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Green Synthesis of Silver Nanoparticles Using Aqueous Orange and Lemon Peel Extract and Evaluation of Their Antimicrobial Properties

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Abstract

Green synthesis of silver nanoparticles (AgNPs) using aqueous extracts of orange and lemon peels, as a reducing agent, and silver nitrate salts as a source of silver ions is a promising field of research due to the versatility of biomedical applications of metal nanoparticles. In this paper, AgNPs were synthetized at different reaction parameters such as the type and concentration of the extracts, metal salt concentration, temperature, speed stirring, and pH. The antibacterial properties of the obtained silver nanoparticles against *E. coli*, as well as the physical and chemical characteristics of the synthesized silver nanoparticles, were investigated. UV-Vis spectroscopy was used to confirm the formation of AgNPs. In addition to green biogenic synthesis, chemical synthesis of silver nanoparticles was also carried out. The optimal temperature for extraction was 65˚C, while for the synthesis of AgNPs was 35˚C. The synthesis is carried out in an acidic environment (pH = 4.7 orange and pH = 3.8 lemon), neutral (pH = 7) and alkaline (pH = 10), then for different concentrations of silver nitrate solution (0.5 mM - 1 mM), optimal time duration of the reaction was 60 min and optimal stirring speed rotation was 250 rpm on the magnetic stirrer. The physical properties of the synthesized silver nanoparticles (conductivity, density and refractive index) were also studied, and the passage of laser light through the obtained solution and distilled water was compared. Positive inhibitory effect on the growth of new *Escherichia coli* colonies have shown AgNPs synthesized at a basic pH value and at a 0.1 mM $AgNO_3$ using orange or lemon peel extract, while for a 0.5 mM $AgNO_3$

using lemon peel extract.

Keywords

Green Synthesis, Silver Nanoparticles, Orange and Lemon Peel Extract, Antibacterial Activity, *Escherichia coli*

1. Introduction

Silver, iron, zinc, gold, platinum and cobalt are the most synthesized metal nanoparticles. In most of cases, the key strategy for biosynthesis of metal nanoparticles is metal salts reduction. Nanoparticles can be prepared from various synthetic and biologically compatible materials (algae, fungi, plant extracts, and essential oils) [1] [2]. Plant materials were recently gathered more attention than microorganism due to low cost and simplicity of control [3].

Anti-microbial resistance is a growing global health concern due to the development of drug-resistant bacteria due to the widespread use of antibiotics. This has resulted in an urgent need for the development of new and effective antimicrobial agents. In this sense, a large number of researchers have increasingly focused their attention on investigating the possibility of biosynthesis of metal nanoparticles using extracts from plants and testing their potential antibacterial activity.

The use of plant extracts for the synthesis of silver nanoparticles is an environmentally acceptable method because secondary metabolites from plants like polyphenols, flavonoids and triterpenes are used as reducing and capping agents instead of aggressive and toxic chemicals in the chemical method [4]. The benefit of green synthesis of metal nanoparticles is that waste streams of costly materials such as silver salts can be recycled, ultimately reducing the overall costs of production [5].

The phytochemicals in plant extracts are key building blocks in the plant-mediated synthesis of AgNPs. These plant extracts contain an abundance of molecules with carboxyl, amino, carbonyl, hydroxyl, and phenol groups and, thus, have the ability to reduce metals such as silver [6]. The major phytochemicals responsible for the bio-reduction process are aldehydes, ketones, flavones, sugars, terpenoids, carboxylic acids, and amides. Antioxidants are strongly implicated as reducing and capping agents in these processes, especially the flavonoids (flavonols, flavan-3-ols), phenolic acids (benzoic, hydroxycinnamic, and ellagic acids), and anthocyanins , while the other plant metabolites such as proteins and chlorophyll are responsible for stabilization of the NPs [7].

In the next part of the paper, only some of the research from a large number of research papers in this field published in the last two years will be presented.

Krishnamoorthy *et al.* [8] used *Argyreia nervosa* plant leaves for the synthesis of AgNPs which showed significant antioxidant, antibacterial activity, inflammatory activity, and good anti-diabetic activity. They highlight that additional *in* *vivo* research focusing on molecular-level investigations is necessary to substantiate these findings and explore the potential of *Argyreia nervosa*-mediated silver nanoparticles as a viable drug for antioxidant, antibacterial, and anti-diabetic purposes.

A research group from India, Magdum *et al.* [9] used for the first time leaves extract of *Decaschistia trilobata* (Dt) an endemic shrub for biosynthesis of AgNPs. They used methanol and water for the extraction of phytochemicals. They quantified total phenolics, flavonoids, terpenoids and alkanoids in both solvents. The results showed that the concentrations of phytochemicals were higher in the methanolic extract. They tested antibacterial properties against Gram-positive *Staphylococcus aureus* (NCIM 2654) and Gram-negative *Escherichia coli* (NCIM 2832), anti-inflammatory and antioxidant properties using FRAP and DPPH methods. UV-Vis spectroscopy confirmed the formation of Dt-AgNPs at a wavelength of around 400 nm. Dt-AgNPs also showed photocatalytic activity in the degradation of textile dyes Rhodamine B and Congo Red, achieving a reduction of these dyes within 10 min, which highlights their potential utility in treating industrial wastewater.

Rama *et al.* [10] prepared AgNPs by green synthesis using *Aegle marmelos* leaf extract as reducing and capping agents. The formation of Ag NPs was confirmed by the Surface Plasmon Resonance centre at 450 nm. AgNPs were spherical with a size range of ~30 - 50 nm.

Furthermore, they showed the cytotoxicity against MDA-MB-231 human breast cancer cells *in vitro*. The MTT assay shows the IC_{50} values at 125 ± 4.26 μg/mL of AgNPs compared to the untreated negative control cells. Besides this, biological activity, they also showed photocatalytic efficiency in degradation of Basic Fuchsin dye within 18 min for 98%.

Abdulazeem *et al.* [11] prepared the AgNPs by using an aqueous extract from broccoli florets (*Brassica oleracea*) at pH of 6 - 7. UV-Vis spectroscopy indicating the surface plasmon resonance (SPR) presence at 425 nm. Interestingly, the silver nanoparticles remained stable at ambient temperature for 25 days without precipitation, retaining their antioxidant and antibacterial properties. In conclusion, the research findings suggest that an aqueous extract of fresh broccoli florets can serve as a viable and environmentally friendly method for producing stable silver nanoparticles with beneficial antioxidant and antibacterial characteristics.

Shereen *et al.* [12] reported the green synthesis of AgNPs using extracts of herbal plant *Swertia chirata prepared in* different solvents such as ethanol, methanol, chloroform, and distilled water. Several characterizing peaks are seen for the synthesis of AgNPs, mainly in the range of 410 - 480 nm. The highest intensity value was observed at the wavelengths of 400 nm and 420 nm (0.390 and 0.440, respectively). They revealed that AgNPs and the plant extracts exhibited a significant zone of inhibition against human pathogenic bacteria (*Escherichia coli*, *S. capitis*, *B. subtilis,* and *Pseudomonas aeruginosa*) as compared to the cefixime and norfloxacin.

Researchers from Ethiopia [13] carried out green synthesis of AgNPs using the leaf extract of *Vernonia amygdalina*. However, they used a binary solvent mixture for extraction methanol and distilled water for better extraction than methanol, ethanol, distilled water and ethanol/distilled water. The UV-Vis spectra showed specific surface plasmon resonance (SPR) absorption peaks between 411 nm and 430 nm which revealed the formation of AgNPs during the synthesis. The synthesized AgNPs showed antibacterial activity against Gram-positive bacteria (*S. pyogenes* and *S. aureus*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*).

Nanotechnology penetrates all areas of human activity, from computers and robotics, electronics, medicine, biomedicine, to the textile industry. Nanoscience is a field of science that measures and explains changes in the properties of matter as a function of size. At the nanoscale, any measured physical property will change continuously with size, often drastically. Nanoscience is promising and the application of nanotechnology is so great that it is predicted to change our world. In the nano-range, the physical, chemical and biological properties of materials are unique. Therefore, nanotechnology provides the tools to create functional and intelligent materials, devices and systems by controlling materials at the nano-scale, using their new phenomena and related properties. Nanotechnology is multidisciplinary by nature. It does not only apply to physics and engineering, but also includes many other disciplines, especially chemistry and biology [14].

Studies on new physical properties and applications of nanomaterials and nanostructures are possible only when nanostructured materials of the desired size, morphology, crystal and micro-structure, and chemical composition are available. Thanks to their unique characteristics, metal nanoparticles, especially silver, have found potential applications such as targeted drug delivery, cancer therapy, biosensor, optical devices, electronic, magnetic, catalysts, water treatment and antimicrobial applications [15].

Nanotechnology has played a key role in the development of new graphene-based nanocomposite materials that enable easy detection of pesticides and heavy metals in water, fruits and vegetables. Graphene shows outstanding electrical, optical and thermal properties for the development of nanosensors for the detection of impurities and toxicants in food [16].

Nanotubes, nanowires and quantum dots are now among the smallest man-made units. The pharmaceutical and biomedical industries have tried to synthesize large supramolecular assemblies and artificial devices that mimic the complex mechanisms of nature or that can potentially be used for more efficient disease diagnosis and better drug delivery. For example, nanocapsules such as liposomes contain drugs that can be selectively released in living organs, or bioconjugated assemblies of biomolecules and magnetic (or fluorescent) nanoparticles that enable faster and more selective analysis of biotissues. Recently, silver nanoparticles were synthesized at room temperature using aqueous extracts of orange peel at different concentrations of 2, 4 and 8% w/v and silver nitrate salt at 400 rpm for 6 hours. The results have shown that the formation of silver nanoparticles increases by increasing the concentration of peel extract with potential antibacterial activity [17].

The functionality of nanomaterials for biomedical applications should ensure the binding of biological molecules to the surface of nanostructures. The very size of nanomaterial particles, which is similar to the size of biological molecules and structures, points to the possible application of nanomaterials in "*in vivo*" and "*in vitro*" biomedical research. Some of the current problems of the application of nanomaterials in nanomedicine are related to the understanding of the toxicity of nanomaterials and their impact on the environment. Nanotechnology is particularly important for medicine because all drugs used to treat diseases target proteins or DNA molecules. Understanding what happens at the nanoscale allows smarter drugs to be made. The ability to work at the nanoscale means that molecules can be seen more easily, enabling early diagnosis of disease. Nanoparticles can act as highly sensitive and specific detectors of bacteria and other cell types and can be used for this type of diagnosis [18].

In this work, the focus is on the synthesis of silver nanoparticles using green technology methods as sustainable nanotechnology. Green nanotechnology provides products and processes that are safe, energy efficient, and reduce industrial waste and greenhouse gas emissions. Silver nanoparticles are interesting materials. There are three main approaches to the synthesis of silver nanoparticles (physical, chemical and biological approach to synthesis) [19].

The synthesis of silver nanoparticles is of great interest to the scientific community due to their wide range of applications. Silver nanoparticles have characteristic physical, chemical and biological properties, which are attributed to catalytic activity, antibacterial effects and great application possibilities in nanobiotechnological research. One of the most significant new properties is the increase in surface area in relation to volume. This characteristic leads to an increased dominance of atoms on the surface, as opposed to those in the interior, causing a change in the mechanical, thermal and catalytic properties of the material. Furthermore, nanoparticle synthesis by green methods is safe for the environment and should be explored and encouraged popularly since various plants' have the high extent to form these nanoparticles [20].

2. Green Synthesis of Nanoparticles

Greener synthesis is economical, environmentally friendly, sustainable, simple and relatively reproducible. It was found that plants are a more ecologically acceptable alternative for the biological synthesis of metal nanoparticles than bacteria and fungi. Various natural sources such as plants, fungi, biomass, yeasts, human cells, algae, bacteria, probiotics, enzymes, vitamins and proteins are used for the green biosynthesis of nanoparticles. Plant parts such as leaf, root, flower, seed, and stem are widely used for the synthesis of metal and metal oxide-based nanoparticles. Plant extracts contain compounds such as bioactive polyphenols, proteins, phenolic acids, alkaloids, sugars, terpenoids, etc., which play the role of reductants in the synthesis process of metal nanoparticles. We can also include amino acids, peptides, NADP reductases, membrane proteins in the reducenses. Then, citric acids, flavonoids, quartzetin, saponin, tannic acid, tartaric acid, enzymes, phyllanthin, functional groups, hydrogenases, dehydrogenases, phenols, polyphenols, reducing sugars, etc. [21].

It is assumed that different plants contain different concentrations and conformations of the aforementioned active biomolecules and that their association with metal ions in water is the main factor in the formation of different sizes and shapes of produced nanoparticles. Synthesis of nanoparticles by reduction of metal salts through plant extracts occurs under mild atmospheric conditions. The work process is incredibly simple. At room temperature, plant extract and metal salt solution are mixed well. The reaction takes place within a few minutes. When the precursor solutions are combined, the biochemical reduction of the salt begins immediately, and the production of nanoparticles is usually indicated by a change in the color of the reaction solution.

2.1. Phases of Nanoparticle Biosynthesis

Plant-mediated synthesis of green nanoparticles can be divided into three phases: activation phase, growth phase, and termination phase. The activation phase is the primary phase in which metal ions are recovered from their salt precursors by the action of plant metabolites or biomolecules (which have the ability to biologically reduce metal ions). Metal ions change from their monovalent or divalent oxidation states to zero-valent states, and then nucleation of condensed metal atoms occurs. This is followed by a growth period in which separate metal atoms join together to form metal nanoparticles. Parallel to the growth progress, the nanoparticles aggregate to form different morphologies such as cubes, spheres, triangles, rods, hexagons, pentagons, and wires. The growth phase increases in the improved thermodynamic stability of the nanoparticles, while the extensive nucleation can result in the aggregation of the synthesized nanoparticles, changing their morphology. The last step in the synthesis of green nanoparticles is the termination phase. Nanoparticles finally get their most promising and stable morphology when they are confined by plant metabolites.

2.2. Factors Influencing the Size and Shape of Synthesized Nanoparticles

The main physical and chemical parameters that influence the synthesis of AgNPs are the reaction temperature, the concentration of metal ions, the content of the extract, the pH of the reaction mixture, the duration of the reaction and stirring speed. Parameters such as metal ion concentration, extract composition and reaction time greatly influence the size, shape and morphology of AgNPs. Many authors have reported on the suitability of the base medium for the synthesis of AgNPs due to the better stability of the synthesized nanoparticles in the base medium. Some other advantages under basic pH are mentioned such as fast growth rate, good yield, monodispersity and improved reduction process. Small and uniform size nanoparticles were synthesized by increasing the pH of the reaction mixture.

2.3. Effect of pH

The pH approximation of the reaction mixture has a large effect on the composition of the nanoparticles. The change in pH leads to a change in the charge in the plant's metabolism and even affects its ability to reduce metal ions during the process, which changes the dimensions, morphology and yield of the synthesized nanoparticles. Several plant metabolites differ in their nature and reduce the abilities of each other. This can also affect the synthesis process of nanoparticles; for example, tryptophan and amino acids such as tyrosine, arginine and lysine are the most powerful reducing agents among all phytochemicals and therefore can reduce a large amount of metal ions in a shorter time.

2.4. Effect of Temperature

Temperature significantly affects the properties of nanoparticles during synthesis. High temperature improves certain crystallinity of the synthesized nanoparticles. Metal ions that have a higher electrochemical potential are likely to be further condensed by plant phytochemicals. For example, the silver cation (Ag^+) has a better ionizing potential than the gold cation (Au^+) due to its smaller size and will therefore condense sooner. The biosynthesis method is safe and offers high-yield nano-sized materials that have good crystalline conformation and appropriate properties. However, a high calcination temperature is required to eradicate the precursor to form crystalline materials.

3. Material and Methods

3.1. Green Synthesis of Silver Nanoparticles

Orange and lemon peels were used as plant materials. For the purposes of the experiment, the peel of each plant material was previously dried. 5% w/v of dried peel was extracted on a magnetic stirrer (Velp Scientifica) in 100 ml of distilled water as a solvent. After the extraction, the sample was filtered and thus a plant extract was obtained, which was used for the further needs of the experiment. The rest was stored in the refrigerator at 4˚C until the next experiment. The synthesis of silver nanoparticles was done with different concentrations of silver nitrate (Lach:Ner) with the use of a plant extract as a reducing agent and stabilizing agent. The extraction was performed at 4 different temperatures 25˚C, 35˚C, 45˚C and 65˚C, and after 10 minutes the obtained extract was filtered through filter paper (Whatman No 4) into a beaker which was then covered with aluminum foil. The stirring speeds used were 250 rpm and 500 rpm. For the purposes of adjusting the pH value, a 5% NaOH solution obtained from 99% NaOH (Lach:Ner) was used.

In an earlier study, the antioxidant capacities of orange and lemon peels were determined using the FRAP and ABTS methods. The antioxidant capacity of orange peel was 5.65 ± 0.12 mmol Fe^{2+}/100g DW determined by the FRAP method, or 1.36 ± 0.09 mg TE/100g DW determined by the ABTS method. The antioxidant capacity of lemon peel was 6.31 ± 0.14 mmol Fe^{2+}/100g DW determined by the FRAP method, *i.e.* 1.50 ± 0.06 mg TE/100g DW determined by the ABTS method [22].

Thus, the experiment was conducted at real pH = 4.7 (orange) and 3.8 (lemon), at neutral pH = 7 and at base-adjusted pH = 10. The pH value was checked using a pH meter (Titroline 7000) and test strips (Filtratech). Silver nanoparticles synthesized in this way were characterized on a UV-Vis spectrophotometer (Perkin Elmer Lambda XLS). In order to demonstrate the antibacterial activity of the obtained solutions, the certified reference material *E. coli* was used for the experiment, which was kept at a specially prescribed temperature of −80˚C in a specially designed chamber (Arctiko) that was calibrated by an authorized institution. *E. coli* was seeded on a substrate (Condalab) that favors the growth and development of bacterial colonies of *E. coli* (TBX). Complete preparation was performed in a laminar chamber (Faster) to ensure completely sterile conditions. Petri plates with agar and certified reference material *E. coli* were placed in an incubator (Memmert) at 44˚C (a temperature that favors the development of *E. coli*) for 24 h. Experiment has been done according to the BAS EN ISO 17025 standard. After the development of bacterial colonies on the plates, previously synthesized solutions of silver nanoparticles were applied to check the antibacterial activity. After applying the solution, the plates were returned to the incubator at 44˚C for 24 h, and the growth inhibition or destruction of individual bacterial colonies was monitored the day after.

3.2. Chemical Synthesis of Silver Nanoparticles

The synthesis of silver nanoparticles was carried out with the help of sodium borohydride as a reducing agent. For the purposes of the experiment, a 2 mM solution of $NaBH_4$ was used, which was prepared from distilled water and $NaBH_4$ (Acros Organics).The sodium borohydride solution was measured into a beaker using a burette, and the beaker was placed in a container with ice for 10 minutes. After cooling, a 1 mM $AgNO_3$ solution was added to the $NaBH_4$ solution at room temperature with stirring on a magnetic stirrer. After the addition of 10 ml of $AgNO_3$, the physical properties of the synthesized silver nanoparticles were examined using laser light. Thus, the passage of laser beams through the obtained solution of silver nanoparticles was monitored in relation to distilled water. The solution of silver nanoparticles synthesized in this way was finally divided into two parts, where a saturated solution of NaCl was added to one part, and then the change in the physical properties of the silver nanoparticles was monitored.

4. Results and Discussion

Aqueous extracts from orange and lemon peel were prepared at different tem

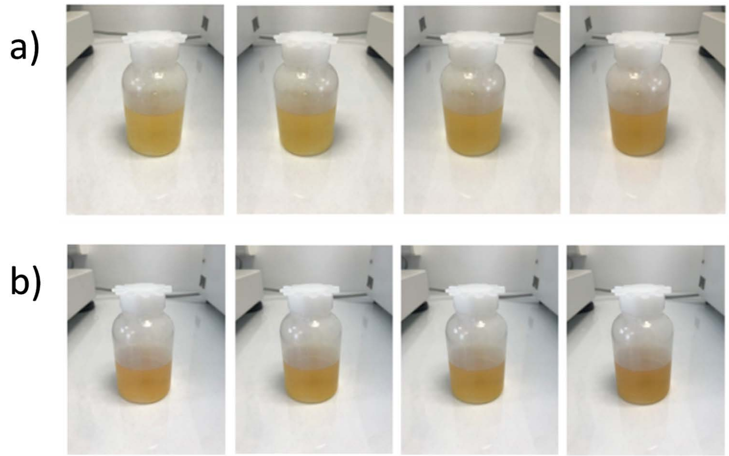


Figure 1. Aqueous peel extracts at different temperatures of a) orange and b) lemon.

peratures: 25˚C, 35˚C, 45˚C and 65˚C (**Figure 1**). In this work, 5% aqueous extracts of orange and lemon peel were prepared at an optimal temperature of 65˚C. The optimal temperature for the synthesis of silver nanoparticles (AgNPs) was 35˚C. The orange peel extract had a pH value of 4.7, while the lemon peel extract had a pH value of 3.8. These are the actual pH values that were further adjusted for the purposes of the experiment using a 5% NaOH solution to pH values of 7 (for the neutral area) and 10 (for the basic area).The optimal time for the synthesis of silver nanoparticles is 60 minutes.

As the temperature increases, the yellow color of the extract becomes darker. For the purposes of the experiment, an extract prepared at the highest temperature was used, because at that temperature the best release of plant material occurs, and such a solution is the most suitable for further use in the process of synthesis of silver nanoparticles. The color of the solution changed from yellow to dark brown after the reduction of Ag^+ by Ag^0 nanoparticles were confirmed that the synthesis of AgNPs. At pH 4, as time passed, the synthesized solutions became darker and darker, which is a consequence of the presence of silver ions in the aqueous solution. In fact, the silver ion (Ag^+) from $AgNO_3$ is reduced in an aqueous solution of citrus extract, accepting an electron from the reducing agent (orange peel extract) to change from a positive valence to a zero valence state (Ag^0), followed by nucleation and growth. This leads to coarse agglomeration into oligomeric clusters to produce silver nitrate particles. The nucleation and growth of silver nanoparticles can be influenced by various reaction parameters, including reaction temperature, pH, concentration, precursor type, reducing and stabilizing agents, and the molar ratio of $AgNO_3$ and orange peel extract. These results are in agreement with previous research [17].

For each type of synthesis, in accordance with different conditions (influence of concentration, pH, mixing speed, and time), UV-Vis spectra were recorded to confirm the formation of AgNPs. All solutions of silver nanoparticles were synthesized at a temperature of 35˚C, because it proved to be the optimal temperature compared to the tested ones (25˚C, 35˚C, 45˚C and 60˚C).

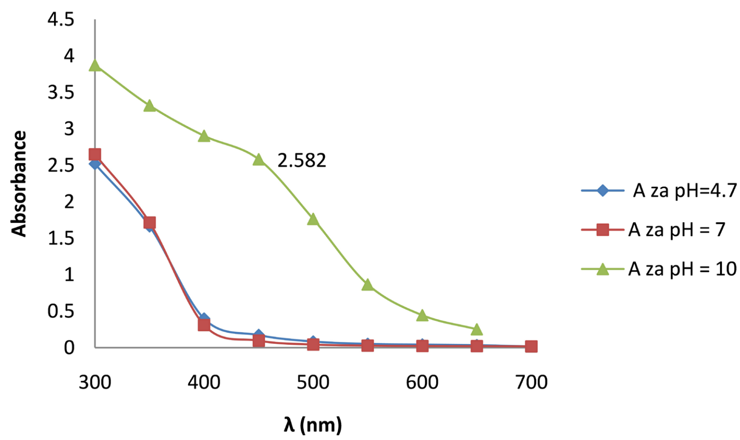


Figure 2. UV-Vis spectrum for different solutions of silver nanoparticles synthesized from orange peel extract as a function of pH value at a concentration of 1mM $AgNO_3$.

Figure 2 shows the UV-Vis spectrum for synthesized AgNPs that showed antibacterial activity against *E. coli*.

The highest absorption maximum with absorption of 2.582 (the surface plasmonic resonance peak) is recorded at 450 nm which indicate successful formation of AgNPs prepared from 1 mM $AgNO_3$. The findings are in agreement with the formation of AgNPs from the aqueous extract of *Mikania cordata* leaves extract [3].

Figure 3 represents the conductivity of solutions of AgNPs prepared from orange peel extract and 1 mM $AgNO_3$ as a function of pH. It can be seen that the conductivity is highest at pH = 10.

In the **Figure 4** it can be seen that the maximum of absorbance peek was recorded around 400 nm and is shifted in the blue region. The absorption value at 450 nm is 0.752, while 1.542 is recorded around 400 nm. In the research carried out by Ishnava [23] has been reported that the maximum of absorption was at 423 nm which indicated the formation of AgNPs synthesized by leaf powder plant materials of *E. axillare.*

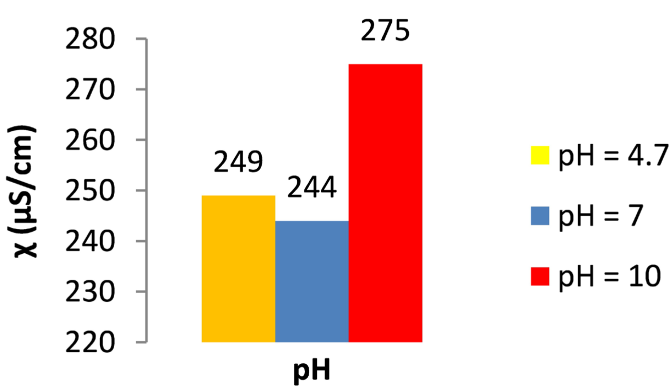


Figure 3. Conductivity of solutions of silver nanoparticles synthesized from orange peel extract and 1 mM $AgNO_3$ as a function of pH.

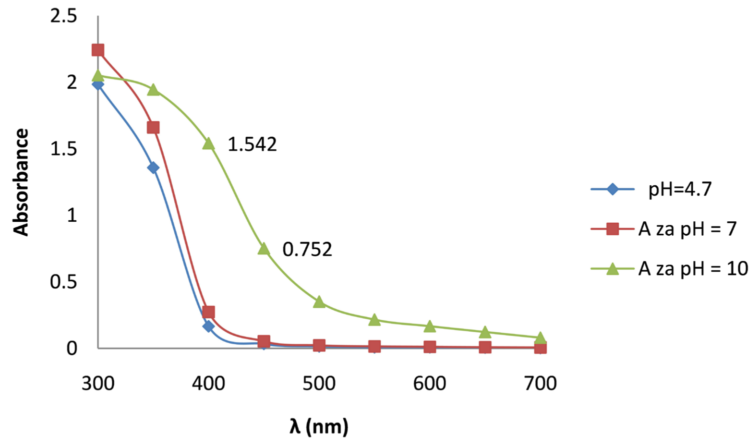


Figure 4. UV-Vis spectrum for different solutions of silver nanoparticles synthesized from orange peel extract as a function of pH value at a concentration of 0.1 mM $AgNO_3$.

In a study conducted by Krishnamoorthy *et al.* [8] it was reported that for different concentrations of $AgNO_3$, the absorption maximum was recorded at 421 nm for 20 mM, and 424 nm for 40 mM $AgNO_3$.

On the other hand, another group of researchers synthesized (AgNPs) by green method using extracts of onion (O), tomato (T), acacia catechu (C) alone, and mixed COT extracts. The synthesized nanoparticles were of the order of 5 - 100 nm crystal structure, and were used as photocatalysts for the degradation of three types of dyes, namely methyl orange (MO), methyl red (MR), and congo red (CR) in the liquid state. COT proved to be the most effective photocatalyst. UV-Vis spectroscopy confirmed the formation of AgNPs with different associated wavelength values for the different extracts used, namely: COT 430 nm; Onion 432 nm; Tomato 450 nm and Acacia Catechu 440 nm [24].

In **Figure 5**, the highest absorption is 2.573 for a solution of silver nanoparticles prepared from 0.5 mM $AgNO_3$ at 400 nm. A peak at 400 nm was

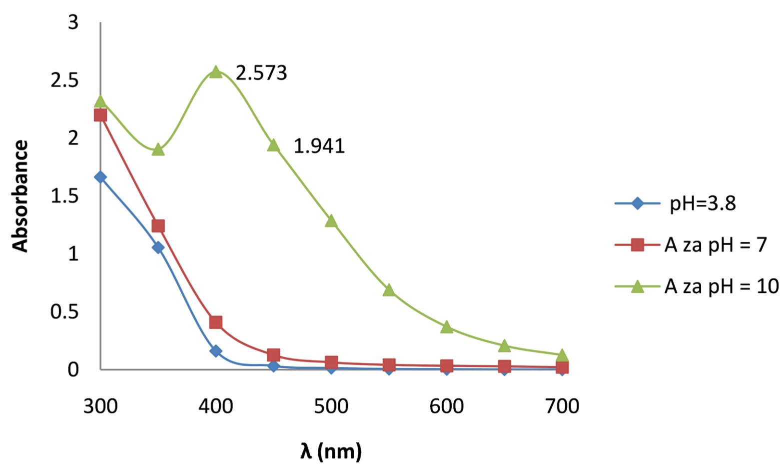


Figure 5. UV-Vis spectrum for different solutions of silver nanoparticles synthesized from lemon peel extract as a function of pH value at a concentration of 0.5 mM $AgNO_3$.

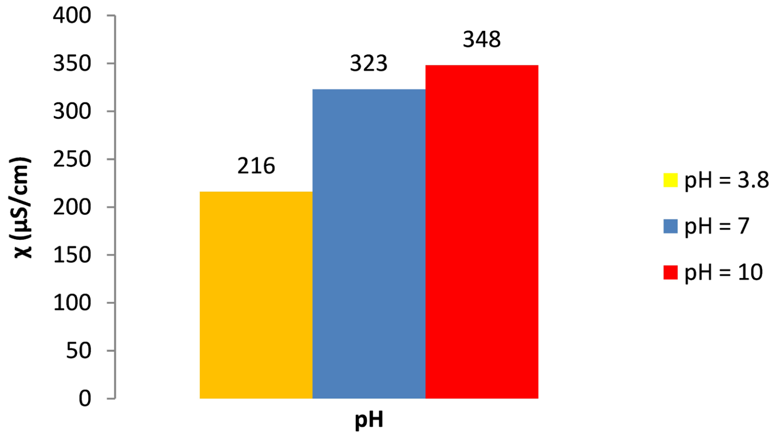


Figure 6. Conductivity vs pH solution of AgNPs synthesized from lemon peel extract and 0.5 mM $AgNO_3$.

observed. The diameter of the peak at a height of about 2 absorption intensity is 2 cm, and at a height of 1.3 cm.

In the research carried out by Melkamu and Bitew [25], revealed that a distinctive SPR peak and maximum absorbance was observed at a wavelength of 406 nm for a concentration of 4 mM $AgNO_3$ solution. The height of the absorption peak of UV-Vis spectrum around 406 nm intensifies as the concentration of silver nitrate increased.

In **Figure 6** in presented conductivity as a function of pH solution of silver nanoparticles synthesized from lemon peel extract and 0.5 mM $AgNO_3$. It can be seen that the conductivity increased linearly with the increase in the pH value.

4.1. Effect of Concentration

When investigating the influence of $AgNO_3$ solution concentration on the formation of silver nanoparticles, three different concentrations were studied: 1mM, 0.1 mM and 0.5 mM with two different extracts (orange peel and lemon peel) each individually, at their actual pH values with a mixing speed of 250 rpm and a reaction time of 60 min. Experimental results showed that silver nanoparticles synthesized with 1 mM $AgNO_3$ and orange peel extract show the highest absorption intensity at 450 nm, which is evidence of the formation of silver nanoparticles. The density of the solution of synthesized silver nanoparticles with different concentrations of $AgNO_3$ did not change in any experiment and was 0.994 g/ml. An increase in the conductivity of each solution of silver nanoparticles with an increase in the concentration of $AgNO_3$ used in the synthesis was recorded. When the experiment was done with lemon peel extract, at pH = 3.8, there is no absorption at 450 nm, but absorption starts already at 400 nm for a solution with 0.1 mM $AgNO_3$.

4.2. Effect of pH

Experimental results showed that pH significantly affects the formation of

AgNPs and that the best area for synthesis is a basic environment (pH = 10). In the AgNP synthesis reaction with 1 mM concentration of silver nitrate and orange peel extract, a broad absorption region containing an absorption maximum at 450 nm and a broad absorption band in the visible part of the spectrum up to 650 nm was obtained, which clearly confirms the formation of AgNPs. The intensity of the absorption maximum is 2.582 at 450 nm. The conductivity of these AgNPs is 275 μS/cm. In order to prove this effect of pH on the formation of AgNPs, experiments were also performed with concentrations of 0.5 mM and 0.1 mM $AgNO_3$. The results obtained with these two concentrations confirm the previous results, with the only difference in the observed blue shift, *i.e.* the shift of the absorption maximum towards the UV part of the spectrum, which indicates a decrease in the size of the nanoparticles. Similar results to the previous ones were obtained with the use of lemon peel extract. In the first part of that experiment, 1 mM $AgNO_3$ and an intense absorption maximum at 410 nm with a wide absorption region in the visible part of the electromagnetic spectrum up to 700 nm at pH = 10 was obtained. A very high conductivity of these AgNPs with a value of 493 μS/cm was recorded. In the second part of the experiment, 0.5 mM $AgNO_3$ and the absorption maximum was recorded at 400 nm with an intensity of 2.573, while at 450 it is 1.941 and covers a wide part of the visible region of the electromagnetic spectrum up to 700 nm. It is interesting that the diameter of the peak at absorption intensity 2 is 2 cm, while at absorption intensity 1, it is 3 cm. This indicates a change in AgNPs particle size with change in concentration of $AgNO_3$. In the third part of the experiment, AgNPs were synthesized from 0.1 mM $AgNO_3$ and show a similar trend as the previous two experiments. Again, it is interesting that at an absorption intensity of 1.5, the diameter of the absorption maximum is 1.5 cm, as in the case of nanoparticles synthesized with 1 mM $AgNO_3$, with a difference in absorption intensity which is in accordance with the changed concentration of $AgNO_3$.

4.3. Effect of Stirring Speed

When investigating the influence of stirring speed on the formation of AgNPs, a concentration of 0.5 mM $AgNO_3$ was chosen for the synthesis of silver nanoparticles and studied at a stirring speed of 250 and 500 rpm. The obtained experimental results showed that there is no significant difference, but that there is a slight preference at 250 rpm. In this part of the research, a difference in the appearance of absorption spectra is observed when orange peel extract is used compared to lemon. Namely, it was shown that the synthesis of AgNPs with lemon peel extract gives a clear peak with absorption maximum at 400 nm and which extends up to 700 nm. It can be concluded that these silver nanoparticles synthesized with lemon peel extract are uniformly distributed and have a unique, probably spherical shape, while those synthesized with orange peel extract are non-uniform or even form aggregates.

4.4. The Influence of Time

The results of the research showed that the reaction time of the formation of nanoparticles significantly affects the number of silver nanoparticles formed, *i.e.* with an increase in the duration of the synthesis reaction; there is an increase in the formed AgNPs particles, which confirms the increase in the absorption maximum at 450 nm.

4.5. Study of the Passage of Laser Light Through Solutions

To confirm with another method that silver nanoparticles were really formed, laser beams were passed through synthesized AgNPs solutions and through distilled water as a comparative sample. Experimental results showed that the laser beam does not pass through distilled water (it is dispersed), while the laser beam passes through a solution containing silver nanoparticles. Silver nanoparticles absorb part of the radiation of laser beams and because of this; the intensity of the laser beam is weakened when it passes through the solution. The formation of silver nanoparticles is detected by the change in the color of the solution to yellowish. A glass containing a synthesized solution of silver nanoparticles with $NaBH_4$ was placed opposite a glass containing distilled water, and a comparison was made of the passage of laser light through the solutions. The formation of a colloidal suspension (nanoparticles) can be detected directly by the reflection of a laser beam from the nanoparticles and indirectly, by changing the color of the solution from colorless to yellow (**Figure 7**).

4.6. Refractometric Tests

For the solutions that showed the formation of silver nanoparticles (basic pH), the 1 mM $AgNO_3$ + 2 mM $NaBH_4$ solution, as well as for the pure extracts of orange and lemon, and distilled water. The refractive index was measured using the Abbe's refractometer. The results showed that there is no significant difference in the reflection of light rays in the solution of synthesized silver nanoparticles. The refractive index for lemon extract was 1.33514, while for the orange extract was 1.33507 and for the AgNPs for different concentrations were in range 1.333501.

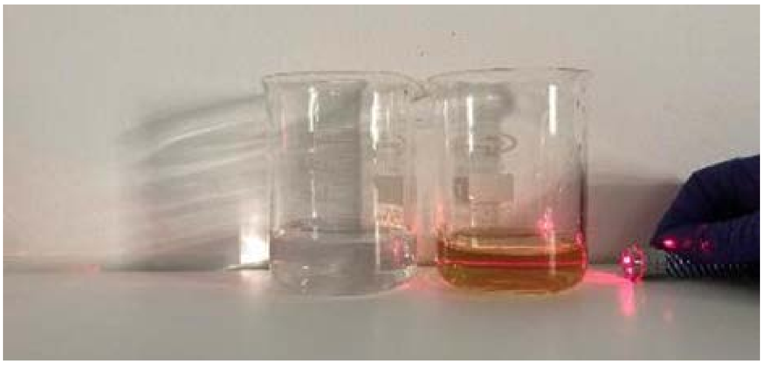
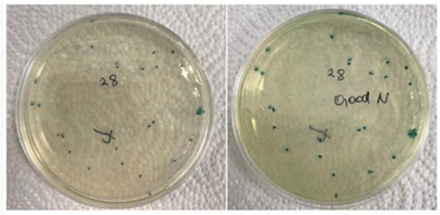
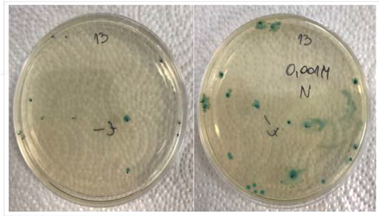


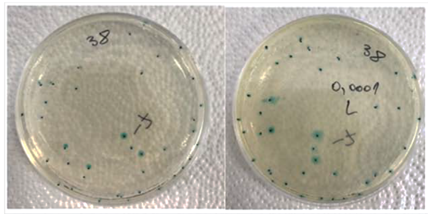
Figure 7. Comparison of the passage of laser light in relation to distilled water (left) and a solution of silver nanoparticles (right).



Bedore and after application of AgNps solution prepared from C($AgNO_3$) =0.1 mM and orange peel extract, pH = 10. Synthesis time 15 min.



Bedore and after application of AgNps solution prepared from C($AgNO_3$) = 0.5 mM and lemon peel extract, pH = 10. Synthesis time 15 min.



Bedore and after application of AgNps solution prepared from C($AgNO_3$) = 0.1 mM and lemon peel extract, pH = 10. Synthesis time 15 min.

Figure 8. Antibacterial activity of AgNPs against *E. coli.*

4.7. Antibacterial Activity of AgNPs against *Escherichia coli*

This type of synthesis shows good results when it comes to inhibiting the further development of *Escherichia coli* (*E. coli)* colonies, which is evident from certain solutions that show a significant inhibitory effect. Silver nanoparticles solutions that show a positive inhibitory effect on the growth of new *E. coli* colonies were synthesized at a basic pH value and at a silver nitrate concentration of 0.1 mM using orange or lemon peel extract, while for a silver nitrate concentration of 0.5 mM using lemon peel extract (**Figure 8**).

5. Conclusion

This paper presents the green synthesis of silver nanoparticles with the help of orange peel as an environmentally friendly way of synthesizing nanoparticles. In addition to having an ecological aspect, this synthesis also has an economic aspect. This synthesis shows the sustainable development of organic waste recycling. UV-Vis spectra showed the formation of silver nanoparticles with an absorption maximum at 450 nm. The experimental results showed a very promising green way of synthesizing silver nanoparticles with minimal impact on the environment. Inhibitory action on the growth of new *E. coli* colonies was shown by silver nanoparticles synthesized at a base medium of 0.1 mM using orange or lemon peel extract, and at 0.5 mM using lemon peel extract.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Gupta, J. (2011) Nanotechnology Applications in Medicine and Dentistry. *Journal of Investigative and Clinical Dentistry*, **2**, 81-88. https://doi.org/10.1111/j.2041-1626.2011.00046.x

[2] Craig, M., Jenner, A.L., Namgung, B., Lee, L.P. and Goldman, A. (2021) Engineering in Medicine to Address the Challenge of Cancer Drug Resistance: From Micro- and Nanotechnologies to Computational and Mathematical Modeling. *Chemical Reviews*, **121**, 3352-3389. https://doi.org/10.1021/acs.chemrev.0c00356

[3] Khatun, M., Khatun, Z., Karim, R., Habib, R., Rahman, H. and Aziz, A. (2023) Green Synthesis of Silver Nanoparticles Using Extracts of *Mikania cordata* Leaves and Evaluation of Their Antioxidant, Antimicrobial and Cytotoxic Properties. *Food Chemistry Advances*, **3**, Article 100386. https://doi.org/10.1016/j.focha.2023.100386

[4] Bratovcic, A. (2023) Green Synthesis of Various Nanostructures Containing Essential Oil and Silver Nanoparticles: Nanocomposites, Nanoemulsions and Nanoencapsules. *International Journal of Engineering Research and Applications*, **13**, 234-242.

[5] Hussain, I., Singh, N.B., Singh, A., Singh, H. and Singh, S.C. (2016) Green Synthesis of Nanoparticles and Its Potential Application. *Biotechnology Letters*, **38**, 545-560. https://doi.org/10.1007/s10529-015-2026-7

[6] Rafique, M., Sadaf, I., Rafique, M.S. and Tahir, M.B. (2017) A Review on Green Synthesis of Silver Nanoparticles and Their Applications. *Artificial Cells, Nanomedicine, and Biotechnology*, **45**, 1272-1291. https://doi.org/10.1080/21691401.2016.1241792

[7] Simon, S., Sibuyi, N.R.S., Fadaka, A.O., Meyer, S., Josephs, J., Onani, M.O., Meyer, M. and Madiehe, A.M. (2022) Biomedical Applications of Plant Extract-Synthesized Silver Nanoparticles. *Biomedicines*, **10**, Article 2792. https://doi.org/10.3390/biomedicines10112792

[8] Krishnamoorthy, K., Jayaraman, S., Krishnamoorthy, R., Manoharadas, S., Alshuniaber, M.A., Vikas, B. and Veeraraghavan, V.P. (2023) Green Synthesis and Evaluation of Anti-Microbial, Antioxidant, Anti-Inflammatory, and Anti-Diabetic Activities of Silver Nanoparticles from *Argyreia nervosa* Leaf Extract: An *invitro* Study. *Journal of King Saud University-Science*, **35**, Article 102955. https://doi.org/10.1016/j.jksus.2023.102955

[9] Magdum, A.B., Waghmode, R.S., Shinde, K.V., Mane, M.P., Kamble, M.V., Kamble, R.S., Jangam, A.P., Pawar, K.D., Sonawane, K.D., Patil, P.S. and Nimbalkar, M.S. (2024) Biogenic Synthesis of Silver Nanoparticles from Leaves Extract of *Decaschistia trilobata* an Endemic Shrub and Its Application as Antioxidant, Antibacterial, Anti-Inflammatory and Dye Reduction. *Catalysis Communications*, Article 106865. https://doi.org/10.1016/j.catcom.2024.106865

[10] Rama, P., Mariselvi, P., Sundaram, R. and Muthu, K. (2023) Eco-Friendly Green Synthesis of Silver Nanoparticles from Aegle Marmelos Leaf Extract and Their Antimicrobial, Antioxidant, Anticancer and Photocatalytic Degradation Activity. *Heliyon*, **9**, E16277. https://doi.org/10.1016/j.heliyon.2023.e16277

[11] Abdulazeem, L., Alasmari, A.F., Alharbi, M., Alshammari, A. and Muhseen, Z.T. (2023) Utilization of Aqueous Broccoli Florets Extract for Green Synthesis and Characterization of Silver Nanoparticles, with Potential Biological Applications, *Heliyon*, **9**, E19723. https://doi.org/10.1016/j.heliyon.2023.e19723

[12] Shereen, M.A., Ahmad, A., Khan, H., Satti, S.M., Kazmi, A., Bashir, N., Shehroz, M., Hussain, S., Ilyas, M., Khan, M., Ilyas Niyazi, H.A. and Zouidi, F. (2024) Plant Extract Preparation and Green Synthesis of Silver Nanoparticles Using *Swertia chirata*: Characterization and Antimicrobial Activity against Selected Human Pathogens. *Heliyon*, **10**, E28038. https://doi.org/10.1016/j.heliyon.2024.e28038

[13] Tesfaye, M., Gonfa, Y., Tadesse, G., Temesgen, T. and Periyasamy, S. (2023) Green Synthesis of Silver Nanoparticles Using *Vernonia amygdalina* Plant Extract and Its Antimicrobial Activities. *Heliyon*, **9**, E17356. https://doi.org/10.1016/j.heliyon.2023.e17356

[14] Kalatar-Zadeh, K. and Fry, B. (2008) Nanotechnology-Enabled Sensors. Springer, Boston, MA.

[15] Dhaka, A., Mali, S.C., Sharma, S. and Trivedi, R. (2023) A Review on Biological Synthesis of Silver Nanoparticles and Their Potential Applications. *Results in Chemistry*, **6**, Article 101108. https://doi.org/10.1016/j.rechem.2023.101108

[16] Bratovcic, A. (2024) Role of Nanocomposites Using Graphene-Based Materials for Food/Toxin-Sensing Applications in Agriculture. In: Mohanta, Y.K., Biswas, K., Mahanta, S. and Muthupandian, S., Eds., *Graphene-Based Nanomaterials*: *Application in Food*, *Agriculture and Healthcare*, CRC Press, Boca Raton, 11. https://doi.org/10.1201/9781003300540-10

[17] Bratovcic, A. (2020) Biosynthesis of Green Silver Nanoparticles and Its UV-Vis Characterization. *International Journal of Innovative Science*, *Engineering & Technology*, **7**, 170-176.

[18] Bratovcic, A. (2022) Application of Natural Biopolymers and Its Derivatives as Nano-Drug Delivery Systems in Cancer Treatment. *World Journal of Pharmaceutical Sciences*, **10**, 232-242. https://doi.org/10.54037/WJPS.2022.100209

[19] Kus, M., Yilmazl Alic, T., Kirbiyik, C., Baslak, C., Kara, K. and Kara, D.A. (2018) Chapter 24—Synthesis of Nanoparticles. In: Hussain, C.M., Ed., *Handbook of Nanomaterials for Industrial Applications*, Elsevier, Amsterdam, 392-429. https://doi.org/10.1016/B978-0-12-813351-4.00025-0

[20] Abada, E., Mashraqi, A., Modafer, Y., Al Abboud, M.A. and El-Shabasy, A. (2024) Review Green Synthesis of Silver Nanoparticles by Using Plant Extracts and Their Antimicrobial Activity. *Saudi Journal of Biological Sciences*, **31**, Article 103877. https://doi.org/10.1016/j.sjbs.2023.103877

[21] Thota, S. and Crans, D.C. (2018) Metal Nanoparticles: Synthesis and Applications in Pharmaceutical Sciences, Wiley-VCH, Weinheim, 15-49. https://doi.org/10.1002/9783527807093

[22] Bratovcic, A., Djapo-Lavic, M., Kazazic, M. and Mehic, E. (2021) Evaluation of Antioxidant Capacities of Orange, Lemon, Apple and Banana Peel Extracts by Frap and Abts Methods. *Revue Roumaine de Chimie*, **66**, 713-717.

[23] Ishnava, K.B. (2024) Biogenic Synthesis of Silver Nanoparticles from the Leaf Extract of *Enicostema axillare* (Poir. ex Lam.)A. Raynal and Its Alpha Amylase Inhibition Activity. *Food Chemistry Advances*, Article 100624. https://doi.org/10.1016/j.focha.2024.100624

[24] Chand, K., Cao, D., Fouad, D.E., Hussain Shah, A., Dayo, A.Q., Zhu, K., Lakhan, M.N., Mehdi, G. and Dong, S. (2020) Green Synthesis, Characterization and Photo-

catalytic Application of Silver Nanoparticles Synthesized by Various Plant Extracts. *Arabian Journal of Chemistry*, **13**, 8248-8261. https://doi.org/10.1016/j.arabjc.2020.01.009

[25] Melkamu, W.W. and Bitew, L.T. (2021) Green Synthesis of Silver Nanoparticles Using *Hagenia abyssinica* (*Bruce*) *J.F. Gmel* Plant Leaf Extract and Their Antibacterial and Anti-Oxidant Activities. *Heliyon*, **7**, E08459. https://doi.org/10.1016/j.heliyon.2021.e08459