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# Phytochemical Study of Endemic Species Helleborus Caucasicus and Helleborus Abchasicus

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**Abstract**

The floristic region of Adjara represents the "Hotpoint" of Caucasians, which is distinguished by the uniqueness of its relict Colchis flora. It represents one of the most powerful refuges in western Eurasia, which is not touched by the chill because of its special geographical location. 176 endemic plants are spread in southern Colchis, of which 45 can be used for some medical treatments. The bioecology and detailed phytochemical content of some medicinal plant populations have not been studied so far. The research objective is to study the phytochemical content of endemic species of *Helleborus Caucasicus* and *Helleborus Abchasicus* that have spread in southern Colchis. The research method for the phytochemical content included the separation analysis, which was performed using UPLC-MS (Waters Acquity QDa detector). Three Steroidal glycosides were isolated from the MeOH extract of the plants of *Helleborus Caucasicus* and *Helleborus Abchasicus*: Hellebrigenin-D-glucose, 20 – Hydroxyecdysone and Hydroxyecdysone – 3 glucoside. Three Steroidal glycosides and Hydroxyecdysone -3 glucoside have been isolated from the MeOH extract of *Helleborus Caucasicus*.

*Keywords:* Phytochemistry; Bioecology; UPLC-MS; Helleborus Caucasicus; Helleborus Abchasicus.

## 1. Introduction

The floristic region of South Kolkheti (Adjara, Georgia) is part of the Caucasus Ecoregion, which is included among the 200 world-renowned ecoregions by the World Wildlife Fund (WWF). These ecoregions are characterized by plant diversity, high levels of endemism, taxonomic uniqueness, and the rarity of biomes globally [1].

Southern Kolkheti (Adjara), in the Caucasus ecoregion, is characterized by the special diversity and originality of the flora, which is present due to the flora complexes rich in plant clusters, relics, and endemic species formed in the third period [2]. There are 1837 species of plants common in southern Colchis, including 176 endemic ones [3]. Among the endemics, the following genera are distinguished by their decorative and medicinal properties: *Helleborus Caucasicus* and *Helleborus Abchasicus* flower in winter-early spring [4]. The genus Helleborus is represented by 2 species: *Helleborus Caucasicus* and *Helleborus Abchasicus* [5].

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*Helleborus Caucasicus* and *Helleborus Abchasicus* (Ranunculaceae) are evergreen, blooming in the autumn-winter-spring seasons, rooted, herbaceous plants, growing on cliffs. Their vegetation begins at the end of November, blooming starts in December, and fruiting is in progress in March-April. Among these species, *Helleborus caucasicus* and *Helleborus Abchasicus* are widely distributed. *Helleborus caucasicus* is an important source of chemical compounds with great medical potential for the treatment of some serious diseases. Glycosides, bufadienolides, monocytes, biocides, and steroid saponins are found in its roots and rhizomes. Among them is 0.1 percent of colerborine P, which has an effect similar to that of stroftineon in the heart. Colerborine P is used for circulatory disorders of quality II and III, most often in chronic heart failure. This has a particularly long and fast effect. In folk medicine in Adjara, the decoction of the root and rhizomes of Helleborus is used, taking into account the dosage due to its toxic properties (1/2 teaspoon of roots in 0.5 l of water) for the treatment of cancer, hemorrhoids, cough, pleurisy, tuberculosis, purulent wounds, dandruff, diseases of the joints, diabetes, urological diseases, diseases of the liver, nervous system, and kidneys; it is also used to lose weight [6-8].

It is the first time that we have studied the detailed phytochemical content of *Helleborus caucasicus* and *Helleborus abchasicus* rootstocks in southern Colchis.

## 2. Methods and Materials

Plant material: the leaves and rhizomes of two species-*Helleborus caucasicus, Helleborus Abchasicus that* were collected in Adjara (Table 1).

**Table 1. Information about test samples**

| # | Test species | Samples collected area | Samples data |
|---|---|---|---|
| 1 | *Helleborus caucasicus* | v. 1 Maisi, Adjara | February 2020 |
| 2 | *Helleborus abchasicus* | s. Kutaisi, Imereti | February 2020 |

Ultra Performance Liquid Chromatography (UPLC)-Preparation of a sample for chromatographic examination of saponins: Various parts of the plant were taken for analysis - the rhizomes and leaves of *Helleborus caucasicus* and *Helleborus abchasicus* as. Raw material of the sample was taken for analysis; Extraction of the crushed sample (2.5 g) was performed with methanol (100% 50-50 ml) three times in an ultrasound bath. The next step intended to filter the extracts by using a vacuum pump. We concentrated methanolic extracts at a temperature of $400^0$C under vacuum conditions until aqueous residue. (In the case of concentrated leaf extract, the sample was further treated with chloroform to remove chlorophyll green pigments). We divided the concentrated water fraction by C18. In the initial stage, the sorbent was conditioned; in particular, the sorbent was activated with methanol and balanced by using water. In the first stage after sampling, we removed unwanted components with water. In the final stage, the research components were eluted with methanol (100%). The resulting eluent was later concentrated to a dry mass. For chromatographic analysis, dry mass extraction was performed by using the mobile phase (acetonitrile: a mixture of methanol). The sample for chromatography was filtered inèto a 0.45 μm filter.

Concentration of analytical samples: *Helleborus caucasicus* rhizomes - g/80 μl (15 g / 1200 μl) and leaves - g/4 ml (15 g/60 ml) and *Helleborus abchasicus* rootstock - g/200 μl (10 g/2000 μl) and leaves - g/4 ml (10 g/40 ml).

## 3. Results and Their Review

The detected Steroidal composition of *Helleborus caucasicus, Helleborus abchasicus* are presented in Table 2.

**Table 2. Steroidal composition of *Helleborus caucasicus, Helleborus abchasicus***

| # | Species name: *Heleborus caucasicus, Helleborus abchasicus* | | | *Helleborus Caucasicus* | | *Helleborus abchasicus* | |
|---|---|---|---|---|---|---|---|
| | | Mass | ESI-MS m/z | Tubers | Flowers | Tubers | Flowers |
| 1 | 20 - Hydroxyecdysone (Ecdysterone)C27H44O7 | 480.3087 | | + | + | + | + |
| 2 | Bufadienolide C24H34O2 | 354.2558 | 503.2[M +Na]+ | + | | + | |
| 3 | Furostan C27H46O | 386.3548 | 355.2 [M + H]+ | + | + | + | + |
| 4 | Hellebrigenin-D-glucoseC30H42O11 | 578.2726 | 431.32 [M+2Na-H]+ | + | | + | |

Four steroidal compounds were isolated from the MeOH extract (the tubers and leaves ) of *Helleborus caucasicus* and *Helleborus abchasicus:* 20- Hydroxyecdysone (Ecdysterone), Bufadienolide, Furostan and Hellebrigenin-D-glucose. All four substances are identified in the extract of the rhizomes, while in the flowers 2 - Ecdysterone and Furostan.

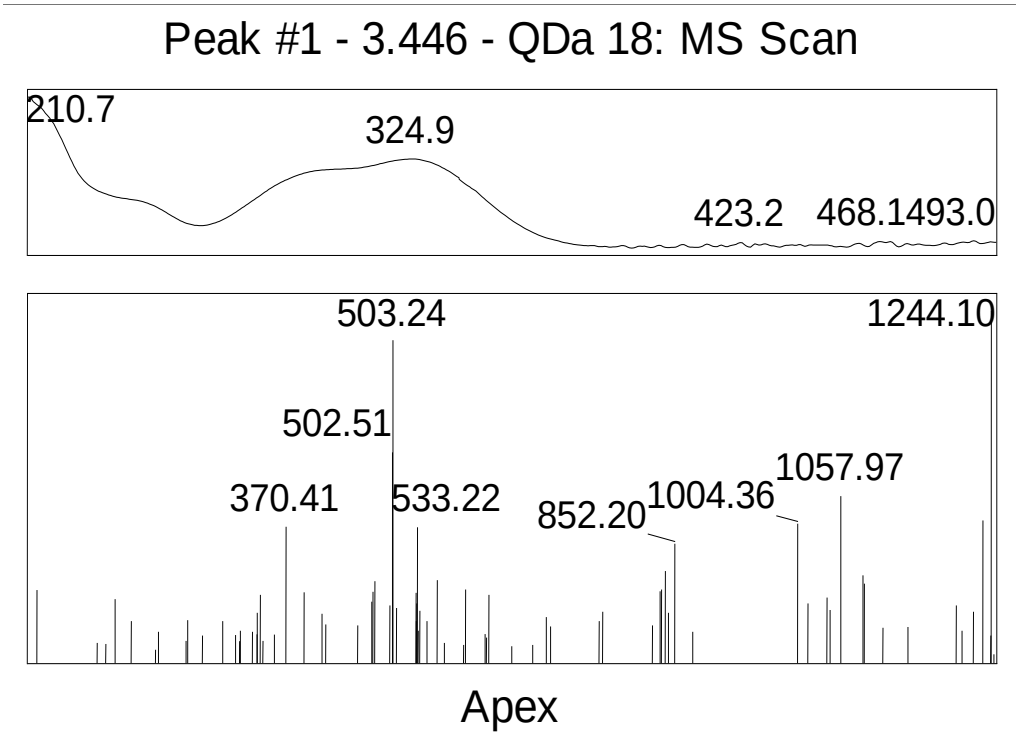


**Figure 1. MS scan ESI-MS m/z: 503 [M+H] +**

The substance 1 - (Figure 1) is retention time 3.446 min, ʎ max324 nm (Table 2); In positive ionization mode, substance 1 mainly showed molecular ions ESI-MS m/z: 503.2 [M +Na]+; according to the obtained results and compounds mass database METLIN (*https://metlin.scripps.edu*) the substance 1 was identified as 20-Hydroxyecdysone (Ecdysterone) [9-11].

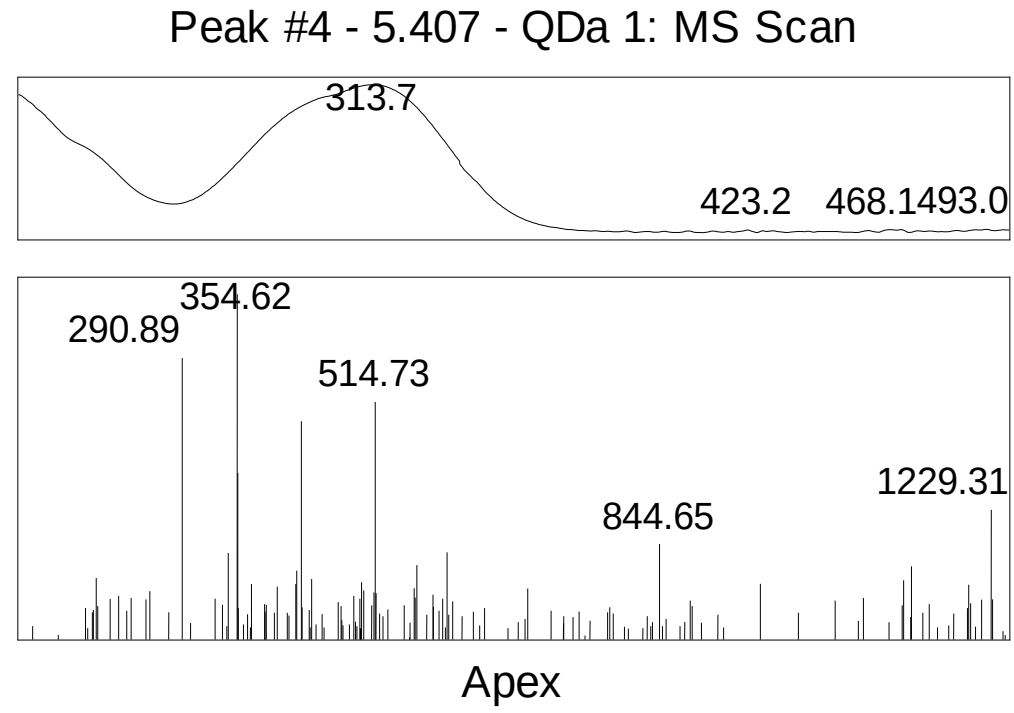


**Figure 2. MS scan ESI-MS m/z: 355 [M+H]+**

The substance 2 - (Figure 2) is retention time 5.407 min, ʎ max 313.7 nm (Table 2); In positive ionization mode, substance 2 mainly showed molecular ions ESI-MS m/z: 355.26 [M + H]+; according to the obtained results and compounds mass database METLIN (https://metlin.scripps.edu) the substance 2 was identified as – Bufadienolide.

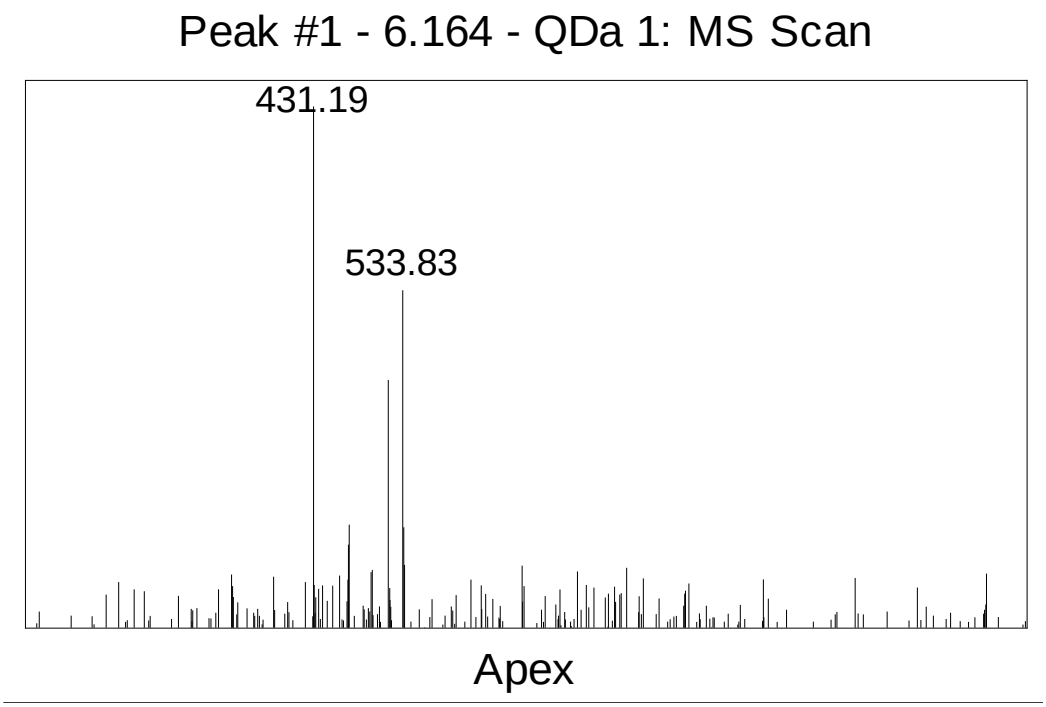


**Figure 3. MS scan ESI-MS m/z: 431 [M+H] +**

The substance 3 - (Figure 3) is retention time 6.164 min; In positive ionization mode, substance 3 mainly showed molecular ions ESI-MS m/z: 431.32 [M+2Na-H]+; according to the obtained results and compounds mass database METLIN (https://metlin.scripps.edu) the substance 3 was identified as Furostan.

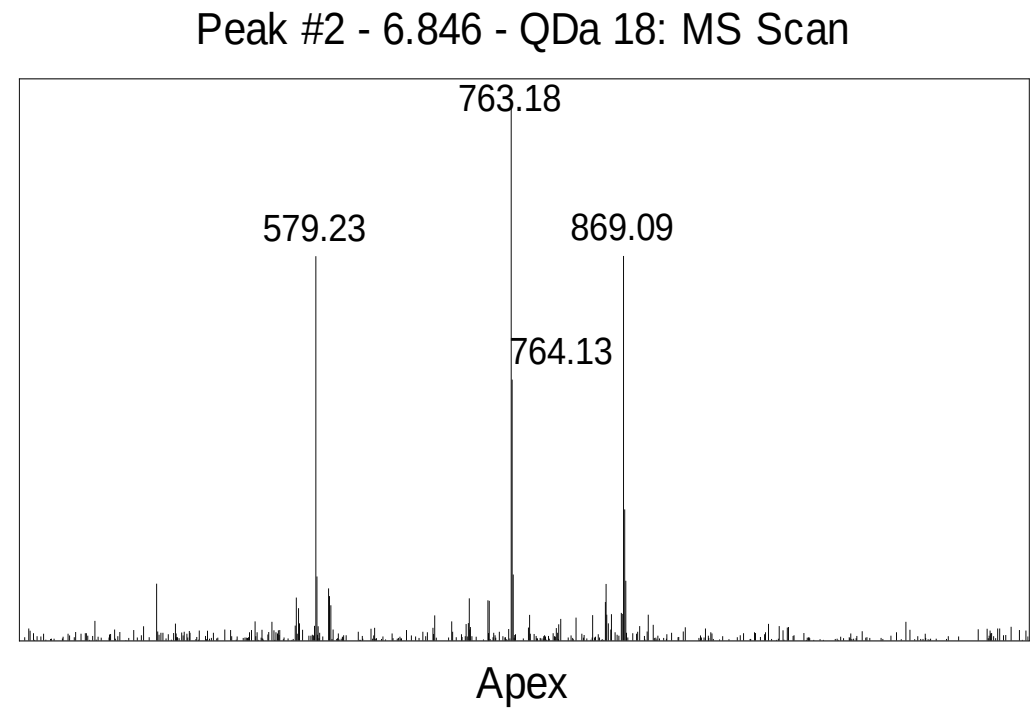


**Figure 4. MS scan ESI-MS m/z: 579+ [M+H]**

The substance 4 - (Figure 4) is retention time 6.164 min; In positive ionization mode, substance 4 mainly showed molecular ions ESI-MS m/z: 579+ [M+H]+; according to the obtained results and compounds mass database METLIN (https://metlin.scripps.edu) the substance 4 was identified as Hellebrigenin-D-glucose.

Four steroidal compounds, were isolated from the MeOH extract of *Helleborus caucasicus* and Helleborus *abchasicus:* 20- Hydroxyecdysone (Ecdysterone), Bufadienolide, Furostan and Hellebrigenin-D-glucose. All four substances are identified in the extract of the rhizomes, while in the flowers 2 - Ecdysterone and Furostan.

Using UPLC-MS/MS, the steroid composition of the plant Helleborus caucasicus and Helleborus abchasicus was studied. In particular, 4 substances have been identified, 2 of which are found in leaves - Ecdysterone and Furostan, and 4 in tubers - Ecdysterone, Bufadienolide, Furostan and Hellebrigenin-D-glucose. Based on the results obtained, it can be concluded that the steroid composition of leaves and tubers of Helleborus caucasicus and Helleborus abchasicus is similar.

## 4. Conclusion

Vegetation of *Helleborus caucasicus* and *Helleborus abchasicus* begins at the end of November, blooming starts in December, fruiting is in progress in March-April. Three Steroidal glycosides were isolated from the MeOH extract of the plants of *Helleborus caucasicus* and *Helleborus abchasicus*- Hellebrigenin-D-glucose, 20 – Hydroxyecdysone and Hydroxyecdysone – 3 glucoside. On the basis of the conducted analysis, it is possible to make a conclusion that three Steroidal glycosides were isolated from the MeOH extract of the plants of *Helleborus caucasicus* and *Helleborus abchasicus*- Hellebrigenin-D-glucose, 20–Hydroxyecdysone and Hydroxyecdysone−3 glucosides. Steroidal glycosides that contribute to the biological activity of the plants, were identified in the *Helleborus caucasicus* and *Helleborus abchasicus*.

## 5. Institutional Review Board Statement

Not applicable.

## 6. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## 7. References

[1] IUCN Standards and Petitions Committee. (2006). Guidelines for Using the IUCN Red List Categories and Criteria. Version 6.2. Prepared by the Standards and Petitions Committee, European Parliament, United Kingdom. Available online: https://nc.iucnredlist.org/redlist/content/attachment_files/RedListGuidelines.pdf (accessed on December 2019).

[2] Manvelidze, Z. K., Memiadze, N. V., Charazishvili, D. S., & Varshanidze, N. I. (2008). Diversity of a Floral Area of Adjara (List of Wild Grown Plant Species). Annals of Agrarian Science, 6(2), 91-93.

[3] Varshanidze, N., Turmanidze, N., Dolidze, K., Zarnadze, N., Diasamidze, I., Epitashvili, T., & Katcharava, T. (2018). Biodiversity of Medicinal Plants Containing Essential Oil and Their Spreading in Adjara. Universal Journal of Agricultural Research, 6(3), 99–104. doi:10.13189/ujar.2018.060301.

[4] Memiadze, N. (2004). Botanical and geographical survey of the endemics of Ajara-Lazeti flora. Bulletin of the Georgian National Academy of Sciences. 169 (2), 341-343.

[5] Dmitrieva, A. (1990). Key to flora of Adjara. Vol. II. Tbilisi: Metsniereba (in Russian).

[6] Jakeli, E., Varshanidze, N., Diasamidze I., Dolidze K., Zarnadze N. (2018). Biodiversity of medical plants of wild flora in Ajara-South Colchis and their usage in folk medicine. 3-rd International Science Symposium "New Horizons in Science", Proceeding Book. At Pristina, Kosovo. 80-96.

[7] Novotny, L., Ghuloom, H. A. A. A., & Al-Hasawi, N. A. (2019). Structural Features and Biological Activities of Bufadienolides. Research Journal of Pharmaceutical Biological and Chemical Sciences, 10(1), 1147-1157.

[8] Tomczyk, M., Gudej, J., & Sochacki, M. (2002). Flavonoids from Ficaria verna Huds. Zeitschrift Für Naturforschung C, Journal of Biosciences, 57(5-6), 440–444. doi:10.1515/znc-2002-5-606.

[9] Guijas, C., Montenegro-Burke, J. R., Domingo-Almenara, X., Palermo, A., Warth, B., Hermann, G., … Siuzdak, G. (2018). METLIN: A Technology Platform for Identifying Knowns and Unknowns. Analytical Chemistry, 90(5), 3156–3164. doi:10.1021/acs.analchem.7b04424.

[10] Muchate, N. S., Rajurkar, N. S., Suprasanna, P., & Nikam, T. D. (2019). NaCl induced salt adaptive changes and enhanced accumulation of 20-hydroxyecdysone in the in vitro shoot cultures of Spinacia oleracea (L.). Scientific Reports, 9(1). doi:10.1038/s41598-019-48737-6

[11] Mamadalieva, N. Z., Böhmdorfer, S., Zengin, G., Bacher, M., Potthast, A., Akramov, D. K., … Rosenau, T. (2019). Phytochemical and biological activities of Silene viridiflora extractives. Development and validation of a HPTLC method for quantification of 20-hydroxyecdysone. Industrial Crops and Products, 129, 542–548. doi:10.1016/j.indcrop.2018.12.041.