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Green Biosynthesis of Silver Nanoparticles were Obtained from the Extract of Pomegranate (*Punica granatum* L.) Leaves by Supercritical Extraction Using Microwave Method

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Abstract

In this study, pomegranate (*Punica granatum* L.) leaf extract and 2% (w/v) aqueous solutions isolated by SFE extraction and microwave extraction were used to create silver nanoparticles (AgNPs). The pomegranate was grown in Turkey's Eastern Black Sea region. $AgNO_3$ solution (0.25, 0.5, and 1 mM) received separate additions of 0.1 and 0.2 mL extract before being microwave-irradiated. Ag nanoparticles made using green chemical techniques were characterized by UV-Visible, XRD, TEM, Zetasizer and FT-IR. By analyzing the plasmon resonance absorption (SPR) spectra by the UV-Visible technique, the ideal circumstances were identified. The face-centered cubic crystalline silver nanostructures' lattice planes (111), (200), (220), and (311) show that the different Bragg reflection peaks occurred at 2 values of 38.1°, 44.3°, 64.6°, and 77.6°. The average particle size of Ag nanoparticles produced by microwave extraction in an aqueous medium was 86.02 ± 0.579 nm, the zeta potential was -14 ± 0.777 mV, and the polydispersity index was 0.257 ± 0.004, according to the results of zeta-Sizer study. The UV-vis absorption spectra of the AgNP solutions, which were kept in a refrigerator, barely altered and remained constant for roughly 4-5 months.

Keywords: AgNPs, FTIR, Pomegranate (*Punica granatum* L.) leaf, Supercritical Fluid Extraction (SFE), TEM, XRD

1. Introduction

Nanotechnology is primarily concerned with materials with diameters ranging from 1 to 100 nm and possessing properties distinct from bulk materials. This update expands the possibilities for nanomaterials in healthcare and environmental remediation [1-3]. Nanotechnology is a combination of technology with science that involves the production or production, design, which is and analysis of nanometer-scale materials [4,5] The ability to manipulate the atoms or molecules in a structure of substances at the nanoscale level allows us to modify their properties various materials for specialized uses [6]. That is why nanotechnology has piqued the interest of numerous scientists and researchers throughout the world and has grown in popularity in both scientific studies and industry [4,7,8]. Currently, nanotechnology involving the environmentally friendly production of nanoparticles that has become an attractive idea that has gained much significance and importance in the past few years because of its excellent facility, clean processing, use of non-toxic chemicals, cost-effectiveness, and environmental and sustainability [4].

Nanomaterials are increasingly being used in combination with anticancer medications, and these molecules have numerous medical applications [9-12]. One of the practical applications of nanomaterials is combining them with anticancer medications so that the medicinal product can be administered to the cell more efficiently while reducing toxicity and adverse effects on healthy cells [9,13-15]. Because of the rising cancer death rate and flaws in radiotherapy and chemotherapy procedures in advanced stages of cancer, it is critical to develop modern cancer control and treatment strategies [16-19]. Nanoparticles with a measurement of 100 nm or less are often used in cancer medical research to target cancer cells when used with anticancer medications [20-22]. The nanomaterials were created using a variety of organisms, for example bacteria, fungi, yeast, and various others, resulting in an environmentally benign and clean process of synthesis [1].

In the biosynthesis process, various plants and components of plants are employed to produce nanoparticles that act as reducing and capping agents.

The synthesis of particles using plant extracts is a type of "green chemistry" that is safe, harmless, and environmentally friendly. Nanoparticles are produced biologically by employing extract-based production and chemicals with reductive action to transform metal ions into the element metal[1]. *Punica granatum* L. has been utilized for treating a variety of ailments for thousands of years throughout various cultures and civilizations [23]. Pomegranate is a well-known fruit in Asian culture. In folkloric medicine, pomegranate has numerous medicinal purposes [9]. Pomegranate is a well-known medicinal plant with many uses in traditional medicine. Punica granatum leaves are used to treat stomach ailments, diarrhea, dysentery, hemorrhages, and conjunctivitis. etc. The production of nanoparticles through plant leaf extracts for therapeutic reasons has recently gained traction [23]. According to prior research, the most significant secondary metabolites in Pomegranate are phenolic compounds such as glycosides, the sterols steroids, resins, flavonoids, alkaloids, lipids, coumarins that and vitamins. Indeed, the variety of chemicals found in Pomegranate are accountable for the plant's extensive range of pharmacological applications [9,24-27]. Silver nanoparticles are one of the most prevalent nanoparticles in this field, and they are thought to be potential anticancer agents. Plants provide a variety of components and biochemicals that can act as stabilizing and reducing agents in the synthesis of green nanoparticles. When compared to alternative biological, physical, and chemical approaches, green synthesis technologies are more environmentally friendly, non-toxic, cost-effective, and stable [28,29]. The production of nanoparticles from plant extract is a low-cost procedure that produces higher yields because to the large amount of phytochemical components in the extract, which can also act as reducing and stabilizing agents, transforming metal ions into metal nanoparticles [28]. Silver nanoparticles (AgNPs) are a type of substance with diameters ranging from 1 to 100 nm and is widely studied [4,28]. Because of their distinctive and appealing chemical, physical, and biological features, there has recently been an increase in devotion to the study of AgNPs and their diverse behaviors [4,30-32].The silver nanoparticle is primarily used for enhancing biomedical applications such as medication administration, wound healing, tissue scafolding, and protective covering. Furthermore, nanosilver has a large accessible surface area, which permits any ligand to connect to it. Silver nitrate is often used as an antibacterial agent. A silver nanoparticle is a novel and emerging antibacterial agent [28]. The physical, chemical, and biological features of such a nanoparticle are significant. Silver nanoparticles have significantly more favorable effects, such as broad-spectrum antibacterial response, non-toxicity, anticancer characteristics, and other therapeutic reasons, as well as the ability to generate distinct, diversified nanostructures and low-cost manufacture [28]. Alternative and sustainable extraction processes have been developed in recent years. These environmentally friendly and efficient approaches yield high-yielding oils, resolving the issues that existing technologies present [33,34]. Some of these technologies include supercritical fluid extraction, aqueous extraction, enzymatic pre-treatment, and extraction aided by ultrasound, microwaves, or electrical pulses [33,35]. Microwave-assisted synthesis is seen as an option to the aforementioned issues due to its multiple advantages over the other synthesis methods, such as low cost, shorter time for processing, increased yield, high purity, and tiny particle size distribution, among others. It also has a high degree of influence over the end product shape and particle size [36]. In this respect, pomegranate leaves extract-mediated synthesis supported by microwave irradiation may serve as a quick and easy way to produce Ag nanoparticles because NP formation and development take place within just a short amount period in the reaction medium, leaves extracts raise NP capping rate, stabilization process is sped up, and AgNP aggregates increase. A supercritical fluid is used as a solvent in supercritical fluid extraction. Carbon dioxide is the most commonly used because of its apolar characteristics. Because the solvent is environmentally friendly, inert, and safe, and it is quickly eliminated after extraction with decompression, it is gaining appeal as a green technology. In comparison to other approaches, SFE solvent has a modulable dissolved potential that can be adjusted by varying the pressure and temperature. Furthermore, supercritical fluids have a larger diffusion coefficient as well as less viscosity and surface tension than liquid solvents, which favors mass transfer [37]. Accordingly, in this study, pomegranate leaves were extracted using the SFE method, which is one of the modern extraction techniques. The method was optimized by performing biosynthesis of silver nanoparticles using microwave method in different volumes of the extract obtained with different concentrations of $AgNO_3$ solution. The current study aimed to produce a low-cost green synthesis of AgNPs utilizing P. granatum leaf extract (PGE). With this study, silver nanoparticles were produced from pomegranate leaves collected in the province of Turkey-Trabzon, which can be used as a basic source for the science and medicine industries, including pharmacology, food, and medicine. The goal of this study was to produce Ag nanoparticles, which has become the focus, according with the fundamentals of green chemistry. The synthesis and characterisation of Ag nanoparticles from pomegranate (Punica granatum L.) leaves extracted with SFE by microwave techniques have not been investigated in the literature. Therefore, the objective of this study was to produce and characterize Ag nanoparticles for the first time from pomegranate (Punica granatum L.) leaf extract obtained by the supercritical extraction method and from Turkey's Trabzon province using microwave-assisted technology. Uv-Vis, FTIR, TEM, XRD, and DLS techniques were then utilized to characterize the silver nanoparticles produced in accordance with green chemistry principles.

2. Materials and Methods

2.1. Chemicals

Silver nitrate ($AgNO_3$ purity 99% Sigma Aldrich) salt was employed as the starting material in this study. The solvent used was deionized water. All compounds used were of analytical quality and were utilized without purification.

2.2. Preparation of Pomegranate Leaf Extract

Pomegranate (*Punica granatum* L.) leaves were gathered in the Eastern Black Sea region (Trabzon, Turkey), dried in an air-conditioned space, and then transported in little pieces under suitable conditions. 20 g of the sample was placed in a high pressure steel container fitted with an Applied Separation supercritical extractor (Spe-ed SFE, USA), a modifier (with co-solvent) pump (Applied Seperation Series 1500), a chiller (Applied Seperation Polyscience), and a pressure pump (Atlas Copco GX-4FF). The sample is enclosed between the glass wool's bottom and the container's top. The extraction was carried out at a pressure of 200 bar and a temperature of 50°C for 2.5 hours, with an ethanol modifier flow rate of 0.5 mL/min.

2.3. The green fabrication of Ag nanoparticles

In this synthesis, 0.1, 0.2 mL of pomegranate leaf extract solution, 20 mL, 0.25 mM, 0.5 mM and 1 Mm aqueous $AgNO_3$ were added, and with a power of 90 W, 1–31 minutes, combined in a microwave extraction equipment (Figure 1). The color of the combination changed rapidly from colorless to light brown. The stability of the produced AgNPs was measured for up to 4-5 months. UV-vis measurements revealed a shift in the SPR of AgNPs. Centrifugation is at 10,000 rpm for 15 minutes (Hermle Z326K) separated the produced AgNPs. The pellets acquired were re-distributed and three times in sterile deionized water to remove any free biomass residue. The cleaned pellets were oven-dried at 50 C for 14 hours before being cleaned off for further character analysis.

2.4. Characterization of AgNPs

UV-visible spectrum analysis, Zeta-sizer, FT-IR ,TEM, XRDanalysis were used to analyze silver nan oparticles.UV-Vis absorption spectroscopy is used to identify nanoparticles with wavelengths that extend from 300 to 800 nm. Metallic nanoparticles produced under specific salt conditions have significant absorption, resulting in a point spectrum in the visible range [38]. UV-visible spectrum analysis was carried out using a Perkin Elmer Lambda 25 UV-visible spectrophotometer. The UV spectral peaks of nanoparticles of silver were reported to be between 400 and 480 nm in the literature [39-41]. The spectrum characterizes the sample substances by providing absorption or transmittance as a function of wavelength [42]. FTIR analysis is a viable, cost-effective, easy, and non-invasive method for identifying the role of biomolecules in the reduction of nanoparticles (silver nitrate to silver) [43]. The produced nanoparticles were analyzed using the FTIR SHIMADZU instrument in the wavelength range of 4000 to 550 cm^{-1}. At the nanoscale level, transmission electron microscopy (TEM) classified and confirmed the crystal structure of material [44,45]. The crystalline AgNPs were studied using a TEM (Hitachi HT -7700). XRD can be used to examine the atomic structures of materials. This technique is useful for determining the both qualitative and quantitative levels of materials. XRD (XRD, Panalytical X'Pert3 Powder) experiments was used to identify and are able to from the size and structure of crystalline nanoparticles [44,45]. XRD charts were measured using Cu-K radiation (=1.54059) at 45 kV and 40 mA in the 10º-80º range. The Debye-Scherrer formula was used to estimate the particle dimension of nanomaterials from XRD data. By using dynamic light scattering (DLS) (Malvern Zetasizer Nano ZSP Analyzer, Malvern, UK), it was possible to determine the size distribution and zeta potential of AgNPs in aqueous suspension.

3. Results and Discussion

The UV-visible absorption spectrophotometer is the most widely used instrument for studying the SPR. The
UV-visible spectroscopy analysis was utilized to observe the sharp and intense SPR peaks, and the aqueous pomegranate extract was employed to synthesis AgNPs. The absorption band's intensity was found to be promising as the consequence of the formation of highly distributed nanoparticles. A correlation between the generation of silver nanoparticles and the manipulation of their size and distribution, as well as their production, was demonstrated to be dependent on the solvent employed for extract synthesis [46]. The biological metabolites included in the extract have a big impact on the size, shape, and optical properties of silver nanoparticles [46]. The reaction solution's color changed from yellowish-brown to reddish-brown in less than 31 minutes, clearly demonstrating the biological reduction of $AgNO_3$ into AgNPs. (Fig. 1a). The color of pomogranate leaf extract went dark once the aqueous $AgNO_3$ solution was added, indicating the reaction had begun. The characterisation of silver nanoparticles based on the SPR (surface plasmon resonance) peak found at 430 nm provided proof that AgNPs had been successfully synthesized. Furthermore, the biosynthesised PLE-AgNPs showed stability for a longer time (4-5 months). It is well known that the silver metal nanoparticle SPR phenomenon makes AgNPs appear dark brown in water [47].

a) b)

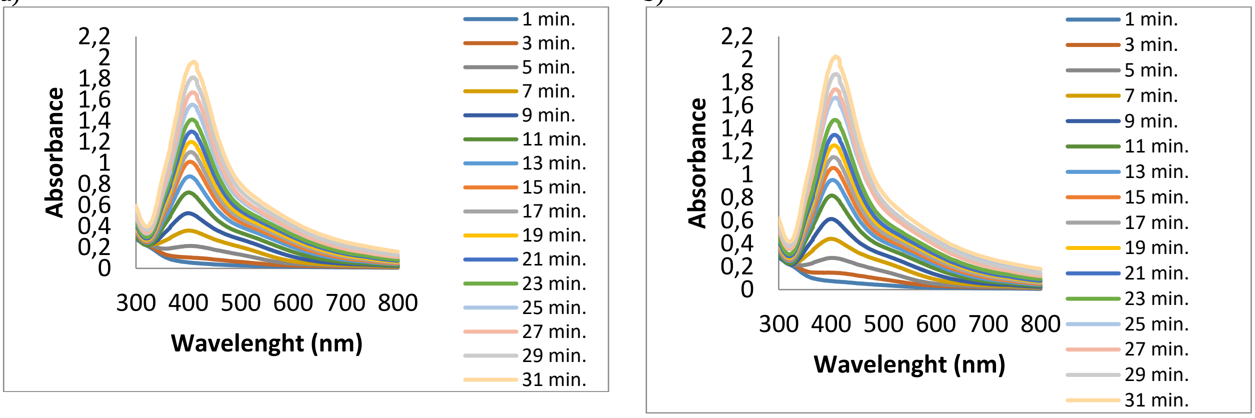

Figure 1. UV-vis spectra of Ag nanoparticles produced by microwave extraction with 0.25 mM $AgNO_3$ and in different amounts of Pomegranate leaves extract [a) 0.1 mL,b)0.2 mL]

a) b)

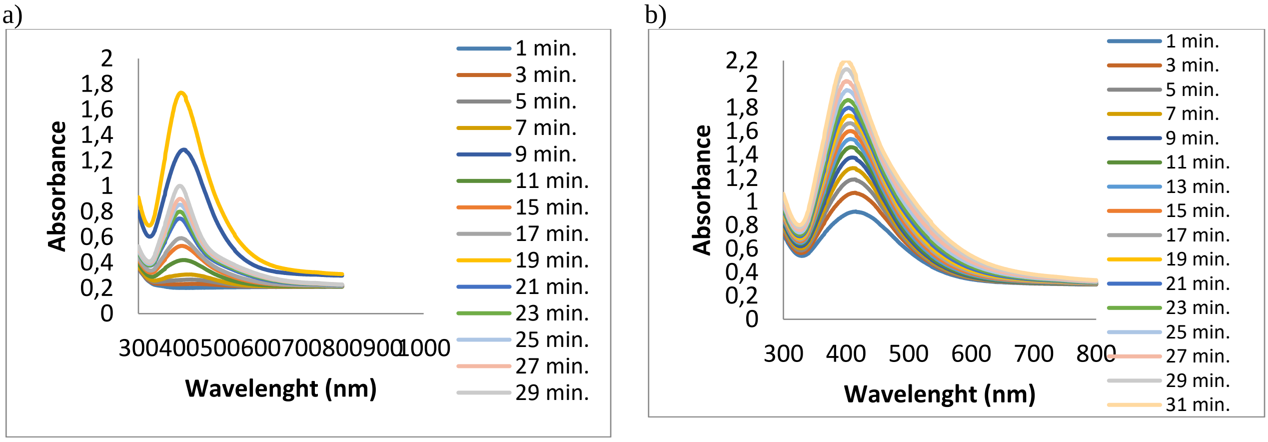

Figure 2. UV-vis spectra of Ag nanoparticles produced by microwave extraction with 0.5 mM $AgNO_3$ and in different amounts of Pomegranate leaves extract [a) 0.1 mL,b)0.2 mL]

a) b)

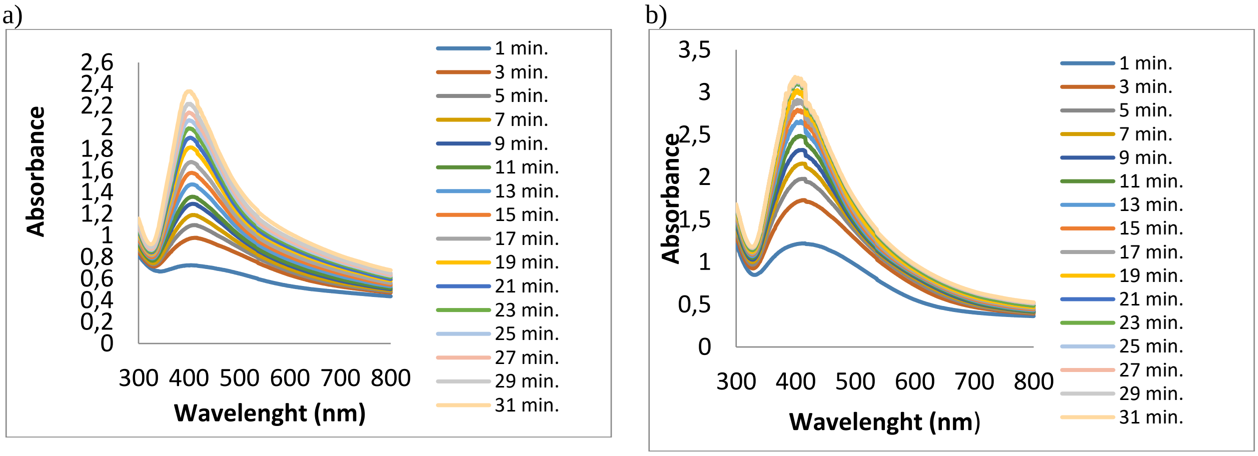

Figure 3. UV-vis spectra of Ag nanoparticles produced by microwave extraction with 1 mM $AgNO_3$ and in different amounts of Pomegranate leaves extract [a) 0.1 mL,b)0.2 mL]

It demonstrates that reducing Ag^+ to Ag^0 by adding PLE to an $AgNO_3$ solution results in the production of silver nanoparticles. A particularly noticeable deepening of color which indicates the reaction's saturation within 31 min, is consistent with little agglomeration and good particle dispersion in the media synthesis process [48]. Size, shape of the metal nanoparticles as well as those of the surrounding medium affect the breadth and frequency of the surface plasmon absorption. The stable position of the absorbance peak at the appropriate wavelength revealed that the generated PLE-AgNPs were not aggregates, which is in line with earlier reports of [47,49,50]. The PLE contains a variety of biological organic molecules that are responsible for the reduction and stabilization of AgNPs.

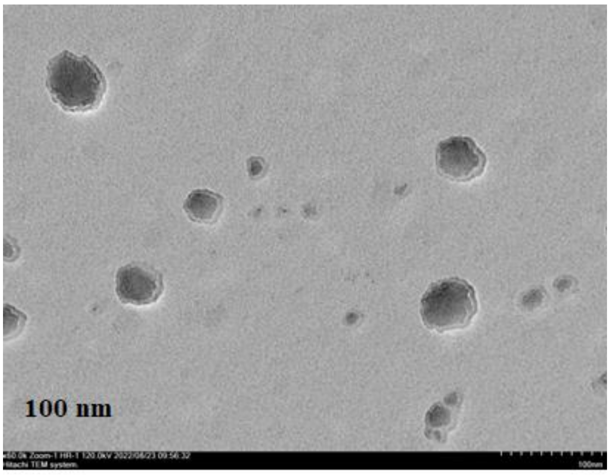

Figure 4. TEM images of Ag nanoparticles synthesized by microwave extraction method with 0.25 mM $AgNO_3$ and 0.1 mL Pomegranate leaves extract (100 nm)

The sample TEM image of the Figure 4 shows a representative TEM picture of the silver nanoparticles that have been found. The monodisperse character of the PLE-AgNPs was shown by the TEM Picture(Fig. 4).

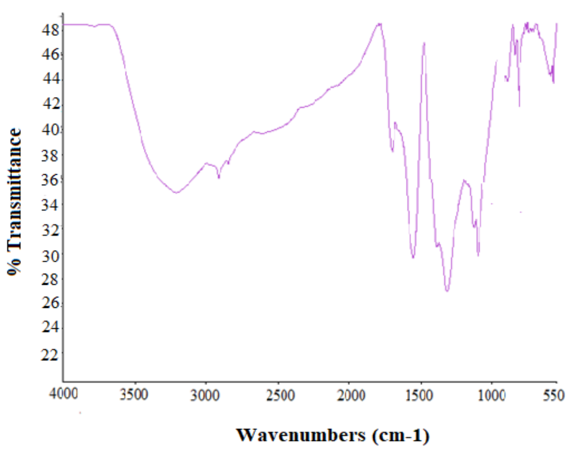

Figure 5. FT-IR spectrum of PLE-AgNPs.

The functional groups involved in the Pomegranate leaves extract reduction of silver into the different AgNPs were identified using FTIR analysis. Strong absorption peaks were visible in the FTIR spectra of the produced AgNPs and the aqueous Pomegranate leaves extract, respectively, at 3210 cm^{-1} 2935 cm^{-1}, 1552 cm^{-1}, 1299 $cm^{-1}$and 1041cm^{-1}. Different functional groups of the Pomegranate leaves extract were likely involved in the formation of the AgNPs, and they play a crucial role in the process of capping and stabilization, which allows for the AgNPs from agglomerating (Figure 5).

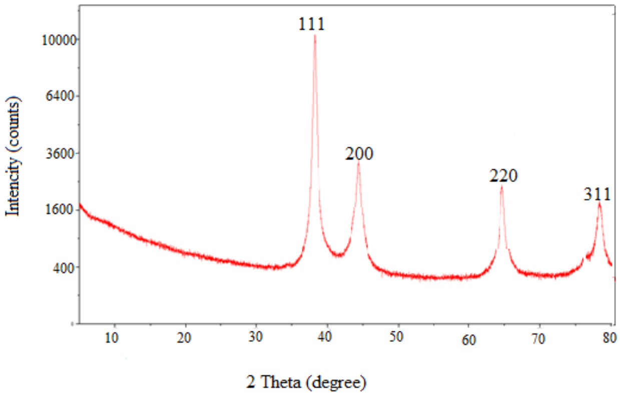

Figure 6. XRD analysis result of Ag nanoparticles synthesized by microwave extraction method with 0.25 mM $AgNO_3$ and 0.1 mL Pomegranate leaves extract (diffraction peaks 2θ=38.1º, 44.3º, 64.6º, 77.6º respectively)

Figure 6 displays the XRD pattern of the produced AgNPs. The diffraction peaks at 2 theta were assigned to the corresponding diffraction signals at 38.1, 44.3, 64.6, 77.6 were provided to the corresponding diffraction signals at 111, 200, 220 and 311 respectively, which show the face-centered cubic crystalline AgNPs (Joint Committee on Powder Diffraction Standards; JCPDS no. 04-0783). Since these peaks are primarily oriented along the (111) plane, the reflection (111) in Figure 6 has a high intensity compared to the other reflections, which shows the presence of silver nanocrystals [51]. Silver's crystalline nature was established by X-ray diffraction (XRD). The findings agree with earlier research revealing comparable AgNP diffraction peaks [51,52].

Over a period of 4-5 months, the stability of the produced PLE-AgNPs revealed no aggregation or significant SPR change. Additionally, in order to determine the charge and stability of the produced PLE-AgNPs, examined the DLS and zeta potential. The zeta potential, which depicts the surface charge of NPs and predicts interactions between NPs, is a significant characteristic that can be used to estimate the long-term stability of NPs in suspension [51]. The zeta potential of dispersion produced PLE-AgNPs in deionized water was -14±0.777 mV and the polydispersity index for gold nanoparticles is 0,257±0.004 (Figure 7(b)). Figure 7(b) shows that PLE-AgNPs have a negative charge.

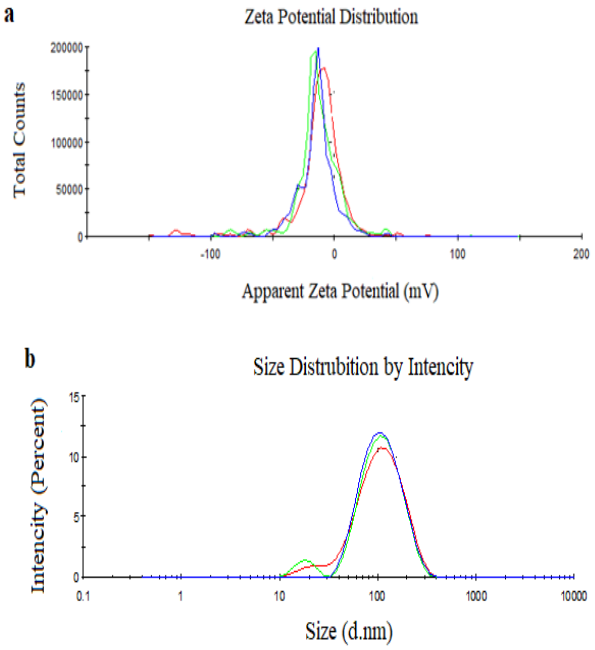

Figure 7. Stability of synthesized PLE-AgNPs up to 3.5-4 months and their (a) zeta potential and (b) particle size distribution determined by DLS.

The repulsion between the particles, which is confirmed by the negative charge on PLE-AgNPs, prevents coalescence and agglomeration, supporting the long-term stability of synthesized AgNPs.

Figure 7(b) shows the DLS size distribution image of PLE-AgNPs. The produced AgNPs are poly-dispersed in nature, as shown by the particle size distribution curve. AgNPs have an estimated 86.02±0.5788 nm average particle size distribution (Figure 7(b)).

4. Conclusion

Silver nitrate ($AgNO_3$) was used as a source of Ag^+ ion for the synthesis of Ag nanoparticles. The color change of the solution from yellow to dark brown showed the formation of Ag nanoparticles. Optimum conditions were determined by measuring plasmon resonance (SPR) absorption spectra. Silver nanoparticles were successfully produced with 0.1 mL (0.25 mM) extract volume. After 7 minutes of microwave application, in the specific resonance band range of 90 W and around 400-460 nm, 0.1 ml pomegranate leaf extract and silver nanoparticles were successfully produced in Figure 1. Pomegranate leaves extract under typical conditions allowed for the quick, simple, economical and successful synthesis of AgNPs in under seven minutes. The pomegranate leaves extract polyphenol molecules worked as stabilizing and reducing agents, respectively. The potential use of synthesized PLE-AgNPs was increased because they were discovered to be stable and showed no symptoms of aggregation during storage for up to 4-5 months. By acting and demonstrating a combined function as a reducing and capping agent for the PLE-AgNPs, the organic phytochemicals present in the leaf extract prevented the use of any hazardous chemicals.

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Author's Contributions

Gönül Serdar: Drafted and wrote the manuscript, performed the experiment and result analysis.

Ethics

There are no declared conflicts of interest for the author. Research ethics and publication requirements are fulfilled by the study.

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