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STUDY OF CHARACTERISTICS OF *PRIMULA VULGARIS* USING RAMAN SPECTROSCOPY

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Abstract. *Primrose, or Primula vulgaris, is a perennial herb that has long been prized for its therapeutic qualities. The entire plant is used for its expectorant, anti-inflammatory, analgesic, antispasmodic, and healing-promoting properties. P. vulgaris is effective in treating rheumatic disorders, gout, insomnia, anxiety, respiratory tract infections, and bronchitis. This study examines the ecological variation of Primula vulgaris from two locations: Dajti Mountain, which is 1023 meters above sea level, and the Tirana Hills, 217 meters above sea level. These locations provide different environmental conditions, making it possible to investigate how these ecological variations might affect the traits of plants. Leaf dimensions (length and width), chlorophyll content were measured, and statistical analysis were performed using Mann-Whitney U test. The findings showed that there was a statistically significant variation in leaf size, with Dajti Mountain plants having shorter leaves. For this study, Raman spectroscopy measurements were performed using a B&W Tek i-Raman Ex Raman spectrometer. We have encountered the presence of Rutin and Tangerine essential oil, from the characteristic peaks present in the Raman spectra. These findings provide insights into how altitude and light exposure shape the morphological, physiological, and chemical traits of Primula vulgaris, revealing that plants in sunnier, low-altitude habitats develop broader leaves and higher concentrations of bioactive compounds, while those at higher elevations exhibit greater chlorophyll content and enhanced photosynthetic efficiency.*

Keywords: *Primula vulgaris, CCM 200, Raman spectroscopy*

1. INTRODUCTION

Primula vulgaris, commonly known as the common primrose or English primrose, is a species of flowering plant in the Primulaceae family [1]. It is a woodland plant species that thrives in habitats rich in humus and low in nutrient soils [2].

The common primrose prefers moist, well-drained soil and partial shade but can also tolerate full sun in cooler climates [1].

Primula vulgaris is a species widely known for its medicinal value. It is traditionally used to treat various ailments because of the beneficial effects of this plant, likely attributed to its phenolic content.

This plant has a long history of cultivation and has been a favorite among gardeners for centuries [1]. The bridge between the traditional medicinal uses of *P. vulgaris* and modern science is built upon the identification of its bioactive phytochemicals. For decades, scientific research has sought to isolate and characterize the compounds responsible for the plant's therapeutic properties. The chemical composition of *Primula vulgaris* can vary depending on factors such as the plant's growth stage, environmental conditions, and geographic location [1].

Since the beginning of the twentieth century, several studies on the phytochemical composition of different species of Primula have been carried out [3].

The extract of *Primula vulgaris* has been found to contain several phenolic compounds, including gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, catechin, vanillic acid, caffeic acid, *p*-coumaric acid, [4] as well as flavonoids and saponins, which are of therapeutic significance [3,4]. The combination of flavonols/anthocyanins and carotenoids contributes to the plant's coloration, ranging from yellow to violet-blue [5].

P. vulgaris leaf and root extracts exhibit antioxidant anti-inflammatory and antimicrobial properties, including strong inhibitory effects against *Escherichia coli*. [6], while the flavonoid isoquercetin is a particularly promising compound, for pharmaceutical and cosmetic applications [7].

The phytochemical profile of *P. vulgaris also* exhibits cardioprotective effects, while its flower extracts have demonstrated selective cytotoxic activity against cervical cancer cells [8,9].

While the presence of these compound classes is well-established, their detailed characterization within the plant matrix often relies on conventional analytical techniques such as High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC), often coupled with Mass Spectrometry (MS) [10]. Although these methods are powerful and provide quantitative data, they come with significant drawbacks. They are inherently destructive, requiring

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the extraction and often complex chemical derivatization of the compounds of interest.

This study leverages the distinct advantages of Raman spectroscopy to conduct a detailed phytochemical investigation of the leaves and flowers of *Primula vulgaris*. The primary objectives are to obtain a comprehensive molecular fingerprint of the plant using a non-destructive approach, and to identify the characteristic Raman spectral signatures of its principal bioactive constituents, including flavonoids and phenolic acids.

2. Materials and Methods

2.1. Plant materials

Plant materials were collected during March 2025 to capture their peak development and flowering period. In order to maintain accuracy plant individuals were collected based on their health and age (same stage of blossoming). *Primula vulgaris* plants were collected in two different and contrasting habitats (Figure 1), the first is situated in a forest area of Dajti Mountain at an altitude of 1023.9 meters above sea level. This area is characterized by a thick layer of leaf litter and exposed limestone rocks.

Figure 1. *P.vulgaris* plants from two different habitats
Left: *P.vulgaris* from Tirana Hills
Right: *P.vulgaris* from Dajti Mountain

P. vulgaris plants were observed growing in clusters in a habitat of limited tree cover allowing filtered sunlight to reach the forest floor. The soil is moist and the presence of a water source was observed while the sloping topography functions as a natural drainage to avoid water logging. The second collection site is located in the Tirana Hills where plants were developed at an altitude of 217 meters above sea level. This is a hillside area has a low herbaceous cover and a sloped terrain exposed to higher levels of sunlight.

2.2. Chlorophyll content assessment and morphometric analyses

Plants were transported to the laboratory for chlorophyll content measurements and further morphometric analysis, including leaf length, and width. Chlorophyll content was evaluated for each plant separately in all their leaves. CCM 200 type chlorophyll content meter was used to measure chlorophyll in the leaves of *Primula vulgaris* plants. After ensuring that the leaves are clean and free of any debris or water droplets that might affect the readings, chlorophyll readings were obtained by positioning the leaf surface between the meter's sensors and recording the values. Each leaf was measured three times, and the average value was used for analysis.

2.3. Application of 1064 nm Raman spectroscopy for non-destructive analysis of Primula vulgaris

Raman analysis was conducted using B&W Tek i-Raman Ex spectrometer. The instrument utilized a wavelength of 1064 nm. Plant leaves and flowers are rich in endogenous fluorophores, such as chlorophylls and carotenoids, which absorb strongly in the visible region of the electromagnetic spectrum [10]. Laser power was adjusted between 45% and 70% based on individual sample characteristics to optimize signal quality, while preventing sample degradation. Measurement times ranged from 50 to 240 seconds, depending on the specific sample type being analyzed. The spectral resolution achieved was between 3 and 13 nm, covering a wavenumber range of 26 to 90 cm^{-1}. More than 100 spectra were collected during this experiment to ensure the reproducibility of the information acquired.

A major advantage of Raman spectroscopy is that it requires minimal sample preparation, allowing for analysis in a state that closely approximates the natural biological condition. Fresh, intact leaves and flowers of *P. vulgaris* were used for the analysis. For direct measurements, the plant material can be placed on a sample holder and analyzed without any pre-treatment.

3. Results and Discussion

3.1. Morphological variation and Raman spectroscopic characterization

Boxplots provided a clear visual indication of the differences between leaves from each area (Figure 2 and Figure 3). These differences are also confirmed by statistical analyses. As a result of data not normally distributed, non-parametric Mann-Whitney U test was used. Results showed that plants from Tirana Hills developed wider and longer leaves in response to higher sunlight exposure. Dajti Mountain plants on the other hand exhibited higher chlorophyll content levels (Figure 4) suggesting enhanced photosynthetic efficiency in partially shaded, humid conditions and higher elevation.

These statistically significant differences suggest that ecological influences such as altitude and light exposure influence photosynthetic capacity and functional traits in different populations of *Primula vulgaris*.

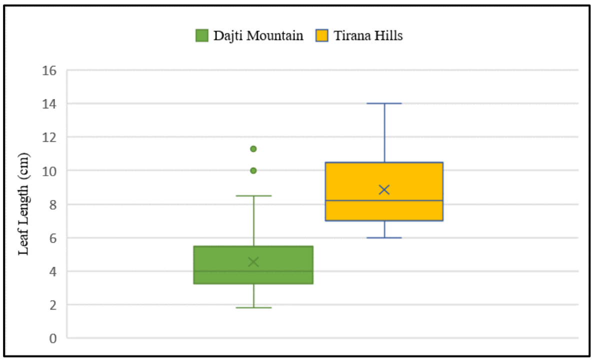

Figure 2. Leaf Length Comparison of *P. vulgaris* from two different locations

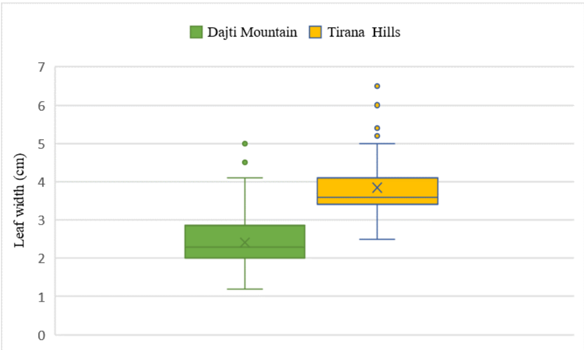

Figure 3. Leaf Width Comparison of *P. vulgaris* from two different locations

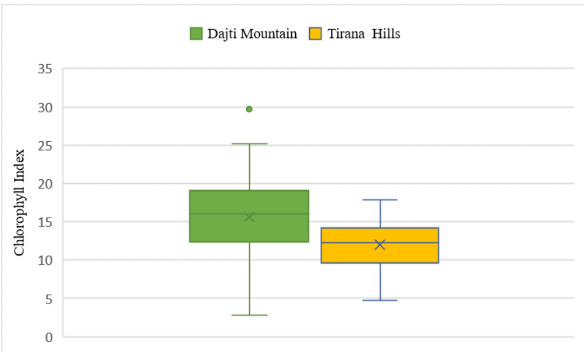

Figure 4. Chlorophyll Index Comparison in *P. vulgaris* from two different locations

The Raman spectra of *Primula vulgaris* flowers and leaves were acquired, revealing distinct peaks indicative of various functional groups and molecular structures (Figure 5 and 6). The Raman spectroscopic analysis of *Primula vulgaris* leaves and flowers yielded rich and complex spectra, characterized by a series of well-resolved peaks across the fingerprint region from 100 to 1700 cm⁻¹. The spectra from both leaves and flowers showed remarkable consistency in the positions of the major peaks, though relative intensities varied, reflecting differences in the concentration of various compounds in these distinct plant organs. A comprehensive table of major Raman peaks, their corresponding wavenumbers, and vibrational mode assignments is presented in Table 1. These assignments highlight the presence of key functional groups such as C-C, C-H, C=O, and characteristic aromatic ring structures.

3.2. Identification of bioactive compounds

The observed Raman spectroscopy peaks in *P. vulgaris* are particularly characteristic of flavonoids, a class of polyphenoloic compounds. This is evidenced by the presence of key vibrational modes commonly found in the core structure of flavonoid compounds, including aromatic ring stretching, $C - H$ bending and $C = C$ stretching. Previously reported flavonoids in *Primula vulgaris* such as quercetin, isorhamnetin, kaempferol, luteolin, and apigenin align with these spectral features. The general Raman signature for flavonoids is characterized by strong bands arising from the vibrations of their core phenyl-benzopyran skeleton, including aromatic ring stretching, C-H bending, and C=C stretching modes [11]. Specific characteristics structural features of these flavonoids correspond to distinct Raman vibrational modes: approximately 996 cm^{-1} (in-plane bending of CH, stretching mode of ring), 1068 cm^{-1} (in-plane bending of CH), 1183 cm^{-1} (bending mode of C-H), 1430 cm^{-1} (in-plane bending of CH_3), 1606 cm^{-1} (stretching mode of C=C in aromatic rings), and 1647 cm^{-1} (which can be attributed to either C=O stretching or C=C stretching in aromatic compounds) [12,13,14]. The presence of rutin is specifically confirmed by the peaks at 1606 cm^{-1} and 1647 cm^{-1} [15].

Table 1. Major Raman Peaks and Vibrational Mode assignments in *Primula vulgaris*

Wavenumber [cm^{-1}]	Assignment
101	(C-C-C) in plane bending [16]
317	out of plane bending (CO), out of plane ben [17]
366	in plane bending (CO) [17]
517	out of plane bending (ring) [17]
598	out of plane bending (CO), stretching (ring) 16a. [17]
735	stretching (CH) + stretching (OH). [17]
850	in plane bending/deformation (CH) [18]
996	in plane bending of (CH), stretching mode (ring) [18]
1068	in plane bending of (CH) [17]
1183	Bending mode of C-H [18]
1430	In plane bending of CH_3 [18]
1450	In plane bending of CH_2 [16]
1493	Bending mode of CH_2 [16]
1606	Stretching mode of C=C in aromatic [18]
1647	Can be either stretching mode of (C=O) or stretching mode of C=C (aromatic) [18]

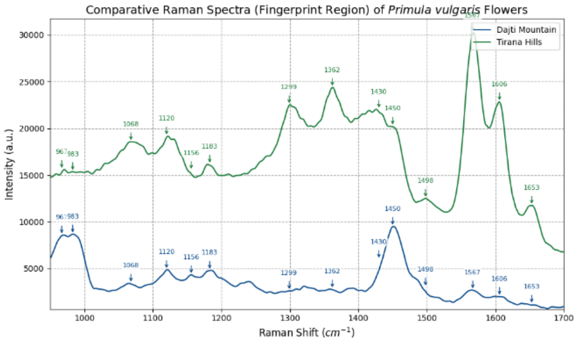

Figure 5. Comparative Raman spectra of *Primula vulgaris* “Flowers”, wavelength 1064nm

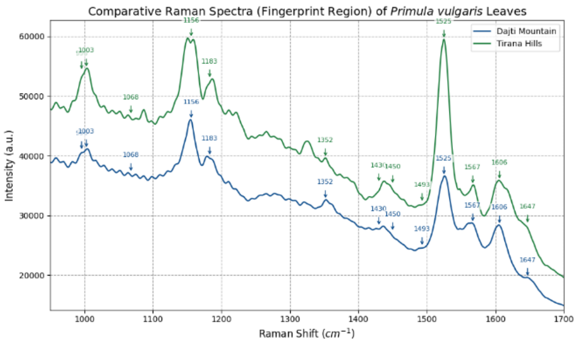

Figure 6. Comparative Raman spectra of *Primula vulgaris* “Leaves”, wavelength 1064nm

Furthermore, the analysis confirmed the presence of common hydroxybenzoic acids, including salicylic acid, protocatechuic acid, syringic acid, and vanillic acid. Key wavelengths justifying their structures include 1667 cm^{-1} (C=O stretching mode of the carboxylic acid group, COOH) [19], 735 cm^{-1} (OH stretching) [19,20,21], 1606 cm^{-1} (C=C stretching in the aromatic ring) [19],[21], ,and 1430 cm^{-1} (in-plane bending of CH_3) [19],[21],[20]. Common hydroxycinnamic acids such as caffeic acid, *p*-coumaric acid, and *o*-coumaric acid were also identified. Their presence is supported by key wavelengths: 1606 cm^{-1} (aromatic ring), 735 cm^{-1} (phenolic OH groups), and 1647 cm^{-1} (C=C in the cinnamic acid side chain) [18].

4. Conclusion

The comparative analysis of *Primula vulgaris* populations from two different locations revealed clear differences in leaf morphology and chlorophyll content affected by environmental conditions. These findings emphasize the impact of ecological factors such as altitude, light availability and moisture on the *P. vulgaris* traits, highlighting the species ability to adapt morphologically and physiologically to environmental conditions. This adaptive capacity is supported by the plants phenotypic plasticity, which enables *P. vulgaris* to grow in a wide range of light conditions [22]. Under controlled growth conditions *P. vulgaris* has demonstrated the ability to adapt to water availability by a reduction mechanism in stomatal conductance and transpiration, however it is not considered a drought tolerant plant [25]. These findings have practical implications for conservation efforts and for optimizing *P. vulgaris* cultivation to enhance its medicinal properties.

In this study, we used Raman spectroscopy as a fast, reliable, and non-destructive method for the phytochemical profiling of *Primula vulgaris*. The analysis of the plant’s leaves and flowers gave us a detailed molecular fingerprint, confirming a complex and therapeutically important mixture of bioactive compounds. Our main findings include the clear identification of spectral features from major polyphenol classes: **Flavonoids**, with strong evidence for quercetin and their glycoside, rutin; **Hydroxybenzoic acids**, the class that contains anti-inflammatory salicylates; and **Hydroxycinnamic acids**, including derivatives of caffeic and coumaric acids. Referring to the superimposed spectra of both flowers (Figure 5) and leaves (Figure 6) the samples measured in the same conditions show the same wavenumber peaks in both locations but clearly with different intensities. The high difference in the relative signal intensity of the compounds found and mentioned in this study shows that the samples collected from the Tirana Hills have a higher concentration of these bioactive compounds in the low-altitude with high-sunlight population.

To address the challenge of spectral overlap identified in this study, where multiple compounds contribute to the same Raman bands, future work should incorporate multivariate data analysis. Advanced chemometric methods, such as Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression, can be used to deconvolve the complex, overlapping spectra. This would allow for a more precise differentiation and semi-quantification of individual compounds within the mixture, unlocking a deeper level of chemical information from the spectral data [23].

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