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Research Article

Phytochemical Analysis of *Polygonum bistorta* L. Subsp. *Carneum* (Koch): Quantitative Analysis of Phenolics, Silver Nanoparticles Synthesis, and Biological Activity

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ABSTRACT

Natural products are significant for food and pharmaceuticals due to their biological effects. The improvement of spectroscopy led to the isolation of bioactive compounds from plants and other organisms, and these compounds have been effectively used as raw pharmaceutical materials. Herein, bioactive compounds in *Polygonum bistorta* subsp. *corneum* aerial part was determined quantitatively by LC-MS/MS. Shikimic acid (mg/g extract) (2.85), catechin (1.88), chlorogenic acid (0.534), quercetin-3-D-xyloside (0.439), and hesperidin (0.355) were found as major products. The aerial part of this plant was employed for silver nanoparticles (AgNPs) synthesis. The aerial of the plant material was heated in deionized water for 2 hours, then filtrated. The filtrate was treated with $AgNO_3$ (1.0 mM) to yield the AgNPs. Advanced spectroscopic and image techniques such as XRD, UV-Vis, Zetasizer, FTIR, and TEM were employed to identify the structure. The particle size of AgNPs was determined as 21.3 nm. DPPH assay and ABTS assay were exploited. AgNPs demonstrated better antioxidant activity than extract.

Keywords: Silver nanoparticles, green synthesis, *Polygonum bistorta* subsp. *carneum*, spectroscopy, natural products.

1. INTRODUCTION

Plants have been used widely for nutrients and pharmaceutical reasons for years.[1-3] After the development of spectroscopy, active molecules in plants have been elucidated, and these compounds have been used for many drug formulations.[4] Moreover, these natural compounds have inspired synthetic chemists to prepare these compounds from readily available chemicals.[5-9] The tendency to use natural products in drugs has risen recently due to the side effects of synthetic compounds.[10]

Nanotechnology is a rapidly developing branch of science with practical usage areas.[11-13] Nanomaterials have a size of 1 to 100 nm and are efficiently used in medicine, pharmacy, agriculture, electronics, and wastewater cleaning.[14-16] The large surface area enables nanoparticles to be used in many fields.[17] There are some methods to produce nanoparticles. The biosynthesis of nanoparticles of metals, metal oxides, and metal composites is cleaner, non-toxic, and harmless to the environment compared to physical and chemical methods.[18-19] Nowadays, metal-based nanoparticles are synthesized from different parts of plants. Plant molecules act as reducing agents.[20] Moreover, the corresponding bioactive secondary metabolites increase the activity of nanoparticles. AgNPs play an essential function in medicine owing to their important physicochemical properties. Silver nanoparticles were demonstrated to have antifungal, anti-inflammatory, antiviral, and anticancer activity.[21-22] The secondary metabolites in the plant extract act as reducing agents. So, the nanoparticles capped, stabilized, and reduced by the bioactive compounds are expected to display good biological activity.

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Polygonum bistorta has been exploited as traditional medicine for treating hematemesis and hemorrhoids. This plant was reported to display antibacterial, anti-inflammatory, and anticancer properties. Phytochemical studies revealed that this plant contained triterpenoids, coumarins, and steroids.[23]

Quantification of active compounds in plants is essential.[24-25] Since the bioactive compounds can be isolated from these plants.[26] Furthermore, the determination of bioactive compounds provides the efficacy and safety of herbal medicines, develops new pharmaceuticals, understands plant metabolism, and improves agricultural practices.[27] Quantitative analysis helps standardize herbal products, which is crucial for consumer trust and regulatory approval.[28] Identifying and quantifying bioactive compounds is a fundamental step in drug discovery.[29] Many pharmaceuticals are derived from plant compounds, and understanding their concentrations can lead to the development of new drugs. Quantifying bioactive compounds helps in developing functional foods and dietary supplements with specific health benefits, such as antioxidants, vitamins, and minerals.[30-31]

Free radicals are highly reactive atoms or molecules.[32] They can cause significant damage to cells, proteins, and DNA by initiating chain reactions.[33] The body has a defense system of antioxidants to neutralize these radicals and prevent oxidative stress. Sometimes, these antioxidants may be insufficient. Hence, natural antioxidants may be critical for the body.[34] Plants produce significant antioxidant compounds.[35] Consuming plants containing antioxidants is vital for good nutrition and protection from diseases caused by free radicals.[36] In addition, due to the cancerogenic effects of synthetic antioxidants used in foods, the tendency to use natural antioxidants instead of synthetic ones has increased in recent years.[37]

This research presents a quantification of the phenolics of *Polygonum bistorta* subsp. *Corneum* aerial part, synthesis of AgNPs using the aerial of this plant, and investigation of their antioxidant activity.

2. MATERIALS and METHODS

2.1. Plant Material

Polygonum bistorta subsp. carneum was collected from Çaykara, Trabzon, in June 2023 (40.55708° N, 40.40457° E) and identified by Prof.Dr. Salih Terzioglu, Karadeniz Technical University.

2.2. Synthesis of Silver Nanoparticles

Polygonum bistorta L. subsp. *Carneum* aerial part (5.0 g) was heated in deionized (DI) water (120 mL) for 2 hours at 45°C at constant stirring, then filtrated. The filtrate was treated with $AgNO_3$ (1.0 mM, 100 mL) for 2 hours at 50°C, yielding the AgNPs. The color change was an essential indication of the formation of AgNPs. The reaction solution was centrifuged for 15 minutes at 15000 rpm, thoroughly washed with DI, and dried by lyophilization.[38]

2.3. Characterization of AgNPs

The synthesized AgNPs were characterized by advanced spectroscopic technique. The maximum absorption of AgNPs was determined by UV-Vis (Hitachi U-2900). The natural compounds responsible for reducing agents were presented by FTIR (FT/IR 4700). Transmission electron microscopy (TEM, Hitachi HT-7700) was utilized to reveal the dimensions and structure of nanoparticles. The particle size and crystalline structure were revealed by XRD (Empyrean, Malvern Panalytical diffractometer). Zeta potential was accomplished by Zetasizer Nano ZSP (Malvern instrument).[39]

2.4. Quantitative Analysis of Bioactive Compounds

Quantification of phenolics was displayed by LC/ESI-MS/MS measurement. The reverse phase column (A Poroshell 120 SB-C18) was employed. The sample (50.0 mg) was dissolved in methanol (1.0 mL), and then 1.0 mL hexane was added and centrifuged. A sample (100 µL) was taken from the methanol phase and diluted by water and methanol (each was 450 µL). Afterward, the solution was filtered and applied to the instrument. Water with formic acid (0.1%) and ammonium formate (5.0 mM) A, and methanol with formic acid (0.1%) and ammonium formate (5.0 mM) B made up the mobile phase. The program was adapted to 0-7 min 40%, 8-15 min 65%, 16-24 min 85%, and 25-30 min 7% for mobile phase B. The capillary voltage was fixed at 4000 V, and the column temperature was adjusted to 40°C. The injection volume was 5.12 µL.[40]

2.5. Antioxidant Activity

The DPPH assay is based on the ability of samples to scavenge the DPPH free radical, a stable organic radical characterized by its deep purple color. The scavenging activity is determined by calculating the percentage of DPPH radicals neutralized by the tested samples. A higher percentage of DPPH scavenged indicates greater antioxidant activity. Silver nanoparticles and extract stock solutions were prepared at a concentration of 1.0 mg/mL. Each extract and nanoparticle solution were then incubated with the DPPH solution for 30 minutes at room temperature. The absorbance was measured using a spectrophotometer.

The ABTS radical cation solution was prepared by reacting ABTS (1.0 mM) with sodium persulfate (1.20 mM) in the dark for 6 hours at room temperature. Subsequently, the solution was diluted with sodium phosphate buffer (0.1 mM, pH 7.4). The extract and nanoparticles were then exposed to this ABTS radical

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cation solution, and the absorbance was measured at 734 nm.[41-42]

2.6. Statistical Analysis

GraphPad Prism (8.00) with one-way ANOVA followed by Tukey multiple comparison test was conducted for statistical analysis of biological activity. The results were specified as mean values and standard deviation ($P < 0.05$).

3. RESULTS and DISCUSSION

3.1. Quantitative Analysis

Polygonum bistorta subsp. *carneum* contains significant bioactive compounds revealing biological activity. The compounds responsible for activity were presented. Quantitative analysis of natural compounds revealed the identification of shikimic acid (mg/g extract) (2.85), catechin (1.88), chlorogenic acid (0.534), quercetin-3-D-xyloside (0.439), hesperidin (0.355), and quercetin (0.234). These compounds have high biological activities (Table 1).

Table 1. Quantitative analysis of natural compounds of *Polygonum bistorta* subsp. *carneum* leaves by LC-MS/MS (mg/g extract).

No	Compound	R.T.	Quantity
1	Shikimic acid	1.312	2.852
2	Gallic acid	3.252	0.013
3	Protocatechuic acid	5.506	0.018
4	Epigallocatechin	6.815	0.003
5	Catechin	6.927	1.881
6	Chlorogenic acid	7.404	0.534
7	Hydroxybenzaldeyde	7.697	0.003
8	Caffeic Acid	7.867	0.010
9	Syringic acid	8.500	0.033
10	Vanillin	8.631	0.004
11	o-coumaric acid	9.401	0.007
12	Salicylic Acid	9.722	0.056
13	Trans-ferulic acid	10.174	0.005
14	Sinapic acid	10.432	0.003
15	p-coumaric acid	11.443	0.001
16	Protocatehuic ethyl ester	11.574	0.001
17	Hesperidin	11.687	0.355
18	Isoquercitrin	11.695	0.049
19	Quercetin-3-D-xyloside	12.519	0.439
20	Kaempferol-3-glucoside	13.030	0.003
21	Fisetin	13.293	0.001
22	Quercetin	14.653	0.234
23	Naringenin	14.797	0.011
24	Kaempferol	16.321	0.153

*R.T.: Retention Time

Shikimic acid (SA), a chemical structure similar to the antiviral drug oseltamivir used against influenza epidemics. SA can be generated via fermentation, synthesis and isolation from some plants. *Illicium verum* is the important source of SA. This compound has many properties such as exfoliating, deodorizing, anti-acne, antibacterial, anti-inflammatory, antifungal, anti-aging.[43] Catechin is the unique molecule due to its diverse therapeutic properties. Catechin is found in *Camellia sinensis* at high concentrations. Catechin has been stated to display significant biological activities such as antidiabetic activity, antihyperlipidemic effects, anti-hypertensive effects, anticoagulant antiplatelet effects, antiulcer effects, bone growth promotion effects, antiosteoporotic effects, antiosteopenic effects, cardioprotective effects, hepatoprotective effect, nephroprotective effects, anticataractogenic effects, antiepileptic effects, anti-Parkinson effects, anti-Alzheimer effect, anti-anxiety effects, regulation of respiratory disorders, antiallergic effects, antimicrobial effects, antioxidant effect.[44]

3.2. Synthesis of Silver Nanoparticles and UV-Vis Measurement

AgNPs were synthesized using the aerial part of *Polygonum bistorta* subsp. *Carneum*. The color change from yellow to dark brown indicated the formation of nanoparticles. The bioactive compounds found in this plant capped, stabilized, and reduced the silver ions. UV-Vis spectroscopic analysis proved nanoparticle formation. The particle size and structure of nanoparticles affect the vibration mode of electrons. Therefore, nanoparticles have characteristic absorption in the UV-Vis region. The absorption was detected at 491 nm, revealing the establishment of AgNPs (Figure 1). Shikimic acid was found as a major compound. So, the mechanism was determined using the shikimic acid (Figure 2).

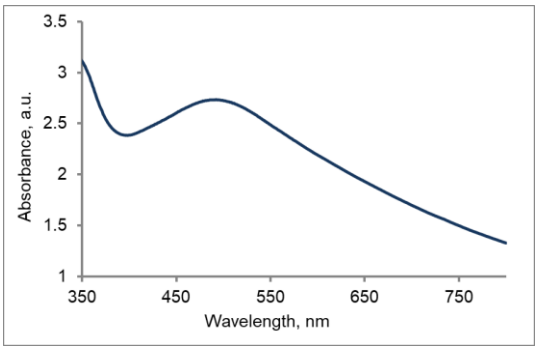

Figure 1. UV-Vis spectrum of AgNPs.

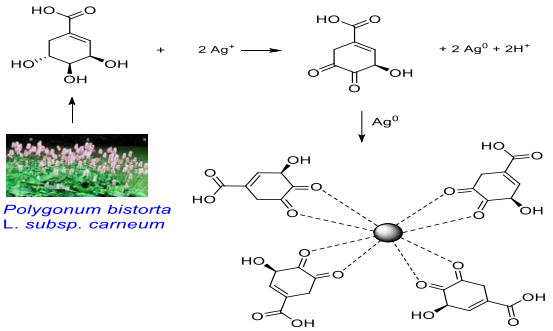

Figure 2. The reaction mechanism of synthesis of AgNPs.

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3.3. FTIR analysis

Fourier Transform Infrared (FTIR) Spectroscopy is a valuable technique for analyzing silver nanoparticles (AgNPs), particularly for identifying functional groups involved in their synthesis, stabilization, and surface modifications. The compounds present in the plant extract were oxidized, and the silver ions were reduced. FTIR measurement revealed the functional groups of the molecules. The absorptions at 3300 cm^{-1} and 2919 cm^{-1} belong to O-H stretching. The peaks at 1639 cm^{-1} and 1518 cm^{-1} attribute to the C=C stretching of alkene and N-O stretching respectively. The signals at 1413 cm^{-1} and 980 cm^{-1} may be due to the O-H bending and C=C bending respectively. The absorption at 795 cm^{-1} may be assigned to the carbon-carbon double bond bending.[41] (Figure 3).

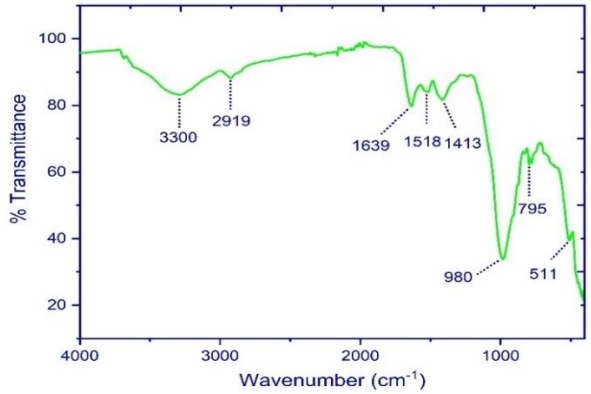

Figure 3. FTIR spectrum of nanostructure.

3.4. TEM Analysis

The morphology of nanostructure was introduced by TEM analysis. The nanoparticles were determined as spherical shape and ranged in size from 5 nm to 14 nm with the average size of 9.6 ± 0.3 nm (Figure 4). TEM analysis revealed that the plants play a significant role in the reduction of silver ions via various types of natural compounds present in the plants. The size distribution of AgNPs is very important since they possess various physical and chemical properties depending on their shape and size.

3.5. XRD Analysis

X-ray diffraction (XRD) is commonly used to analyzed silver nanoparticles (AgNPs) to determine their crystallographic structure, phase composition, and particle size. The crystalline structure of silver nanoparticles (AgNPs) was analyzed using X-ray diffraction (XRD) measurements. The signal at the angles of 38.16°, 44.32°, 64.46°, 77.43° and 81.55° indexed to lattice planes [111, 200, 220, 311, and 222] confirmed the face-centered cubic structure (Figure 5). The molecules on AgNPs may cause the undefined peaks.[45] Scherrer equation (Eq. 1) was used to determine the average size of the AgNPs.

$$D = 0.9\,\lambda/\beta cos\theta \quad (1)$$

The diameter of the silver nanoparticles was shown with D, θ is the Braggs angle (degree), λ is the wavelength of the x-ray radiation source, β indicates the angular line with half maximum intensity as radian.

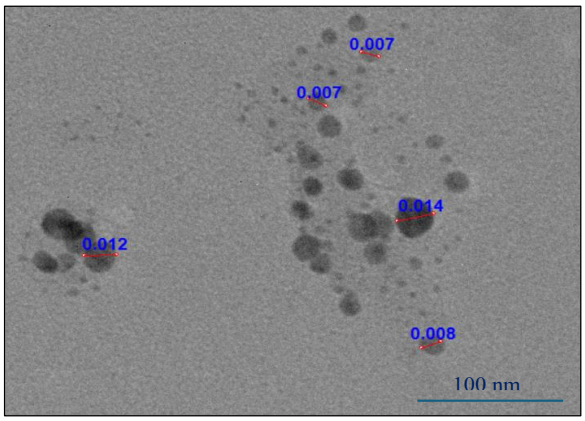

Figure 4. TEM image of AgNPs.

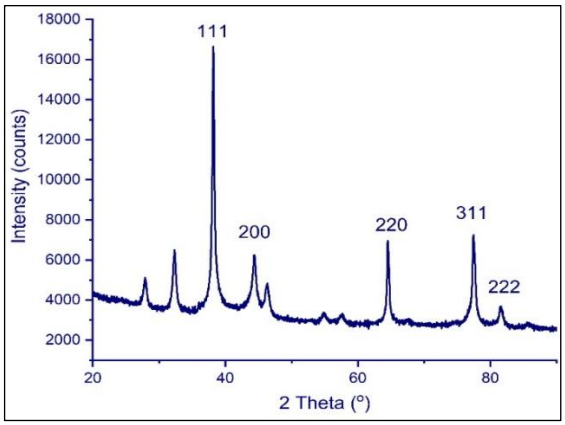

Figure 5. XRD pattern of AgNPs.

3.6. Zeta Potential

Zeta potential indicates the stability of nanoparticles. The green synthesized AgNPs have a zeta potential of -27.6 mV, indicating that the nanoparticles are stable. Electrostatic repulsion between negative charges indicates that silver nanoparticles are stable in colloidal solution. High negative values indicate anionic stability. Negatively charged surfaces prevent nanoparticle aggregation and provide shape and size control (Figure 6).

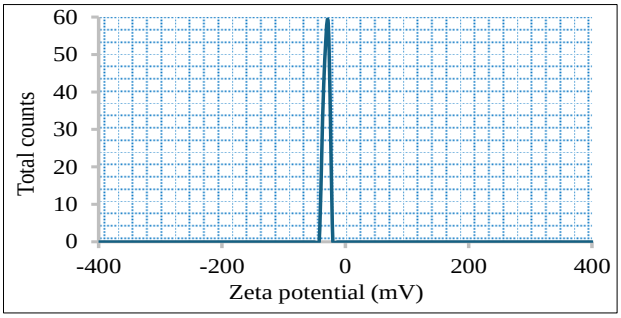

Figure 6. Zeta potential of AgNPs.

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3.7. Antioxidant Activity

Antioxidants in plant extracts are compounds that help protect cells from oxidative damage caused by free radicals. Many plants contain natural antioxidants, which can be classified into different groups based on their chemical structures. Some of the most common antioxidants found in plant extracts include polyphenols, carotenoids, vitamins, alkaloids.[46]

Antioxidant activity of *Polygonum bistorta* L. subsp. *Carneum* aerail part was investigated using the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays. Due to the large surface area to volume ratio of AgNPs, they exhibited high antioxidant activity. AgNPs displayed excellent DPPH activity with a value of 12.12 ± 0.24, and extract activity was reported to be 15.16 ± 0.13, which was lower than the AgNPs. However, standard BHT and BHA activities were reported as 13.23 ± 0.20 and 6.28 ± 0.19, respectively. In ABTS activity, the same trend was observed. $ABTS^{\bullet+}$ activities of AgNPs and extract were reported as 10.12 ± 0.14 and 14.22 ± 0.21 respectively (Table 2).

Table 2. Antioxidant activity of AgNPs, extract, and standards (IC_{50}, µg/mL).

Samples	$DPPH^{\bullet}$ effect	$ABTS^{\bullet+}$ effect
AgNPs	12.12 ± 0.24^{b}	10.12 ± 0.14^{c}
Extract	15.16 ± 0.13^{d}	14.22 ± 0.21^{d}
BHT	13.23 ± 0.20^{c}	9.13 ± 0.12^{b}
BHA	6.28 ± 0.19^{a}	7.13 ± 0.14^{a}
Trolox	6.83 ± 0.15^{a}	9.88 ± 0.18^{b}

*A statistical analysis was carried out. Different letters indicate a significant difference in mean values ($p < 0.05$).

Silver nanoparticles were synthesized using various plants, which revealed considerable antioxidant activity. *Lactuca anatolica* root was used for the synthesis of silver nanoparticles that displayed antioxidant activity.[47] Silver nanoparticles (AgNPs) synthesized using plant extracts often exhibit greater biological activity than the extracts themselves. This enhanced activity can be attributed to several factors. Nanoparticles have a high surface-area-to-volume ratio, which improves their interaction with biological targets, such as bacteria or cancer cells. The bioactive compounds from plant extracts can cap and stabilize AgNPs, enhancing their antibacterial, antioxidant, and anticancer properties. AgNPs can penetrate cells more effectively than crude plant extracts, leading to improved biological efficacy.[48]

4. CONCLUSION

Polygonum bistorta subsp. *carneum* aerial part was used for synthesis of AgNPs. Due to the bioactive compound contents of this plant, the silver nanoparticles capped, stabilized and reduced by the corresponding compounds are expected to show considerable biological activity. Quantitative analysis resulted in the determination of shikimic acid, catechin, and chlorogenic acid as the major products. Silver nanoparticles were synthesized by eco-friendly, cheap, fast, and scalable method. The extract and AgNPs displayed excellent antioxidant activity. Therefore, the plant extract and AgNPs have the potential to be used in the food and pharmaceutical industries.

Conflict of Interest

The authors declare that there is no conflict of interest.

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