers have discovered many plant species and components that contain antioxidant and antimicrobial compounds, such as nitrogen, amino acids, polyphenols, and sugars [24-25]. These compounds act as capping agents for the synthesis of nanoparticles [26]. Generally, metal nanoparticles are manufactured in two ways: bottom-up (fabrication of material from atom to bottom atom) and top-to-bottom (cutting of bulk material to obtain nano-sized particles).

AgNPs made from plant leaf extracts are generally stable even after a month and do not undergo any changes [27].

AgNP synthesis from guava and Sidr extracts has recanted plants because of their natural ability to reduce toxic and hazardous chemicals (figure 3). During the synthesis of nanoparticles, the observed dark brown color for Guava and Sidr indicates the formation of Gu and Sd-AgNPs [28]. Moodley et al. and Kumavat & Mishra also confirmed that the formation of plant extract-AgNPs was investigated by the color change to brown and dark brown after adding aqueous plant extract to $AgNO_3$ solution [29-30].. Generally, surface plasmon resonance (SPR) for metallic nanoparticles and quantum confinement are the main mechanisms that impart color to nanoparticle suspension [31,]

3.1.1 XRD analysis:

The X Ray Diffraction (XRD) patterns of sider and guava AgNPs (Figure4) revealed clear peaks of AgNPs, indicating the crystalline nature of the biosynthesized AgNPs, as confirmed by the corresponding peaks with respect to Bragg's model of diffraction given by the Joint Committee on Powder Diffraction Standards (JCPDS). The obtained diffraction silver nano peaks at (38.274, 38.178), (44.185, 44.345), and (64.671, 64.7995). were assigned to the (111), (200), and (220) planes, respectively. This corresponds to JCPDS Joint Committee on Powder Diffraction Data No. 04-0783. The prepared samples had a cubic structure, and the average crystallite sizes (D)were 42.6548 nm and 27.89016 nm, respectively. Scherrer's equation [26-28]

$$\boldsymbol{D} = \frac{\mathbf{0.9}\boldsymbol{\lambda}}{\boldsymbol{\beta}\mathbf{cos}\boldsymbol{\theta}} \ldots\ldots\ldots\ldots\ldots \quad (1)$$

where λ = 0.154 nm, θ is the diffraction angle, and β is the full width at half maximum, which was used to calculate the crystallite size (Table 1).

Table 1: 2-theta, FWHM and Average (D, nm) of Ag NPs prepared using ziziphus and guava leaves extracts. Ziziphus-Ag Nanoparticles (42.6548 nm) and Guava-Ag Nanoparticles (27.89016), with best Nanoparticles.

.Leaves	Metal	2-theta (2θ°)	(hkl)	FWHM (β)	Crystallite size (D, nm)	Average (D, nm)
Ziziphus	Ag	38.274	(111)	0.130	64.68836	42.6548
		44.185	(200)	0.336	25.51822	
		64.671	(220)	0.249	37.75783	
Guava	Ag	38.178	(111)	0.251	33.49421	27.89016
		44.345	(200)	0.295	29.08132	
		64.799	(220)	0.446	21.09496	

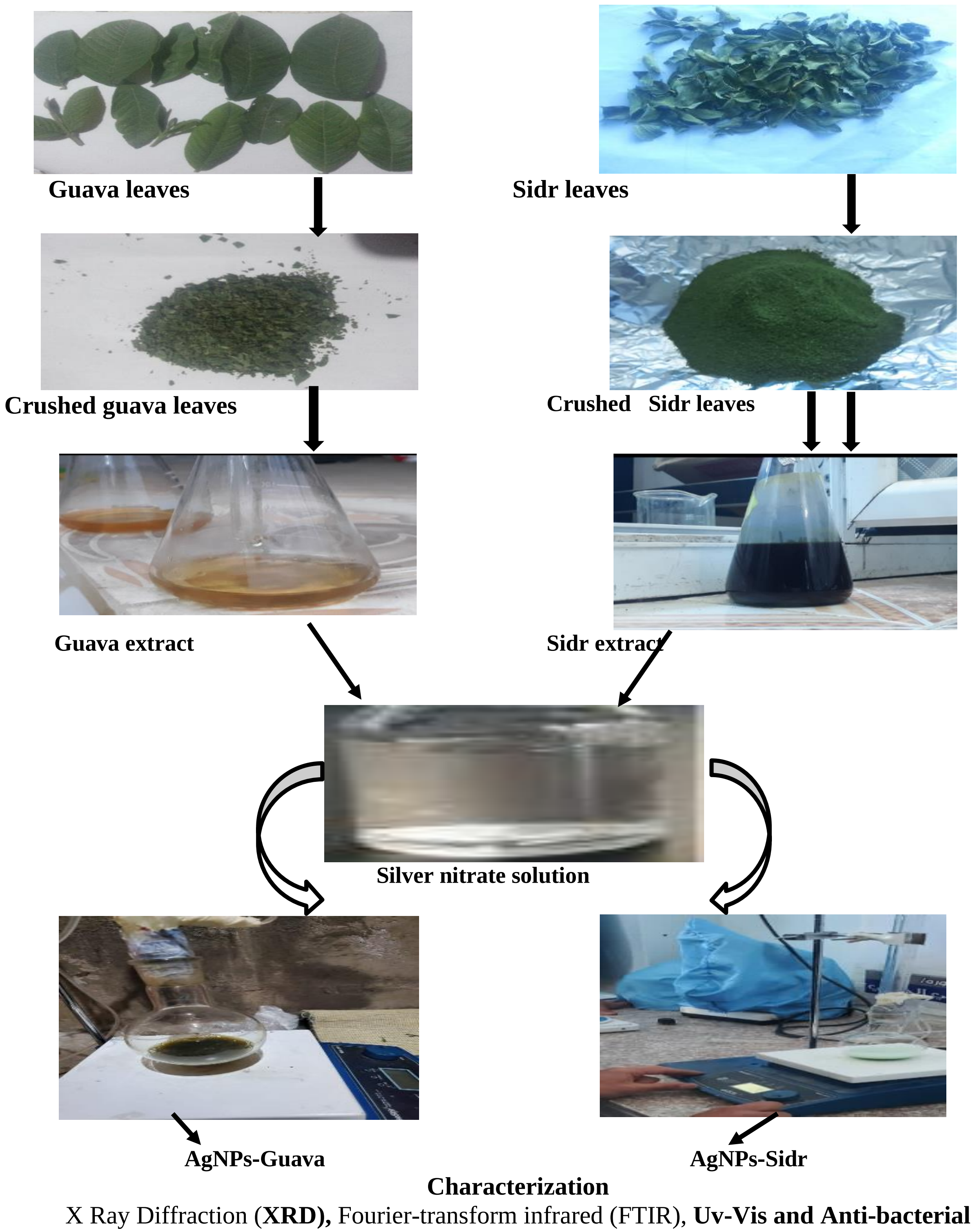


Figure. 3: plant leaves extract-based biosynthesis of silver nanoparticles

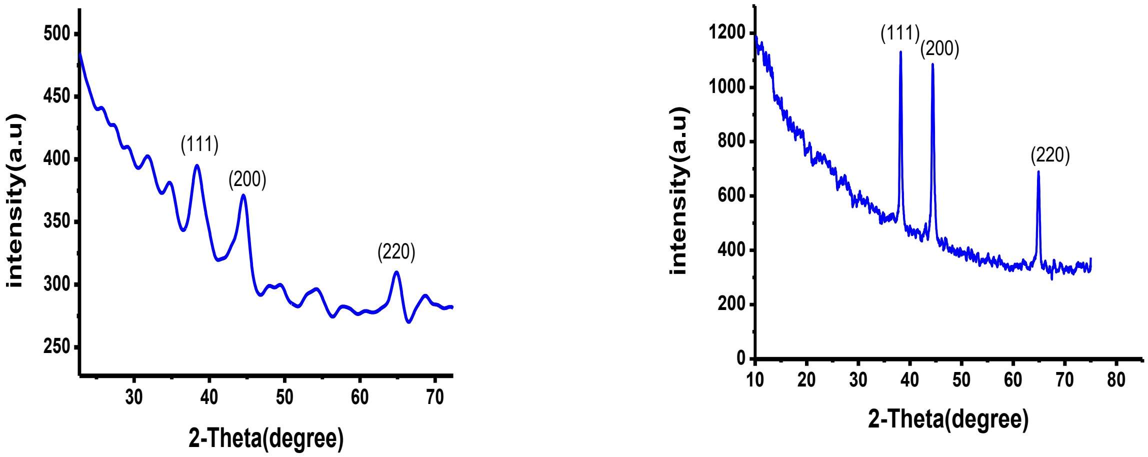


Figure. 4: XRD pattern of Ag NPs prepared using (a) *Ziziphus* and (b) *Guava* leaves extracts

3.1.2 FT-IR analysis of ziziphus leaves extract and AgNPs :

FT-IR spectroscopy was used to determine the presence of functional groups responsible for the formation of Ag. The dried powder of the aqueous leaf extract and Ag with leaf extract samples were analyzed using FT-IR spectroscopy in the range 4000–400 cm^{-1} as shown in Figure (5)

Fourier-transform infrared (FTIR) spectrum for the dried powder of aqueous leaf extract showed peaks at ~620, 1060, ~1383 and ~1630 cm^{-1} which correspond to alkyl-halide (C-Br), C-O stretch, C-H and C=C stretching, respectively [21]. This confirmed the presence of phytochemicals, such as alkaloids, flavonoids, polyphenols, saponins, and tannins in the aqueous leaf extract of *Ziziphus mauritiana* Lam. [24-27,32-35]. Medium-intense bands of Ag stretching were observed at approximately 500 and 557 cm^{-1}. The formation of Ag was further confirmed by Fourier-transform infrared (FTIR) spectroscopy. A wide vibrational band in the region of ~3400cm^{-1} was also observed, which corresponds to O-H stretching, indicating the presence of moisture [36]. The prepared Ag exhibited bands at 1383 cm^{-1}(C–H) bending, indicating the presence of phytochemicals on the surface of the synthesized Ag. Fourier-transform infrared (FTIR) analysis also verified the presence of phytochemicals in *Ziziphus mauritiana* Lam.

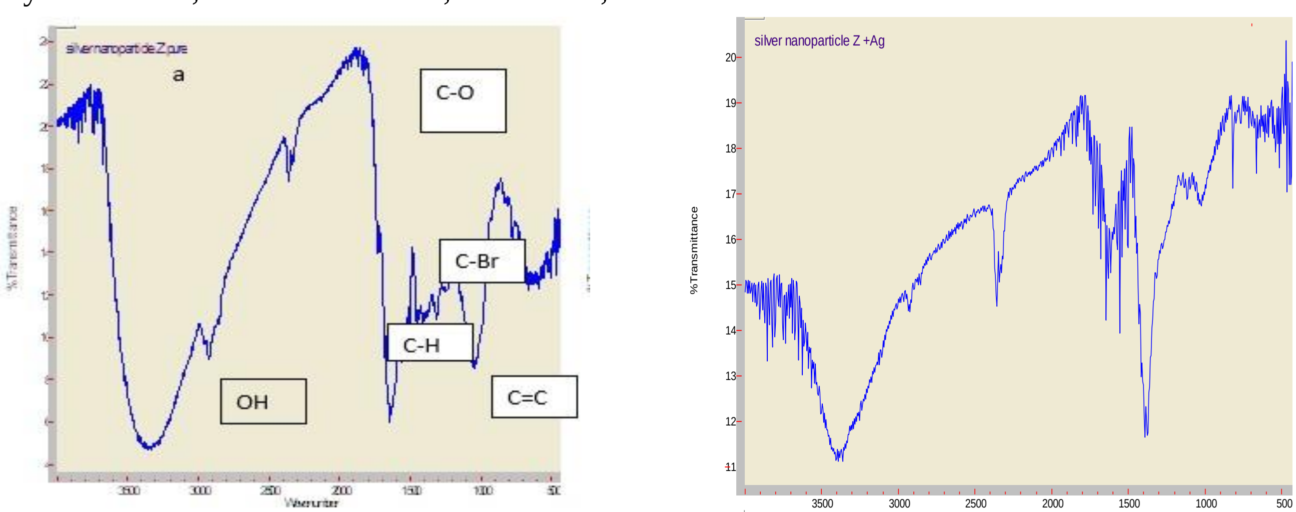


Figure. 5: FT-IR analysis of (a) *Ziziphus* leaves extract (b) *Ziziphus*-AgNPs 3.1.3 FT-IR analysis of guava leaves extracts and AgNPs:

FT-IR spectroscopy was used to determine the presence of functional groups responsible for the formation of Ag. The dried powder of the aqueous leaf extract and Ag with leaf extract samples were analyzed using FT-IR spectroscopy in the range 4000–400 cm^{-1} as shown in (figure 6). Previous studies have shown that guava leaf water extract contains mainly tannins, eugenol, and flavonoids, such as quercetin. The Fourier-transform infrared (FTIR) spectrum of guava leaf extract showed a strong broad band at 3400 cm^{-1} (-OH) and a shoulder at 1620 cm^{-1} (amide), 1450 cm^{-1} (C-C=C), 1363 cm^{-1} (N-O), 1040 cm^{-1} (C-O), 835 cm^{-1} (alkenes), and 702 cm^{-1} (aromatic rings), representing the different functional groups present in the guava leaf extract. FTIR peaks similar to those of the extract with respective shifts due to the attachment of the extract biomolecules on the AgNP surface []. It should be mentioned that the band at 3440 cm^{-1} appeared sharper in AgNPs than in the extract because of the consumption of–OH groups in the reduction process. showed bands at 1383cm^{-1}(C–H) bending, indicating the presence of phytochemicals on the surface of the synthesized Ag.

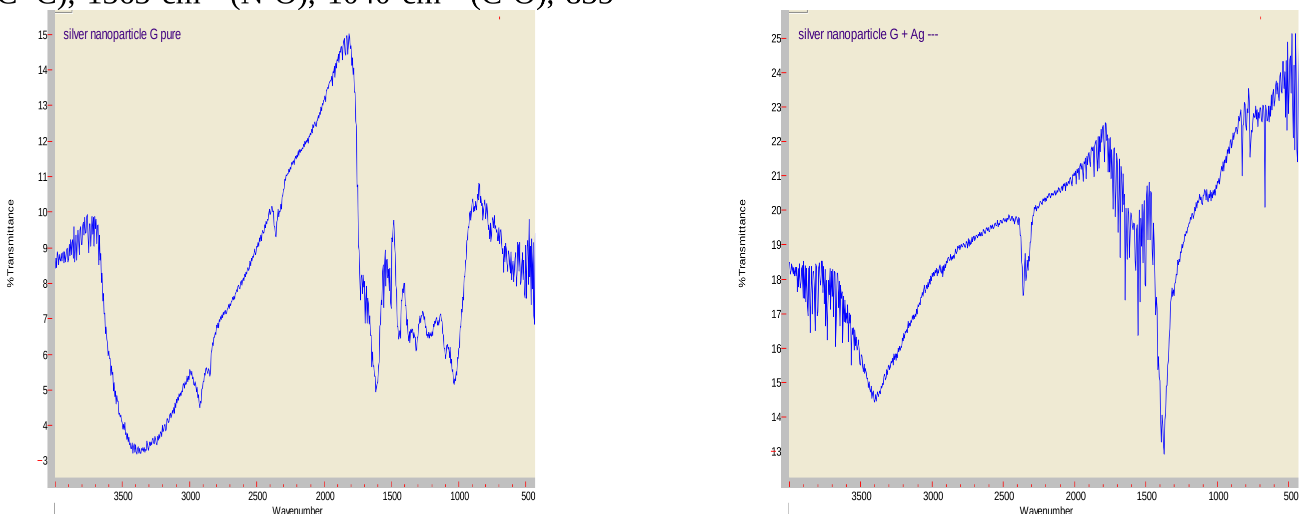


Figure. 6: FT-IR analysis of (a) *Guava* leaves extract (b*) Guava*-AgNPs

3.1.4 UV-visible analysis of Ag NPS using ziziphus and guava leaves extracts:

UV-Vis spectroscopy was used to confirm the formation of Ag-NPs from ziziphus and guava. It is clear that Ag-NPs show absorption at (261.3, 359.80 nm), [37] and 665.8 nm) respectively, as shown in (**figure7)**

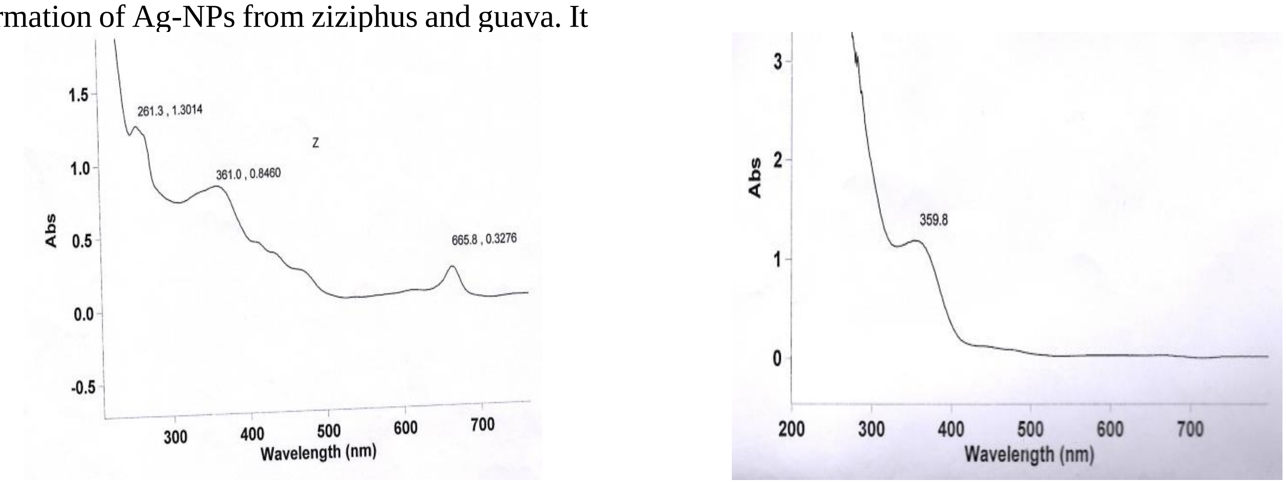


Figure. 7: UV-visible spectra of Ag NPs prepared using (a) *Ziziphus* and (b) Guava leaves extracts.

3.1.5 Antibacterial Activity:

The antibacterial activity of the biosynthesized Silver Nanoparticles (Ag-NPs) using *guava* leaves and ziziphus leaf extracts at different concentrations (25–100 mg/mL) was evaluated against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia* coli, and *Klebsiella pneumonia*) bacteria using the disc diffusion method [38-39]. The results are summarized in (Table 2).

Selective plate images and counted ZOI values are shown in (Figures 8). As can be seen, the ZOI increased with Ag-NPs concentration increased and are higher against *S. aureus* than *E. coil* and *K. pneumonia*; however, even at the highest Ag-NPs concentration, the activity is still below that of the Ciprofloxacin standard drug. As the NPs concentration increased, more growth factors, including reactive oxygen species (ROS) and metal ions, were generated. These factors apparently drive the activity of the target NPs. Notably, at 100 mg/mL, the activity of the Ag-NPs against gram-positive bacteria (*S. aureus*) approached the values of ciprofloxacin, the results of this investigation are consistent with those found in the literature, as reported by A. Nurul et al., 2019[40]. Typically, the bioactivity of a material can be ascribed to various factors, including the nature of the material, particle size, shape, and charge, as well as the application conditions, such as concentration and exposure time. The mechanism underlying the antibacterial activity of the drug involves one or more of the following steps:

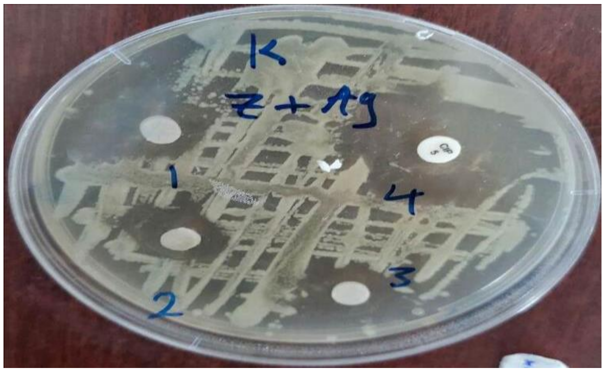
1) Attachment of NPs onto the bacterial surface disturbs the wall function.
2) The release of metal ions such as Ag, which can penetrate cells and affect organ function.
3) Generation of reactive oxygen species (ROS), which cause damage to proteins and nucleic acids, leading to cell death.

Therefore, it can be concluded that this biogenic method is effective for producing Ag NPs with desirable properties for potential biomedical applications.

Table 2: Diameters of inhibition zone for *E. coli, S. aureus and Klebsiella bacteria*

Concentration	25mg/ml	50mg/ml	100mg/ml	25mg/ml	50mg/ml	100mg/ml	25mg/ml	50mg/ml	100mg/ml
bacteria / *compounds*	*E. coli*			*S. aureus*			*K. pneumonia*		
Guava Leaves Extract	0mm	0mm	0mm	0mm	0mm	0mm	0mm	0mm	0mm
AgNPs (Guava)	15mm	14mm	15mm	18m.m	16 mm	23mm	12 mm	20 mm	21 mm
Ziziphus Leaves Extract	0mm	0mm	0mm	0mm	0mm	0mm	0mm	0mm	0mm
AgNPs (Ziziphus)	14mm	15mm	20mm	16 mm	19mm	21mm	14mm	16mm	19 mm
Antibioticciprofloxacin	24mm	26mm	27mm	23mm	25mm	32mm	24mm	25mm	27mm

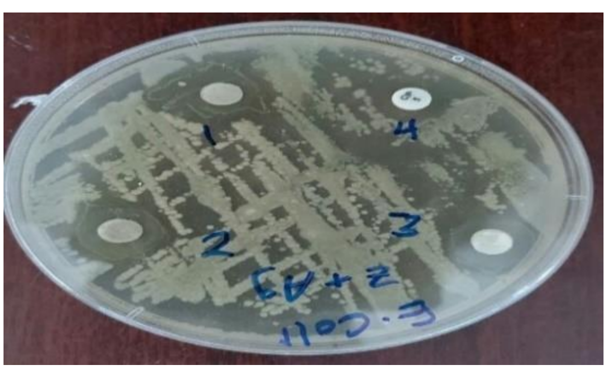
Zone inhibition(mm): zero mm means no activity of both leafs' extracts (Guava and Sidr), but Silver Nanoparticles of Guava and Sider (G-SNPs) have activity at different concentration (25,50, and 100 mg/ml) against E. *coli, S, aureus, and K, pneumonia*



A

B

C



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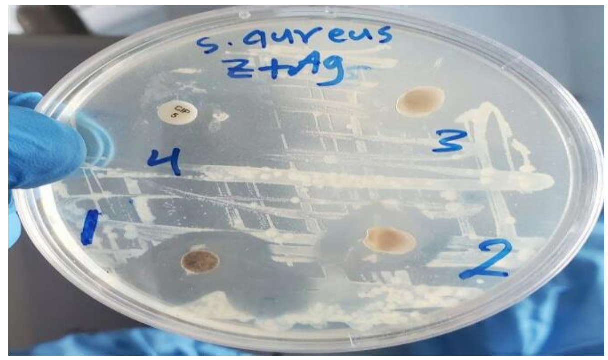

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Figure. 8: Zone inhibition at different concentration (25, 50, and 100mg /l) for Guava and Sidr leaves extracts without inhibition (0 mm), and G-S AgNPs with inhibition ranging from (14 to 27 mm).

a. Ag-Nps for *Ziziphus*
b. *Ziziphus* leaves extract against K. *pneumonia*.
c. Ag-Nps for Guava
d. Ag-Nps for *Ziziphus* against E. coli.
e. *Ziziphus* leaves extract
f. Ag-Nps for *Ziziphus* against *S.aureus*

4. Conclusion:

In this paper, we described an effective green method of silver nanoparticles (Gu-Sd-AgNPs) using Guava and sdir leaves extract as a reducing and stabilizing agent. The plant extracts phytochemical components of both Guava and sidr were characterized using the Fourier-transform infrared (FTIR) analysis, which are responsible for the reduction. Greenly synthesized and extracted powder (Gu-Sd-AgNPs) were characterized by X Ray

Diffraction (XRD) method is an environmentally friendly approach compared to chemical synthesis methods. The synthesized (Gu-Sd-AgNPs) wass showed antibacterial activity with a clear zone of inhibition against gram positive and gram-negative bacteria. while the extracts did not show any activity. This study highlights the possibility of using AgNPs in medical applications and may pave the way for the prepared compound to be widely used in the medical field.

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