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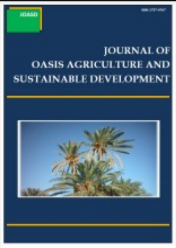

JOURNAL OF OASIS AGRICULTURE AND SUSTAINABLE DEVELOPMENT

www.joasdjournal.org

JOASD

p-ISSN: 2724 – 699X
e-ISSN: 2724-7007

Response of *Pelargonium graveolens* under variance of geographical distribution

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Article info

Article history:
Received: 27 August 2024
Accepted: 10 November 2024

Keywords: Antioxidant activity, growing region, *Pelargonium graveolens*, phytochemical composition, total phenolic content.

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Conflict of Interest: The authors declare no conflict of interest.

Abstract

The present study evaluated the influence of habitat characteristics on the phytochemical composition and antioxidant properties of *Pelargonium graveolens*. Among the different sites assessed, Mahdia area showed the highest protein and fat content at 6.8% and 3.7%, respectively. Additionally, plants from the same region exhibited the highest energy value of 412.1 kcal/100g. *Pelargonium* originating from Kairouan region displayed elevated levels of Mg and Ca, followed by those collected from Sejnane region. Notably, the highest iron content was observed in plants harvested from Mahdia. These variations were attributed to several factors such as geographical location, altitude and average rainfall. Extract from Sejnane demonstrated the highest levels of secondary metabolites, with total phenolic content at 388 mg GAE/g and total flavonoid content at 80 mg QE/g. Regarding antioxidant activity, *Pelargonium graveolens* from Sejnane and Kairouan, exhibited the most potent scavenging activity against DPPH and ferric reducing antioxidant power. These results underscored the species' potential as a valuable natural source of bioactive antioxidant compounds within its ecological niche.

1. INTRODUCTION

Recent researches have increasingly on medicinal and aromatic plants as a promising source of bioactive compounds due to their abundant secondary metabolites, including terpenoids, flavonoids, and phenolic acids (Chassagne et al. 2019; Chaachouay and Zidane, 2024). These compounds are widely acknowledged as natural antioxidants with the potential to combat oxidative stress, a factor implicated in numerous chronic diseases (Muscolo et al. 2024; Rahman et al. 2024). The antioxidant properties of polyphenols, in particular, have been highlighted for their diverse biological functions including antifungal, antimicrobial, antiviral and anti-inflammatory effects, showcasing their potential for therapeutic applications (Aourahoun et al. 2019; Manso et al. 2022). The demand for natural antioxidant alternatives is growing as synthetic antioxidants are increasingly scrutinized due to links with adverse health effects, including damage to vital organs such as the circulatory system, kidneys and stomach and initiation of cancerous diseases (Choe and Yang, 1982).

Pelargonium graveolens, an aromatic plant belonging to the Geraniaceae family, is widely cultivated and highly regarded in traditional medicine across many cultures. It has been used for centuries to treat a variety of ailments, such as diarrhea, bronchitis, fever, tuberculosis, malaria and gastroenteritis (Saraswathi et al. 2011; Tahan and Yaman 2013). Modern studies have further revealed that both extracts and essential oil from this species exhibit notable antibacterial, anti-inflammatory and spasmolytic activities, expanding its applications in contemporary pharmacology (Boukhatem et al. 2013; Moyo and Staden 2014). This pharmacological potential was partly attributed to the species richness in bioactive phytochemicals including oxygenated coumarins, gallic acid-derivatives, flavonoids, phenolic and hydroxycinnamic acid-derivatives, compounds known for their therapeutic efficacy (Colling et al. 2010). In addition, a recent study on the same plant revealed notable differences in essential oil composition and bioactive compound concentration with salt-stressed plants showing higher levels of phenolic compounds and potent antioxidant activity (Lahmar et al. 2024).

Despite its recognized importance, research on Tunisian *Pelargonium graveolens* remains relatively limited, especially regarding how ecological factors and geographical variability affect its bioactive profile. Given the plant's medicinal relevance and ecological adaptability, understanding how environmental conditions influence its phytochemistry is essential. This study aims to address this gap by investigating *Pelargonium graveolens* collected from three distinct Tunisian regions. We seek to determine how differences in geographical habitat, soil composition, and climate impact the nutrient composition, mineral content, phenolic content, and antioxidant potential of this species. This comprehensive analysis will enhance the sustainable use of the species in pharmacological applications.

2. MATERIAL AND METHODS

2.1. Plant collection and preparation

Aerial parts of *Pelargonium graveolens* were harvested during the full flowering stage across three distinct regions in Tunisia: Sejnane (1), Mahdia (2) and Kairouan (3), each located at different elevations as indicated in Table 1. Three samples were obtained from each growing region. After collection, the plants underwent air-drying in the laboratory, were finely ground using an electric grinder (IKA MF 10 Basic) and subsequently stored in dark plastic containers at room temperature until further analysis.

Table 1. Geographical and climatic data collected for the sampled ecological regions

Growing location	Altitude (m a.s.l.)	Rainfall (mm/ year)	Min T°C	Max T°C
Sejnane	230	462.8	10.4	29.6
Mahdia	12	273.9	15.4	25
Kairouan	68	260.9	8.5	34

2.2. Extraction of plant material

The preparation of the hydroalcoholic extracts required a soaking of 50 g of each plant sample in 200 mL of a methanol–water solution (80:20, v/v) at 25°C during three days, followed by a centrifugation at 150 g for 15 min (Lahmar et al. 2017). The resulting residue was filtered and subsequently concentrated using a rotary evaporator (Buchi R-210) at 40°C.

2.3. Determination of proximate composition

The protein content was assessed following the Kjeldhal method. Crude fat content was determined gravimetrically using the Soxhlet method. Moisture content was determined by subjecting samples to oven drying at 103°C. Ash content was determined by incinerating plant powders in a muffle furnace at 550°C until a consistent weight and whitish color were attained. Carbohydrate content was calculated by subtracting the sum of protein, fat, ash, and moisture from the sample's dry weight, considering it as 100% according to Ferris et al. (1995). Total energy was estimated in kcal by multiplying the percentages of protein, fat, and available carbohydrate by the factors 4, 9, and 4, respectively (Guil-Guerrero et al. 1998).

2.4. Analysis of mineral content

Dry plant samples weighing 0.5 g were subjected to ashing procedure at 550°C, followed by a digestion with 10 mL of 2.8% nitric acid overnight (Farahat and Linderholm 2015). Magnesium (Mg), iron (Fe), and calcium (Ca) concentrations were determined using a flame atomic absorption spectrophotometer (Perkin Elmer A Analyst 200). Mineral concentrations were expressed in milligrams per kilogram based on dry weights.

2.5. Assessment of total phenolic and flavonoid contents

Total phenolic content was determined using the Folin-Ciocalteu method described by Singleton et al. (1999). An 800 µL aliquot of the plant extract was mixed with 50 µL of Folin-Ciocalteu reagent (2N), followed by the addition of 150 µL of sodium carbonate solution. Absorbance was measured at 765 nm using a UV-VIS spectrophotometer after 2 h of incubation at 37°C. Results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight. Total flavonoid content was determined using aluminum chloride according to Lamaison and Carnat (1991), with results expressed as milligrams of quercetin equivalents (QE) per gram of dry weight.

2.6. Antioxidant activity assay

DPPH scavenging activity was measured as described by Naik et al. (2003) with slight modifications. One milliliter of DPPH solution

(10^{-4} M) was mixed with 1 mL of diluted plant extract, incubated in the dark for 20 min. Absorbance was measured at 517 nm against a blank. Butylated hydroxytoluene (BHT) served as the positive control, with results expressed as IC_{50}, indicating the extract concentration required to inhibit 50% of free radicals. Ferric reducing antioxidant power (FRAP) was evaluated according to Oyaizu (1986) mehod. Absorbance was measured at 700 nm. Results were expressed as EC_{50} indicating half maximal effective concentration and referring to BHT as a standard.

2.7. Statistical analysis

Independent samples of each item were analyzed in triplicate. Statistical analyses of the data were performed using the SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA). Data were expressed as the mean ± standard deviation and analyzed by one way ANOVA. Significance level was determined ($p < 0.05$) and significant difference was determined using Duncan's Multiple Range Test.

3. RESULTS & DISCUSSION

3.1. Analysis of chemical composition

Table 2 presents the proximate composition, including protein, fat, moisture, carbohydrate, and energy value of *Pelargonium graveolens* collected from various localities. Plant growing in Mahdia region was the highest rich in protein and fat content, showing 6.8 and 3.7%, respectively. However, displaying respectively values of 8.4 and 87.8%, the studied plant growing in Kairouan region was considered as the richest in ash and carbohydrate content. Moreover, the present findings presented an energy value quasi-equal in Mahdia and Kairouan.

Differences were noted in the nutrient composition across the three plant samples. According to literature, a high moisture content was mainly correlated to a salt tolerance of the sampled species (Lahmar et al. 2024). However, in saline environments, plants tend to accumulate more water in their tissues to counteract the osmotic stress caused by excess salt. This increased moisture helps maintain cellular functions and turgidity, allowing the plant to survive under challenging conditions (Hasanvand et al. 2017; Naveed et al. 2021). Additionally, salt stress triggers the production of osmolytes, antioxidants, and other protective compounds that help the plant cope with the negative effects of salinity (Ben ElHadj Ali et al. 2020). The described correlation promoted physiological adaptation to salinity conditions, and favorized the preservation of water within plant tissues (Gupta and Huang 2014; Feng et al. 2016).

Notably, lipids and proteins extracted from plants hold considerable economic value. The scarcity of protein resulting from various diseases, coupled with the escalating costs of animal products, has spurred researchers to explore novel alternative protein sources (Lonnie et al. 2018). *Pelargonium* presents an opportunity as an environmental asset for producing value-added protein supplements, potentially mitigating waste production (Abdel-Khalek and Mattar 2022). The presence of a considerable ash content was a crucial parameter for food quality assessment, often indicative of a substantial mineral deposit in each food sample (DeBuchananne and Lawson 1991; Lahmar et al. 2023). The elevated total carbohydrate content in such plant sample contributed to its high energy value (Zhelev et al. 2022). Consequently, *Pelargonium* could serve as a valuable animal fodder. These chemical analysis results may be influenced by various factors such as geographic location, bioclimatic conditions, soil composition, plant age and vegetative cycle. Overall, the investigation confirmed that *Pelargonium graveolens* possesses significant nutritional value across the examined regions, establishing it as a valuable energy source.

3.2. Analysis of mineral content

Table 3 illustrates the mineral concentrations of *Pelargonium graveolens* across various Tunisian localities. Macroelements, such as Ca (calcium)

Table 2. Nutrient composition values at different localities

Growing location	Protein (%)	Lipids (%)	Moisture (%)	Ash (%)	Carbohydrate (%)	Energy (kcal/100g)
Sejnane	5.7±0.9^{a}	3.1±0.2^{c}	2.8±0.2^{c}	6.2±0.7^{b}	83.4±0.5^{b}	384.3±0.9^{a}
Mahdia	6.8±0.4^{b}	3.7±0.6^{b}	4.8±1.3^{a}	1.8±0.1^{ab}	83.6±0.9^{a}	412.1±0.1^{c}
Kairouan	6±0.7^{ab}	3.4±0.4^{a}	3.1±0.5^{a}	8.4±0.9^{b}	87.8±0.2^{a}	406±0.1^{b}

and Mg (magnesium), along with microelements like Fe (iron), were examined. The highest levels of Mg and Ca were found in *Pelargonium* plants from Kairouan, with values of 22.6 and 48 mg kg^{-1}, respectively. This was followed by plants from Sejnane, which contained 21 mg kg^{-1} of Mg and 33.3 mg kg^{-1} of Ca. However, the highest Fe content, at 7.6 mg kg^{-1}, was observed in *Pelargonium* collected from Mahdia. Indeed, *Pelargonium* species, accumulated a variety of essential minerals in different vegetative parts, and their mineral composition can be influenced by environmental factors, stress, and nutrient management. Several studies revealed varying levels of essential minerals in different plant parts. Gâlea et al. (2015) reported significant concentrations of Mn (manganese), Zn (zinc), and Fe in the roots, stems, and leaves of *P. roseum*, with Fe being particularly notable, reaching 606 mg kg^{-1} in the leaves and 314.7 mg kg^{-1} in the lignified stems. Considered as an important microelement in the plant, Fe can have a potential role in the medicinal properties of the studied plant. Similarly, Jawzal et al. (2024) demonstrated that *P. graveolens* contained a variety of minerals, including Cu (copper), Mn, Co (cobalt), Ni (nickel), Pb (lead), Mg, Fe and Ca, with Mg and Ca found in significant amounts.

Table 3. Concentrations of minerals (mg kg^{-1}) at different localities.

Growing location	Magnesium (Mg)	Calcium (Ca)	Iron (Fe)
Sejnane	21±0.9^{c}	33.3±1.3^{b}	7.2±0.1^{a}
Mahdia	18.8±0.3^{b}	20.7±0.7^{c}	7.6±0.5^{a}
Kairouan	22.6±0.8^{ab}	48±0.6^{a}	6.1±0.6^{c}

Ca stands as a fundamental element for plant growth (Ahmed et al. 2021). It fortified bones, recognized for its anticoagulant properties and played a vital role in tissue formation (Kumar et al. 2018). Meanwhile, Mg played a crucial role in photosynthetic reactions and it contributed to the production of high-quality plants serving as a co-factor for activation of diverse enzyme systems, phosphate metabolism and plant respiration (Chen et al. 2018; He et al. 2020). Mg is essential for phosphorylation reactions and associated with robust antioxidant activity (Lukaski 2004). Additionally, plants rich in iron contributed to the enhancement of hemoglobin and red blood cell levels (Hossain et al. 2021). *P. graveolens* presented significant levels of essential minerals crucial for maintaining overall health. The mineral composition was mainly attributed to the physiological properties (Mahdavi et al. 2010; Konczak and Roulle 2011) combined to geographical locality, altitude and average rainfall, as observed by Felhi et al. (2016). The concentration and variety of mineral elements found in the studied plant presented a crucial role in evaluating their nutritional value and impact on human health. Present findings hold significant importance for both health professionals and consumers alike.

3.3. Phenolic compounds content and antioxidant activity

The differences in total phenolic and flavonoid contents among *Pelargonium graveolens* plants highlighted the influence of environmental factors on secondary metabolite production (Table 4). In this study, extract from Sejnane region exhibited higher total phenolic content (388 mg GAE/g) and flavonoid content (80 mg QE/g), suggesting the significant impact of specific local environmental conditions on polyphenol biosynthesis. In a related analysis of the same plant gathered from a different Tunisian region, the leaves extract contained slightly lower concentrations of bioactive compounds, with total phenolic content at 142.7 mg GAE/g DW and flavonoid content at 52.3 mg RE/g DW (Ben El Hadj Ali et al., 2020). Previous research supported this finding, indicating that polyphenol accumulation is often driven by abiotic and biotic factors as well as genetic traits, and extraction techniques (Lahmar et al. 2017; Kumar and Kumar Sharma 2018). Additionally, *Pelargonium graveolens* collected from Sejnane and Kairouan displayed the most potent scavenging activity for DPPH (2-2- diphenyl-1-picrylhydrazyl) scavenging activity as well the ferric reducing antioxidant power (Table 4). In fact, noted antioxidant activity levels, were positively correlated with these phenolic and flavonoid contents, corroborating findings that higher phenolic levels generally enhance antioxidant potential (Lahmar et al. 2023). As well it was reported that an increased DPPH inhibition was associated with higher reducing sugar levels and Maillard reaction product formation (Incedayi et al. 2016; Xing et al. 2024). Compared to the present results, the ethyl acetate extract of *Olea europaea*, specifically the Chemlali variety, demonstrated an IC_{50} of 70 μg/mL using the DPPH assay, indicating significant antioxidant activity. The results, however, may depend on the solvent used in the extraction process, underscoring the influence of extraction solvents on antioxidant potency (Khelouf et al. 2023).

networks, enhancing the plant's overall defense response to abiotic stressors (Salam et al., 2023). Thus, understanding the phenolic composition of *Pelargonium graveolens* under varied environmental conditions is critical for optimizing its medicinal properties, as these compounds are directly linked to antioxidant activity, bioavailability, and the potential therapeutic effects of the plant.

Table 4. Total phenolic and flavonoid contents, IC_{50} values of the DPPH free radical scavenging and EC_{50} values of the FRAP assays at different localities.

Growing localities	Phytochemical analysis		Antioxidant activity	
	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH IC_{50} (μg/mL)	FRAP EC_{50} (μg/mL)
Sejnane	388±1.2^{ab}	80±0.6^{a}	28.7±0.5^{b}	29.4±0.7^{b}
Mahdia	110±0.7^{b}	20±0.2^{c}	78±0.1^{ac}	56.6±0.9^{ab}
Kairouan	312.6±0.9^{c}	72±1^{a}	30.9±0.5^{a}	39.7±0.2^{c}
Butylated HydroxyToluene	-	-	37±0.6^{b}	35.1±0.1^{ab}

Phenolic compounds are essential secondary metabolites that plants produce as a defense mechanism against environmental stressors, and attacks by pathogens or herbivores (Lattanzio, 2013; Al-Khayri et al. 2024). Plants under stress often exhibited increased levels of these metabolites as part of a biochemical adaptation that helps them tolerate adverse conditions (Piccolella et al. 2018; Salam et al. 2023). In addition to abiotic factors, the geographic and climatic variation between habitats further affects polyphenolic profiles, influencing the bioactivity of these compounds. Studies on *Pelargonium* and other medicinal plants have shown that the composition and concentration of phenolics vary with altitude, light exposure, and seasonal shifts, thereby impacting the antioxidant properties of extracts derived from plants in different regions (Ben Haj Yahia et al. 2019; Lahmar et al. 2024).

Phenolic compounds' production often increased in response to these stresses, activating a cascade of oxidative processes that result in heightened free radical production within the plant cells (Feng et al. 2020; Ali et al. 2023). These radicals were neutralized by phenolic antioxidants, which reduce oxidative damage, thus playing a central role in the plant's adaptive response to environmental challenges (Muscolo et al. 2024). These findings suggested that phenolic compounds can not only serve as antioxidants within the plant system but also contribute to potential health benefits when used in medicinal and nutraceutical applications (Tirado et al., 2022).

Moreover, recent research indicates that phenolic compounds may interact with other metabolites to form complex antioxidant networks, enhancing the plant's overall defense response to abiotic stressors (Salam et al., 2023). Thus, understanding the phenolic composition of *Pelargonium graveolens* under varied environmental conditions is critical for optimizing its medicinal properties, as these compounds are directly linked to antioxidant activity, bioavailability, and the potential therapeutic effects of the plant.

4. CONCLUSIONS

This study offers valuable new insights into the phytochemical profile, polyphenol concentration, and antioxidant capacity of *Pelargonium graveolens* cultivated across various regions in Tunisia. The present analysis revealed regional differences in these parameters, suggesting that geographical and environmental factors considerably influence the plant's nutrient composition. Specifically, plants growing in Mahdia region demonstrated superior nutritional value, with significantly higher mineral content, particularly Mg and Ca, in samples from Kairouan site. Notably, plants collected from Sejnane exhibited the highest total polyphenol content, which correlated strongly with enhanced antioxidant activity ($p < 0.05$), underscoring the potential of *P. graveolens* as a robust source of natural antioxidants. These findings highlight the impact of habitat on bioactive compound accumulation, supporting the species' use in health-promoting applications and justifying its cultivation in diverse Tunisian regions for optimized therapeutic potential.

Author contributions

Imen Lahmar and Lyubov Yotova contributed to the study conception and design. Material preparation, data collection and analysis were performed by Imen Lahmar and Lyubov Yotova. The manuscript was drafted by Imen Lahmar. All authors commented, read and approved the submitted version of the manuscript.

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