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Bellevalia Pseudolongipes PLANT: COMPREHENSIVE ANALYSIS OF THE ELEMENTAL COMPOSITION AND TOTAL PHENOLIC AND FLAVONOID CONTENTS

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Abstract: *Bellevalia pseudolongipes* is a recently described plant species, whose mineral and biochemical composition have not yet been reported. Thus, this study examined the mineral composition and total phenolic and flavonoid contents of the *B. pseudolongipes* plant. Elemental analysis revealed that the plant contained high levels of calcium (22379.556 ± 025 ppm) and potassium (19721.157 ± 005 ppm). Phenolic compound analysis demonstrated a high total phenolic content (0.24 ± 0.004 mg gallic acid equivalent/g sample), thus highlighting the antioxidant capacity of the plant. Additionally, the assessment of flavonoid content (0.043 ± 0.001 mg catechin equivalent/100 g sample) indicated the potential use of the *B. pseudolongipes* plant as a source of antioxidants. These findings underscore the value of *B. pseudolongipes* as a natural resource that is rich in antioxidant, mineral, and phenolic content, while also providing a crucial foundation for researchers interested in exploring its pharmacological, medical, and industrial potential. The results of this study contribute to our understanding of the biological and health values of plants, thereby providing a useful tool for the development of products that are derived from natural sources and innovative solutions that contribute to human health.

Keywords: *Bellevalia pseudolongipes*, Elemental content, Total phenolics, Total flavonoids

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1. Introduction

Plants play a crucial role in ecosystems and contribute to their overall biodiversity. They also provide medical, economic, and environmental benefits. Trace elements are minerals found in small quantities in living tissues, and their excess or absence can result in a range of health issues (Akınoğlu and Erdal, 2024; Yolbaş, 2024a).

Plant secondary metabolites can be classified into terpenes, phenolics, and nitrogenous compounds (Tekin, 2022). Among the bioactive compounds that are present in plants, phenolic compounds have attracted considerable attention owing to their antioxidant, antimicrobial, and anti-inflammatory properties. These compounds are essential metabolites in almost all plant parts and protect plants against biotic and abiotic stresses (Borowska and Szajdek, 2003; Dietrich, 2004; Yolbaş, 2024c). Consequently, determining the biochemical composition and mineral content of plants is of great significance (Kandemir et al., 2022; Jegadeeshwari et al., 2023).

Flavonoids are a class of low-molecular-weight phenolic compounds that are commonly found in the leaves, flowers, and fruits of plants (Okar et al., 1997; Sghaier et al., 2011). They can trap free radicals (Miller and Ruiz-Larrea, 2002) and exhibit diverse biological effects, such as anti-inflammatory, anti-allergic, and antiviral properties (Shi et al., 2001; Yolbaş, 2024b; Saraçoğlu, 2024b). Although the human body cannot produce flavonoids, they can be obtained from fruits and vegetables (Kılıç, 2020); the beneficial effects of plant-derived flavonoids have been reported previously (Panche et al., 2016).

With the advancement of instrumental analytical techniques, numerous elements can be determined more accurately in a short period. Trace elemental analysis, which is the most important research technique in analytical chemistry, has attracted attention owing to its ability to provide insights into the functions of trace elements in various fields, such as high-purity materials, geochemistry, environmental pollution, pharmaceuticals, and their effects on the human body and metabolism (Saraçoğlu, 2024a).

Bellevalia is a genus of plants in the Asparagaceae family that comprises 74 species (Johnson, 2003). *Bellevalia pseudolongipes* has recently been described and illustrated as a new species from the Siirt province in southeastern Anatolia, Türkiye. It is morphologically similar to *B. longipes*, but differs in morphological features and chromosome number (Karabacak et al., 2014). Several studies have been conducted on the biochemical contents of the *Bellevalia* species and their

therapeutic properties (Yildirim et al., 2013; Savio et al., 2019; Ouelbani et al., 2020), and researchers have even investigated the biochemical properties of *B. pseudolongipes* (Balos, 2021). However, no studies have evaluated its mineral content; therefore, determining the mineral composition of this new species, *B. pseudolongipes*, provides insights into the biodiversity of the genus *Bellevalia*.

For the first time, this research aims to analyze the elemental composition of the *B. pseudolongipes* plant and measure its total phenolic and flavonoid contents using methanol extraction. This study will help us understand its potential health benefits and industrial applications by providing missing information regarding its biochemical profile. The results presented in this study will form the basis for understanding the biological activities of the plant and identifying potential areas of use.

2. Materials and Methods

2.1. Sample Collection

Fifty *B. pseudolongipes* plants that grew in their natural habitat were collected from the Pervari district of Siirt province in early May 2023. The collected plant samples, including their bulbs, roots, stems, flowers, and leaves, were dried whole in a dark room at 24 °C for one month. The dried *B. pseudolongipes* samples were then ground into a powder under the same conditions and stored in a closed container in a dark room until the analysis (Yolbaş, 2023).

Figure 1. The *B. pseudolongipes* plant.

2.2. Extract Preparation

A powdered plant sample (0.2 g) was mixed with 5 mL of 75% methanol (Merck, Darmstadt, Germany) containing 0.1% phosphoric acid (Merck, Darmstadt, Germany). The mixture was then homogenized for 30 s at 600 rpm using an Ultra-Turrax homogenizer (MS3-MaxiHomo35, Osaka, Japan). Subsequently, the sample was centrifuged at 2500 rpm (Archer LC-05 A, Istanbul, Türkiye) for 10 min at 24 °C. The resulting supernatant was incubated in an ultrasonic water bath at 25 °C for 15 min. The extraction procedure was conducted twice, and the obtained extracts were combined. The final extract volume was standardized to 10 mL using methanol, and the resulting extract was placed in 100 µL tubes and stored in the refrigerator (5 °C).

2.3. Total Phenolic Content

For the determination of the total phenolic substances, the method developed by Çapanoğlu et al. (2013) was employed, with slight modifications. The *B. pseudolongipes* extract (100 µL) was mixed with ultrapure water (900 µL, 18.2 MΩ, Arium Pro Ultraclean Water System, Sartorius, Göttingen, Germany) and Folin–Ciocalteu reagent (5 mL, 0.2 M). The blend was vigorously shaken and left undisturbed for 8 min. Subsequently, a sodium carbonate solution (5 mL, 7.5%) was introduced, and the mixture was vortexed for 20 s and stored in the dark at 22–24 °C for 2 h. The absorbance was recorded at 765 nm using a Biochrome Libra S70 double-beam spectrophotometer (Cambridge, UK). Quantification was conducted using the gallic acid standard to establish the calibration curve, and analyses were performed in triplicate.

2.4. Total Flavonoid Content

For the determination of the total flavonoid substances, the method developed by Zhishen et al. (1999) was employed, with slight modifications. The *B. pseudolongipes* extract (0.4 mL) was transferred to a 10 mL volumetric flask, and 4 mL of distilled water was added. Next, a $NaNO_2$ solution (0.3 mL, 5%) was added, and the mixture was allowed to rest for 5 min. Subsequently, an $AlCl_3$ solution (0.3 mL, 10%) was introduced, and the mixture rested for 6 min. Finally, a NaOH solution (2 mL, 1 M) and 3 mL of distilled water were added, and the mixture was shaken. The absorbance was measured at 510 nm using a Biochrome Libra S70 double-beam spectrophotometer (Cambridge, UK), with pure water used for the blank reading. Calculations were conducted using the catechin standard to establish the calibration curve. All analyses were performed in triplicate.

2.5. Elemental Analysis

2.5.1. Sample preparation

For the analysis using inductively coupled plasma mass spectrometry (ICP–MS), powdered *B. pseudolongipes* samples (1 g each) were placed in individual microwave digestion Teflon vessels (CEM brand MARS 6 One Touch microwave oven, Matthews, NC, USA). A concentrated nitric acid solution (65%, 10 mL, Merck, Darmstadt, Germany) was added to each sample, and a blank sample containing only nitric acid (65%, 10 mL) was prepared. The vessels were then sealed, placed in the microwave oven, and digested. The temperature was increased from room temperature (22–24 °C) to 210 °C within 25 min and was maintained at 210 °C for 15 min. The samples were cooled to 22–24 °C and transferred to volumetric flasks (50 mL). Ultrapure water was added to attain the final volume.

2.5.2. ICP–MS analysis

The ICP–MS calibration solutions (Table 1) were obtained by diluting commercially available multi-element standards with Suprapur nitric acid (1%, Millipore Sigma, Burlington, MA, USA) and ultrapure water. Appropriate sample dilutions were performed, and the solutions were analyzed using a NexION 2000 B ICP mass spectrometer (PerkinElmer, Waltham, MA, USA) equipped with a quartz fogger, cyclonic fog chamber, and an integrated automatic sampling device under the operating conditions summarized in Table 2. Yttrium (^{89}Y) was used as the internal standard. A wash solution containing Suprapur nitric acid (1%) and ultrapure water at the concentrations specified in Table 1 was prepared for clean-up. The samples were injected into a cyclonic fog chamber using Ar gas via a peristaltic pump. A large quantity of helium gas was also used to prevent interference. The Syngistix software for ICP–MS version 2.2 (PerkinElmer, Waltham, MA, USA) was used for the device settings, data collection, and analysis. Analyses were conducted in triplicate.

Table 1. Calibration curve standard solutions

Analytes	Std1 (ppm)	Std2 (ppm)	Std3 (ppm)	Std4 (ppm)	Std5 (ppm)	Std6 (ppm)	Internal standard
Li, B, Al, Ti, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Nb, Mo, Ru, Pd, Ag, Cd, Sn, Ba, Hf, Ta, W, Au, Pb, U	0.5	1	5	25	50	00	^{89}Y
Na, Mg, K, Ca, Fe	25	50	2.50	1250	2500	5000	

Table 2. ICP–MS operating conditions

Parameter	Description/Value
Nebulizer	MEINHARD plus Classic Type C
Spray chamber	Glass cyclonic (baffled), 4 °C
Torch	One piece w/ 2.5 mm Quartz Injector
Injector	2.0 mm i.d.
Nebulizer flow	Optimized for < 2% oxides
RF (Radio frequency) power	1600 W
Cones	Ni
Replicates	3
Dwell time	50 ms
Aerosol dilution	Set to 2.5×
Sample delivery rate	350 µL/min
Rinse time	45 s
Nebulizer gas flow rate	0.93 L/min
Deflector voltage	Approximately 12 V
Analog stage voltage	Approximately 1750 V
Pulse stage voltage	1100 V
Discriminator threshold	26
Sample tubing (orange-yellow)	Flared PVC pump tubes 0.51 mm/0.89 mm
Internal standard tubing (orange-red)	Flared PVC pump tubes 0.19 mm/0.91 mm
Peristaltic pump speed	35 rpm
Alternating current (AC) rod offset	Approximately 4

3. Results and Discussion

3.1. Elemental Analysis Results

Elemental analysis of the *B. pseudolongipes* plant was conducted using the ICP–MS method with microwave-assisted dissolution. According to the results, the concentrations of 34 different elements (Al, As, Au, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Ga, Hf, K, Li, Mg, Mn, Mo, Na, Nb, Ni, Pb, Pd, Rb, Ru, Se, Sn, Sr, Ta, Ti, U, V, W, and Zn) were determined (Table 3). Within the detection limits, the element with the highest concentration was Ca (22379.556 ± 0.025 ppm), whereas the element with the lowest concentration was Ru (0.524 ± 0.004 ppm). The concentrations of B, Na, and Mg were 137.436 ± 0.016, 5670.721 ± 0.000, and 4246.435 ± 0.003 ppm, respectively.

Organisms require not only protein, carbohydrates, and vitamins but also micronutrients for their normal growth and biological functioning (Onat et al., 2021). These micronutrients play crucial roles in various enzymatic reactions in metabolism and are vital for living organisms (Şap, 2012). In particular, copper plays a critical role in bodily functions and is one of the fundamental building blocks of hair, skin, internal organs, and bones (Kahvecioğlu et al., 2003). Zinc is required by various enzymes and hormones, thereby influencing their functions, and is involved in the renewal of cells and tissues (Özkaya et al., 1991). Iron is a functional component of hemoglobin found in red blood cells that is crucial for oxygen transportation (Yolbaş, 2023). Magnesium is essential for several metabolic processes, particularly in ensuring the proper spread of sodium, potassium, and calcium in cell membranes via cellular pumps (Wilkie and Cordess, 1994). These findings demonstrate the critical role of micronutrients in the healthy functioning of living organisms.

Various plant species have been subjected to numerous

elemental analyses using different methods. However, this is the first study on the *B. pseudolongipes* plant regarding elemental analysis, and significant findings have been obtained when compared to studies on elemental determination in other plants. The presence of metals in plants is dependent on environmental factors, such as the physical and chemical structure of the soil and the potential of plants to absorb metals from the soil (Zurera-Cosano et al., 1989; Erdoğrul et al., 2005). Therefore, when comparing the element analysis results of *B. pseudolongipes* with those of other plants, similarities and differences were apparent.

The presence of K and Ca in high concentrations in the digest indicated that this plant might contribute to regulating cellular functions, maintaining water balance, promoting bone health, and participating in other vital functions (Kuyumcu, 2009; Tosun, 2009). Owing to the significant amounts of zinc and selenium, this plant could protect the body against free radicals and increase resistance to various diseases (Atlihan et al., 1990). Heavy metals, such as Pb, Cu, and Cd, were detected at concentrations of 20.106 ± 001, 13.628 ± 005, and 1.172 ± 013 ppm, respectively (Karaaslan, 2009). These values were higher than expected because the area where the plant samples were collected is close to the highway and human settlement. Investigating plant elemental distribution is essential because it helps determine their mineral and nutritional contents.

This study was subject to limitations, including the inability to comprehensively evaluate the pollution effects on the sampled area and the restriction of samples collected from a limited region. In future studies, it is essential to collect samples from diverse geographical regions and conduct a more extensive investigation into the effects of pollution. This preliminary study represents a pioneering effort in this field and offers crucial findings that will serve as a reference for future research.

Table 3. ICP–MS elemental analysis results

Li 7 Helium KED High (ppm)	B 11 Helium KED High (ppm)	Na 23 Helium KED High (ppm)	Mg 24 Helium KED High (ppm)	Al 27 Helium KED High (ppm)
8.954±030	137.436±016	5670.721±000	4246.435±003	3309.567±004
Cr 52 Helium KED High (ppm)	Mn 55 Helium KED High (ppm)	Fe 57 Helium KED High (ppm)	Co 59 Helium KED High (ppm)	Ni 60 Helium KED High (ppm)
11.688±001	60.959±004	2427.598±001	2.167±003	26.334±002
Se 82 Helium KED High (ppm)	Rb 85 Helium KED High (ppm)	Sr 88 Helium KED High (ppm)	Nb 93 Helium KED High (ppm)	Mo 98 Helium KED High (ppm)
18.237±052	4.929±002	453.384±014	1.138±001	1.609±007
Sn 118 Helium KED High (ppm)	Ba 138 Helium KED High (ppm)	Hf 180 Helium KED High (ppm)	Ta 181 Helium KED High (ppm)	W 184 Helium KED High (ppm)
1.710±011	44.328±011	1.120±021	1.690±002	1.936±001
K 39 Helium KED High (ppm)	Ca 43 Helium KED High (ppm)	Ti 48 Helium KED High (ppm)	V 51 Helium KED High (ppm)	Au 197 Helium KED High (ppm)
19721.157±005	22379.556±025	50.890±006	7.410±001	2.127±002
Cu 63 Helium KED High (ppm)	Zn 66 Helium KED High (ppm)	Ga 69 Helium KED High (ppm)	As 75 Helium KED High (ppm)	Pb 208 Helium KED High (ppm)
13.628±005	445.185±001	9.479±002	1.563±024	20.106±001
Ru 102 Helium KED High (ppm)	Pd 106 Helium KED High (ppm)	Ag 107 Helium KED High (ppm)	Cd 111 Helium KED High (ppm)	U 238 Helium KED High (ppm)
0.524±004	1.309±002	1.479±011	1.172±013	5.361±000

3.2. Total Phenolic Content

Oxidative damage contributes to various diseases, including cancer, hypertension, atherosclerosis, bronchitis, asthma, diabetes, Parkinson's disease, liver diseases, Down syndrome, aging, and rheumatism, by affecting cellular components and altering nucleic acid bases in DNA (Matés and Sánchez-Jiménez, 2000). Phenolic compounds are significant phytochemicals that confer antioxidant activity to plant materials (Pizzale et al., 2002; Fattahi et al., 2012). Epidemiological studies have demonstrated the protective effects of plant sources against reactive oxygen species. This protection is attributed to compounds such as vitamin C, vitamin E, carotenoids, glutathione, flavonoids, and phenolic acids

present in fruits and vegetables (Halvorsen et al., 2002). In this study, the total phenolic content of *B. pseudolongipes* was 0.24 ± 0.004 mg gallic acid/g sample. Phenolic compounds occur naturally in plants and possess antioxidant properties. For example, *Bellevalia saviczii* is a plant with anti-rheumatic and anti-inflammatory properties located in Iraq. The anti-inflammatory effects of a certain compound, dracol, which inhibits intracellular Ca^{2+} release and suppresses cytokine secretion, have been observed in this plant (Savio et al., 2019). Additionally, *Bellevalia gracilis* exhibits a high antioxidant activity (Yildirim et al., 2013). Therefore, the phenolic content observed in *B. pseudolongipes* underscores its ability to mitigate oxidative stress at the cellular level by increasing the antioxidant capacities of cells.

Balos (2021) have previously reported variations in the phenolic content between the bulb, leaf, and flower components of *B. pseudolongipes*, where the highest phenolic content was observed in the maceration extraction method (ethanol extract) of the flower. Tekin (2022) investigated the phenolic content in different parts of the *B. sasonii* plant. Their results revealed that the highest phenolic amount was determined in the onion extract of the plant, whereas the lowest was observed in the stem extract. Different extraction methods and the selection of solvents employed significantly impact the bioactive compounds obtained. Therefore, selecting the appropriate extraction method and solvent is of great importance.

3.3. Total Flavonoid Content

Flavonoids are known for their antioxidant properties, which may help prevent several diseases by reducing oxidative stress at the cellular level. They are also known for their anti-inflammatory properties. The total flavonoid content of *B. pseudolongipes* was 0.043 ± 0.001 mg catechin/100 g sample, indicating its antioxidant capacity and health-enhancing characteristics. Catechin glycosides, particularly C3'G (cyanidin-3-glucoside), exhibit potential as stable catechin precursors (Raab et al., 2010).

The limitations of this study included factors such as method standardization and validation, the flavonoid–antioxidant relationship, the applicability of previous studies, comparability between plant parts, and constraints in sample collection. These limitations are crucial for interpreting and generalizing the study's findings.

Previous studies have reported significant differences in the flavonoid content between different plant parts of both the *B. pseudolongipes* and *B. sasonii* plants. The results of Balos (2021) revealed that the highest amount of flavonoids was observed in the bulb extracted by a traditional extraction method, while the results of Tekin (2022) revealed that the highest content was in the leaf extract, followed by the flower, bulb, and stem extracts.

The results of these studies provide valuable guidance for the effective use of herbal resources and the selection of appropriate plant components for potential therapeutic applications. Combining these results with the current study can assist in the pharmacological evaluation and potential therapeutic applications of *B. pseudolongipes* plants; however, further research is required to understand their biological effects.

4. Conclusion

This study examined the elemental composition and the total phenolic and flavonoid contents in the methanol extract of *B. pseudolongipes* plants. Elemental analyses revealed that the plant is particularly rich in potassium and calcium. Consequently, it is a valuable natural product that could serve as a crucial source for developing novel drugs. This plant is also rich in phenolic compounds, and its high flavonoid content further underscores its high antioxidant capacity. Therefore, it possesses an antioxidant potential that can help protect the body from free radicals. Considering the positive effects of phenolic components on human health, the *B. pseudolongipes* plant holds promise for its utilization in natural resource-based medicines and functional foods. However, further comprehensive studies are required to fully elucidate its potential health benefits. Thus, the *B. pseudolongipes* plant is a valuable natural health resource with promising potential for application in human nutrition and health.

Author Contributions

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

	İ.Y.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C= Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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