

Phytochemical Analysis, FTIR Characterization, and in Vitro Evaluation of Synthesized Silver Nanoparticles from *Annona Squamosa* Bark

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Abstract: The current study reports the synthesis of silver nanoparticles (AgNPs) from the bark extract of *Annona squamosa* (commonly known as custard apple) and their evaluation against human breast cancer cell lines. The bark extract, known for its potent antioxidant properties, was used for the green synthesis of AgNPs. The study involved the characterization of the synthesized nanoparticles through various analyses, including phytochemical screening, Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), energy-dispersive X-ray analysis (EDAX), X-ray diffraction (XRD), UV-visible spectroscopy, free radical scavenging assay (DPPH), and cell viability assays against breast cancer cell lines. The results showed that silver nanoparticles were rapidly synthesized within one hour by adding the *Annona squamosa* bark extract to an ethanolic solution containing silver nitrate (1 mg/mL). The structural properties of the nanoparticles were confirmed by XRD analysis, while FTIR and UV-visible spectroscopy provided additional confirmation of their composition and stability. The antioxidant activity analysis revealed significant free radical scavenging potential of the synthesized AgNPs, with a percentage inhibition of 65.7 $\mu\text{g/mL}$ against DPPH. Additionally, the synthesized nanoparticles exhibited dose-dependent cytotoxicity against breast cancer cells, with an IC₅₀ value of 9.7 $\mu\text{g/mL}$. The percentage of apoptotic cells was observed to increase in a dose-dependent manner. These findings demonstrate the potential of *Annona squamosa* bark-derived silver nanoparticles as a promising agent in cancer therapy.

Keywords: *Annona Squamosa*, XRD, FT-IR.

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I. INTRODUCTION

Nanotechnology is a rapidly advancing field in science, involving the design, manipulation, and imaging of nanostructures within 1–100 nm (Kumar et al., 2017; Balachandar et al., 2019). Nanoparticles (NPs) are small particles composed of matter that exhibit unique transport and physical properties. They are chemically active, enabling encapsulation, dissolution, and attachment (Pourzahedi and Vance, 2017). Biological synthesis of nanoparticles using plant extracts offers advantages such as stability, controlled growth, and eco-friendliness. Silver nanoparticles (AgNPs) are among the most widely studied due to their varied applications in medicine, including drug delivery, cancer therapy, and diagnostics (Ahmed and Swami, 2016).

➤ Synthesis of Nanoparticles

Nanoparticles can be synthesized using chemical or green methods. Common techniques include co-precipitation, chemical reactions, microwave radiation, and the sol-gel method. Green synthesis, utilizing plant extracts, is economical, eco-friendly, and less toxic compared to chemical methods (Korkmaz and Ceylan, 2020). Two primary approaches to nanoparticle synthesis are: Top-down: Breaking down bulk materials into smaller structures. Bottom-up: Building nanomaterials from atoms or molecules (Gopinath et al., 2013). Noble metals like silver, gold, and platinum are often used due to their nano-scale properties.

Nanomedicine involves using nanomaterials for disease monitoring, prevention, diagnosis, and treatment. Nanoparticles enhance drug delivery by increasing circulation time and targeting efficiency. Their size and

properties are crucial for therapeutic applications (Marimuthu et al., 2020).

Medicinal plants are a promising alternative for synthesizing nanoparticles due to their bioactive compounds such as flavonoids, polyphenols, saponins, and alkaloids. These compounds exhibit antimicrobial, antioxidant, and anti-cancer properties. Green synthesis using plant extracts is cost-effective, eco-friendly, and safe, making it an excellent substitute for chemical methods (Radinia and Hasan, 2018).

Silver nanoparticles possess unique electrical, optical, thermal, and chemical properties compared to bulk materials. They are synthesized using physical, chemical, and biological methods. Green synthesis is preferred for its eco-friendliness and ability to produce stable nanoparticles at a lower cost (Marimuthu et al., 2011). Silver nanoparticles are widely used in medicine, healthcare, food industries, and biomedical devices due to their unique properties. They serve as antibacterial agents, cancer therapeutics, wound dressings, and coatings for medical devices, enhancing their efficacy and durability. Green synthesis is a simple, safe, and energy-efficient method for nanoparticle production. It uses natural reducing agents like plant extracts to synthesize nanoparticles with minimal environmental impact (Sivapriyajothi et al., 2014).

Chemoprevention aims to control, suppress, or reverse cancer progression using natural compounds. Medicinal plants in India, rich in bioactive molecules, play a significant role in cancer prevention by inhibiting cell proliferation, inducing differentiation, and promoting apoptosis (Shirwaikar et al., 2004). *Annona squamosa* L., belonging to the family Annonaceae, is commonly known as custard apple or sugar apple. It possesses various medicinal properties and is widely used in herbal medicine (Alali et al., 1999).

Annona squamosa is a novel source of mono-tetrahydrofuran ring acetogenins, which exhibit cytotoxic selectivity against human pancreatic carcinoma cell lines (Hopp et al., 1997). According to Chavan et al., the bark demonstrates significant antioxidant properties and is used in medicine for treating degenerative diseases, including cancer.

II. MATERIALS AND METHODS

➤ Quantitative Analysis of Phytochemical Constituents in *Annona Squamosa*

The phytochemical analysis of *Annona squamosa* revealed the presence of flavonoids, phenols, terpenoids, alkaloids, and saponins, quantified in mg/g (Zodrow et al., 2009).

➤ Synthesis of Silver Nanoparticles

To synthesize silver nanoparticles (AgNPs), 50 mL of 1 mM silver nitrate was combined with 5 mL of plant extract. Silver ions (Ag^+) were reduced to metallic silver upon reacting in ambient conditions. This was evidenced by a color change from translucent to reddish-brown, indicating the formation of silver nanoparticles (Yeruva et al., 2008).

➤ Characterization of Silver Nanoparticles

Various techniques were employed to characterize the synthesized nanoparticles:

- *UV-Visible Spectroscopy:*

This technique was used to assess the optical properties of AgNPs. After mixing the extract with silver nitrate, UV-visible spectroscopy measurements were taken after 24 hours, showing absorption in the 350–500 nm range (Franco-Molin et al., 2010).

- *X-Ray Diffraction (XRD):*

XRD was used to determine the crystalline structure and grain size of the AgNPs (Kim MR and Choi HK, 2009).

- *Fourier Transform Infrared Spectroscopy (FTIR):*

FTIR analysis identified the functional groups involved in nanoparticle formation, with an absorbance range of 4000–400 cm^{-1} (Wang and Ding et al., 1997).

- *Scanning Electron Microscopy (SEM):*

SEM was used to confirm the size and shape of the nanoparticles (Ragasa et al., 2005).

- *Energy-Dispersive X-Ray (EDX) Analysis:*

EDX was employed to analyze the elemental composition of the AgNPs (Hofilen et al., 2002).

➤ Antioxidant Activity Analysis: DPPH Assay

The antioxidant capacity of the AgNPs was evaluated using the DPPH assay. Ethanolic DPPH solution (0.05 mM) was mixed with AgNPs at varying concentrations (20–100 $\mu\text{g}/\text{mL}$). The reaction was allowed to stand at 4°C in the dark, and absorbance at 540 nm was measured spectrophotometrically after five minutes. Ethanol served as the control, and the percentage inhibition of DPPH activity was calculated using the formula:

$$\text{Inhibition (\%)} = \left(\frac{A - B}{A} \right) \times 100$$

Inhibition (%) = (A - B) × 100

Where *A* is the absorbance of the control, and *B* is the absorbance of the sample. The concentration required for 50% inhibition (IC_{50}) was determined by plotting inhibition percentages against concentrations (Deepti and Sushila et al., 2015; Ahmed et al., 2016).

III. RESULT AND DISCUSSION

➤ Qualitative Analysis of Phytocompounds from *Annona Squamosa* Bark

Table 1 presents the evaluation of phytochemical constituents in *Annona squamosa* bark extract using various solvents and assays. The analysis revealed the presence or absence of specific metabolites in the extract. The bark extract exhibited a high concentration of phenols, xanthoproteins, terpenoids, flavonoids, cardiac glycosides, saponins, leucoanthocyanins, tannins, carbohydrates, alkaloids, anthocyanins, steroids, proteins, glycosides, coumarins, and anthraquinones. Several studies have reported

the antitumor properties of *Annona squamosa* seeds (Deepti R, etal). In the current study, the bark extract demonstrated

significant antioxidant activities, which may contribute to preventing various diseases, including cancer.

Table1 Qualitative Analysis of Phytochemicals from the Ethanolic Extract of *Annona Squamosa* Bark Extract

Test Number	Test	Observation	Result
1.	Terpenoids	Reddish with brown	+++
2.	Flavonoids	Yellow color	+++
3.	Saponins	Formation of Emulsion	+++
4.	Tannin	Green precipitate	+++
5.	Alkaloids	Yellow color precipitate	+++
6.	Steroids	Reddish brown ring	+++
7.	Glycosides	Violet into blue into green color	+++
8.	Phlobatanins	Red precipitate	+
9.	Proteins	White precipitate	+++
10.	Conmarin	Yellow precipitate	+++
11.	Emodin	Red color	+++
12.	Anthroquinone	Pink, violet, red color	+++
13.	Anthocyanin	Pinkish red to bluish violet color	+++
14.	Carbohydrate	Reddish violet ring formation	+++
15.	Lauconthocyanin	Organic layer into red	Absent
16.	Cardiac glycosides	Formation of violet or browning	+++
17.	Xanthoprotein	Blue black color	+++
18.	Phenols	Reddish orange color	+++

A – Absence, + - Trace, ++ - Moderate, +++ - Strong

➤ *Quantitative Investigation of Annona Squamosa Bark Extract*

Figure 2 illustrates the quantitative phytochemical analysis of *Annona squamosa* bark extract. The results revealed that the extract contains a higher concentration of tannins compared to other compounds, such as saponins, alkaloids, terpenoids, phenols, and flavonoids, Several

studies have reported that the phytoconstituents of *Annona squamosa* exhibit significant antioxidant activity. In the present study, we investigated key phytochemical constituents, including saponins, tannins, terpenoids, alkaloids, phenols, and flavonoids, which demonstrated potent activity, particularly in inhibiting cell proliferation.

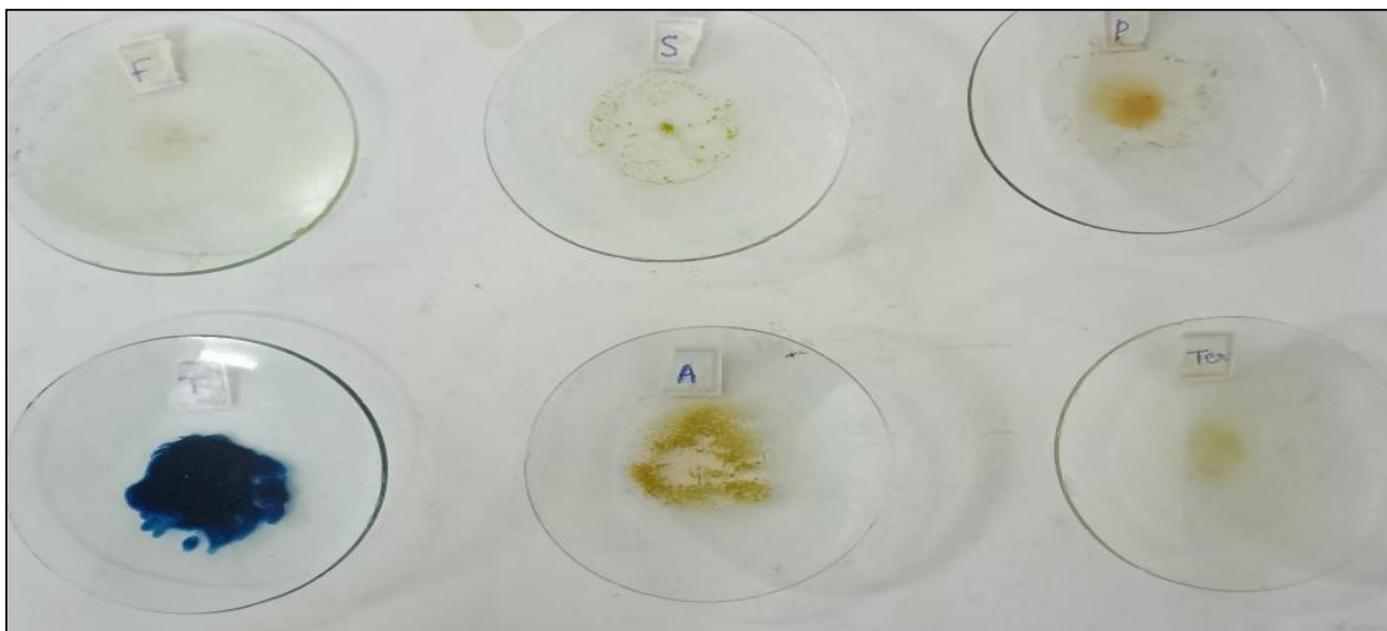


Fig 1 Quantitative Analysis of Secondary Metabolites from *Annona Squamosa* Bark

➤ *Visual Color Chang and Shown the UV Spectroscopy:*

In this experiment, the presence of silver nanoparticles was indicated by color changing from colorless to reddish brown upon adding the plant sample’s ethanol extract to the

glass vial containing AgNO₃. UV spectra of the sample connected to the published literature showed the presence of a plasma resonance band in the 443.4 nm range.

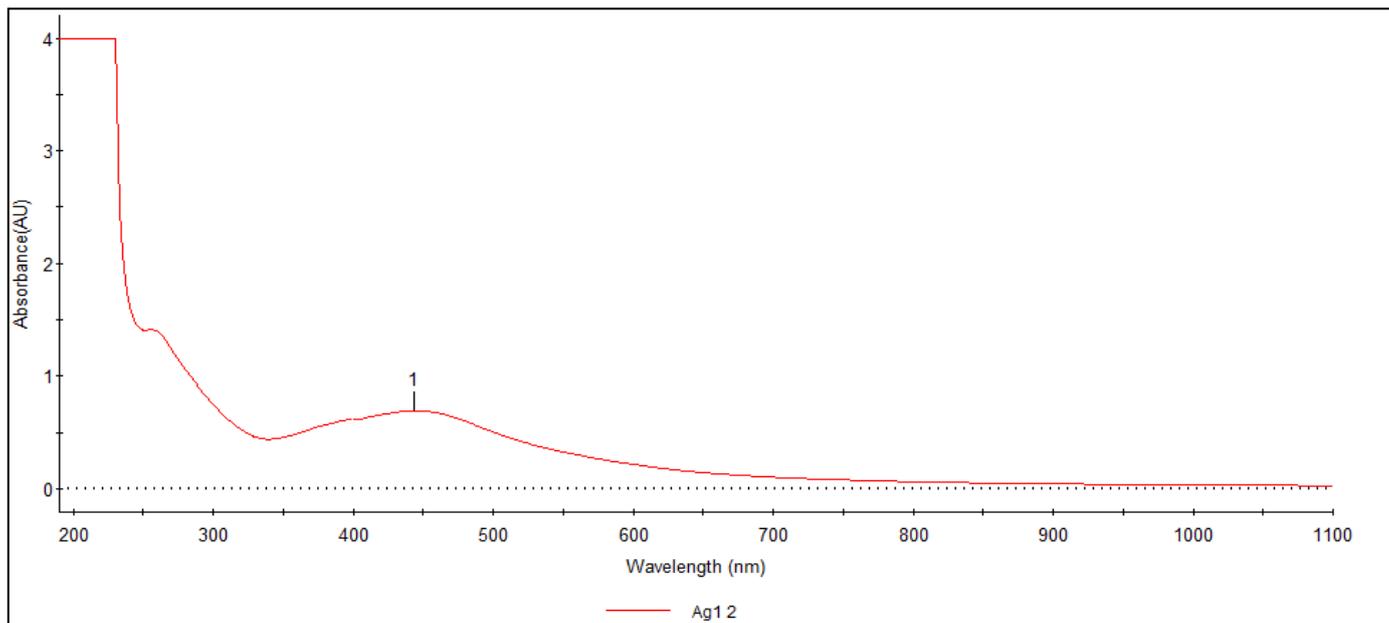


Fig 2 UV – Vis Spectrum of Synthesised Nano Particles Using Medicinal Plants

Name	No	peak (nm)	peak (AU)
Ag1	1	443.4	0.693

IR spectra. Tables and figures are display the FT-IR spectra of silver nanoparticles that were produced utilizing flax and graph seeds.

➤ *Functional Group Determination Using FT-IR Spectroscopy*

Figure 1 showed that the The functional group involved in the reduction of the silver ions it is by ethanol extract’s FT-

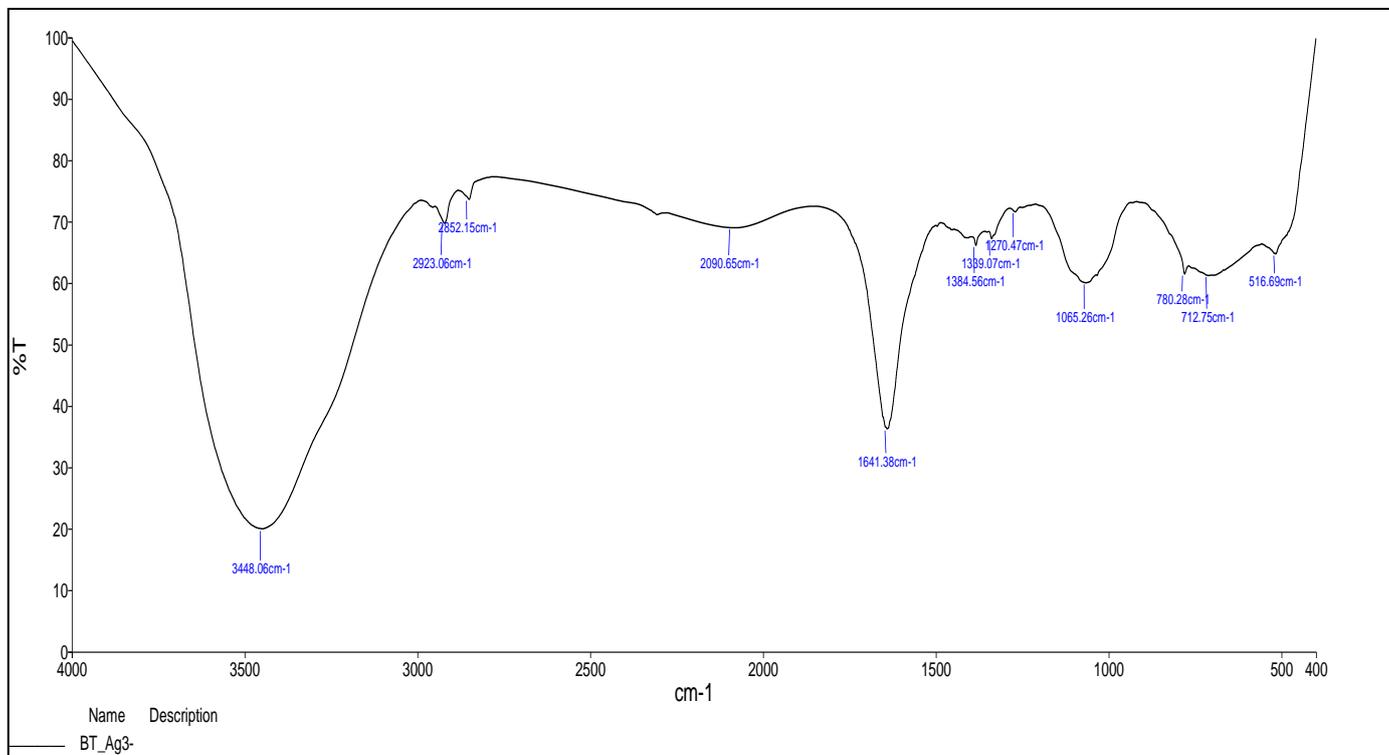


Fig 3 FTIR Analysis of Synthesized Silver Nanoparticles

➤ *X-Ray Diffraction (XRD):*

The typical peaks (at 2θ) were visible in the acquired XRD pattern for silver nanoparticles made utilizing medicinal

herbs. The XRD planes (111), (200), (220), and (311) are shown in the table. The produced silver nanoparticles, all of the miller indices revealed a face- centered cubic shape.

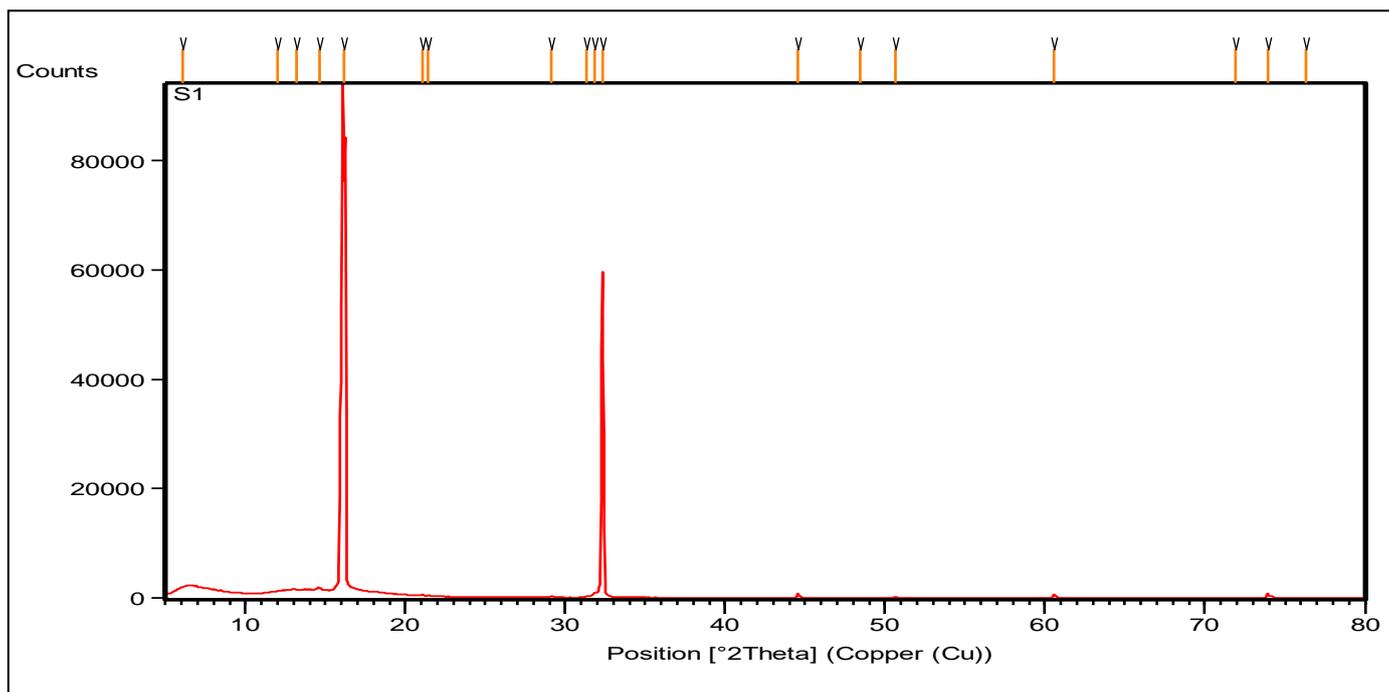


Fig 4 Shows XRD Patterns of Silver Nanoparticles Synthesized Using Medicinal Plants:

➤ *SEM Image:*

The size and shape of the resulting silver nanoparticles are predicted from the SEM image. The average size of

nanoparticles is approximately 200 nm in diameter, and their forms are spherical and cubic. Their size fall within the range of 76-85 nm.

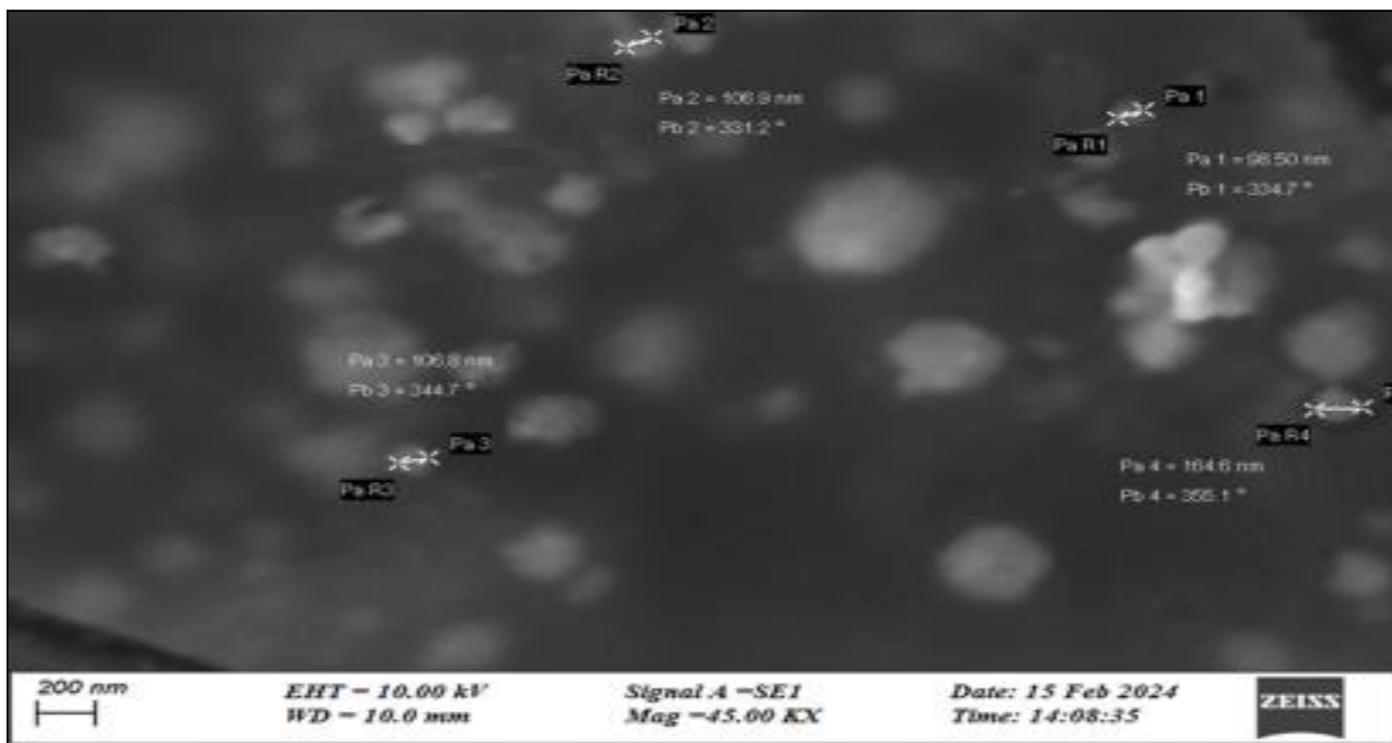


Fig 5 SEM Photograph for Silver Nanoparticles Using Medicinal Plants:

➤ *EDX Analysis:*

The elemental signature of silver nanoparticles was verified by investigation using an energy dispersive X-ray (EDX) spectrometer. The energy in KeV is displayed on the horizontal X-axis, while the vertical Y-axis display the total number of X-ray counts. Because biomolecules are bound to

the surface of the silver nanoparticles, the EDX spectrum collected for the particles is displayed in figure 4 along with an extra peak of oxygen, copper, and sulfur. According to EDX spectra, flax seed reduces silver nanoparticles, which have a 62.19% silver weight percentage.

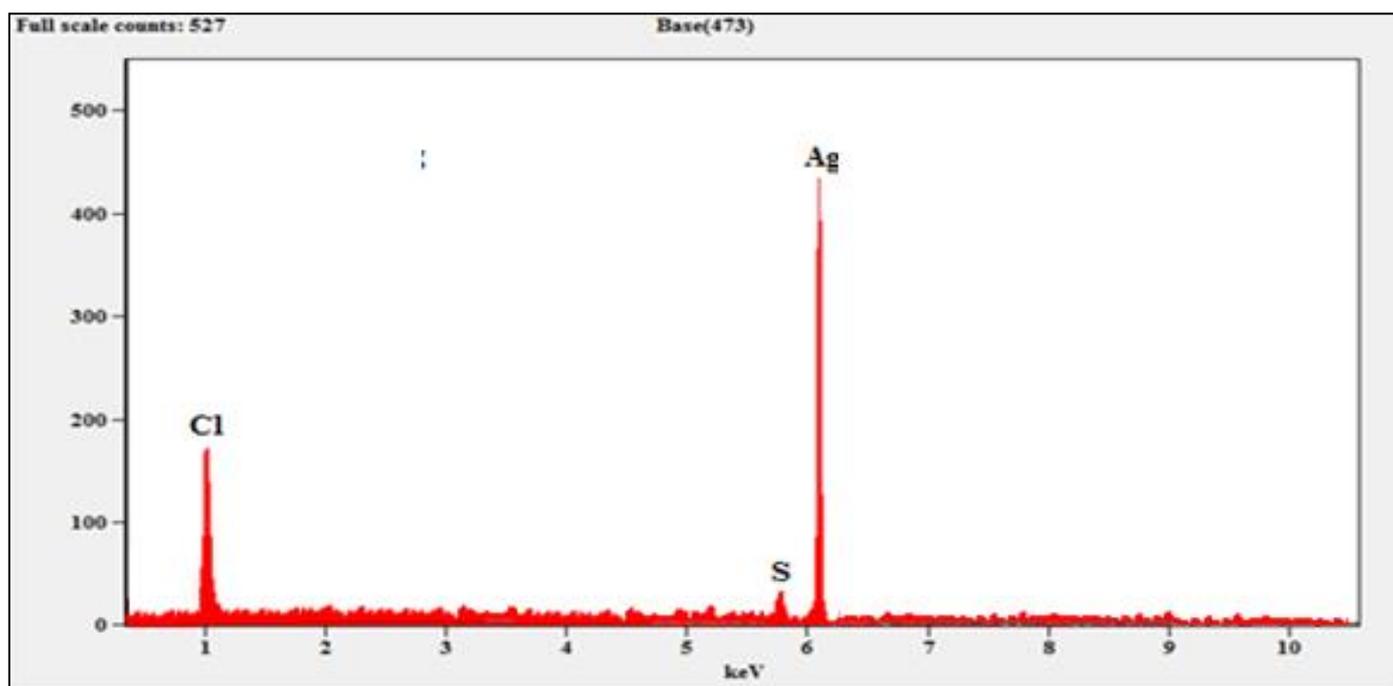


Fig 6 EDX Spectrum of Synthesized Silver Nanoparticles

IV. CONCLUSION

The environmentally friendly synthesis of silver nanoparticles provides a sustainable alternative, reducing risks associated with conventional physical and chemical methods. The synthesized nanoparticles were characterized using UV-visible spectroscopy, which revealed a peak intensity at 440.90 nm. EDX analysis confirmed the elemental composition, while FTIR analysis identified the functional groups present. SEM analysis indicated that the nanoparticles exhibited spherical and cubic morphologies, and XRD analysis verified their crystalline nature. Antioxidant analysis demonstrated significant inhibition at 100 µg/mL, with DPPH (65.7 µg/mL), hydrogen peroxide (51.48 µg/mL), and metal chelating (60.93 µg/mL). Additionally, the silver nanoparticles synthesized from *Annona squamosa* bark showed promising anticancer activity against the MCF-7 breast cancer cell line, with an IC₅₀ value of 47.64 µg/mL. These findings underscore the potent antioxidant and anticancer properties of *Annona squamosa* bark extract.

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