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Evaluation of the Effect of Different Extraction Temperatures on the Synthesis of Silver Nanoparticles from *Ocimum basilicum* (Basil) Plant

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Abstract: The eco-friendly green synthesis of silver nanoparticles (AgNPs) using *Ocimum basilicum* (basil) extract at varying extraction temperatures (40, 60, 80, and 100°C) was investigated to determine the optimal conditions for nanoparticle formation. Analysis methods such UV-Vis spectrophotometry, Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Transmission Electron Microscopy (TEM) confirmed the crystalline, spherical nature of AgNPs and identified phytochemicals acting as capping and reducing agents. Notably, it was found that the extraction temperature influenced both the DPPH radical scavenging activity and the structural properties of AgNPs. TEM analysis revealed that higher extraction temperatures led to increased nanoparticle formation efficiency but also resulted in wider size distribution. The crystallite sizes of AgNPs synthesized at different extraction temperatures were determined to be 12.45 nm, 18.77 nm, 17.76 nm, and 16.03 nm, respectively, using the Scherrer equation. The hydrodynamic sizes of the AgNPs ranged between 158.1 and 333.7 nm. The study highlights the critical role of extraction temperature in the synthesis process, proposing 40°C as the optimal temperature for achieving efficient and environmentally friendly synthesis of AgNPs with enhanced biological activities.

Ocimum basilicum (Fesleğen) Bitkisinin Farklı Ekstraksiyon Sıcaklıklarının Gümüş Nanopartikül Sentezine Etkisinin Değerlendirilmesi

Anahtar Kelimeler
Yeşil sentez, Ekstraksiyon, *Ocimum basilicum*, Gümüş nanopartikül

Öz: *Ocimum basilicum* (fesleğen) özütü kullanılarak çeşitli ekstraksiyon sıcaklıklarında (40, 60, 80, ve 100°C) çevre dostu yeşil sentez yöntemi ile gümüş nanopartikülleri (AgNPs) sentezi araştırıldı. Nanopartikül oluşumunun optimal koşullarını belirlemek için UV-Vis spektrofotometri, Fourier Dönüşümlü Kızılötesi Spektroskopi (FTIR), X-ışını Kırınımı (XRD) ve Geçirimli Elektron Mikroskobu (TEM) gibi analiz yöntemleri kullanıldı. Bu yöntemlerle AgNPs'lerin kristalin ve küresel yapısı doğrulanmış ve kaplama ile indirgeyici ajan olarak hareket eden fitokimyasallar tespit edilmiştir. Özellikle, ekstraksiyon sıcaklığının hem DPPH radikal süpürme aktivitesini hem de AgNPs'nin yapısal özelliklerini etkilediği bulunmuştur. TEM analizi, daha yüksek ekstraksiyon sıcaklıklarının nanopartikül oluşum verimliliğinin artmasına yol açtığını ancak aynı zamanda daha geniş boyut dağılımına yol açtığını ortaya çıkardı. Farklı ekstraksiyon sıcaklıklarında sentezlenen AgNPs'lerin kristalit boyutları Scherrer denklemi kullanılarak sırasıyla 12.45 nm, 18.77 nm, 17.76 nm ve 16.03 nm olarak hesaplanmıştır. AgNPs'nin hidrodinamik boyutlarının 158.1 ile 333.7 nm arasında değişmiştir. Çalışma, ekstraksiyon sıcaklığının sentez sürecindeki kritik rolünü vurgulayarak, gelişmiş biyolojik aktivitelere sahip AgNP'lerin verimli ve çevre dostu sentezini için 40 °C'yi en uygun ekstraksiyon sıcaklığı olarak önermektedir.

1. INTRODUCTION

Nanotechnology is an interdisciplinary field of research and innovation taht focused on building constructing materials and devices at the atomic and molecular scale, typically within the size range of one nanometer (one billionth of a meter, or 10^{-9} meters) [1]. Nanoparticles are recognized as essential building blocks within nanotechnology, holding the potential to revolutionize a broad spectrum of applications [2]. These miniature particles, with sizes ranging from 1 to 100 nanometers (nm), exhibit unique physical, chemical, and biological properties that differ significantly from those of bulk materials. Due to their small size, nanoparticles possess a high surface area to volume ratio, resulting in heightened reactivity and interaction with biological systems [3]. Nanoparticles, which include metallic (such as gold, silver, and platinum), metal oxide (including titanium dioxide, zinc oxide, and iron oxide), quantum dots, carbon-based (fullerenes, carbon nanotubes, graphene), ceramic, polymeric, and lipid-based varieties, offer a diverse range of applications from catalysis, medical imaging, and drug delivery to cosmetics, electronics, energy solutions, and pharmaceuticals, due to unique properties like conductivity, optical characteristics, mechanical strength, and magnetic or electronic behaviour [5]. Silver nanoparticles (AgNPs), among others, exhibit a multitude of biological properties that render them promising candidates for a variousbiomedical applications. Their diverse properties include antimicrobial activity against a broad spectrum of pathogens, including bacteria, fungi and viruses [5]. AgNPs exert their antimicrobial effect through diverse mechanisms, such as disrupting of microbial cell membranes, interfering with key cellular processes and including oxidative stress leading to cell death [6]. In addition, silver nanoparticles exhibit remarkable anti-inflammatory properties by modulating the production of pro-inflammatory cytokines and attenuating the activation of inflammatory cascades. This anti-inflammatory effect is promising for the treatment of various inflammatory conditions, thus promoting tissue repair and wound healing [7].

Silver nanoparticles can be synthesized by various methods, including physical (plasma spraying, thermal reduction and electrospinning under inert gas atmosphere), chemical (chemical reduction, sol-gel method, microemulsion) and biological (plant extracts, microorganisms, proteins or polysaccharides) methods [8]. The use of plant extracts in the biological synthesis of AgNPs has gained significant attention for several reasons. Plant extracts contain bioactive molecules such as phenolic compounds, flavonoids, alkaloids and terpenoids, which can serve as reducing agents, stabilizing agents and capping agents during the synthesis process. These compounds facilitate the reduction of silver ions into silver nanopartcicles. Additionally, the use of plant extracts offers a green and eco-friendly approach to nanoparticle synthesis compared to conventional chemical methods, which often involve the use of hazardous chemicals and harsh conditions. The green synthesis route using plant extracts not only reduces the environmental impact but also eliminates the need for energy-intensive processes, making it a sustainable and cost-effective alternative [9]. However, there are some important points to consider when synthesizing nanoparticles from plant extract. The conditions of plant extraction in nanoparticle synthesis crucial, given the pivotal as both reducing and stabilizing agents in the green synthesis process [10]. The extraction method and conditions—including temperature, time, and pH—must be optimized to maximize the extraction of active compounds without degrading their properties, as these factors directly impact the nucleation, growth, and morphology of the nanoparticles [11]. Among them, extraction temperature is a critical parameter in the process of extracting phytochemicals from plant materials, as it directly influences the efficiency, yield, and quality of the extracted compounds. Furthermore, the temperature at which phytochemicals are extracted significantly affects their antioxidant capacity, notably their effectiveness in DPPH radical scavenging activity, which in turn influences the biosynthesis and functional quality of nanoparticles derived from these extracts. This underscores the interconnected impact of extraction temperature on both the phytochemical yield and the subsequent synthesis of biologically active nanoparticles [12]. Higher temperatures generally increase the solubility of phytochemicals in solvents, allowing for more efficient extraction, yet there is a risk of thermal degradation of heat-sensitive compounds [13]. Many different temperatures are used in plant extraction. Previous studies on the synthesis of AgNP using plant extract are shown in Table 1. This paper presents a comprehensive study on the synthesis of AgNPs using *Ocimum basilicum* extract at different extraction temperature (40, 60, 80 and 100 ^{o}C). This research investigates the influence of temperature variations on synthesis efficiency and the properties of produced nanoparticles by harnessing the natural reducing and stabilizing properties of plant extract. Biosynthesized AgNPs were characterized using UV–Vis spectrophotometer, FT-IR, XRD, and TEM analysis. The 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of each extract was determined

Table 1. Green synthesis of silver nanoparticles using plant extracts

Plants	Extraction temp. (^{o}C)	Extraction time	Sahape-Size	References
Allium cepa L	25	4h	Cubic-150-200 nm	[14]
C. prophetarum	80	3h	Polymorphic-below 150 nm	[15]
Tectona grandis seed	80	20 min	Oval,spherical-10–30 nm	[16]
Sambucus ebulus	55	4h	Spherical-18.6 nm	[17]
Moringa oleifera	100	30 min	Spherical-10-25nm	[18]
Selaginella myosurus	80	5 min	Spherical-15-95nm	[19]
Guettarda speciosa	60	30 min	Spherical-30-35 nm	[20]

2. MATERIAL AND METHOD

2.1. Materials

Silver nitrate ($AgNO_3$, 99.8%), ethanol (C_2H_6O), and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma-Aldrich. The plant materials required for the synthesis were sourced from the area's Marketplace

2.2. Preparation Process of The Herbal Extracts

10 grams of the dried plant were weighed and added to 100 mL of pure water. Based on the extraction temperatures most commonly used in the literature, it was allowed to stand for 1 hour at 40, 60, 80, and 100°C**.** After the extraction process, the mixture was allowed to cool to room temperature. Subsequently, the samples were filtered and stored at 4°C [21].

2.3. Biosynthesis of AgNPs

100 mL of 0.01 M silver nitrate solution was prepared in four separate beaker 10 mL of plant extract was gradually added to each. The mixture was left to react for 24 hours. At the end of the reaction time, the samples were centrifuged at 10.000 rpm. The pellet was washed several times with ethanol to remove impurities. It was then left to dry at 50 ºC in the oven [18].

2.4. DPPH Radical Scavenging Assays

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay relies on the reduction of the DPPH radical (a stable free radical with a violet colour) in the presence of a hydrogen-donating antioxidant, leading to a colour change from violet to yellow. The DPPH free radical scavenging capacity was applied with some modifications to the Molyneux (2004) method [22]. Initially, the plant was extracted at 40, 60, 80 and 100 °C for 1 hour. The measured 0.1 mL extract was then transferred into a clean test tube and ethanol was added to bring the total volume of the solution up to 3 mL. 1 mL of 10^{-4} M DPPH (2,2-diphenyl-1-picrylhydrazyl) solution was added and the mixture was vortexed, followed by incubation for 30 minutes at room temperature in the dark. Absorbance were measured against ethanol at 517 nm with a UV-Vis spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan). Measurements were made in three replicates. Results are given as percentage of DPPH radical scavenging. % inhibition was calculated with the following formula:

$$\%Inhibition = \frac{(Abscontrol - Abssample)}{Abscontrol} \times 100$$

2.5. Characterization Analysis

Morphological structure was examined using transmission electron microscopy (TEM, Hitachi HighTech HT7700). Crystal structure was evaluated with an X-ray diffractometer (XRD, PANalytical Empyrean), across a range of 2θ angles from 20° to 80°.Functional groups responsible for NP reduction and stability were identified through Fourier transform infrared spectroscopy (FTIR, Bruker VERTEX 70v). Zeta potential and hydrodynamic size distribution were measured using a Malvern Zetasizer Nano ZSP. DPPH scavenging activity of the extracts was measured using UV-Vis spectrophotometer (1601, Shimadzu, Kyoto, Japan).

3. RESULTS AND DISCUSSION

3.1. Colour Change Observation

The synthesis of AgNPs utilizing plant extracts was visually monitored. Initially, the solution displayed a brownish-purple to purple colour, indicating of the presence of plant extracts alone. Upon the addition of silver ions, a gradual color transition from gray to gray-brown was observed, signifying the formation of AgNPs [23] (Fig 1). This colour change serves as a qualitative indicator of silver nanoparticle formation, attributable to the SPR effect of AgNPs [24].

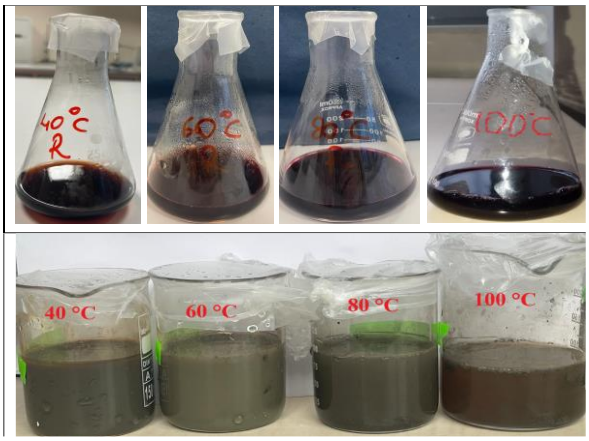

Figure 1. Plant extracted at different temperatures (40, 60, 80 and 100 ºC and AgNP formation (reaction time 24 hours)

3.2. DPPH Radical Scavenging Results

In Figure 2, percentage the %inhibition values are presented depending on the extraction temperature. It was observed that DPPH radical scavenging activity increased with higher extraction temperatures. The % inhibition values at 40, 60, 80 and 100 ºC were found to be 33%, 39%, 59% and 74.5%, respectively. The extraction of compounds from plants and their subsequent antioxidant activity are significantly influenced by temperature. Higher temperatures usually increase the solubility of many compounds, including antioxidants, in the solvent. Elevated temperatures enhance the efficiency of the extraction process as they can dissolve and extract more compounds from the plant material by breaking down the cell walls, thereby releasing more phytochemicals into the extraction solvent.

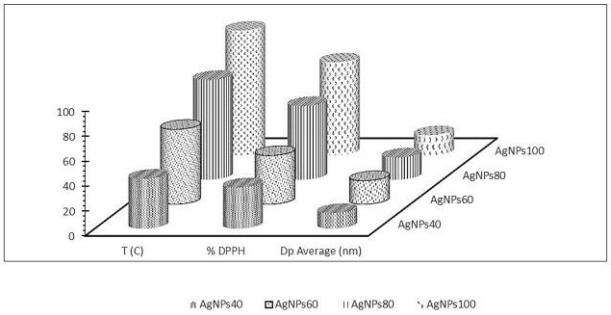

Figure 2. DPPH radical scavenging activity of basil extracted at different temperatures

3.3. TEM Results

TEM analysis enabled a detailed investigation of morphological, structural, and dimensional characteristics of the nanoparticle (NP) samples. The findings revealed a predominance of spherical nanoparticles, with sizes primarily ranging from 5 to 100 nanometers (Fig 3). A wide size distribution is generally observed in nanoparticle synthesis using plant extracts This variability can be attributed to the diverse bioactive compounds present in plant extracts, each with its own unique reducing capacities. As a result, nanoparticles of various sizes and shapes are formed. Dhir et al. (2024) and Iravani (2011) have noted that the use of plant extracts in NP synthesis leads to wide variations in size distribution [25-26]. Additionally, it was also noted that nanoparticles tended to agglomerate. A significant finding was that the efficiency of nanoparticle formation was positively influenced by higher extraction temperatures, indicating the crucial role of thermal conditions in the synthesis and properties of nanoparticles. TEM has led to significant insights into the influence of crystalline structures within individual particles on TEM image contrast. It has been observed that particles containing multiple AgNPs crystallites exhibit a significantly higher degree of electron scattering than particles with a single crystalline structure. This enhanced scattering effect is attributed to the presence of multiple interfaces and boundaries within the particle resulting from the aggregation of different crystallites. Consequently, such particles appear darker in TEM images as the electron beam is more obstructed when passing through the particle [27].

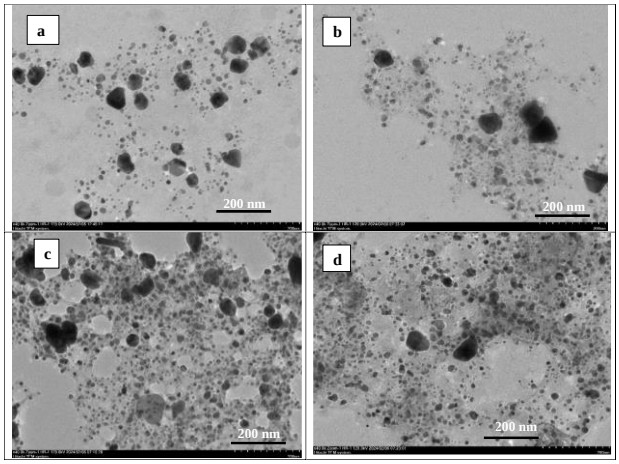

Figure 3. TEM image of AgNPs synthesis from different extraction temparature a) 40 ˚C b) 60 ºC c) 80 ºC d)100 ºC

3.4. XRD Results

The crystalline structure of AgNPs was characterized using X-ray diffraction (XRD) analysis. The obtained XRD pattern is presented in Figure 4. The diffraction peaks observed at 2θ values are indexed to the (111), (200), (220), and (311) planes, respectively, of face-centered cubic (fcc) silver, according to the standard Joint Committee on Powder Diffraction Standards (JCPDS) file No. 04-0783 [28]. These peaks confirm the crystalline nature of the synthesized silver nanoparticles. A high XRD peak indensity has been observed in the AgNPs synthesized from the plant extract obtained at 40 and 60 °C. This indicates that these nanoparticles possess a more regular crystalline structure [29]. The additional peaks indicated by asterisks in the XRD pattern may suggest the crystallization of a bio-organic phase on the surface of the AgNPs [30]. The average crystallite size was calculated using the Scherrer equation;

$$D = \frac{K\lambda}{\beta \cos\theta}$$

where: D is the mean size of the crystallites (often in nanometers), K is the shape factor (a dimensionless constant, typically taken as about 0.9), is the wavelength of the X-ray radiation (in meters),β is the full width at half maximum (FWHM) of the peak in radians, θ is the Bragg angle (in radians). AgNP synthesis conducted with extracts obtained at 40, 60, 80, and 100°C, the crystallite sizes were calculated to be 12.45, 18.77, 17.76, and 16.03 nm, respectively. The results suggest that the controlled synthesis parameters significantly influence the crystallite size, thereby impacting the physical and chemical properties of the materials.

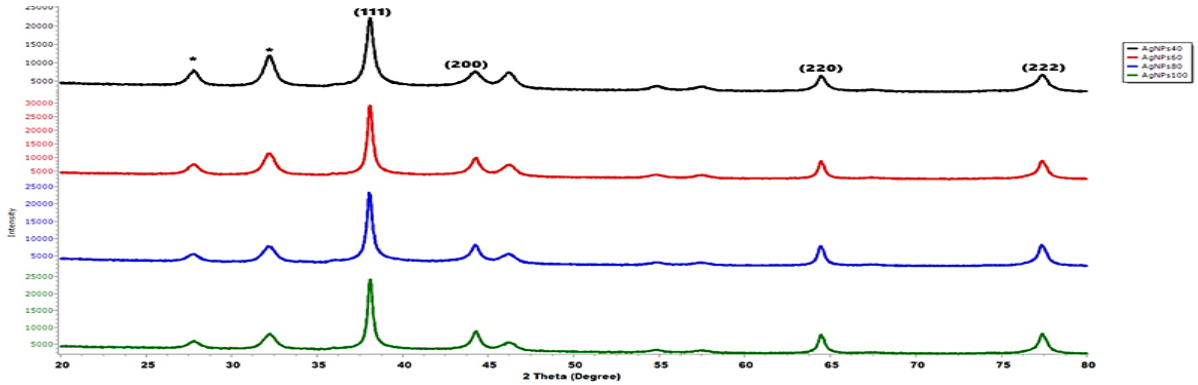

Figure 4. XRD graph of AgNPs (black line 40 ºC, red line 60 ºC, blue line 80 ºC, green line 100 ºC)

3.5. FT-IR Results

FTIR spectroscopy was utilized to identify the functional groups present on the surface of the synthesized AgNPs and to elucidate the mechanism behind the reduction of silver ions to AgNPs using plant extract. The FTIR spectra were recorded in the range of 4000-400 cm^{-1}(Fig 5). The presence of a peak in the 2928 cm^{-1} region signifies C-H stretching vibrations, indicating aliphatic hydrocarbons' presence. This could relate to fatty acids or other hydrocarbon chains in the plant extract, possibly playing a role in stabilizing the silver nanoparticles. A strong peak around 1650 cm^{-1}, indicative of C=O stretching vibrations typical of carbonyl groups in carboxylic acids, esters, and amides, suggests that compounds with carbonyl groups. emphasizing the potential of phytochemicals as dual-functioning reducing and capping agents in the environmentally friendly production of nanoparticles.

3.6. Hydrodynamic Size and Zeta Potential Results

The analysis of hydrodynamic size and zeta potential provides crucial insights into the physical stability and surface charge characteristics of nanoparticles. The hydrodynamic size, determined through dynamic light scattering (DLS) techniques, provides information on the effective diameter of nanoparticles in a suspension, reflecting both the core particle size and any surface-bound layers or molecules [34]. The results from this analysis indicated that the nanoparticles had an average

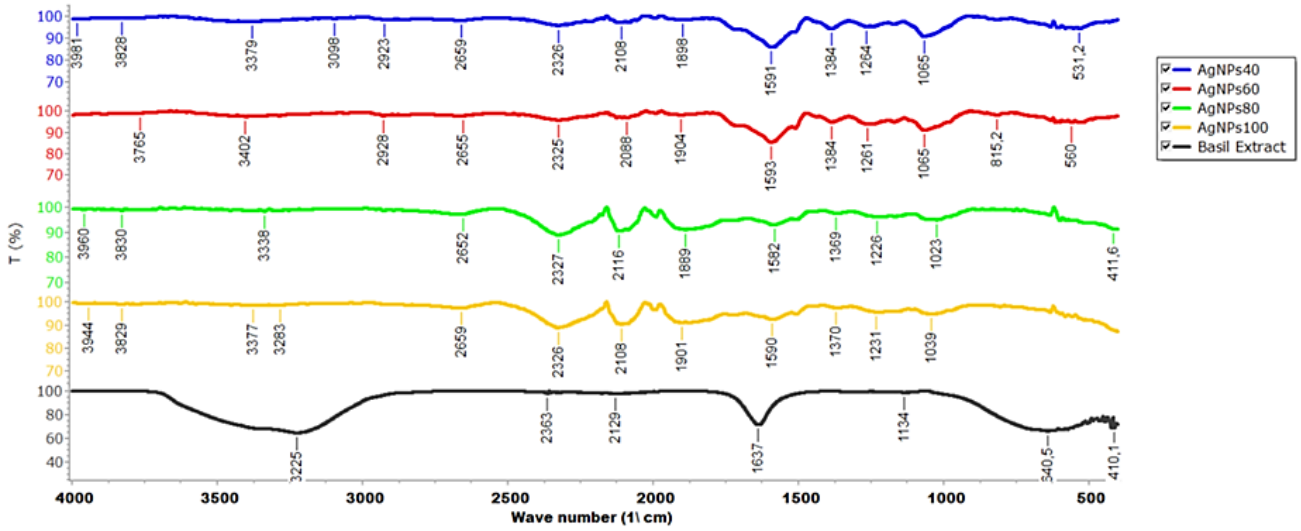

Figure 5. FTIR spectrum of Basil extract and synthesized AgNPs

The peak at around 1591 cm^{-1} can indicate C=C stretching vibrations, and it may also correspond to N-H bending vibrations in amides of the aromatic rings. The peak at ~1370 cm^{-1} indicates C-N stretching from amines, and the peak at ~1040-1070 cm^{-1}, showing C-O stretching, suggests the plant extract's nitrogen compounds aid in the synthesis and stabilization of AgNPs [31-33]. The FTIR results suggest that the phytochemicals present in the plant extract, such as phenolics, alcohols, aliphatic compounds, proteins, and nitrogen-containing compounds, play a crucial role in the bioreduction of silver ions to nanoparticles and their subsequent stabilization. These findings offer valuable insight into the green synthesis of AgNPs using plant extracts, hydrodynamic diameter within the range of 158.1 and 333.7(Fig 6 and Table 2). Nanoparticles synthesized with extracts prepared at 80°C have shown a wide size distribution. The polydispersity index (PDI) quantifies the distribution of particle sizes within a sample [35]. It provides insight into the uniformity of the particles, with a lower PDI indicating a more uniform size distribution. While nanoparticles synthesized with extracts prepared at 80 °C had high PDI values, the lowest PDI was observed at 60 °C. High temperatures can increase the solubility of bioactive components in plant materials [36-37]. With increasing solubility, more reducing agent passes into solution, which can lead to the formation of a denser nanoparticle. Higher temperatures may enhance the

solubility of phytochemicals in the extract, leading to a more rapid reduction of silver ions and accelerated growth of nanoparticles. This rapid growth can result in larger nanoparticles.

Table 2. Zeta potential, size and PDI values of synthesized AgNPs

Samples	Temperature (°C)	Z-Ave. size (nm)	PdI	Zeta potential (mV)	Mobility (µmcm/Vs)	Conductivity (mS/cm)
AgNPs40	25	158,1	0,263	-21,5	-1,686	0,00729
AgNPs60	25	230,4	0,233	-25,7	-2,014	0,012
AgNPs80	25	333,7	0,401	-21,6	-1,692	0,0142
AgNPs100	25	294,9	0,27	-16,2	-1,27	0,00587

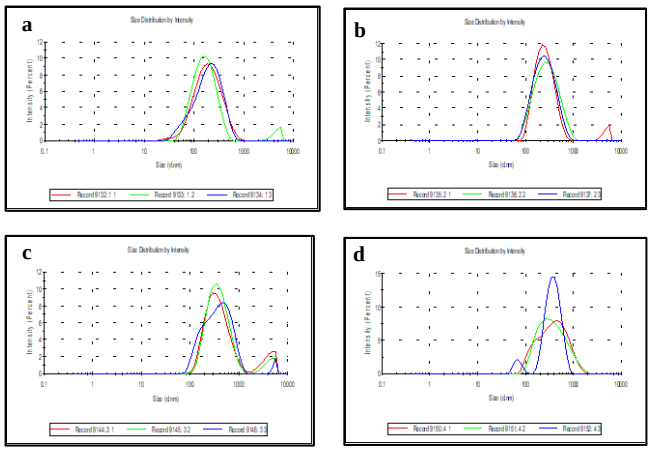

Figure 6. Hydrodynamic size distribution of AgNPs a) 40 ˚C b) 60 ºC c) 80 ºC d)100 ºC

4. DISCUSSION

This study investigated the impact of different extraction temperatures (40°, 60, 80, and 100°C) on the eco-friendly synthesis of silver nanoparticles (AgNPs) using *Ocimum basilicum* (basil) extract. It was observed that higher extraction temperatures increased DPPH activity likely due to increasing the solubility of phytochemicals in basil extract. However, despite the higher efficiency in nanoparticle formation, higher extraction temperatures led to larger size distrubition, lower stability, and aggregation. The study underscores the critical influence of extraction temperature in the synthesis process, proposing 40°C as the optimal temperature for achieving efficient and environmentally friendly synthesis of AgNPs with enhanced biological activities.

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