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ORIGINAL ARTICLE

Biosynthesis and evaluation of metallic silver nanoparticles (AgNPs) using *Ajuga macrosperma* (Ghonke ghas) leafextract, along with anticancer activity

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ABSTRACT
The term "nanotechnology" refers to the development and application of materials whose component parts are, by convention, up to 100 nm in size and exist at the nanoscale. It alludes to a young branch of research that deals with the creation and synthesis of diverse nanomaterials. Among the various metallic nanoparticles, one of the most significant and fascinating nanomaterials for biological implementations is silver nanoparticles (AgNPs).The plant extract of Ajuga macrosperma was used in this investigation to perform green production of silver nanoparticles. Because of their distinct qualities, silver nanoparticles have been a research topic. The production of brown hue during the reduction of Ag^+ to Ag^0 was observed and produced silver nanoparticles exhibit an absorption band in the UV-vis. spectrum at 337 nm. Using zeta potential, FTIR, XRD, SEM, UV-Vis, and EDX, silver nanoparticles were characterised after the reaction. The FTIR study's findings demonstrated that the synthesis and stabilisation of the AgNPs were caused by the presence of terpenoids and flavonoids. According to SEM analyses, the cubic shape of the metallic silver nanoparticles particles having a size range of 10 to 50 nm was evident. Produced silver nanoparticles had a promising instability, as evidenced by the XRD data, which revealed that they had a fcc structure and a -29.06 mV zeta potential value. The silver nanoparticles produced through green synthesis could be applied to the treatment of various cancers. The synthesized AgNPs had IC50 values of 1.20, 4.69, 2.45, and 8.90 g/ml against the MCF-7 (breast cancer), HeLa, PC-3 (prostate cancer), and A549 (lung cancer) cell lines, respectively.

Keywords: biosynthesis, silver nanoparticle, characterization, anti-cancer properties**.**

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INTRODUCTION
As time goes on, every sector will come to rely more and more on developments in nanotechnology. Due to its cheap cost and benign impact on the environment, biosynthesis of nanoparticles has lately gained favors. Because of its unique physical and chemical features, nanotechnology is becoming vital to fields as diverse as industry, medicine, the environment, and health care. [1].
Nanoparticles, which have a diameter of just 10^9 nm, may be transported and characterized much like larger particles but on a much smaller scale. In general, chemical, physical, and biological techniques have been employed to produce metallic nanoparticles [2, 3]. Synthesis techniques that rely on chemicals or physical processes are often costlier and result in waste that is harmful to the environment. Plant-based synthesis methods have expanded to include the biosynthesis of nanoparticles. Researchers are interested in metal oxide nanoparticles because of their distinct properties and potential uses in a variety of physical and chemical fields. Nanoparticles composed of metals and metal oxides have advanced medical imaging, diagnosis, and therapy in recent years. Silver, copper, and gold are now the most widely used metals in the world. Silver nanoparticles (AgNPs) also work well as agents against angiogenesis,

inflammation, blood clots, and viruses. In collaboration with other researchers, Studied that zinc oxide shows promise as a photo catalyst due to the size of its band gap energy and the durability of zinc nanoparticles. Metallic nanoparticles show promise include: [4] Nano generators; [5] gas sensors; [6] biosensors; [7] solar cells; [8] site visitors; [9] photo detectors; and catalyst [10]

The plant-based synthesis route for making nanoparticles is used in many different ways today. A fascinating experiment was carried out utilising leaf extracts from Zea mays, Basella alba, Sorghum bicolor, Saccharum officinarum, Oryza sativa and Helianthus annus [11]. They came to the conclusion that H. annus had the greatest potential of all the examined plant leaf extracts to the quick reduction of silver ions.

Anticancer Activity

Breast carcinoma is the most prevalent cancer in women, , which accounts for over 1.7 million confirmed cases internationally. The human epidermal receptor 2 (HER2), progesterone receptor (P.R.), and oestrogen receptor (E.R.) are typically used to diagnose the condition despite its complexity [12]. 15% of breast tumors overexpress HER-2, and more than 70% of these instances can be treated with anti-hormonal and anti-HER2 drugs. Because few recognized therapeutic drugs have yet to be produced for this aggressive variant of breast cancer [13] and lung cancer, untargeted chemotherapy is the sole treatment option left for the remaining patients whose tumors express none of these sites. These patients are known as triple-negative breast cancers (TNB).

Synthesized from the plant Syzygium cumini, silver nanoparticles were investigated for their anti-diabetic and cardio-protective capabilities. This research found that silver nanoparticles generated from S. cumini were effective in reducing the heart stress caused by glucose. Researchers determined that protecting cell membranes and decreasing oxidative stress were the underlying mechanisms of action [14] .

MATERIAL AND METHODS

Plant material collection

The plant material was collected by hills land of district Pauri Garhwal Uttarakhand. The plant was authentified as Ajuga macrosperma (Ghonke ghas.) family- Lamiaceae by Dr. Neelu Singh (Taxonomist). National Tropical Research Institute of Forest, Jabalpur, Madhya Pradesh, Species number is 11251.

Scientific Classification of plants

The tropical areas of India, Nepal, and China are home to the perennial plant known as Ajuga macrosperma Wall. Ex Benth. The mainland of China is home to two distinct varieties of A. macrosperma: var. macrosperma and var. thomsonii. In traditional Chinese medicine, A. macrosperma var. macrosperma is utilized for the treatment of nephritis, as well as for the reduction of fever and the elimination of phlegm. .

Figure 1; *Ajuga macrosperma* plant

Classification

Kingdom	Plantae
Phylum	Tracheophyta
Order	Lamiales
Class	Magnoliopsida
Family	Lamiaceae
Genus	Ajuga
Species	Macrosperma wall, ex. Benth

Method for preparation of leaves extract.

After being harvested from their natural habitat in Kotdwara, *Ajuga macrosperma* were washed with in distilled water to eliminate any remaining dust. After washing and shorting the leaves, they were air dried in the sun for 8 to 9 days before being ground into a powder. A grinder was used to reduce the dried leaves to a fine powder, which was then kept for further use in the test (*Ajuga macrosperma* leaves powder, see in figure 2).

In a 500 ml round- bottom flask, 7 gram of *Ajuga macrosperma* leaves powder was combined using 100 ml pure water and boiled for 50 minute, at 80 C to extract the desired substance. After waiting 1 hour, the watery extract solution becomes a dark brown, indicating that the extract had formed. After allowing the extract to cool to ambient temperature, It was refined via Whatman No. 1 filter paper. (3 in figure)

Figure 2; *Ajuga macrosperm*a leaves powder

Figure 3; Leaf extract of *Ajuga macrosperma*

Synthesis of silver nanoparticles

In order to create silver nanoparticles (AgNPs) from the leaf extract of Ajuga macrosperma plant, a 0.1M silver nitrate solution was made in a 100 ml beaker by adding 25 ml of ionised water and stirring continuously. 25 ml of silver nitrate solution were combined with 7 ml of Ajuga macrosperma leaf extract, and the solution was warmed at 80 °C for one hour and 30 minutes. The solution's brown colour changed to a bright light brown. (Shown in figure 4) The existence of synthesized AgNPs was confirmed by UV-visible spectroscopy in the 200–800 nm range. After cooling, the mixture was brought to room temperature. The resultant solution was centrifuged at 15000 rpm for 20 minute to obtain the Ag-NPs pellets. The pellets were washed twice with distilled water to remove impurities. The Ag-NPs were characterized for other studies.

Figure 4; synthesis of silver nanoparticles

Characterization

The U.V. Vis., FTIR, EDAX, and SEM techniques were utilised to characterize the synthesized silver nanoparticles (AgNPs). The UV-Vis double-beam bio-spectrophotometer UV-3900 from Shimadzu was used to measure the absorbance spectra of the sample at wavelengths between 200 and 800 nm. Using a Nano Size Particle Analyzer and laser diffractometry, 34.13 d (nm) silver nanoparticles were measured. Shimadzu's I.R. Affinity 1A FT-IR spectroscope, which has a scan range of 4000 to 500 cm^{-1} was used to record the functional group of the nanoparticles. Energy dispersive X-analysis (EDAX) by using the (AMETEK team V.4.3 EDS detector) instrument and morphological analysis of silver nanoparticles was done using SEM (JEOL JEM 2100, Japan).

RESULTS AND DISCUSSION

UV-Visible spectroscopy

Silver nanoparticles (AgNPs) biomolecule-assisted synthesis is being studied using a special method called extraordinary U.V.-visible absorption spectroscopy. Metallic silver nanoparticles are manufactured extracellular, metal ions are reduced into nanoparticles and stabilized by a number of alkaloids and biomolecules that are reductive found in the plant leaf extract. A technique to examine the reduction of precursors that has taken place in the 350–490 nm regions is UV–visible spectroscopy. The absorption peak of a manufactured silver nanoparticle is at 377 nm (Figure 5). An absorption peak between 300 and 450 nm, which is identical to the features of the UV-visible spectra of silver oxide nanoparticles, was observed as a result of the stimulation of surface Plasmon interactions in the silver nanoparticle solution. The production of AgNPs is indicated by a maximum absorption at 377 nm. Solomon et al. (2007) discovered that Ag particle size and plasmon maxima indicated that U.V. spectra were in the shorter wavelength range (384-414 nm) with particles ranging from 10 to 14 nm.

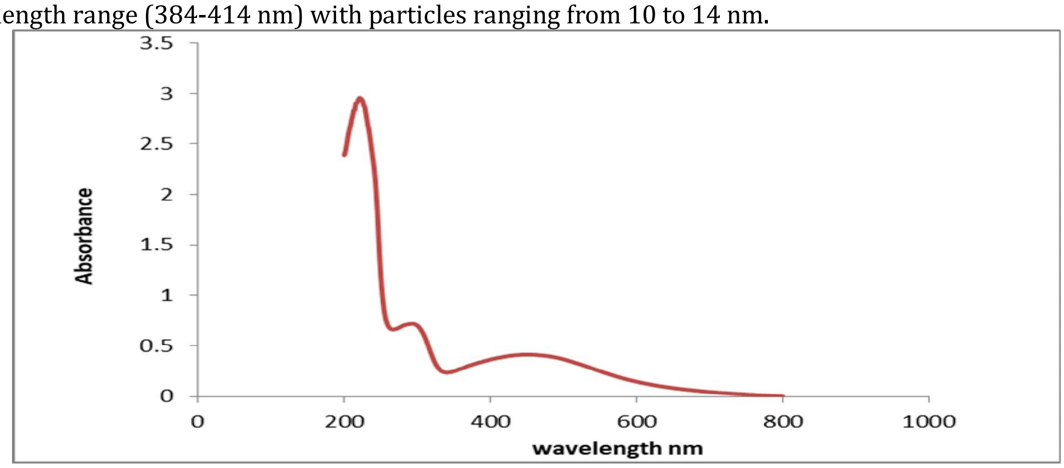

Figure 5 ; Shows the ultraviolet-visible spectra of silver nanoparticles made from Ajuga macrosperma plant extract.

Fourier transformed infrared (FTIR)

FTIR is a method that is more frequently used than I.R. spectroscopy. The FTIR patterns of free groups and functional groups bound to the metallic nanoparticle surfaces differ. The produced Ag nanoparticle and the Ajuga macrosperma extract were subjected to FTIR measurements in order to look for any potential changes in functional group bonds that might have occurred during the reduction process. Figure 5 depicts the FTIR spectra for extracts of Ajuga macrosperma, which revealed numerous distinct bands near 3738.05, 2304, 1514, 1222-1070, and 681 -cm-1. The absorption pleatu at 3738.05 cm^{-1} is correlated with the stretching vibrations of the hydroxyl (OH) functional group of polyphenolic substances. The primary cause of the biomolecule-aided formation of AgNPs in ajuga macrosperma extract is the existence of phenolic (-OH.) groups of terpenoids and flavonoids. The C-O and C-C stretching correlate to other peaks at 2304 and 1514 cm1, respectively. The functional groups of polysaccharides and ester carbonyl are represented by the absorption peaks at 1222 and 1070, respectively. Peaks caused by silver nanoparticles below at 681 cm^{-1}.

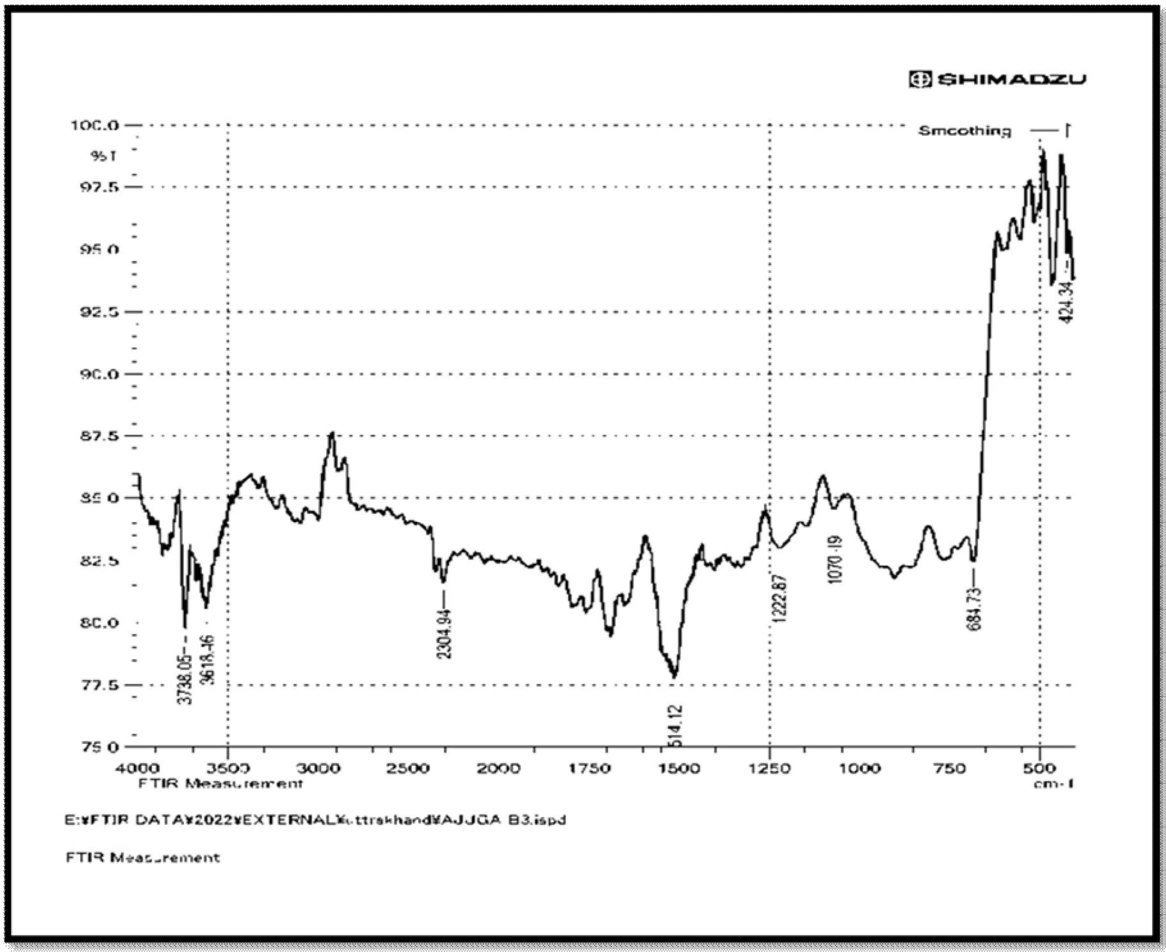

Figure 6; FTIR spectra of silver nanoparticles using plant extract of *Ajuga macrosperma*

XRD analysis of silver nanoparticles (AgNPs.):

The analysis method known as X-ray diffraction is widely used to study crystal and atomic structures, qualitatively identify different compounds, gauge the degree of crystallinity, resolve chemical species quantitatively, determine particle sizes, and identify isomorphous replacements, among other things. Figure (7) displays the X-ray diffraction spectrum of the AgNPs that were created utilising the leaf extract from Ajuga macrosperma. A 1 ° per minute scan was used to record the scope in a 2 theta/theta range between 20–80 degrees at an X-ray wavelength of 1.54 nm. The crystal phase of silver nanoparticles is depicted by the indexed diffraction peaks in the picture silver XRD data reference number 01 087-0719 matches the cliffs. The three show peaks of fcc structure of silver nanoparticles are 111, 200, and 64.72. Debye-Sherrer formula to predict the crystalline size of AgNPs.

$$D= 0.89/\cos$$

Where
B = is the line broadening at half the maximum intensity (FWHM) in radians
theta is the Bragg angle, and 0.89 is the shape factor, gamma is the X-ray wavelength, and these values are all expressed in radians. According to the Debye-Sherrer equation,\ the silver nanoparticle's crystalline size was around 23.28 nm.

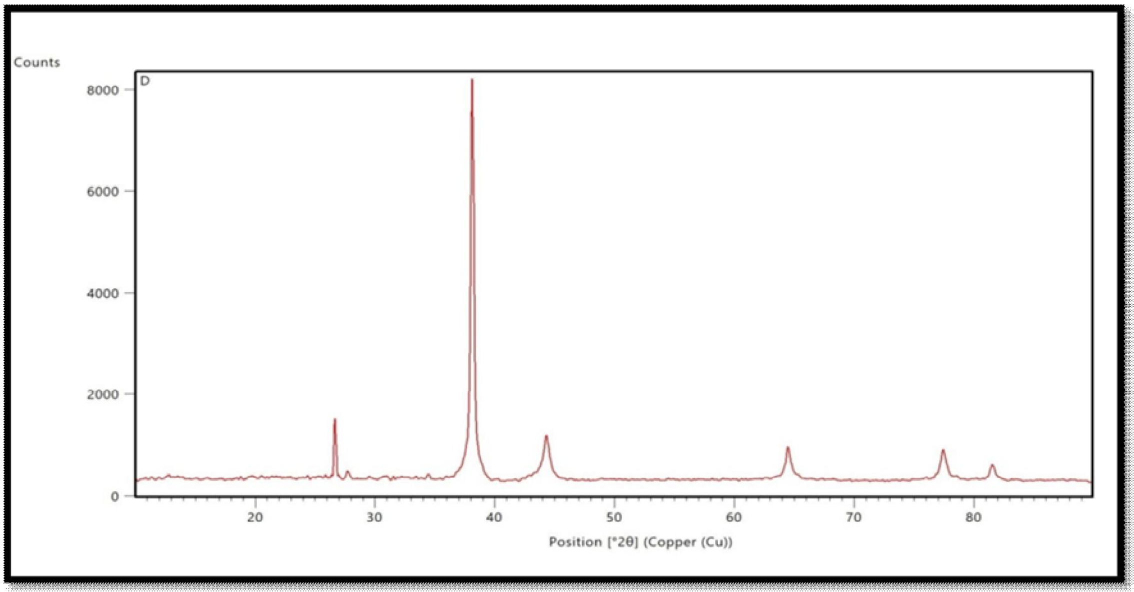

Figure 7; displays the X-ray diffraction spectrum of the AgNPs that were created by employing the leaf extract from the *Ajuga macrosperma* plant.

Scanning electron microscopy (SEM)
It is an effective method for obtaining images of any material surface with a resolution of as little as 1 nm. Secondary electrons with energies under 50 eV are created as a result of an incident electron beam's interaction with the specimen. SEM can provide details regarding the samples' purity of nanoparticles. The anatomy of the produced silver nanoparticles was studied to use a scanning electron microscope. It was demonstrated that the particles are predominantly cubic in shape. Literature reported *Pinus desiflora, Diospyros kaki, Ginko bibola, Mangalia kokus,* and *Platanus orientalis* cubic silver nanoparticles [15]. SEM images show that the silver nanoparticles synthesized by *Ajuga macrosperma* cubic shape and size of the silver nanoparticles at a wave length of 10 to 50 nm. In The first image, 8(a), shows a 2m with a magnification of 20.000 K X and the second nanoparticles ranging from 10 to 50 nm. Using image 8(b), which shows the 2 m with 10.000 KX magnifications, a similar result of silver nanoparticles was reported.

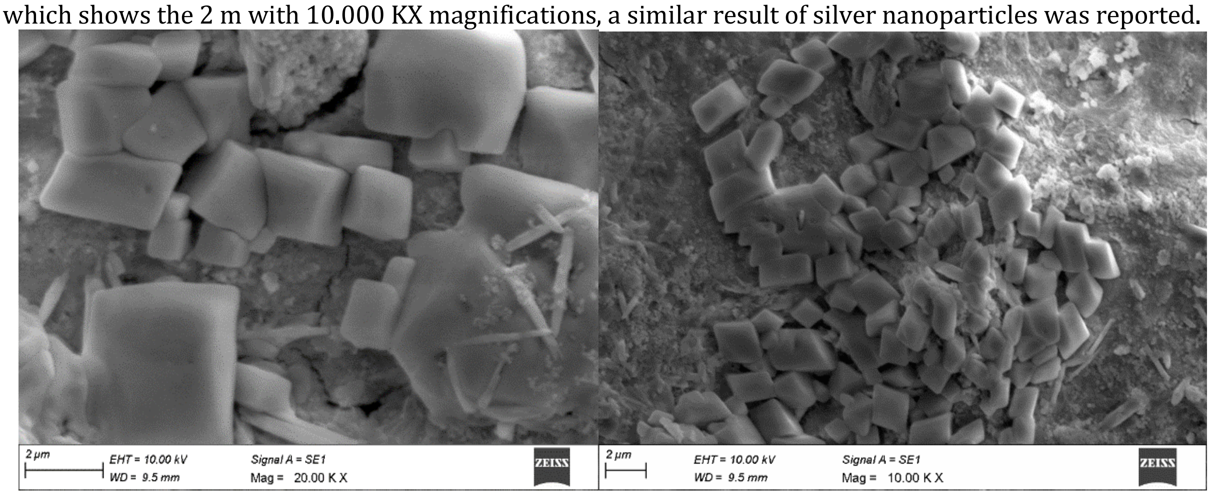

Figure 8; (a) Silver nanoparticle SEM results utilising *Ajuga macrosperma* plant extract using 20 K X(b) Silver nanoparticle SEM results utilising plant extract from *Ajuga macrosperma* using 10 K X

EDX (Energy-dispersive X-ray)

EDAX is an X-ray technology for determining the elemental composition. EDX spectra of silver nanoparticles is given in Figure 9, confirming the presence of Ag in the nanoparticles system. The EDAX spectrum image shows the elemental composition, which is present in the Ag NPs and in fig 8. It displays four firm peaks, which are identified as silver. Other elements discovered at smaller amounts included C, O, Na, Cl, and K.

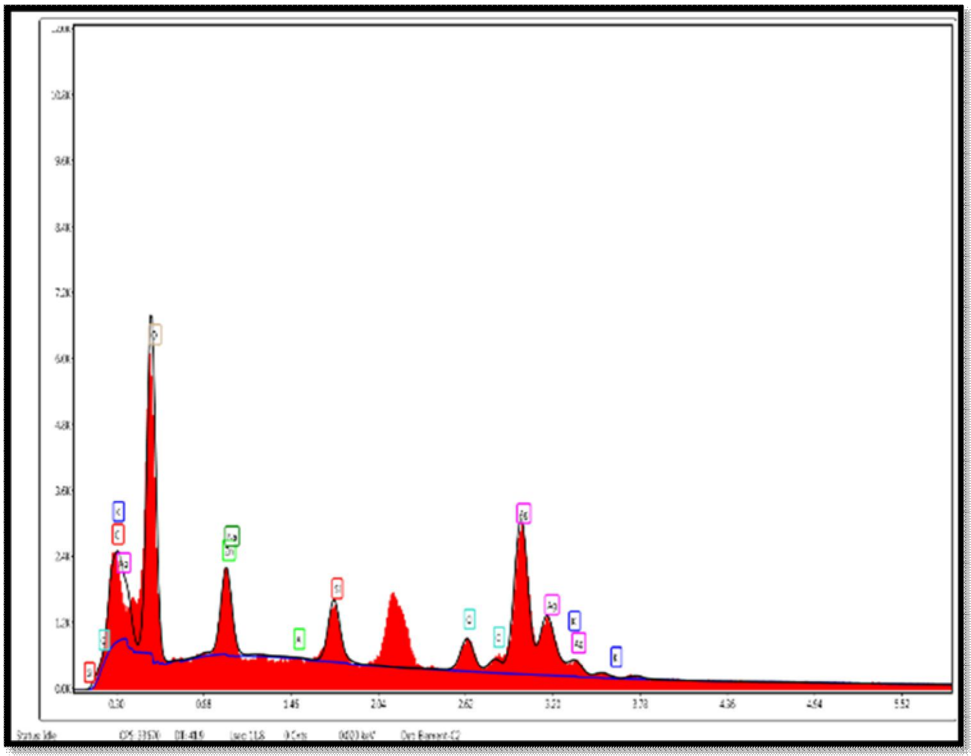

Figure 9; EDAX result of silver nanoparticles synthesized by leaf extract of *Ajuga macrosperma*

Zetasizer's examination of AgNPs' particle size

The synthesized AgNPs had an average size of 34.13 nm and 1.000 PDI value. The single peak seen suggests that the produced silver nanoparticles were of good **quality (**figure 10). The produced silver nanoparticles appear to be polydisperse in nature, according to PDI values.

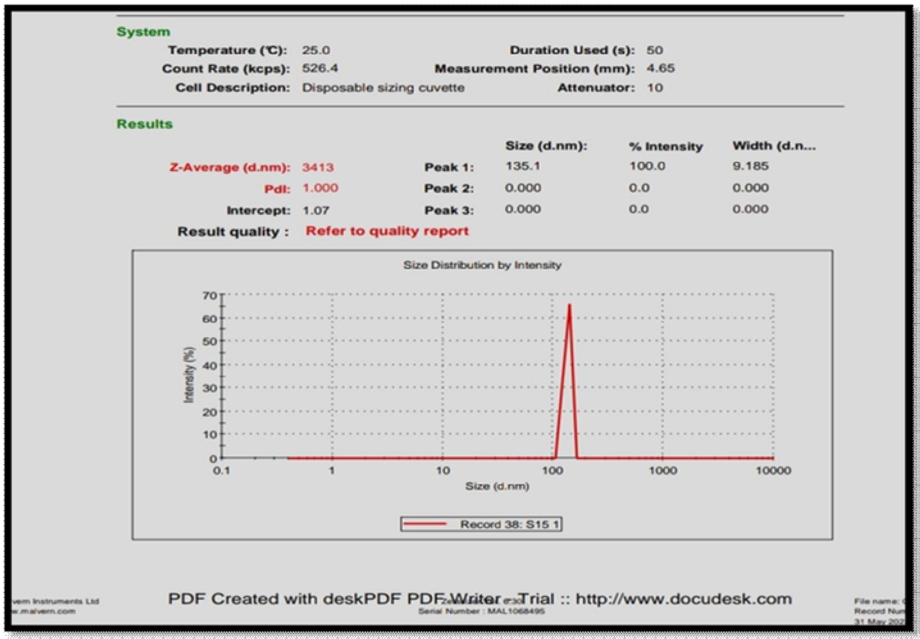

Figure 10; particles size distribution of AgNPs solution synthesized by Ajuga macrosperma

Analysis of the zeta potential

The Vander Waals force of attraction causes nanoparticles to assemble when they are disseminated in water. Aggression can be avoided, though, if the particles become charged during dispersion and repellent electrostatic forces prevail over attractive Van der Waals forces. The increased magnitude of zeta potential, which is regarded as a gauge of surface control on nanoparticles, enhances the stability of their dispersion.

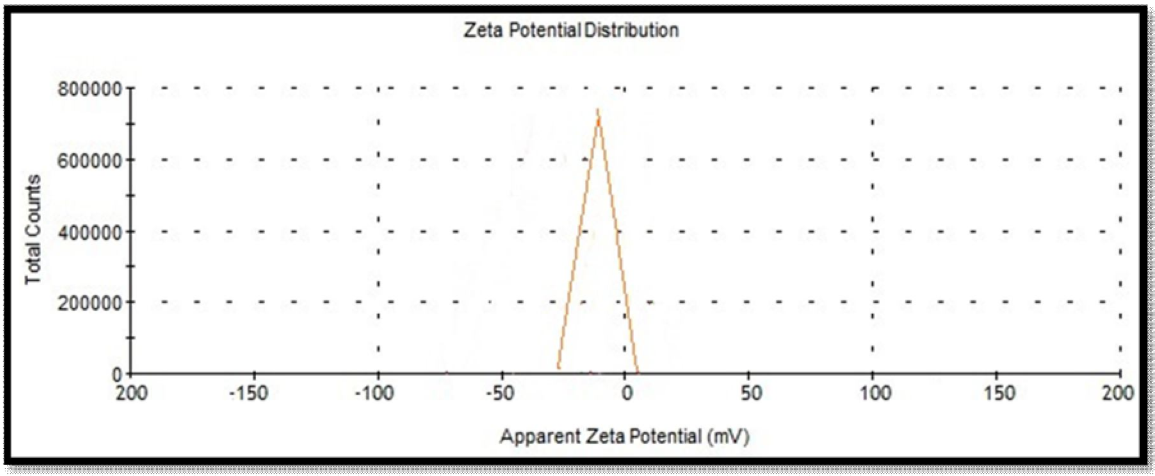

Figure 11; Zeta potential curve of AgNPs solution synthesized by Ajuga macrosperma

Ag nanoparticles dispersed in water were found to have a zeta potential of -29.06 mV. Zeta potential at its maximum obviously indicates great stability.

Anti-cancer activity

General Method

Cell growth inhibition assay

Anticancer activity: The anticancer efficacy of leaf extracts from *Ajuga macrosperma* was evaluated using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide tetrazolium (MTT) reduction assay. According to the manufacturer's instructions, cell titer was measured using the cell titer 96 aqueous non-radioactive cell proliferation assay (Promega, WI, USA). In a proliferation or cytotoxicity experiment, the amount of live cells may be measured colorimetrically using the Cell titer 96aqueous one-solution assay. In this solution, both the MTS chemical and the PES electron-coupling reagent may be found. A coloured formazan product that is soluble in tissue culture media is produced when cells bio-reduce the MTS chemical (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphonyl)-2H-tetrazolium). The cells were seeded at a density of 5,000 per well in growth media onto 96-well plates and allowed to adhere overnight. Subsequently, the cells were exposed to chalcones and their derivatives at the given doses (100, 33.33, 11.11, 3.70, 1.23, and 0.41 g mL-1). Twenty microliters of cell Titer 96 aqueous solution were poured into each well after 72 hours of treatment. The plates were put back into the incubator and the lights were turned off for a period of two hours. Absorbance was determined at 490 nm against a 690 nm standard using a Spectra Max 340 microplate reader (Molecular Devices, USA). It was repeated three times to ensure accuracy.

Cancer cell cytotoxicity

Commercial anti-cancer medications cisplatin and doxorubicin were used as the standard of comparison for the cytotoxicity of the produced chalcones and their derivatives toward cancer cells. Using the PC-3 (Prostate Cancer Cell Line), Hela, A459 (Human Lung Cancer Cell Line), and MCF-7 cells (Human Breast Cancer), preliminary screening using the MTT assay revealed that all of the complexes could suppress cell viability with IC50 values in the micromolar range of Cisplatin 13 M and Doxorubicin 4.1 M being used as standards. Synthesized AgNPs had IC50 values of 1.20, 4.69, 2.45, and 8.90 g/ml against the HeLa, PC-3 (prostate cancer), and A549 (lung cancer) and MCF-7 (breast cancer) cells line, respectively.

CONCLUSION

When it comes to green synthesis, producing AgNPs is straightforward. Without using harmful chemicals, we could synthesize silver nanoparticles from aqueous leaf extract of *Ajuga macrosperma* in a quick, simple, cost-effective, and Ecologically benign manner. As demonstrated in this study, the *Ajuga* *macrosperma* leaf extracted solution synthesis is a new practical approach for preparing silver nanoparticles utilizing inexpensive precursors. Green silver nanoparticles are ideal theranostic agents as the global demand for nanotechnology and nanomedicine grows exponentially. Because of their low toxicity and excellent biocompatibility, they are effective therapeutic agents for malignant diseases such as cancer.

CONFLICTS OF INTERESTS

There aren't any conflicts of interest, according to the writers.

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