PLASMA-CHEMICAL PREPARED AQUEOUS GRAPE POMACE EXTRACT AS GREEN REDUCTANT FOR THE SYNTHESIS OF SILVER NANOPARTICLES: ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY

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ABSTRACT

A simple and eco-friendly method for silver nanoparticles (AgNPs) synthesis employing the aqueous extract obtained from grape pomace by plasma-chemical extraction technique was developed. The reduction of silver ions in solution was monitored using UV - Visible absorption spectroscopy. The synthesised nanoparticles were characterised using transmission electron microscopy (TEM) and dynamic light scattering measurement (DLS). The effect of silver ions concentrations and temperature on the synthesis of silver nanoparticles and average particle size was investigated. The surface plasmon resonance of AgNPs around 430 - 460 nm was observed. The sizes of the spherical silver particles were found to be in the range of 33 - 65 nm. X-ray diffraction analysis confirmed the presence of AgNPs. The study elucidates that AgNPs synthesized in such a way exhibit potential antibacterial activity against Gram positive and Gram-negative bacterial strains tested. The nanoparticles show enhanced antioxidant properties compared to the extract.

Keywords: silver nanoparticles, plasma-chemical extraction, antioxidant activity, antibacterial properties.

INTRODUCTION

Metal nanoparticles usually exhibit uncommon physical, chemical and biological properties compared to their macroscopic equivalents. Different types of metallic nanoparticles have been prepared, including silver nanomaterials that have gained considerable attention due to their high performance in many scientific fields, such as optics, catalysis, and biosensing [1]. In particular, silver nanoparticles (AgNPs) have been used as an excellent antimicrobial and antioxidant agent and as a catalyst for accelerating some chemical reactions [2, 3].

Silver nanoparticles can be synthesized using different approaches, such as electrochemical methods, decomposition, microwave-assisted techniques, and wet chemical procedures [4 - 6]. However, the chemicals used in those methods are usually flammable and highly toxic, which limits their applications in many areas because they may pose risks to human health [7]. Therefore, there is an increasing need to promote a safe and environmentally friendly procedure to synthesize metallic nanoparticles without using toxic chemicals [8]. Recently, the development of effective green chemistry methods has received much attention as an alternative approach to synthesize metal nanoparticles, which can eliminate or minimize the generation of toxic or hazardous waste materials and establish a sustainable process [9]. A fundamental key to the strategy of green synthesis is the use of nontoxic chemicals, renewable materials, or environmentally benign solvents.

Grapes is one of the world’s largest fruit crop with a global production of 68 million tons [10, 11], at the same time about just 38 million tons of which is processed. Annually around 2.5 million tons of grape pomace (GP) is generated. Grape pomace mainly consists from peels (skins), seeds and stems. According to [12] they correspond to 20 - 25 % of the grape weight crushed to produce a wine. However, an analysis of the pertinent literature revealed challenging issues and shortcomings limiting the advancement of the green synthesis. Major
issues are associated with the source/type and concentration of plant extracts and waste materials.

The formation of NP with the use of plant extracts, as a rule, has two stages: preparation of the extract and its subsequent use as a reducing/stabilizing agent. Maceration of solid material with different solvents is the most common method to prepare extracts [13, 14]. These techniques often involve several extraction steps, so take a lot of time. Different alternative extraction techniques have been studied in an effort to increase a yield, including e.g. ultrasound-assisted extraction from grape stems, superheated liquid extraction from vine shoots, or fluidized-bed extraction from grape canes, between others [15 - 18]. Some scientists additionally influence the reaction mixtures directly at the stage of the NP formation by different types of radiation. Thus, a variety of radiation sources emitting in the gamma, UV or Vis and microwave ranges are used to synthesize Ag-NPs in the presence of plants extracts [19 - 21]. Among these, plasma-assisted extraction has low instrumental requirements and allows a simultaneous treatment of various samples in less time [22, 23].

Among plasma-chemical discharges, contact non-equilibrium low-temperature plasma (CNP) is a promising option from the point of view of practical application [24, 25]. Plasma discharge is generated between the electrode in the gaseous phase and a liquid surface, where another electrode is located [26]. Therefore, chemical transformations on the phase interface are conditioned by the combined effect of: an electrochemical oxidation-reduction; initiated photolysis reactions, the UV radiation; a flow of charged particles from the gaseous phase to the surface of the liquid medium [27, 28]. These factors may increase the efficiency of extraction and concentration of the resulting extracts, and as a consequence, the efficiency of further synthesis of AgNPs.

The main goal is to determine the synthesis conditions and specific properties of silver nanoparticles obtained by green method with the use of the grape pomace extract prepared by plasma-chemical extraction and study their antimicrobial and antioxidant activity.

**EXPERIMENTAL**

**Materials**

Silver nitrate (99.8 %, Kishida), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (95 %, Merck Co.Ltd). Aqueous solutions of precursor were prepared using ultrapure water (Direct-Q UV, Millipore) and were utilized as starting materials without further purification.

**Preparation of Grape Pomace Water Extract**

Grape pomace (GP) was stored in plastic bags at 4°C until treatment. The grape pomace was dried at 100°C for 48 h and grounded to obtain a fine powder. The bidistilled water (40 ml) was added to 1 g of dry GP powder and stirred. The resulting mixture was placed in a plasma-chemical reactor. The scheme and the principle of the plant operation for the plasma-chemical reactor are given in works [29 - 31]. The mixture was treated CNP discharge for 5 minutes (at the amperage of I = 120 mA and P = 0.8 MPa), cooled and filtered. The freshly obtained GP water extract was used immediately after filtration. Further, such extracts are mentioned as the plasma-chemically obtained grape pomace water extract (PC GPWE). As a control sample, aqueous extract obtained by traditional methods (T GPWE) was used. The traditional grape pomace extract was obtained by boiling of 1 g of grape pomace powder into 40 ml for 15 min, followed by cooling and filtration. The freshly prepared extracts were used for further experiments.

**Synthesis of silver nanoparticles AgNPs**

AgNO₃ was dissolved in bidistilled water to prepare the solutions with concentrations in the range 0.025 - 6.0 mmol/L. In a typical reaction procedure, 40 ml of grape pomace extract was added to 40 ml solution AgNO₃ under stirring during 0.1 min. The final product was as a colloidal dispersion. The obtained mixture was heated at 80°C for an appropriate time. The obtained mixture was kept for 24 h at an environmental temperature of 24 ± 1°C. The color change of the mixture of “AgNO₃-PC GPWE” to the brown indicates [1] the formation of AgNPs. The strong SPR band at 400 - 450 nm in UV-Vis spectra additionally confirms the formation of AgNPs. The AgNPs obtained by chemical synthesis were centrifuged at 5000 rpm for 5 min. The dried powders were used for a further characterization.

**Characterization techniques**

Spectra of colloidal solutions were obtained using the spectrophotometer UV-5800PC and quartz cuvettes in the wavelength range of λ = 190 - 700 nm (FRU, China).

Particle size was determined by the particle size
analyzer Zetasizer Nano-25 (Malvern Instruments Ltd., Malvern, England). The measurement error does not exceed 1.0 nm in dispersed systems with particle sizes from 1 nm to 10 µm. Microphotographs of nanoparticles were obtained on electron microscope JEOL TEM (Model 100 CX II; Tokyo, Japan).

AgNPs suspensions were diluted in deionized water 1:3 (v:v) and then dried overnight on the sample holder. Analyses were carried out in a Carl-Zeiss Libra 120 microscope using HV = 80 kV.

Antioxidant activity assay
The antioxidant activity of AgNPs was measured by using the DPPH Radical Scavenging Assay method. For each analysis, 1 mL of AgNPs solutions or GPWE was added to 1 mL of freshly prepared DPPH solution (1 mM in methanol). Then the mixture was incubated at room temperature in the dark for 30 min, afterwards the absorbance of the solution was measured at λ = 517 nm. The methanolic solution of DPPH without additives was used as control. The DPPH free radical scavenging activity was calculated using the following Eq. (1):

$$\text{DPPH scavenging effect (\%)} = \left( \frac{A_0 - A_t}{A_0} \right) \times 100$$  \hspace{1cm} (1)

where $A_0$ is the absorbance of the control solution and $A_t$ is the absorbance of the sample.

Assay for antimicrobial activity of Ag nanoparticles against microorganisms
The disk diffusion method was used to study the antibacterial activity of the synthesized silver nanoparticles. Staphylococcus aureus and Escherichia coli were used as model test strains for Gram-positive and Gram-negative bacteria, respectively. The bacterial suspension (About $10^4$ colony forming units (CFUs) of freshly cultured microbial cells) was spread on nutrient agar in Petri plate to create confluent lawn of bacterial growth. The wells of 5 mm were prepared by borer. The solutions of different AgNPs concentrations (15 µL) were poured into each well. The well without silver nanoparticles was treated as control (water and extract). After 24 h incubation at 37°C, the dimensions of the inhibition zones around the samples were measured in five directions, and the average values were used to calculate the circle zone area.

RESULTS AND DISCUSSION
It is well known that AgNPs exhibit strong absorption band due to the surface plasmon resonance (SPR) [32 - 33]. The formation of AgNPs was monitored by UV-Vis absorption spectroscopy.

The results of obtaining silver nanoparticles using the plasma-chemically obtained extract as a reducing and stabilizing agent are represented in Fig. 1. It is evident that the exposure of mixture of AgNO$_3$ (different initial concentration) and extract (fixed volume) for 24 hours at room temperature leads to the formation of silver nanoparticles, with a characteristic peak at 430 - 460 nm [31 - 32]. It was established that increasing the initial concentration of silver nitrate in the reaction mixture contributes to an increase in absorption intensity, indicat-

![Fig. 1. UV-Visible spectra of silver nanoparticles synthesised at room temperature with PC WGPE at different concentration of AgNO$_3$ (a) and T WGPE (b).](image-url)
The formation of silver nanoparticles requires a larger number of nanoparticles. It should be noted that with an increase in the concentration of silver from 3.0 to 6.0 mmol/L, there is no significant increase in absorption intensity. This may be due to the limited reproducibility of the components of the plasma-chemically obtained extract. At the same time, with the use of a traditionally produced extract (AgNO$_3$ 3.0 mmol/L), after 24 hours of exposure, a weak peak SPR is observed at $\lambda = 450$ nm, which is characterized by low concentration of AgNPs synthesized. However, the intensity of the peak is significantly smaller than if the plasma-chemically obtained extract was used. Thus, the obtained data proves that the plasma-chemically obtained extract is more efficient in comparison with the traditionally obtained silver nanoparticles.

The influence of the duration of plasma discharge action during extraction on the further formation of silver nanoparticles has been investigated. (Studies were carried out at an initial concentration of AgNO$_3$ 3.0 mmol/L). It was established that an increase in the duration of extraction from 1 to 5 minutes under conditions of plasma discharge contributes to an increase in the absorption intensity, and hence the concentration of formed nanoparticles. When using the extract obtained by the 10 minute processing it can be noticed that the peak of the formed particles is slightly lower and wider. This indicates the formation of nanoparticles with a large broad size-distribution.

The influence of the temperature on the formation of silver nanoparticles with the use of a plasma-chemically obtained extract was studied. Fig. 1(a) shows UV-Vis spectra of silver nanoparticles synthesized at room temperature (25°C) with PC WGPE at different concentrations of AgNO$_3$. Fig. 3 shows the UV-Vis absorption spectra of AgNPs obtained at different concentrations of AgNO$_3$ (0.25 - 6.00 mmol/L) and in the presence of fixed amount of plasma-chemical obtained aqueous extract (AgNPs synthesized at 80°C, $\tau = 10$ minutes).

The influence of the temperature of nanoparticles formation on the average size of formed nanoparticles

<table>
<thead>
<tr>
<th>GPW Extract</th>
<th>Temperature of synthesis, °C</th>
<th>C AgNO$_3$, mmol/L</th>
<th>Average particle size $d_{AgNPs}$, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC GPWE</td>
<td>25</td>
<td>0.25</td>
<td>65.3±1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.0</td>
<td>73.0±0.7</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>0.25</td>
<td>27.0±1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.0</td>
<td>33.0±1.0</td>
</tr>
</tbody>
</table>

Table 1. Results from the particle size analyzer.

Fig. 2. Influence of the duration of the plasma discharge during an extraction on the formation of silver nanoparticles (exposure at room temperature for 24 hours).

Fig. 3. The UV-Vis absorption spectrum of silver nanoparticles synthesized using PC WGPE at heating for different concentrations of AgNO$_3$.  

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given by the DLS analysis was investigated. The results are shown in Table 1. It was found that the average particle size decreases with increasing temperature of synthesis. The zeta potential of the AgNPs was observed to peak at around \( -25 \) mV, which was evidence for the stability of the nanoparticles. This negative potential was attributed to the presence of a good capping layer around the nanoparticles formed by the bio-organic components of the extract [34].

The morphology of the synthesised AgNPs were investigated using transmission electron microscopy (TEM) analysis (Fig. 4). The AgNPs were spherical in shape with same size distributions. Fig. 4 (a) shows that the particles at concentration \( \text{AgNO}_3 \) 6.0 mmol/L are with relatively broad size distribution. The broad size distribution of particles in TEM image are in accordance with the UV-Vis spectral study. UV-Vis absorption spectra (Fig. 3) shows that the SPR band at 6.0 mmol/L in compare the band at 3.0 mmol/L contains wide peaks and indicated on broad size distribution of particles.

The crystalline nature of the synthesized AgNPs was investigated by XRD and the corresponding XRD diffractogram is shown in Fig. 5. The conditions of experiment were as follows: \( \tau = 10 \) minutes; \( C(\text{AgNO}_3) = 6.0 \) mmol/L; \( t = 80^\circ\text{C} \). The XRD peaks at 11.62º, 27.81º, 32.16º, 38.1º, 46.1º, 54.55º, 66.74º, and 76.84º, 79º correspond to 100, 210, 122, 111, 200, 511, 142, 220, and 311, 420 crystalline planes for cubic crystalline structure of metallic silver (JCPDS card number 04-0783). The intensity of peaks reflected the high degree of crystallinity of the silver nanoparticles [35]. The remaining unassigned peaks at 40.1º, 50.2º could be due to the
crystallization of phytochemicals present in the extract on the surface of AgNPs [36].

The antibacterial activities of synthesized AgNPs were analyzed against Gram positive Staphylococcus aureus and Gram negative Escherichia coli. AgNPs exhibited potential antibacterial activity. Increasing PC GPE-AgNP concentration led to an increased zone of inhibition for all selected species, whereas PC GPE alone did not show any measurable antibacterial activity against selected microbial pathogens. In the present study, zone of inhibition was found to be highest 17 mm against E.Coli and lowest 13 mm against S.Aureus (Table 2).

The bactericidal property of AgNPs is mainly due to the release of silver cations (Ag⁺) and these Ag⁺ ions are attach to the bacterial cell wall due to electrostatic attraction. Moreover, the Ag⁺ ions are not only interacting with the surface of a membrane but can also penetrate inside the bacteria [37 - 39].

Antioxidants are compounds that protect cells against damaging effects of reactive oxygen species. As well they can neutralize free radicals before the last can do harm and also undo some damage already caused to specific cells. Many plants are known to possess free radical scavenging molecules, such as phenolic compounds, terpenoids, vitamins and some other endogenous metabolites, that show high activity antioxidant activity [40]. In the present study, antioxidant activity of the PC GPWE and AgNPs synthesized by GPWE were investigated by DPPH scavenging assay. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts. The results revealed the existence of effective radical scavenging activity for both plant extract and biosynthesized AgNPs (Table 3).

The highest radical scavenging activity (81.5 %) was observed for AgNPs at the initial AgNO₃ concentration in the range of 6.0 mmol/L. The DPPH radical scavenging activities of AgNPs increased gradually with the initial concentration of AgNO₃. However, AgNPs demonstrated the higher inhibition rate compared to GPWE, that confirm the potential benefits of AgNPs as antioxidative agents.

**CONCLUSIONS**

We have reported an easy, simple, and environmentally friendly approach for the synthesis of AgNP mediated by PC GPWE as a reducing and capping/stabilizing agent. Nanoparticles have been prepared by the chemical reduction method from an aqueous solution of silver nitrate in the presence of PC GPWE. The effect of temperature on the synthesis of silver nanoparticles was carried out at room temperature (25°C) and 80°C. The formation of silver colloidal solutions is characterized by the presence of peak λₘₐₓ = 430 - 460 nm in the spec-

Table 2. Zone of inhibition of synthesized silver nanoparticles against bacterial pathogens.

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Zone of inhibition (mm)</th>
<th>Control</th>
<th>PC GPWE</th>
<th>C AgNO₃, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Sterilized water</td>
<td></td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>(de-ionized)</td>
<td></td>
<td></td>
<td>PC GPWE</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td></td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± standard deviation of 3 replicates.

Table 3. Antioxidant activity of AgNPs compared to GPWE.

<table>
<thead>
<tr>
<th>Tested sample</th>
<th>Scavenging (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC GPWE</td>
<td>30.1</td>
</tr>
<tr>
<td>AgNPs</td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>51.3</td>
</tr>
<tr>
<td>6.0</td>
<td>81.5</td>
</tr>
</tbody>
</table>
trum. Through transmission electron microscopy silver nanoparticles were observed to be spherical with up to 50 nm diameter. The formation of silver metal particles is confirmed by X-ray diffraction analysis. The antibacterial studies showed that the AgNPs have a potent antibacterial effect against the tested microbial pathogens.

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