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Characterization, Antioxidant, and Anticancer activities of Silver Nanoparticles using *Serenoa repens*

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ABSTRACT

Using the aqueous extracts from Serenoa repens fruits, silver nanoparticles (Ag NPs) have been synthesized simply, cheaply, and eco-friendly. Human prostate cancer antioxidant activity, as well as AgNP toxicity, were evaluated. The aqueous extract has been utilized as a reducing and stabilizing agent in a case when making AgNPs, which had been characterized with the use of a variety of methods, including ultraviolet-visible spectroscopy (UV-Vis), energy-dispersive X-ray (EDX), scanning electron microscopy (SEM), and Fourier transform infrared (FTIR). In the UV-Vis spectrum, the aqueous medium that contains AgNPs had shown an absorption peak at about 350nm. FTIR spectra showed that biomolecules have been responsible for capping and reduction agents of Ag NPs. The EDX spectrum indicates the presence of silver. Additionally, antioxidant activity has been assessed using the DPPH test. The percentage inhibition values concerning ascorbic acid, Serenoa repend extract, and AgNPs at 100 µg/mL were found to be 72.68, 80.08, and 75.3 µg/mL, respectively. It was discovered that NPs could stop large prostate cancer cells from growing.

KEYWORDS: Green synthesis, antioxidant activity, anticancer, silver nanoparticles, *Serenoa repens*

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1. Introduction

It is used for disease treatment, prevention, identification, and health enhancement in the quickly developing field of nanomedicine. There are numerous applications for it (1). NPs are minuscule particles that are often much smaller than one to one hundred nanometers and are just some nanometers in size. Polymers, metals, ceramics, and composites are just a few of the many materials that could be used to create such particles (2–3). Those particles are widely utilized in the fields of microbiology, electronics, medicine, biotechnology, materials engineering and science, and the environment as a result of their huge surface-to-volume ratio, which is their principal property (4-6). As illustrated in Fig 1, NPs can be classified as either organic (carbon NPs, such as fullerenes) or inorganic (magnetic NPs, semiconductor NPs, like zinc oxide, and Nobel metal NPs, such as silver) (7).

Metal NPs can be produced through constructive or destructive means of reducing metals to nonmetric sizes. Cadmium (Cd), cobalt (Co), aluminum (Al), copper (Cu), iron (Fe), gold (Au), silver (Ag), lead (Pb), and zinc are some of the metals used in the manufacture of NPs. NPs are superior to metals in terms of their bulk properties (9). As a result, new approaches for the synthesis of NPs could be developed that require less severe reaction conditions, ecologically friendly conditions, and reasonably priced reagents (10). The synthesis regarding metal NPs using microorganisms and

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plants has gained popularity recently because the traditional chemical and physical techniques are more expensive and harm the environment. Concerning green synthesis, phytochemicals contained in plant components like fruit peels, leaves, callus, bark, and roots are studied as potential bio-reductants and capping agents for NP synthesis, making them useful (11). AgNPs are a significant constituent of noble metal NPs. AgNPs' unique properties make them suitable for usage in composite fibers, electronic components, antibacterial applications, and biological sensor materials. With the use of various chemical and physical methods, AgNPs were produced and stabilized (12-14). Because of their remarkable stability and unique biological and catalytic properties, studies have shown that AgNPs have remarkable antioxidant and antibacterial activity (11,12,15). Because of such properties, AgNPs could be good choices for recycling and catalytic applications, according to Manjari et al. (2018) (16). Lately, scientists have investigated extracts from a number of plants to produce AgNPs. Yet, common food plants are often greatly sought after for significant scientific initiatives because of their extensive range and accessibility (17). For many years, people have used a range of medicinal plants to heal illnesses worldwide. Furthermore, the phytochemicals that are derived from plants vary substantially (18). Because of their many therapeutic and biological qualities, reduced cost, and higher safety margins, herbal medicines are quite popular for primary healthcare in both developing and developed countries (19). The extracts of plants like Mollugo nudicaulis, Lippia citriodora, Azadirachta indica, Prunus armeniaca, and Desmodium gangeticum (L.) DC were used successfully in the biosynthesis regarding AgNPs (20-24). Citrus sinensis (25) Alternanthera dentata, (26) Mangifera indica (27) Ocimum Tenuiflorum, (28) Magnolia kobus, (29) Malva parviflora, and (30) Murrayya koenigii (31).

Saw palmetto (Serenoa repens (W.Bartram) Small, Arecaceae) is a small palm that grows to a height of seven to ten feet and is native to the Southeast region of the US. The dried fruits of such plants turn black when they are ripe. They have long been used by Native Americans for the treatment of genitourinary disorders. 30 Studies on the pharmacology and phytochemistry of SrE began in the 1870s and focused on the lipophilic compounds that had a multi-factorial effect on benign prostatic hyperplasia (BPH), which has been majorly explained through their pro-apoptotic, anti-inflammatory, and anti-androgenic activities (32–34). Approximately 85% of the complex mixtures that comprise Serenoa repens extracts are free fatty acids or esterified fatty acids (with 5-6% triglycerides and 2% methyl-ethyl esters). The primary forms of free fatty acids are myristic (10%), palmitic (10%), oleic (30%), and lauric (30%) acids. Along with esterified or free fatty acids, fatty alcohols (0.8 to 1.1%) and triterpenes (1%) were detected. Tocopherols, carotenoids, hydrocarbons, and volatile chemicals (1%), among other trace components, were indicated (35,36). The most common method for assessing Serenoa repens extracts is gas chromatography-mass spectrometry (GC-MS). Using this technique, certain components are altered to aid in identification, like fatty acids being more volatile and sterols and other esterified substances becoming saponified. For assessing biological qualities of such samples as strong anticancer and antioxidants agents, we present here the manufacture of Serenoa repens aqueous extract and an environmentally friendly method for synthesizing silver nano-solution utilizing such plant extract. Our objective is to discover substitute chemo-therapeutic drugs.

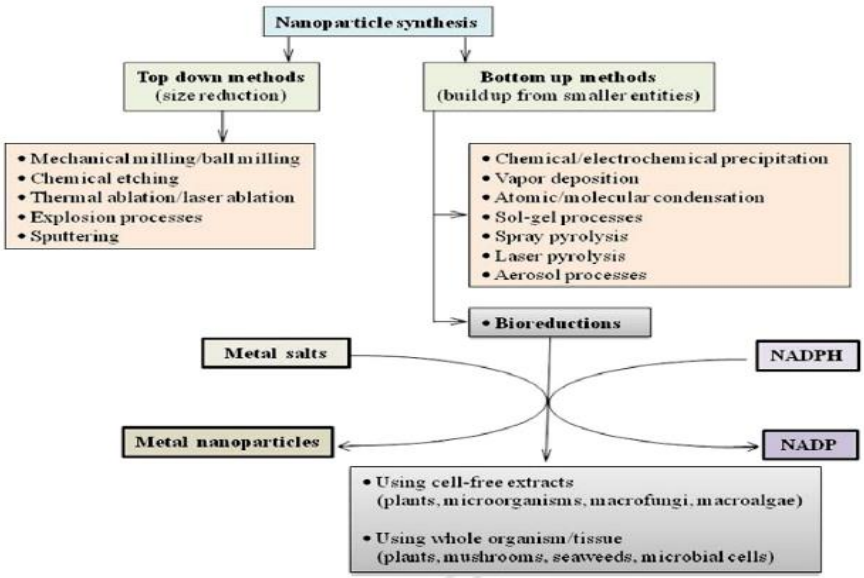

Figure 1. Different methods to make NPs and cofactor-dependent bioreduction adapted from (Mittal *et al*., 2013) (8).

2. Methods and Materials

2. 1. The Collection of *Serenoa repens* fruits:

In Nasiriyia City, Thi-Qar, Iraq, samples of Serenoa repens fruits have been gathered from the local market. They have been cleaned after breaking ground in an electric grinder.

2. 2. Methods:

2. 2.1. Preparation of *Serenoa repens* fruits Extract:

100 ml of deionized water and 25 g of Serenoa repens fruits were put in a 250.0 ml flask, and the mixture was cooked for 10 mins. The components were allowed to come to room temperature before being filtered through Whatman No1 filter paper. AgNPs were made from the ensuing clear extract from Serenoa repens fruits.

2.3. AgNPs Synthesis:

Ten milliliters of a 25% Serenoa repens fruit aqueous extract, which was incubated at the temperature of the room with shaking under dark conditions, were added gradually to 90 ml of 1mM silver nitrate (AgNO3) in a 250.0ml conical flask. The reaction solution was inspected for half an hour, and the color of the AgNO3 solution was monitored to determine if it changed from colorless to brown. AgNPs solution was centrifuged at 15000 rpm for 30 mins. The resulting particle has been dispersed once more in the deionized water, and the supernatant has been discarded. The pellet has been centrifuged two or three times to remove any materials off the surface of AgNPs (37).

2.4. AgNPs Characterization:

The produced AgNPs were analyzed with the use of a UV-Vis spectro-photometer UV1700 (Shimadzu, Tokyo, Japan) that operates in a 250nm–750nm scanning range. FTIR was used to analyze synthetic AgNPs to determine biomolecules that have been the reason behind AgNP reduction. A Shimadzu model operating in the 400–4000 cm^{-1} wavelength range was used. EDX (6490 LA) and SEM, JEOL JSM-6490A have been utilized for examining the morphology and chemical structure of produced NPs. For EDX, a 20.0 kV acceleration voltage was employed. The TEM approach, also known as the transmission electron microscope, offers crystallographic as well as morphological details on NPs. The JEM-HR-2100; JEOL, Japan, has been utilized to carry out TEM analysis.

2. 5. Potential Biological Characteristics:

2. 5. 1. Antioxidant Activity Procedure:

A colorimetric DPPH free radical test has been utilized for evaluating the antioxidant activity of the AgNPs and *Serenoa repens* aqueous extract. 0.0039g of DPPH has been dissolved in 100 ml of methanol to create a solution of 0.1 mM DPPH. 2.0 milliliters of AgNPs at concentrations in the range of 20-100µg/mL was combined with a 1.0 milliliter solution. The samples' absorbance (concentrations) has been measured at 517nm after 30 mins. Using ascorbic acid was the standard procedure. Regarding its radical scavenging activity, the sample with the highest absorbance of reacted combination is identified (38). The radical scavenging activity of the sample has been expressed as a percentage of the inhibition of free radicals with the use of the next formula:

% Inhibition of DPPH = $(Ac-As)\ Ac$ x100

In this case, Ac represents the absorbance of the control (blank, without AgNPs), and *As* represents absorbance in the presence of AgNPs.

2. 5. 2. Cell Cultures Maintenance:

The Iranian National Cell Bank provided PC3, a human cell line for prostate cancer (Pasteur Institute, Iran). The cells have been cultured in Gibco's RPMI1640 media supplemented with 10% FBS and antibiotics (100.0µg/ml streptomycin and 100U/ml penicillin). Trypsin/EDTA (Gibco) and phosphate-buffered saline (PBS) solution have been used to passage the cells, which were then maintained at 37 ° Celsius in humidified air with 5% CO_2.

2.5.3. MTT cell viability assay:

Cell viability and proliferation have been evaluated by using MTT [3-(4, 5-dimethylthiazol-2yl)-2, 5-di-phenyltetrazolium Bromide] (Sigma-Aldrich) assay. Trypsin has been utilized for breaking down the cells, which were after that collected, adjusted to a 1.4 104cells/well density, and after that planted into 96-well plates with 200µl of new media per well over 24 hours. Cells have been treated to 600–7.4µg/ml of chemicals for a whole day at 37° Celsius in 5% CO2 once a monolayer had formed. Following a 24-hour treatment period, 200 µl/well of MTT solution (0.5mg/ml in PBS) has been added, and the supernatant has been disposed of. The monolayer culture has been left intact in the original plate, and this plate has been incubated for an additional 4 hours at 37° Celsius. MTT solution: Following the collection of cell supernatant, 100µl of di-methyl sulfoxide has been applied to every one of the wells. The cells have been incubated at 37 Celsius on a shaker until the crystals have been completely dissolved. The vitality regarding the cells was measured with the use of absorbance at 570nm utilizing ELISA reader (Model wave xs-2, Bio-Tek, US). The concentration of chemicals that led to 50% cell death (IC-50) has been determined with the use of the proper dose-response curves.

3. Results and Discussion:

3.1. AgNPs Characterization

3.1.1. UV–Vis spectroscopy analysis

The synthesis of AgNPs in the aqueous solution has been seen through the recording of absorption spectra throughout a wave-length range of 300–700nm (Fig 2). Adding plant extract caused the silver nitrate solution to turn dark brown, signifying AgNP formation. On the other hand, when plant extract was not applied, no color change was seen. The detection of a broad, single, strong SPR peak in the UV-Vis spectrum at 350 nm confirmed the synthesis of AgNPs. Based on earlier studies, AgNPs showed an SPR peak between 330 and 500 nm, which may be associated with spherical NPs (39). SPR patterns and characteristics of Ag NPs are significantly influenced by their medium dielectric constant, surface adsorbed particles, and stabilizing molecules (40).

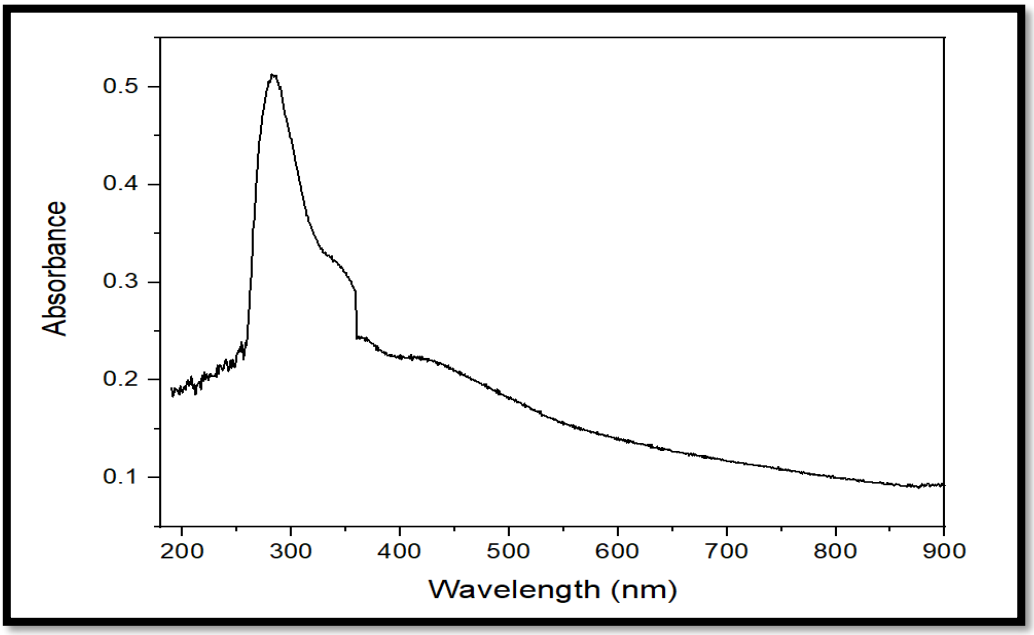

Fig2. UV–vis absorption spectrum regarding biosynthesised AgNPs with the use of *Serenoa repens* aqueous Extract.

3.1.2. FTIR analysis

For stabilizing and reducing AgNPs, the extract of the plant comprises alcohol, phenol, carboxylic acid, amine, terpenoids, and alkaloids. The main factors for the production of AgNPs are secondary metabolites (41). Investigations into possible bio-reducing agents that could be present in the AgNPs have been carried out utilizing FT-IR spectroscopy. It was suggested that the conversion of Ag+ to AgNP is brought on by the existence of phenolic compounds and AgNPs flavonoids in the leaf broth of G. sepium (42). In A. indica, it was demonstrated by Shankar *et al*. (43). and Tripathy *et al*. (44) that phenolic substances have carboxyl and hydroxyl groups that can bind metals. The stretching vibration of the O-H bonds in alcohols and phenols is associated with absorption bands at 3405 cm-1, the stretching vibration of aliphatic C-H bonds with bands at 3127cm-1 and 2830cm-1, the stretching vibration of carbonyl (C=O) with bands at 1607cm-1, and the stretching vibration of phenols with bands at 1400cm-1 and 1367cm, according to FTIR results. The C-N vibration at 1107 cm is where the -1band could be found. The asymmetric vibration of C-O at 1081 cm is where the -1 band could be found. The C-O vibration symmetry at 759 cm is associated with 1 band. The -1 band is caused by the vibration of the C-C skeletal system (45).

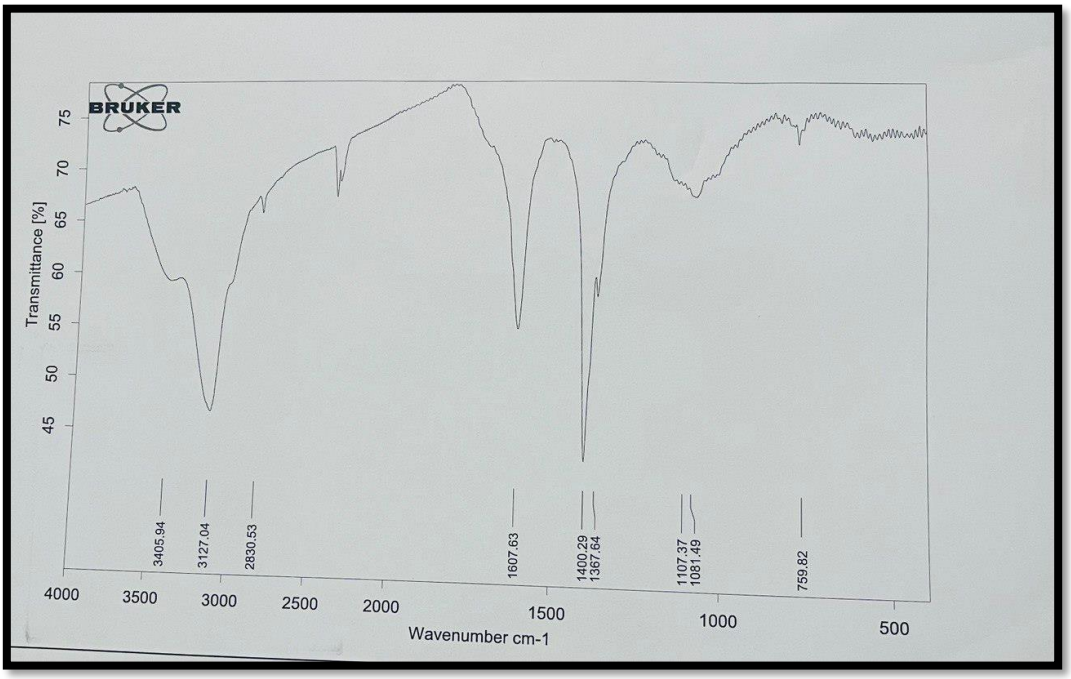

Figure 3. FT-IR spectrum of synthesized AgNPs

3.1.3. TEM, SEM, and EDX analysis

Fig 4 displays the structure and size of AgNPs as determined by SEM examination. SEM images show AgNPs and fully consumed Ag+ ions. AgNPs generated in the sample had a final size of 84.93 nm. The differences in Ag particle size could be explained by Ag aggregating throughout the SEM study preparation. The surface topography, composition, and other characteristics of AgNPs, like their electrical conductivity, are all precisely revealed by SEM images. The results further demonstrated the spherical shape of the particles. The spherical AgNPs have appropriate applications in medical and pharmaceutical manufacturing. Our results are consistent with the results that have been reported by Allemailem *et al*. (46). AgNPs that have been produced from Ajwa-date seed extracts were shown to be mainly spherical, with a diameter ranging between 15 and 80 nm. TEM micrographs of generated AgNPs are presented in Fig 5. A moderate range of particle sizes, as well as a spherical shape, have been observed in almost all AgNPs. According to the size distribution, most of the NPs were in the 8–90 nm range (23). Fig 6 shows the artificial NPs' compositional analysis (spectrum EDX). EDX spectrum was used for decoding the signals that corresponded to elemental silver (10%) and other related substances such as nitrogen, carbon, sodium, oxygen, potassium, chlorine, and so on. The reason for the decreased intensity regarding signals is the phytochemical components found in Serenoa repens fruits. EDX confirms the production of AgNPs by revealing a strong signal in the silver region. Metal silver nanocrystals usually present an optical absorption peak of about 3 keV as a result of the SPR. 45

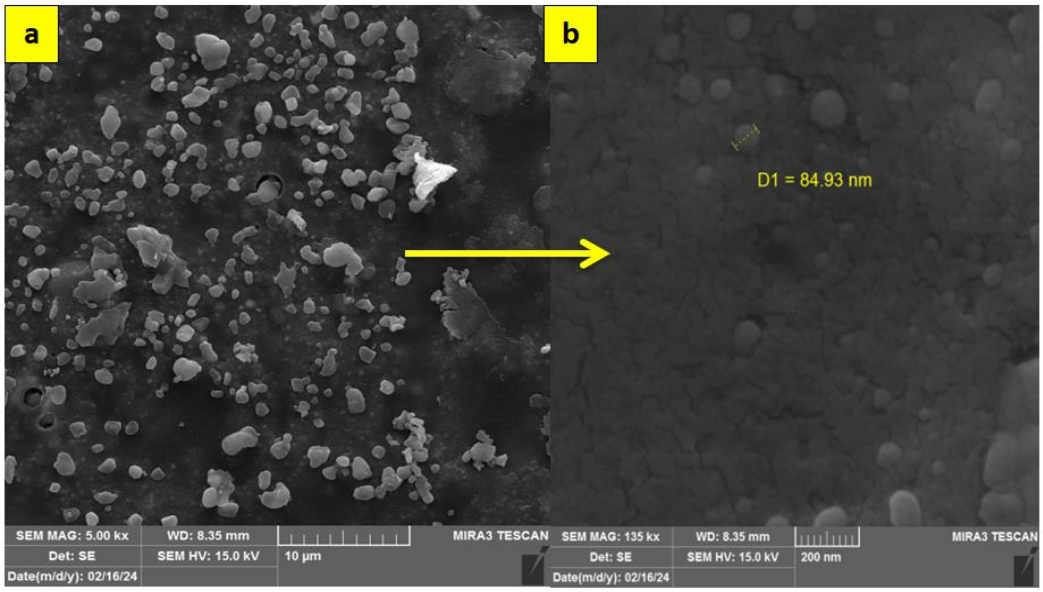

Figure 4. SEM images (a & b) of the synthesized AgNPs.

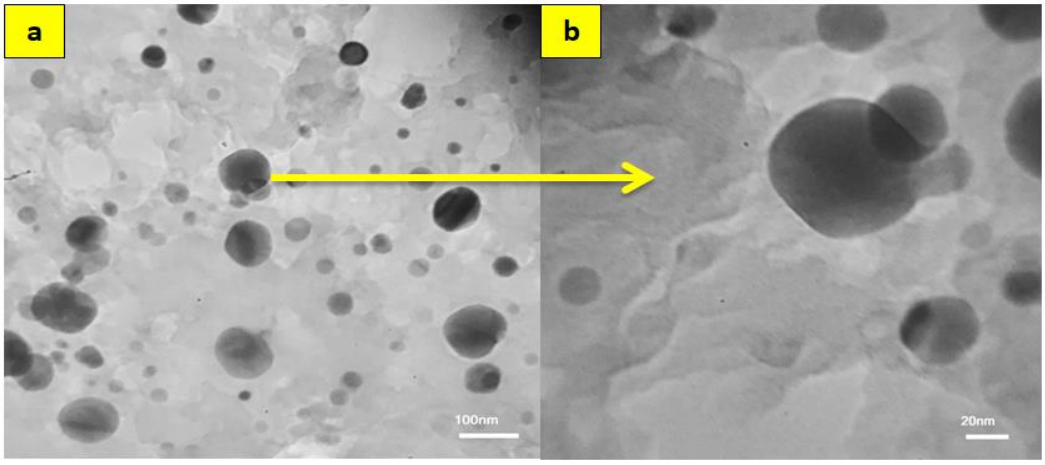

Figure 5. TEM image (inset is high-resolution TEM image) of the synthesized AgNPs.

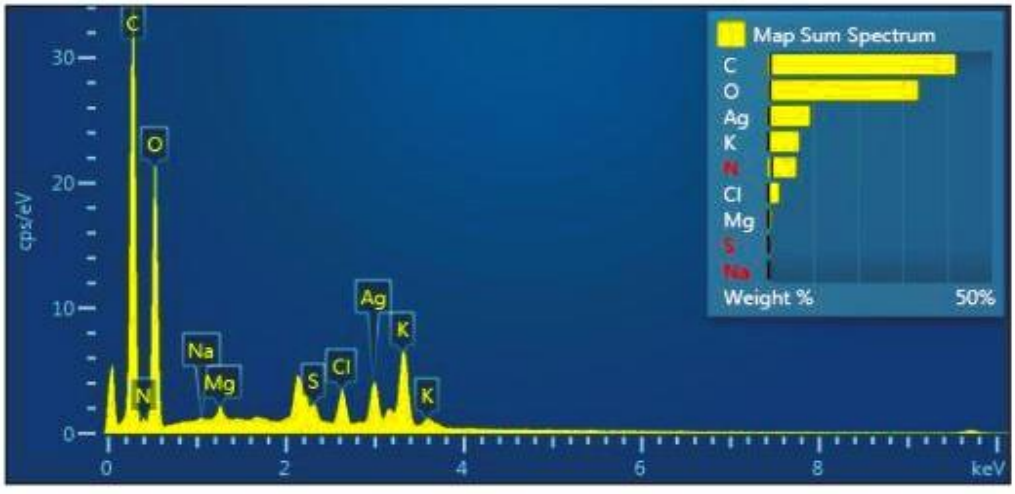

Figure 6. EDX of the synthesized Ag-NPs.

3.2. AgNPs Antioxidant Activity

With the use of the DPPH approach, we assessed the antioxidant activity regarding AgNPs as well as the extract to ascertain the degree of the scavenging effect of the Serenoa repens extract. The radical scavenging activity (RSA) % was used to depict the final numbers. Color changes were used to assess how well the AgNPs reduced DPPH; the methanol solution turned violet, while the control group displayed no color change. It was transformed into a yellow substance called diphenyl picrylhaydrazine (48). The extracts' ability to scavenge free radicals is demonstrated by their decreased absorption of DPPH. This technique was used to measure antioxidant activity through tracking variations in DPPH absorbance at 517 nm. The existence of hydroxyl groups in the plant could be responsible for the high ability of some chemicals (phenolic compounds or flavonoids) to scavenge free radicals. 47 In the DPPH scavenging experiment, AgNPs performed noticeably better than ascorbic acid as well as Serenoa repens extract as a reference. The radical scavenging activity of AgNPs that have been produced from Serenoa repens extract has been 80.08%, which was the highest test result. At 100µg/mL, the antioxidant activity of aqueous extract was 75.3%, whereas conventional ascorbic acid was 72.68% (Fig 9). Flavonoids, proteins, carbohydrates, and phenolic compounds are some examples of phytochemicals containing a -OH group. Ag NPs that contain a lot of hydroxyl groups may be antioxidants. (50,51).

3.3. Anticancer Activity of AgNPs

The anticancer (cytotoxicity) activity regarding the generated Ag NPs has been evaluated in the PC3 (human prostate cancer cell line) with the use of MTT assay. In addition, many dosing concentrations of the generated AgNPs (7.4, 22.22, 66.66, 200, and 600 µg/mL) were administered to PC3, a human prostate cancer cell line. After a day, the treated cells have been examined for alterations in nuclear morphology (Fig10(a–f)). Cell viability has been reduced considerably in the presence of green synthetic AgNPs in comparison with the control. The phytoconstituted synthesized Ag NPs' IC50 value has been determined to be 354.23 ± 4.26µg/mL following a 24-hour treatment. The human prostate cancer cell line PC3 had a viability rate of 46.23 at 600 µg/mL following a 24-hour treatment with AgNPs. Numerous investigations have shown that the remarkable cytotoxicity (i.e., anti-cancer) of MNPs produced increased physiologically in tandem with the NP concentration (52,53). The results might indicate that Serenoa repens-assisted nano silver could be of lethal action to prostate cancer cells via an apoptosis-dependent mechanism. Oxidative stress, cell shrinkage, coiling, and physiological reactions are all morphological changes brought on by an intracellular suicide procedure called apoptosis. One possible explanation is that AgNPs alter cells by interfering with the cellular function of biological proteins.

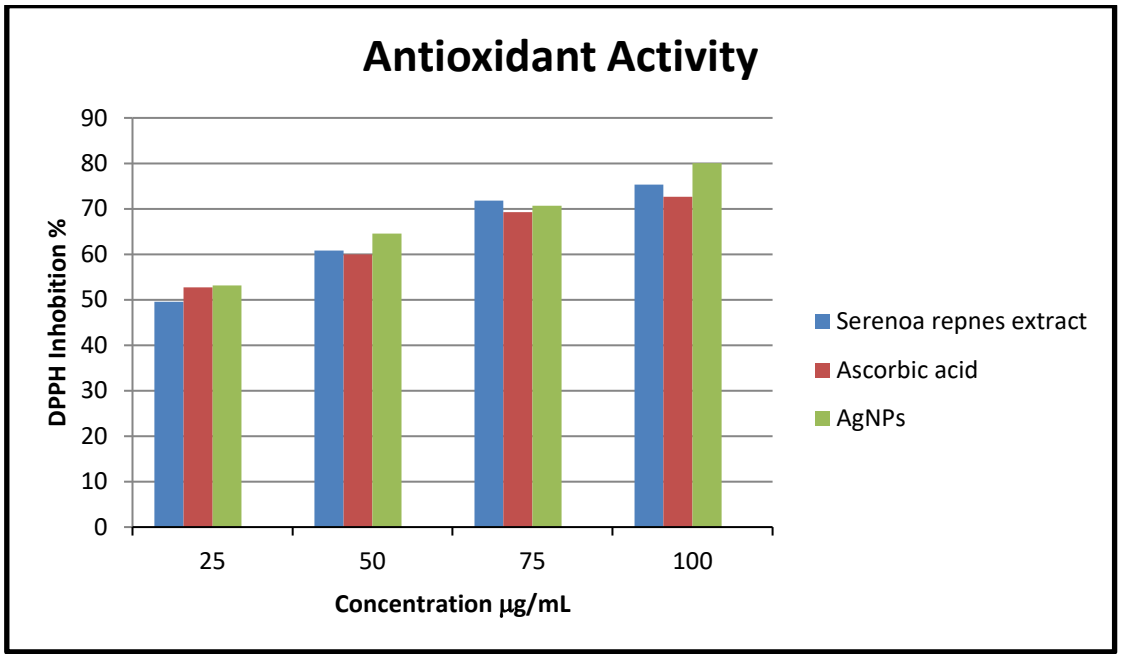

Figure 7. DPPH free radical scavenging activity of the AgNPs at various concentrations compared with the standard (i.e., ascorbic acid) and extract *Serenoa repens*

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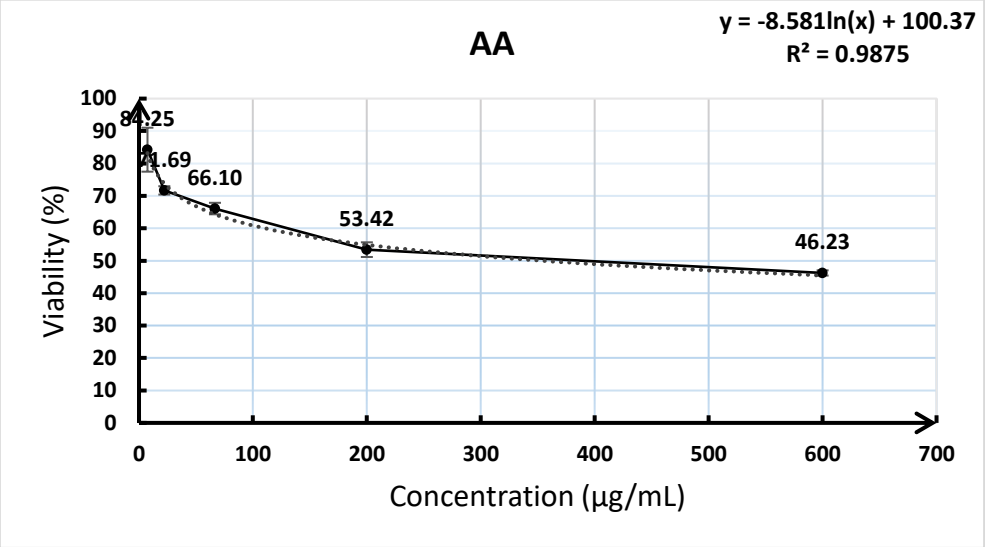

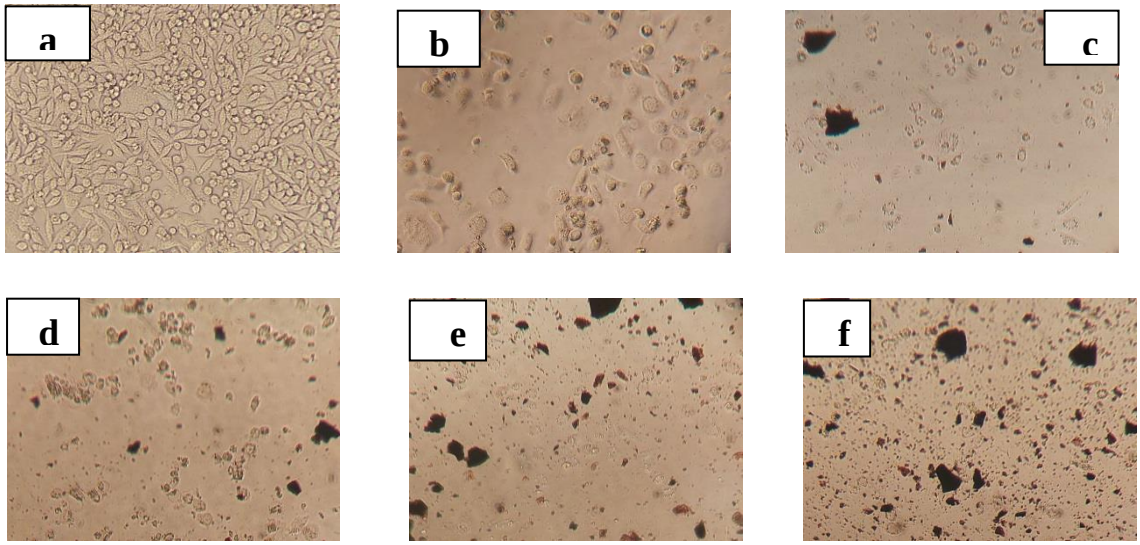

Figure 8. Anticancer activity of Ag NPs, (a) Control (PC3); (b) 7.4; (c) 22.22; (d) 66.66; (e) 200; (f) 600µg/mL.

Conclusion

To conclude, Serenoa repens extract has been utilized for creating AgNPs, which had an average size of 80.93nm. TEM, FTIR, UV-vis spectroscopy, EDX, and SEM methods have been utilized for examining Ag NPs. AgNPs derived from green materials have shown notable cytotoxicity as well as antioxidant activity against the PC3 cell line, suggesting possible medical applications.

Conflict of interest: None

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