Synthesis of Silver Nanoparticles in Walnut Waste Extract by Pulsed Plasma Method

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Synthesis of Silver Nanoparticles in Walnut Waste Extract by Pulsed Plasma Method

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Abstract: *Silver has better antibacterial properties in nanoparticle form. Phenolic and flavonoid compounds from plants and crops are the largest group of phytochemicals that account for most of the antimicrobial activities. In this study, silver nanoparticles (AgNP) were synthesized in walnut extract by the pulsed plasma method, in order to enhance the antibacterial properties and to reduce the total number of processes in the synthesis of nanoparticles and extraction of phenolic and flavonoid compounds from walnut waste. The antibacterial properties of the synthesized nanoparticle were analyzed by the standard Kirby-Bauer method with Escherichia coli (gram-negative), Staphylococcus aureus (gram-positive) and Shigella (gram-negative) bacteria. The phenolic compound concentration in the sample was found to be 0.886 mg/g by using the total phenol calibration curve. The amount of flavonoids was found to be 0.362 mg/g. The antibacterial activities were analyzed for mixture of AgNPs and walnut extract, AgNPs synthesized in walnut extract and the extract itself.*
Keywords: walnut waste; pulsed plasma; nanoparticle synthesis; antibacterial activity

1. INTRODUCTION

As a result of the emergence of infectious diseases caused by various pathogenic bacteria and the development of antibiotic resistance caused by the use of broad-spectrum antibiotics [1-2], pharmaceutical companies [3] and researchers have been researching new antibacterial agents in recent years [4-15]. Nanomaterials have become novel antimicrobial agents with their unique chemical and physical properties and surface area-to-volume ratio. Nanotechnology is used in science and technology to produce new materials at the nanoscale and is becoming a rapidly developing field [16]. Due to the development of species resistant to metal ions and antibiotics, antibiotics that have a strong effect against them and eliminate their resistance have attracted the interest of researchers [17]. Many different metal nanomaterials have been synthesized in the literature. Among them, copper and silver stand out because they have good antimicrobial activity against bacteria, viruses, and other eukaryotic microorganisms [17-18].

The large area of fruit and nut forests in the Arslanbab River Valley is the largest in the world and covers an area of more than 77,500 hectares. Up to 20,000 tons of walnuts are collected in Arslanbab every year. Arslanbab nut forest is a national reserve. One of the characteristics of walnuts in Arslanbab walnut forest is that they are not cultivated, but grow and reproduce on their own. As the average mass of the nut and its essence is 8.8 g, 4.3 g, 50 % of the mass of the nut consists of the shell, and without processing it, 10,000 tons of nut shells are thrown into the environment as waste every year. Converting these wastes into useful wastes without dumping them into nature requires methods that prevent environmental pollution [19-21]. Prevents environmental pollution by synthesizing nanoparticles with antibacterial properties from walnut shell extract and converting the waste walnut shell into a useful product [22].

The literature on the synthesis of nanoparticles with walnut waste extract, which forms the basis of the study, is limited. However, several studies have indicated that walnut waste extract contains various biological and chemical components and can be used as an environmentally friendly solvent due to these properties [23-26].

By synthesizing nanoparticles using the pulsed plasma method, using the extract of walnut waste as a liquid medium in the synthesis process, it aims to increase the efficiency of the method and the antimicrobial properties of nanoparticles by performing two processes at the same time [27-34].

2. EXPERIMENTAL PART

2.1. Walnut sample collection

Sample material was collected from walnut trees that grow in the territory of the Kyrgyz-Turkish Manas University Jal campus. After collection, the sample was cleaned from dirt and other impurities with clean running water. After drying, the sample was crushed to obtain smaller fractions. As a result, 90 grams of sample was taken. The goal is to increase the contact area and improve the extraction process. We used a regular portable grain grinder for grinding. The alcoholic extract was filtered through Watman #1 filter paper.

2.2. Determination of total phenolic content

Determination of total phenolic content (TPC) was carried out by the Conde-Hernández method with some modifications. Briefly, 0.4 mL of the extracted solution was mixed with 2 mL of ultrapure water and 0.4 mL of Folin-Chocalto reagent, then incubated at room temperature (25 °C) for 5 min and mixed with 2.5 mL of 20% Na_2CO_3 solution, kept at room temperature for 2 hours. The sample was ready for qualitative analysis in a UV-vis spectrophotometer. GA was used as the reference standard and results are expressed as "mg" of gallic acid equivalents (GAE) per "gram" of sample.

2.3. Preparation of gallic acid calibration standards

Dissolve 0.5 g of gallic acid in 10 mL of ethanol, then make up to 100 mL with water (5 g/L or 5000 mg/L final concentration). Dilute 1, 2, 5, and 10 ml to 100 ml with water to prepare standards with concentrations of

50, 100, 250, and 500 mg/L, respectively. It can be stored for up to 2 weeks at 4°C.
Standards retain 98% of their potency for 2 weeks when kept sealed in a refrigerator (4°C), but this potency only lasts for 5 days at room temperature. Commercial gallic acid is usually pure, but can be recrystallized from water if necessary.
For dilution, the formula: $C_1\ V_1 = C_2\ V_2$ is used.

1. C_a=50mg/L
5000mg/L*V_1 =50mg/L*100 ml V_1 =1 ml
To prepare a solution with a concentration of C_a=50mg/L, take 1 ml of a solution with a concentration of 5000 mg/L and add it to 100 ml with water.

2. C_a=100mg/L
5000mg/L*V_1=100mg/L*100ml V_1 =2ml
To prepare a solution with a concentration of C_a=100 mg/L, take 2 ml of a solution with a concentration of 5000 mg/L and add water to 100 ml.

3. C_a =250mg/L
5000mg/L* V_1=250mg/L*100ml V_1=5ml
To prepare a solution with a concentration of Ca=250 mg/L, take 5 ml of a solution with a concentration of 5000 mg/L and add water to 100 ml.

4. C_a =500mg/L
5000mg/L* V_1=500mg/L*100ml V_1=10ml
To prepare a solution with a concentration of Ca=500 mg/L, take 10 ml of a solution with a concentration of 5000 mg/L and add water to 100 ml.

2.4. Total flavonoid content

Total flavonoid content (TFC) Feng et al. determined with some changes through the method. Briefly, 0.5 ml of alcoholic extract was taken and 2 ml of ultrapure water and 1 ml of $NaNO_2$ solution (5%) were added to it. After the sample stood for 6 minutes, it was mixed with 1 ml (10%) $Al(NO_3)_3$ solution, to which 3 ml NaOH solution (4%) was added after 6 minutes. When all reagents were added, the solution was incubated at room temperature (25 °C) for 15 min. Flavonoids (TFC) were analyzed using a spectrophotometer in the absorption spectrum at 510 nm. Rutin was used as reference standard and results are expressed as mg of rutin equivalents/gram of sample (RE). The standard experimental curve is:

$$y = 0.0945 * X + 0.0276\ \ (R^2 = 0.9986)$$

2.5. Preparation of rutin calibration standards

For calibration, 1.0 g of rutin was taken. Put it in a 1-liter flask and add 1 liter of water. Then a solution with a concentration of 1000 µg/ml was prepared. Five 50 ml flasks were taken for the calibration schedule. They were injected with 2.5, 5, 10, 15, 20 ml of 1000 µg/ml rutin solution. They were filled with water up to 50 ml. Then rutin solutions with concentrations of 0.05, 0.1, 0.2, 0.3, 0.4 mg/ml were prepared. 0.5 ml of those solutions was taken and poured into 15 ml test tubes. Then 2 ml of water, 1 ml of 5% NaNO2 were added and kept for 6 minutes. Then, 1 ml of 10% $AlCl_3$ was added and left for 6 minutes. Then, 3 mL of 1M NaOH was added and left to stir for 15 minutes. It was taken at a wavelength of 7510 nm with the help of spectrophotometry.

2.5. Synthesis of silver nanoparticles from walnut peel extract

Synthesis was carried out using pulsed plasma equipment. 99.9 % silver electrodes with a total mass of 3 g in the form of rods were submerged in the previously obtained walnut peel extract. The equipment should be prepared, the electrodes should be fixed in the form of V and they should be inserted into the extract. Then the pulsed plasma was applied for an hour.

2.6. Determination of antibacterial properties of the extract containing silver nanoparticle

Antibacterial activity of nanoparticles in the sample was studied against Escherichia coli (gram-negative), Staphylococcus aureus (gram-positive) and Shigella flexneri (gram-negative) bacteria by well diffusion method. Using a sterile metal rod, Plate Count agar and bacterial suspensions on top of it are evenly placed in Petri dishes. 0,1 ml of sample is poured into each well on Petri dish. After 24 hours of incubation at 37°C, the susceptibility of bacteria was determined by measuring the diameter of inhibition zones.

3. RESULTS OBTAINED AND THEIR DISCUSSION

Results of determination of phenols and flavonoids. First, we determined the content of phenols and flavonoids in the walnut peel using a UV-Vis spectrophotometer that is shown in Fig.1.

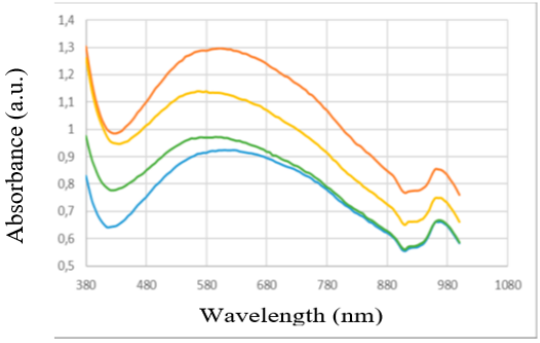

Fig. 1. Absorption spectra of total phenolic compounds in the sample.

According to the obtained data, the presence of flavonoids was confirmed. A calibration graph using "Gallic" acid was established as shown in the Fig. 2

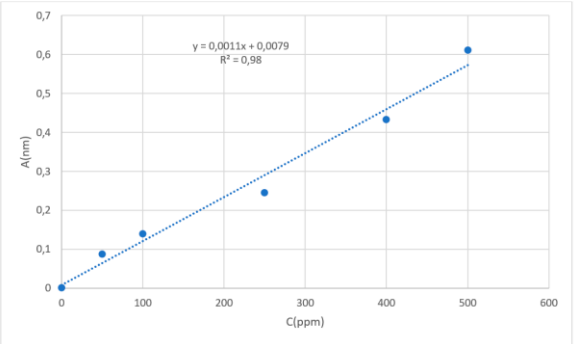

Fig. 2. Gallic acid calibration graph

As a result of the calibration, the following equation was obtained:

$$C_{GA} = \frac{absorbance - 0.0523}{4.15}$$

To find the concentration (mg/g) of the sample from the concentration of gallic acid in the diluted extract, the following equation was created, taking into account that 10 times dilution, 10 g of sample was taken, and the volume of the extract was 100 ml:

$$C_{sample} = \frac{CC_{GA} \cdot 10 \cdot 100}{10}$$

Using this equation, the concentration of phenol in the sample was found to be as in the Table 1.

Table 1. Concentration of total phenolic compounds

Sample	Optical density (nm)	Concentration (pmm)	Concentration (mg/g)
Phenols	0,971	875,5455	0,886

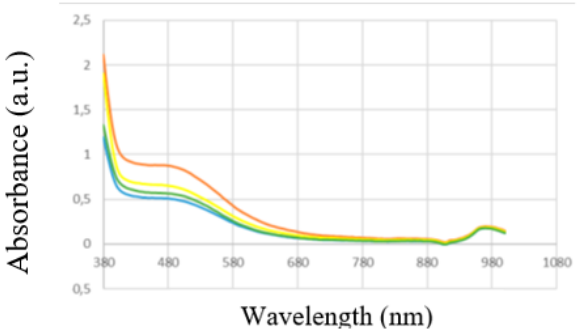

Fig. 3. Absorption spectra of total flavonoid compounds in the sample

According to the received data, the existence of flavonoid compounds was also confirmed. A routine calibration graph has been set up.

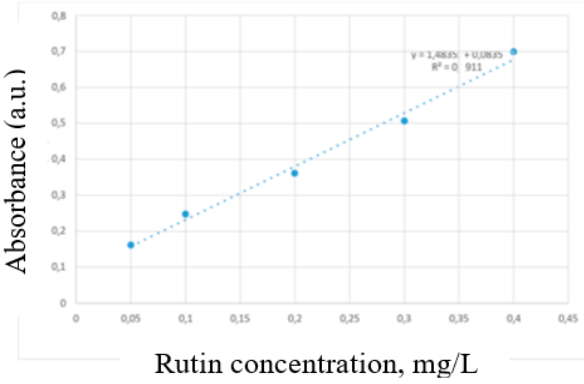

Fig. 4. Rutin calibration graph

As a result of the calibration, the following equation was created:

$$C_{rutin} = \frac{absorbance - 0.0835}{1.4835}$$

Using this equation, the retention of flavonoids in the sample was found as shown in the Table 2.

Table 4. Concentration of total flavonoid compounds

Sample	Optical density (nm)	Concentration (pmm)	Concentration (mg/g)
Flavonoids	0,971	875,5455	0,886

After the nanoparticles were synthesized, we again checked the content of phenols and flavonoids in them using the method described above.

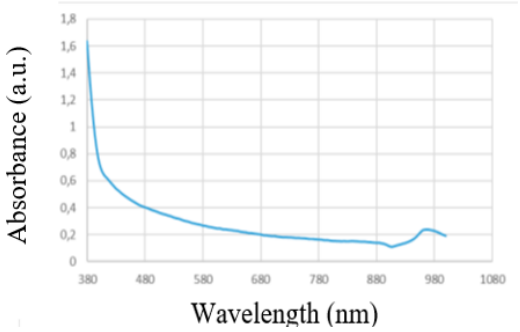

Fig. 5. TFC content after synthesis of AgNPs

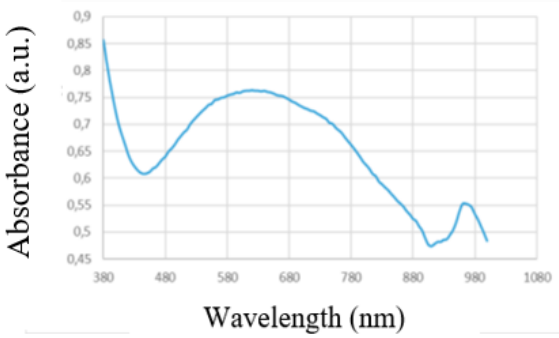

Fig. 6. TPC content after synthesis of AgNP

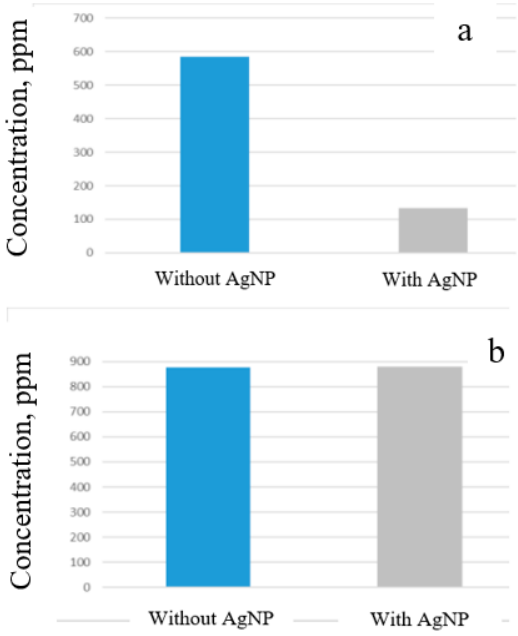

Fig 7. Changes in (a) TFC and (b) TPC content after synthesis of AgNPs

According to the graphs, the content of total phenols after synthesis of AgNPs did not change much compared to before synthesis. We also obtained the absorption spectrum. And the content of total flavonoids

changed relatively, other peaks and absorption spectra were obtained. As mentioned in the literature, silver ions are recovered by flavonoids and precipitate as nanoparticles. But in fact, we cannot say whether flavonoids are reduced or not, because the absorption spectrum of TFC is around 480-510 nm, while the absorption spectrum of AgNPs is 400-480 nm. They may overlap with each other, according to the literature already mentioned.

Results of antibacterial properties of extract with silver nanoparticle.

In the study of antibacterial properties, we used the following samples:

- No. 1. Extract + AgNPs (simple mixing);
- No. 2. AgNPs synthesized in extract by the pulsed plasma method;
- No. 3. Pure extract;

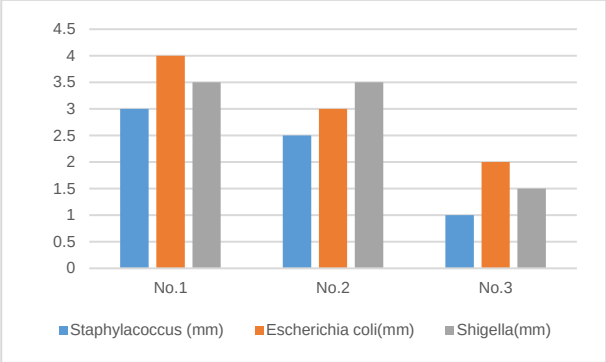

Fig. 8. Antibacterial properties of the samples

The best results were obtained by the mixture of the extract and AgNPs. Addition of the extract and the AgNPs contributed to the antibacterial properties. Antibacterial activity was lower in sample #3. On the other hand, the sample obtained by the pulsed plasma in walnut extract showed relatively lower antibacterial properties, which we attribute to high plasma temperature. During pulsed plasma synthesis, high temperature plasma lead to partial decomposition of substances that show antibacterial properties contained in extract solution. The results of the sample No. 3 indicate that the antibacterial activity of the extract and the AgNPs improved each others activities.

4. CONCLUSION

The composition of total phenols and flavonoids was studied during the work. According to the quantitative analysis, the following values were obtained: the average concentration of total phenols was 876 ppm (0.886 mg/g), and the average concentration of flavonoids was 362 ppm (0.362 mg/g).

The synthesis of silver nanoparticles by the pulsed plasma inside the walnut extract can be utilized for combination of extraction process of the walnut peel and the synthesis of AgNPs at the same time. By this way, we can simultaneously synthesize nanoparticles and perform extraction of walnut peel components, which provide us green route for recycling the walnut waste materials and enhancing antibacterial activities of AgNPs by addition of phenolic and flavonoid compounds extracted from walnut peel. In the future, systematic research will be conducted using the shell and leaf membrane inside the walnut.

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