Phytofabrication of silver nanoparticles using sorrel tree leaf extract for anticancer activity

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Abstract: Sorrel tree leaf extract reduced silver nanoparticles (AgNPs) in the lab. The extract's phytochemicals slowed and stabilised silver nanoparticles. Characterising nanoparticle size, shape, and morphology required many approaches. UV-visible, FT-IR, XRD, and SEM were used. All synthesised nanoparticles were spherical and monodisperse. FTIR spectrum stretching vibrations showed green extract stabilises silver nanoparticles. Further examination indicated that these small particles were Sorrel tree (ST) extract silver nanoparticles (ST-AgNPs). Biological synthesis improves silver nanoparticle effectiveness. By optimising these parameters, ST-AgNPs may combat lung cancer (A549 cell line) and breast cancer (MCF7 cell line) more effectively. A549 cells had a 49.52 g mL-1 IC50, whereas MCF7 cells had 78.40. The MTT experiment demonstrated that AgNP concentration correlated with cytotoxicity in both cell lines. Biosynthesized ST-AgNPs are highly anti-cancer. Spherical and monodispersed nanoparticles with significant cytotoxicity against cancer cells were synthesised using Sorrel tree leaf extract as a reducing agent.

Keywords: *Sorrel tree leaf, silver nanoparticles, ST-AgNPs, anticancer activity*

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Introduction:

Based on their chemical makeup, biological characteristics, and medical applications, nanoparticles (NPs) are divided into three primary groups in medicine: metallic, non-metallic, and metal composite nanoparticles.[1–5] Nanoparticles' special qualities have made them widely used in a variety of high-tech fields, notably in technology and medicine.6–8 In the process of making NPs, reducing agents including citrate, hydrazine, and sodium borohydride are often utilised. These substances, however, have the potential to harm the environment and be poisonous to both people and animals. On the other hand, NPs are stabilised and kept from aggregating using capping agents.[9,10]

Proteins, polymers, and surfactants are often utilised as capping agents.[11–14]

Due to their dual function as capping and reducing agents, the utilisation of dissolvable plant extracts has shown remarkable promise in the environmentally friendly production of nanoparticles (NPs).[15–18] These extracts are made up of a wide range of natural substances, such as terpenoids, phenolic acids, and avonoids, all of which have noteworthy capping and reducing capabilities. For instance, a natural reducing agent, such as green tea, ginger, or garlic extract, is mixed with silver nitrate during the manufacture of silver nanoparticles (AgNPs). By serving as a capping agent, the extract stabilises the silver ions by reducing them to AgNPs throughout this process. Different plants may produce NPs with various characteristics, opening up a variety of uses in electronics, catalysis, and medicine.[19–23]

Along with their application studies, developing the green synthesis of metallic nanoparticles is an important study field.[4,24–26] Several techniques, including chemical, photochemical, electrochemical, radiation, and biological syntheses, may be used to create silver nanoparticles. A sustainable, environmentally benign, and economically viable method for creating nanoparticles is provided by the developing field of phyto-nanotechnology, which has several benefits for use in high-tech applications such as medicine.[3,7,27,28] Green chemical practises and the use of natural reducing agents produced from plants or microbes provide a potentially effective way to advance technology while preserving the environment.[29,30]

AgNPs are often used as signal boosters, optical receptors, battery intercalating materials, polarising filters, sensors, bio-labelling materials, anticancer, antioxidants, and antibacterial agents. In the battle against cancer, AgNPs have been found to reduce toxicity, enhance surface

plasmon resonance, and boost electrical resistance. AgNPs has properties that make them ideal for use as agents against cancer, infection, and microbes as well as in the filtration of drinking water.

Silver nanoparticles have emerged as a potential therapy option in this case because of their ability to target cancer spots and concentrate at a higher concentration there.

The size of the nanoparticles may also be carefully controlled during production to optimise their adsorption at the appropriate location. One of the key advantages of using silver nanoparticles as an anticancer drug is their strong cytotoxicity towards cancer cell types, especially for smaller-sized particles.

It has been shown that smaller silver nanoparticles exhibit more cytotoxicity than larger ones. Additionally, these nanoparticles have unique chemotherapeutic properties, such as the capacity to cause cancer cells lacking the p53 gene to undergo programmed cell death, which depends on apoptosis. This is a huge advantage over conventional cancer therapies, which cannot result in cell death in such cells.[31] The capacity of silver nanoparticles in the size range of 5-35 nm to induce apoptosis via the mitochondria and targeted drug delivery systems has also been shown in recent studies, further stressing their therapeutic potential as an alternative to chemotherapy. By using the properties of silver nanoparticles, it may be possible to build effective and targeted cancer medicines with minimum damage to healthy cells. The goal of the present study was to validate the efficacy of an ecologically friendly phyto-synthesis for silver nanoparticles (AgNPs) using Artemisia tschernieviana extract (ATE). The AgNPs derived from A. tschernieviana extract were highly cytotoxic to the HT29 colon cancer cells and promoted apoptosis. These findings demonstrate the potential of AgNPs produced by plants for application as anticancer agents. Furthermore, Cr-doped ZnO nanostructures displayed a blueshift in UV emission and a notable drop in deep level emission. The band gap widened with Cr doping as a result of size reduction and doping effects. The developed nanostructures also shown promise for the eradication of bacteria.[32,33]

In the present study, Sorrel tree (ST) extract was used in a bio-reduction approach to produce AgNPs. The technique was simple to utilise, the synthesised nanoparticles were stable, and they were in the nanometer range. Despite the fact that several research have been conducted on the green synthesis of AgNPs using leaf extract, there are few studies on the potential anticancer and antibacterial effects of AgNPs synthesised utilising wild and indigenous species. ST, an oxaloacetate-family plant, shrub, or small tree, is found in the tropics and subtropics. There are around 12 different species in India, and beverages made from the leaves are

reportedly used to cure diabetes and irritated rectums as well as being antibacterial and antiscorbutic. Additionally, syphilis, coughs, colds, itches, and skin rashes may all be treated using a paste prepared from the leaves.

AgNPs research has expanded the possibilities for using nanoparticles in a variety of fields. The standard synthetic processes may have a greater negative effect on the environment than the green synthesis of AgNPs utilising plant extracts. An easier and more practical way for synthesising stable AgNPs in the nanometer range is provided by the current work on the bio-reduction process employing ST extract. Further research on the possible anticancer and antibacterial action of AgNPs employing native and wild species might be very informative.

2. Experimental

2.1 Materials

Acetone and silver nitrite (AgNO3) were purchased from Sigma-Aldrich (AR grade). The extraction was performed using a Soxhlet equipment and double-distilled water. A digital centrifuge, well microtiter plate, MCF-7 cell line, microscope, CO2 incubator, and gyratory shaker were additional pieces of equipment.

2.2 Preparation of aqueous extract from the leaves of ST

The Sorrel tree leaves were carefully picked up from the Navanagar neighbourhood of Hubballi, Karnataka, India, and cleaned before being dried in the shade. They were finely ground using a mortar and pestle after they had dried fully, yielding roughly 50 g of dry powder. The powder was added to the thimble after a thorough cleaning and drying of the Soxhlet extractor. The extraction was carried out at a temperature of 80 °C after a round-bottomed bottle holding 100 mL of acetone was heated to boiling point and decanted. At least 20 cycles were necessary for the extract to flow from the thimble to the roundbottom ask. The extracted substance had a green hue, suggesting the presence of phytochemicals. After filtering the ST extract using Whatman No. 1 filter paper to eliminate any contaminants, it was kept at a lower temperature to preserve its efficacy. This careful procedure made sure that the ST extract was of the highest calibre and prepared for use in further studies or applications.

2.3 Synthesis of silver nanoparticles (ST-AgNPs)

A normal laboratory process was used to create a 1 mM concentrated silver nitrate solution, which was then used to synthesise the silver nanoparticles using Sorrel tree extract. The silver nitrate solution and ST extract were then combined in a 1:5 ratio while being well stirred. Immediately after combining, the mixture's coloration changed from bright green to chrome yellow, which over time became brown. The ST-AgNPs were then produced in a powdered,

dry state for further research after the combination was allowed to stay stable at room temperature for 24 hours (Fig. 1 and 2). This first finding served as the basis for nanometrological confirmation of the synthesised ST-AgNPs. In order to guarantee that the nanoparticles possessed the requisite qualities and features required for their future use in biomedical applications, such as anticancer and antibacterial applications, it was crucial to confirm the synthesis of the nanoparticles.

Fig 1: Sorrel tree leaves

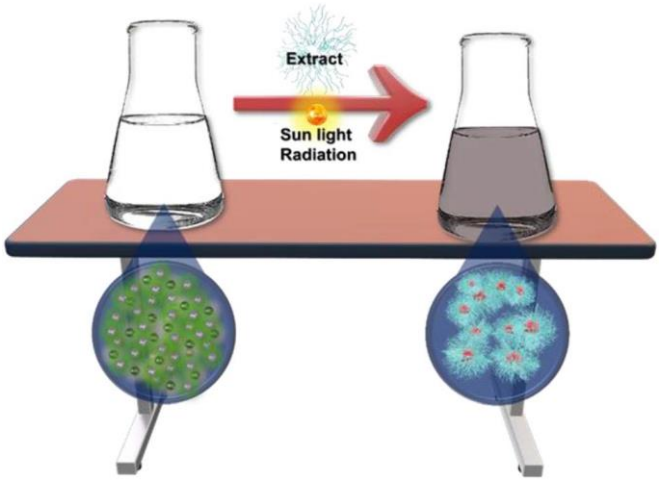

Fig. 2 Schematic representation of the synthesis of ST-AgNPs.

3. Results and discussion

In the field of medicine, using nanoparticles has recently emerged as a potential strategy, notably for the treatment of cancer. One of the various types of nanoparticles, silver nanoparticles, have generated attention due to their unique properties and potential use in the treatment of cancer.

This research looked at the biosynthesis of silver nanoparticles made from ST extract (ST-AgNPs) and assessed its potential impact on cancer cell lines.

3.1 Phytochemical analysis

Numerous phytochemicals were discovered to be present in the leaf extract of ST in water and to be significantly involved in decreasing and capping AgNPs. Diverse secondary metabolites that show potential for a range of biological and industrial uses were discovered via qualitative research. SI 1 of the ESI contains a description of these findings. Ag+ ion reduction to Ag0 atoms was a step in the biosynthesis of AgNPs from the ST extract. The special mix of phytochemicals in the extract may function as stabilisers and reducing agents, preventing the nanoparticles from clumping and promoting uniform dispersion in an aqueous media. The potential of Sorrel tree leaf extract as a sustainable and environmentally friendly substitute for traditional chemical procedures for the manufacture of silver nanoparticles is highlighted in this work. Natural plant extracts may make nanoparticle production less harmful to the environment while also offering a scalable, affordable method for creating nanoparticles with the appropriate qualities.

3.2 Nanometrology of silver nanoparticles

A UV-160V spectrophotometer was used to monitor the synthesis using UV-visible spectroscopy. This approach measured the samples' ultraviolet and visible absorption spectra, revealing the synthesised nanoparticles' electronic transitions and chemical composition.

FTIR analysis was performed on dried ST-AgNPs after synthesis to detect biomolecules. FTIR spectra indicated nanoparticle production and stability functional groups and chemical bonds. XRD, SEM, EDX, and TEM characterised the nanoparticles. SEM and TEM observed nanoparticle size, shape, and distribution, while EDX analysed their elemental composition.

XRD determined the nanoparticles' crystallographic orientation and lattice spacing. These analytical methods characterised the Sorrel tree aqueous leaf extract-synthesized ST-AgNPs. These findings explain the green production of AgNPs using natural extracts and their prospective uses in medicine, electronics, and catalysis.

3.3 UV-visible spectroscopy analysis

Silver, gold, platinum, and copper noble metal nanoparticles have distinctive optical features thanks to SPR. When free electrons oscillate in response to a certain frequency of light radiation, nanoparticles absorb the light energy. Metal nanoparticles only display this behaviour as a result of the close proximity of their conduction and valence bands, which allow electrons to easily move between them. Noble metal nanoparticles that have been synthesised have a distinctive SPR absorption peak.

Controlling the morphology, size, and form of metal nanoparticles is necessary during synthesis. The SPR absorption peak's relationship to particle size, the dielectric medium, and

chemical conditions is explained by the Mie theory. As the size of the nanoparticles diminishes, the SPR peak moves towards shorter wavelengths. Design and use of metal nanoparticles are impacted by this.

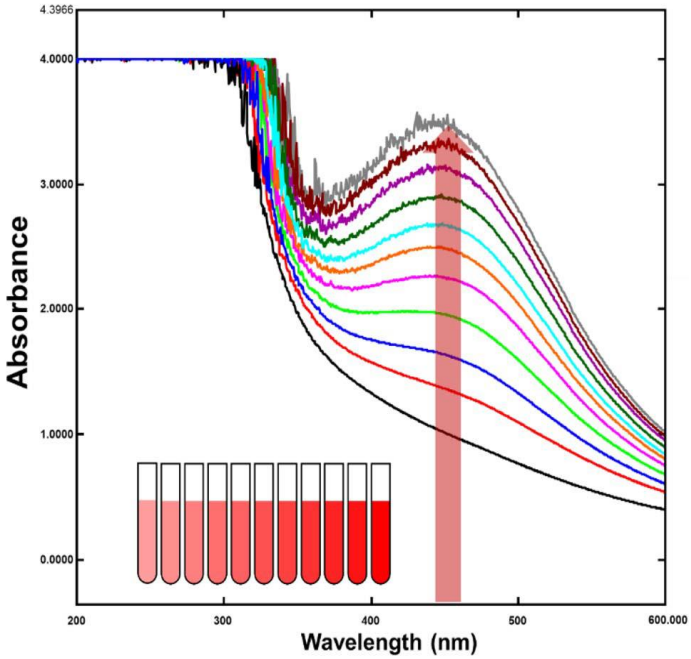

Fig. 3 UV-visible spectrum of ST-AgNPs.

3.4 X-ray diffraction analysis

XRD examination determined the chemical composition and crystalline nature of ST-AgNPs. This method is often used to study nanoparticle crystal structures. The XRD investigation identified and estimated the ST extract material's crystalline structure and AgNP particle sizes. XRD spectra showed that ST-AgNPs were crystalline, and their oxidation status changed over time. Fig. 4 showed nanometric silver nanoparticles. Based on strong peaks at 45.1° and 64.7°, the Debye–Scherrer formula computed the average particle size. XRD showed five primary diffraction peaks for the (111), (200), (120), (202), and (311) planes. TEM scans confirmed these peaks in AB-biosynthesized AgNPs. The nanoparticle size greatly affected the XRD peak patterns, demonstrating the need to manage particle size during synthesis.

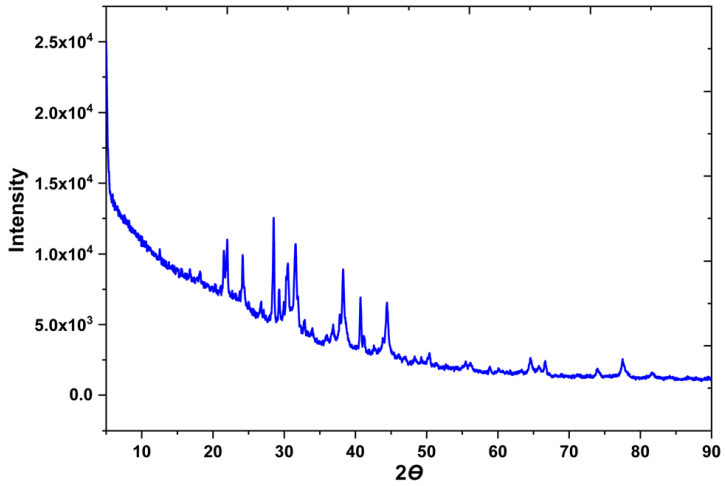

Fig. 4 XRD diffractograms of the AB-AgNPs.

3.5 Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy helps analyse plant extracts' silver reduction. Infrared light is used to assess plant extract nanoparticle surface chemistry. Infrared radiation absorption and transmittance may identify a sample and its phytochemicals' functional atoms and chemical bonds. Fig. 5 shows plant-based ST-AgNP synthesis.

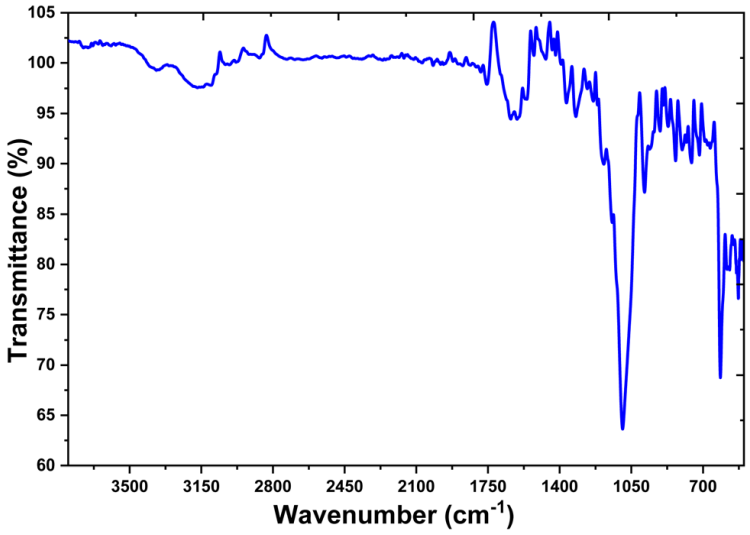

Fig. 5 FTIR spectrum of AB-AgNPs.

3.6 Scanning electron microscopy (SEM) analysis

ST-AgNP morphology determines their physicochemical characteristics and applications. SEM was used to analyse the ST-AgNPs' morphology. The 3–5 nm ST-AgNPs were evenly dispersed

and spherical. Nanotechnology and healthcare need these tiny dimensions. EDX showed a high silver absorption peak at 3 keV.

Silver, the main component of ST-AgNPs, was high in this peak. SEM microscopy confirmed the homogeneous distribution of ST-AgNPs in Fig. 6. Synthesised ST-AgNPs showed promise for drug delivery, biosensing, and imaging.

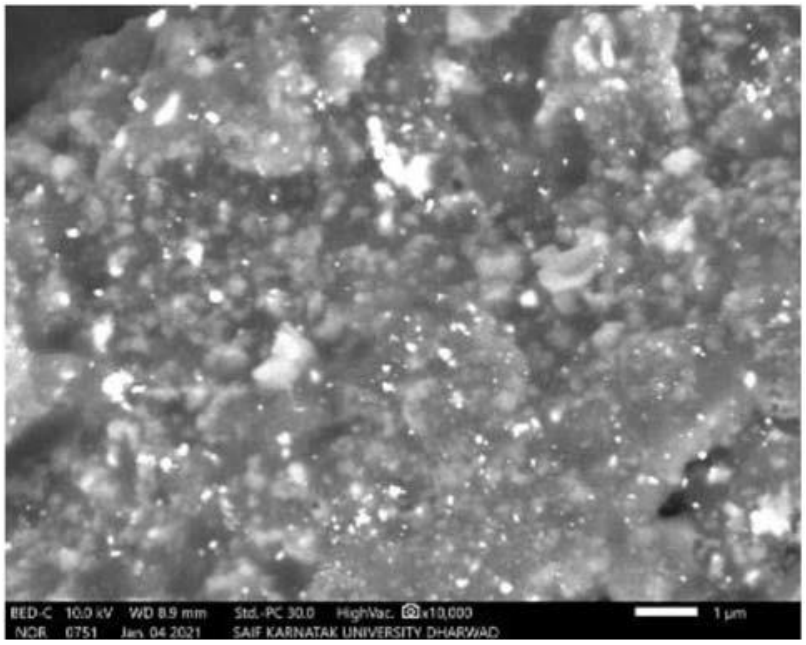

Fig. 6 SEM images of the ST-AgNPs samples.

3.7 Transmission electron microscopy (TEM) analysis

TEM is used to analyse the shape and size range of silver nanoparticles (AgNPs). TEM's strong resolving power enables nanoparticles to be detected and imaged on a photographic plate. TEM examination properly characterises AgNP morphology, revealing their size and form. Electrons must pass through a thin sample for TEM investigation. Nanoparticle topography, shape, size, and morphology may be studied using this instrument. TEM showed ST-AgNPs were spherical and crystalline. ImageJ software showed these nanoparticles were under 5 nanometres.

ST-AgNPs displayed polycrystalline borders in the TEM picture. As illustrated in Fig. 7, each grain of the synthesised ST-AgNPs has multiple lattice planes oriented in various orientations, confirming their polycrystallinity. The SAED pattern showed concentric bright-colored rings (111), (200), (220), and (311), which matched the XRD planes, suggesting a face-cantered cubic lattice (fcc). TEM characterises nanoparticle size, shape, and morphology. TEM has helped researchers grasp ST-AgNPs' characteristics by showing their polycrystalline structure

in great detail. New nanoparticle uses in health, electronics, and environmental research need such understanding.

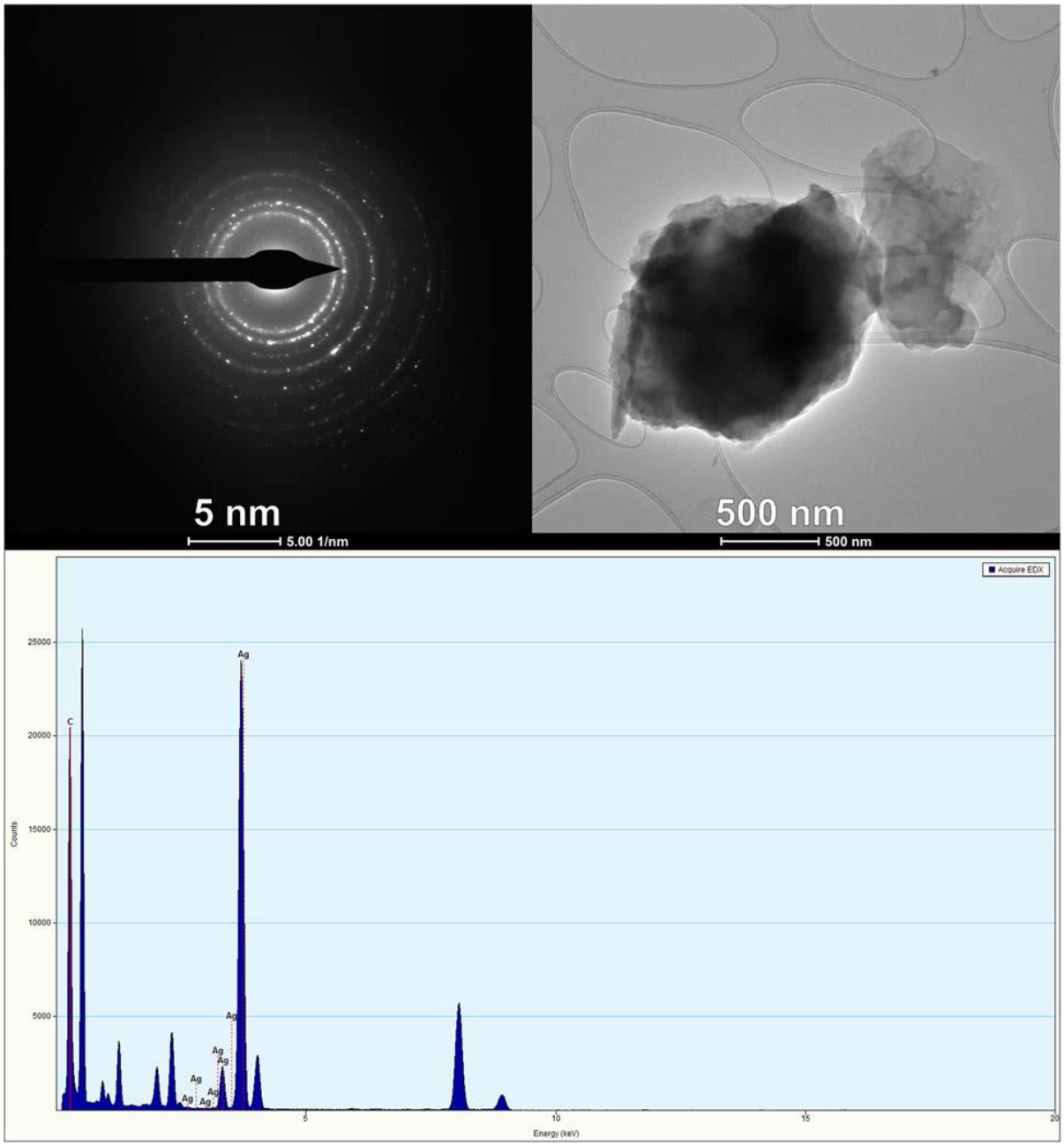

Fig. 7 TEM images of ST-AgNPs and TEM-EDX of the Ag content in ST-AgNPs.

3.8 Mechanism of the anticancer activity of AB-AgNPs

Cancer cell apoptosis involves several routes. ROS-induced apoptosis is a typical mechanism. Silver nanoparticle size affects ROS production. Apoptosis is caused by a three-step signal transduction pathway. First, ST-AgNPs attach to the receptor protein (p53) to receive a chemical signal and transport it to the cell's nucleus. Tumour suppressor p53 controls cell

division and proliferation. 50% of malignant cancer cells contain p53 mutations, whereas normal cells have low amounts. Primary receptor proteins activate other second messenger proteins.

These second messengers boost the nucleus and cell message. ATP energy stimulates metabolic enzymes. Signal transduction pathways activate enzymes. The third stage, cellular response to activation, disrupts mitochondrial transmembrane potential. Suppressing cell respiration causes cell death. Inducing cancer cell apoptosis using silver nanoparticles is promising study. Silver nanoparticles' impact on healthy cells and therapeutic uses need further investigation. [34–36]

The ecological amalgamation of Mentha arvensis, or "corn mint," has produced silver nanoparticles (AgNPs) with promising results. Synthesised AgNPs may promote cytotoxicity in breast cancer cells, particularly via inducing caspase-9-mediated apoptosis in MCF-7 cells.[37] Targeted medication delivery of silver nanoparticles causes selective cancer cell killing. Silver nanoparticle concentrations vary in normal and malignant cell lines. [35-43]

Conclusion:

ST extract synthesises silver nanoparticles safely and environmentally. UV-vis, FTIR, XRD, and SEM analysed the ST-AgNPs. FTIR tests showed that the phytochemicals of the AB extract have hydroxyl and carbonyl groups, which reduced and capped the nanoparticles. SEM examinations confirmed the development of nanoclusters with particle sizes from 50 to 90 nm, corresponding with XRD findings that demonstrated a face-centred cubic (fcc) structure, as confirmed by standard JCPDS data. Under optimised settings, the ST-AgNPs were tested to inhibit lung cancer (A549 cell line) and breast cancer (MCF7) cell proliferation. A549 and MCF7 cells had IC50 values of 49.52 and 78.40 mgmL−1 for ST-AgNPs at the lowest doses. MTT experiment confirmed ST-AgNPs' dose-dependent cytotoxicity on both cancer cell lines. Thus, biosynthesized ST-AgNPs may fight cancer.

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