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Research of Pyrrolizidine Alkaloids in Senecio platyphyllus Using a UPLC-MS Method

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Abstract: The aim of the study was to study the chemical composition of the Georgian endemic plant Senecio platyphyllus, in particular, to develop methods for the identification and quantification of pyrrolizidine alkaloids using ultrahigh-performance liquid chromatography-mass spectrometry (UPLC-MS). Senecio platyphyllus is rich in pyrrolizidine alkaloids (platyphylline, seneciphylline, sarracine, as well as the N-oxide form of platyphylline) and their content is present in all anatomical parts of the plant. It is known for its spasmolytic activity and is widely used in the therapy of pathologies of the cardiovascular and respiratory systems. The supercritical water extraction (SWE) method was used to prepare the samples, using water containing 2% sulfuric acid, which ensured maximum extraction of alkaloids. The extraction was performed twice, each with a 10-minute cycle, which was optimally selected considering both yield and analysis time efficiency. Qualitative and quantitative analysis of pyrrolizidine alkaloids was performed using a UPLC-MS system (Waters UPLC Acquity QDa), which provided simultaneous, highly sensitive and specific determination of sarracine, senecifilline, platyphylline, and platyphylline N-oxide, based on calibration curves of standard compounds.

Keywords: Senecio platyphyllus, Pyrrolizidine alkaloids, Platyphylline , UPLC-MS

Introduction

Senecio platyphyllus (Harishubla) is an endemic plant of Georgia and Turkey, notable for its high content of pyrrolizidine alkaloids, particularly platyphylline, seneciphylline and saracine. Traditionally, a preparation obtained from it (platyphylline hydrotartrate) has been used to treat abdominal smooth muscle spasms and to dilate the pupil. However, classical extraction technologies employ reagents that are harmful to the environment. Current research focuses on “green” extraction methods (e.g., high-pressure water extraction) and process control by UPLC, which ensure environmental friendliness and high product quality. For the determination of pyrrolizidine alkaloids, ultra-high-performance liquid chromatography combined with mass spectrometric detection (UPLC-MS, Waters UPLC Acquity Qda) was used; this method enabled the simultaneous identification of the alkaloids saracine, seneciphylline, platyphylline and its N-oxide (Aşçı et al., 2018; Usta et al., 2009; Yayli, 2008).The genus Senecio (Asteraceae) is one of the most speciose taxa and is characterized by broad chemical diversity and biological activity. Pyrrolizidine alkaloids (PAs) are particularly important in species of this genus; they are regarded as the principal toxic components and are responsible for hepatotoxicity (Lu et al., 2021). These compounds are widely distributed in the plant kingdom (approximately 3–5%), but their

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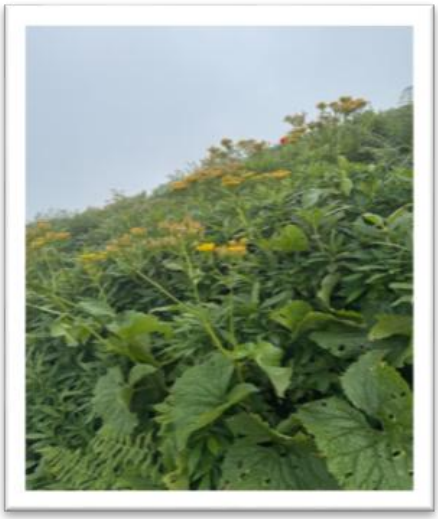
occurrence in Senecio species attracts special attention from both toxicological and chemotaxonomic perspectives. (Lu et al., 2021; Sadgrove, 2022). Modern studies actively employ high-performance analytical methods, especially liquid chromatography coupled with mass spectrometry (LC-MS, UPLC-MS), for rapid and sensitive identification of pyrrolizidine alkaloids. It has been established that these compounds can be identified by their characteristic fragmentation patterns, such as ions at m/z 120, 122, 124, 138, 140 and 142, which indicate the presence of various types of saturated/unsaturated, lactone or monoester alkaloids (Lu et al., 2021). This approach considerably accelerates analysis and improves method accuracy, which is important for comprehensive characterization of plant chemical profiles. Differentiation of Senecio species is often difficult due to morphological similarity; therefore metabolomic approaches gain particular importance. UPLC-MS–based metabolomics is effectively used to distinguish closely related species, for example Senecio scandens and S. vulgaris, which is especially relevant given their differing toxicities (XiongLi et al., 2011). Such approaches support both pharmacognostic identification and quality control of plant material.Phytochemical studies show that Senecio plants contain a wide range of secondary metabolites, including flavonoids, terpenoids, steroids, tannins, saponins and glycosides, although pyrrolizidine alkaloids remain the main focus. For example, senecionine and seneciphylline have been identified in Senecio vulgaris, and their structures were confirmed by HPLC and FTIR methods (Kamil Hamid et al., 2016). These compounds play important roles in plant defense and in toxicity.It is noteworthy that the chemical profile of Senecio is not limited to alkaloids. Studies indicate that sesquiterpenes (e.g., eremophilanes, bisabolols), flavonoids and other natural compounds are widespread in this genus and often co-occur with alkaloids (Sadgrove, 2022). In some species the sesquiterpene content even surpasses that of pyrrolizidine alkaloids, reflecting a high level of chemical diversity and research gaps, particularly for African species (Sadgrove, 2022). Advances in extraction technologies for bioactive compounds have made significant contributions to the study of Senecio chemical composition. Modern methods such as ultrasonic-assisted extraction (UAE), pressurized liquid extraction (PLE) and microwave hydrodiffusion (MHG) provide high yields of alkaloids, including integerrimine and senecionine (Confortin et al., 2021). These technologies not only increase extraction efficiency but also enable identification of a larger number of compounds. Senecio species also exhibit notable biological activities. For instance, extracts of Senecio tenuifolius showed strong antibacterial activity against Staphylococcus aureus, with active fractions identified by GC-MS (Manubolu et al., 2013). Additionally, essential oils of S. glaucus and S. vernalis demonstrate antioxidant, antimicrobial and cytotoxic activities, attributable to high contents of monoterpenes and sesquiterpenes (Bagci & Kilic, 2012; Balpinar & Okmen, 2019). Chemotaxonomic studies indicate that essential-oil composition of Senecio species may be used for species classification. For example, the main components reported in S. Vernalis β-phellandrene, 1,8-cineole and caryophyllene oxide reflect that species' chemical specificity (Bagci & Kilic, 2012). Moreover, phenolic compounds and alkaloids found in different extracts determine antioxidant and antimicrobial activities (Balpinar & Okmen, 2019). In conclusion, species of the genus Senecio constitute an important subject of pharmacological and toxicological interest. Pyrrolizidine alkaloids remain the most critical constituents of this genus, although other bioactive compounds also play significant roles. Use of modern analytical methods and extraction technologies allows deeper investigation of their chemical composition and biological activities, which is necessary both for safety assessment and for discovery of new bioactive compounds (Lu et al., 2021; Bagci & Kilic, 2012; Balpinar & Okmen, 2019). Unfortunately, in the context of Georgia, recent studies employing modern methods have not been conducted.

Plant Material and Sample Preparation

Senecio platyphyllus (locally known as Kharishubla) is an endemic plant species of Georgia, distributed in the high-mountain region of Adjara. Plant material was collected at the peak of its vegetative period, during the flowering stage, from July through September. The collection site was the high-mountain area of Adjara in western Georgia, specifically Mount Gomi (Fig. 1), at an altitude of approximately 2031.3 m above sea level, which ensures the ecologically clean origin of the samples.

After collection, the plant material was divided into two parts for processing and storage. One part, intended for chemical analyses, was stored at (–25 °C) in order to preserve the original chemical composition of the plant material and to prevent the degradation of biologically active compounds. The second part was subjected to processing. Using a specially developed methodology, extraction procedures were carried out on this portion. The aim of the extraction was the efficient isolation and concentration of the main biologically active constituents, particularly pyrrolizidine alkaloids (platyphylline, seneciphylline and saracin), from the different parts of the plant (roots, stem, leaves, flowers) for subsequent phytochemical analysis and preparation of pharmaceutical formulations.



Dec Degs 41.830524° 42.164656°
Dec Min Secs 41°49'49.9"N 42°09'52.8"E
Figure. 1 Gomi mountain

Instrumentation and Analytical Methods

The study of pyrrolizidine alkaloids was carried out using UPLC-MS chromatographic methods. An analytical column (Phenyl 3.5 µm, 4.6 × 150 mm) and a mobile phase system consisting of 0.2% formic acid–acetonitrile water in gradient mode were used. For the determination of pyrrolizidine alkaloids, ultra-high-performance liquid chromatography coupled with mass spectrometric detection (UPLC-MS, Waters UPLC Acquity QDa) was employed. This method enabled the simultaneous identification of the alkaloids saracin, seneciphylline, platyphylline and its N-oxide, as well as their quantitative determination using reference standards.

Results and Discussion

A study was carried out on the qualitative and quantitative composition of pyrrolizidine alkaloids in all anatomical parts (flower, leaf, stem, root) of Kharishubla (Senecio platyphyllus). Using ultra-high-performance liquid chromatography (UPLC-MS), the following compounds were identified and quantitatively determined:

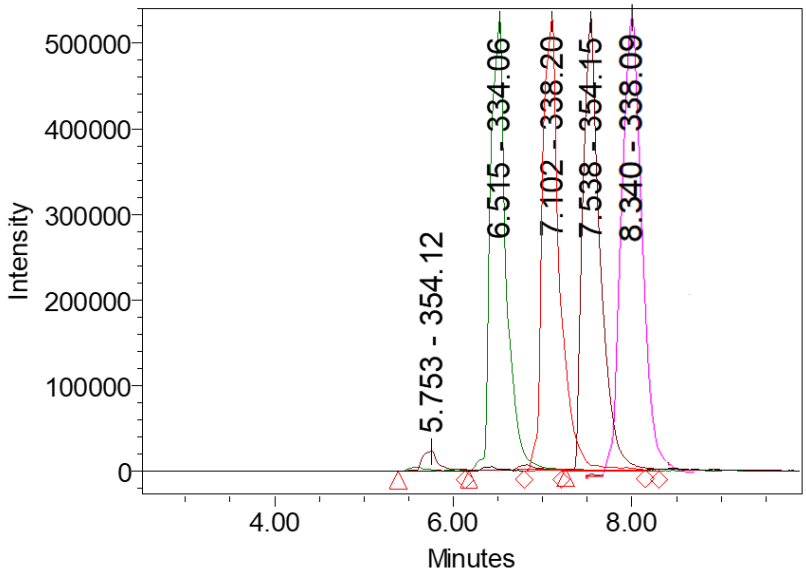


Figure 2. Chromatograms

Substances 1 seneciphylline ESI-MS-m/z 334.06 [M+H], MF: $C_{18}H_{23}NO_5$ MW: 333.4 g/mol IUPAC Name: (1R,4Z,7R,17R)-4-ethylidene-7-hydroxy-7-methyl-6-methylidene-2,9-dioxa-14 azatricyclo[9.5.1.014,17] heptadec-11-ene-3,8-dione. (Retention time – 6,515 min)

Substances 2 saracine ESI-MS-m/z 338.20 [M+H], MF: $C_{18}H_{27}NO_5$ MW: 337.4 g/mol IUPAC Name: [(1*S*,7*R*,8*R*)-7-[(*Z*)-2-methylbut-2-enoyl]oxy-2,3,5,6,7,8-hexahydro-1*H*-pyrrolizin-1-yl]methyl (*Z*)-2-(hydroxymethyl)but-2-enoate. (Retention time – 7,102 min)

Substances 3 platyphylline ESI-MS-m/z 338. 41 [M+H], MF: $C_{18}H_{27}NO_5$ MW: 337.4 g/mol IUPAC Name: (1R,4Z,6R,7R,11S,17R)-4-ethylidene-7-hydroxy-6,7-dimethyl-2,9-dioxa-14-azatricyclo[9.5.1.014,17]heptadecane-3,8-dione. (Retention time – 8.340 min)

Substances 4 platyphylline N-oxide ESI-MS-m/z 354.15 [M+H], MF: $C_{18}H_{27}NO_6$ MW: 353.4 g/mol IUPAC Name: (1R,4Z,6R,7S,11S,17R)-4-ethylidene-7-hydroxy-6,7-dimethyl-14-oxido-2,9-dioxa-14-azoniatricyclo[9.5.1.014,17]heptadecane-3,8-dione. (Retention time – 7,538 min)

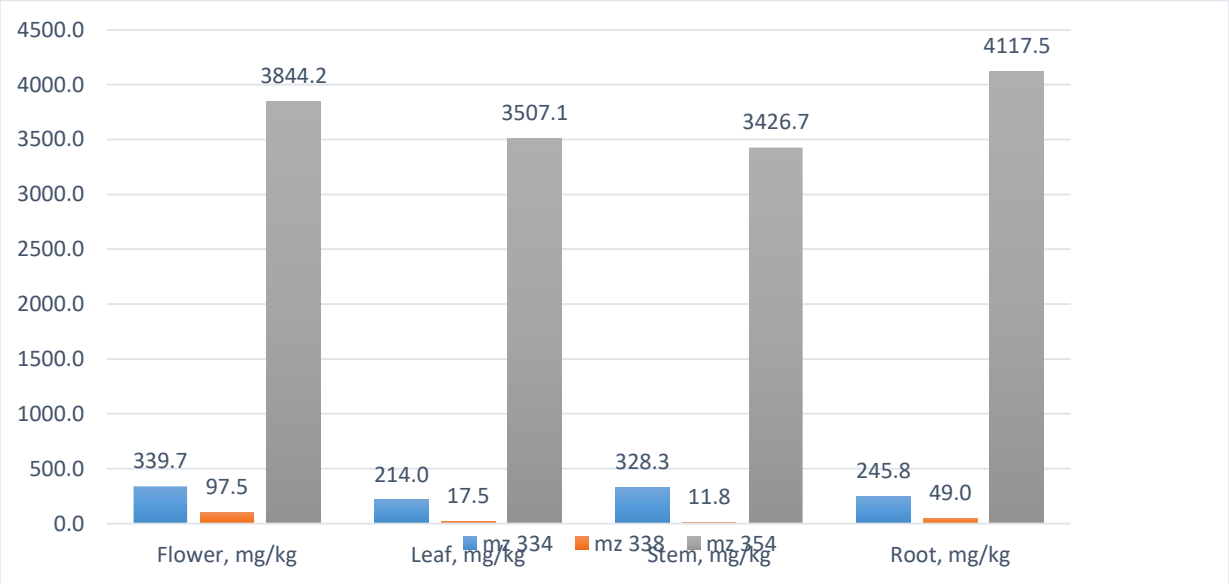


Diagram 1. Quantitative determination of pyrrolizidine alkaloids in Kharishubla (Senecio platyphyllus)

Different levels of pyrrolizidine alkaloids, namely seneciphylline, saracin and platyphylline N-oxide, were detected in various parts of Senecio platyphyllus, indicating the high alkaloid potential of this plant. The N-oxide form of platyphylline is the dominant pyrrolizidine alkaloid and is distributed throughout all parts of the plant, with the highest concentration found in the root (4117.5 mg/kg) and relatively lower levels in the other parts (leaf 3507 mg/kg, stem 3426 mg/kg). The highest content of seneciphylline is observed in the flower (339.7 mg/kg), followed by the leaf (328.3 mg/kg) and the root (245.8 mg/kg), while the stem has the lowest concentration (214.0 mg/kg).

Conclusion

Pyrrolizidine alkaloids of Kharishubla (Senecio platyphyllus) collected from the Gomi Mountain location were studied using supercritical water extraction (SWE) and the UPLC-MS method. The composition of pyrrolizidine alkaloids was compared in all anatomical parts of the plant. Optimal extraction conditions were developed, which increased the yield of the target compounds.Based on the results, it was established that the highest content of platiphylline was recorded in the root, seneciphylline and saracin in the flower.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

Conflict of Interest

* The authors declare that they have no conflicts of interest.

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