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Helleborus - phytochemistry and antimicrobial properties. A review

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Abstract The genus *Helleborus* is part of the *Ranunculaceae* family and includes over 20 species in spontaneous flora, of which *H. purpurescens* (common asparagus) and *H. odorus* (green asparagus) are also found in Romania. This study highlights the richness in phytocompounds of roots and rhizomes, respectively the antimicrobial and pharmacological activity of the two species of Helleborus. The main compounds identified in Helleborus extracts were cardiac glycosides, thionines, saponins, ecdisteroids, resins, lactones and minerals. *H. purpurascens* is rich in glycosides, polyphenolic compounds and tannins. Thionines have antimicrobial and antiviral activity, saponins have an antiproliferative, immunomodulatory, antihepatotoxic, cardiac effect. Glycosides have hemolytic and narcotic action (hellebore) and together with alkaloids and saponins have influenced the activity of the nervous system. Helleborus is also used as an anti-inflammatory, being known for its effectiveness in this condition, the product Boicil, used for a long time in our country. *Helleborus odorus* L. contains bufadenolides, flavonoids and phenolic heterosides, has an antiparasitic, purgative role and many applications in the veterinary field.

Key words

antimicrobial activity, phytoconstituents, *H. purpurescens, H. odorus,* pharmacological properties

Helleborus is native to Europe and Western Asia. The plant belongs to the genus *Helleborus,* family *Ranunculaceae,* class *Magnoliopsida,* division *Magnoliophyta* [18, 68]. Spânzul is a perennial plant with a well-developed rhizome and root system, large, palmate leaves and flowers with reduced petals, often transformed into nectariferous cones, arranged in racemose inflorescences [69].

The name Helleborus comes from the Greek word "elein" - wound and "bora" - food. Most species are concentrated in the Balkans and the Carpathians [35]. In Western countries, asparagus is known as the winter rose or the Christmas rose, an inappropriate name because roses belong to the Rosaceae family. It grows spontaneously in forests and pastures [39]. It multiplies by seeds and vegetatively. In vitro propagation is of interest for commercial production [18]. Micropropagation seems to be a difficult process, influenced by a wide range of factors, such as genotype, type of explants, phytohormones in culture media and environmental factors etc.

The genus *Helleborus* includes over 20 perennial species in the spontaneous flora. They are considered medicinal [18; 59; 69]. Among the most widespread species we mention: *Helleborus niger* L., *Helleborus cyclophyllus* L., *Helleborus dumetorum* L., *Helleborus odorus* L., *Helleborus orientalis* L., *Helleborus purpurascens* L., *Helleborus thibetanus* L., *Helleborus viridis* L. [68].

H. purpurescens is one of the most common species in eastern Europe, especially in the Carpathian Mountains [15]. There are two species in the spontaneous flora of Romania: *Helleborus purpurascens* L. (common asparagus) and *Helleborus odorus* L. (green asparagus) [8; 52]. *H. purpurescens* is a plant that grows on calcareous soils in deciduous and coniferous forests. Instead, *Helleborus odorus* L., grows in the southern part of Romania [46]. Helleborus is an ethnopharmacological species used since ancient times in Southeast Europe. Helleborus roots, rhizomes and leaves have traditionally been used for a wide range of ailments since the time of Hippocrates [35]. Although it contains toxic substances, its medicinal benefits are important and recognized, and research on its chemical and pharmacological composition is imperative. For example, *H. purpurascens* has been used for rheumatic pain, cardiovascular and nervous system problems [55]. In Europe and the USA, asparagus is still used today as a medicinal or ornamental plant. Although important steps have been taken regarding the biological activity of asparagus extracts, studies continue because many are still unknown regarding the composition and chemical structures of *Helleborus purpurascens* L. compounds [4; 24; 33; 66]. Based on this fact, the purpose of this paper is to present the chemical composition, pharmacological benefits and antimicrobial activity of *H. purpurescens* L and *H. odorus* L species, found in

our country. Various databases (USDA, Springer, Google Scholar, PubMed), books, and scientific articles were used for this paper.

Material and Method

Phytoconstituents

Modern analytical methods have made it possible to isolate and investigate in detail the structure and role of various chemical compounds in flax, but also the action of primary and secondary metabolites on microorganisms and the human body, subjects that are insufficiently studied [7]. In general, the extraction of compounds from Helleborus species is done by standard methods. According to Wittmann, (2008) [63] chromatography and mass spectrometry have proven to be effective techniques for identifying compounds in asparagus. For example, Franz (2018) [15] identified amino acids in asparagus by the GC-MS technique. Gregory et al. (2021)**,** performed qualitative chemical analyzes using HPTLC and quantitative determinations using HPLC.

A first report on the general chemical composition of asparagus dates back to 1943, when Karrer isolated cardiac glycoside (hellebine) from *H. niger* L. Other classes of compounds from different Helleborus species have been isolated since the 1970s [61; 62].

The essential components identified in Helleborus extracts include cardiac glycosides, thionines, saponins, ecdisteroids (Figure 2), resins, lactones and minerals. Cardiac glycosides are present in angiosperms [14]. The same author mentions that one of the first compounds isolated from the underground part of different Helleboreus species was heleborin.

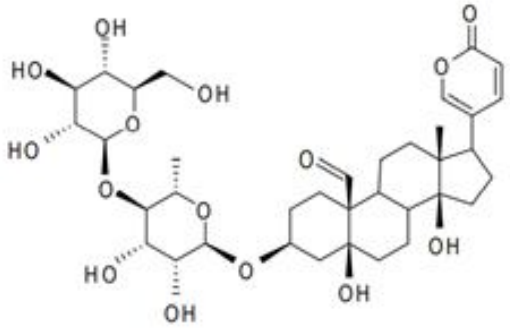

Figure 1. The structure of the helebrin [11, 35]

Cardiac steroids (helebrin) (Figure 1), cysteine-rich proteins (hellethionin) and some saponins [15] have been identified in Helleborus species in south-eastern Europe.

Chemical analyzes have shown that *H. purpurascens* L. is rich in glycosides, polyphenolic compounds and tannins [19, 49]. Muhr et al., (1995) [43] studied the chemical composition of the roots and rhizomes of *H. purpurascens* L, and showed that helebrine is found in higher concentrations in this plant compared to other species of asparagus. Saponins (Fig. 3) are steroids with great structural diversity, which explains their action on the human body, microorganisms and viruses.

Among the flavonoids Pilut et al., (2021) [46] mention the high concentration of quercetin, epi-Catechin and kaempferol from the extracts studied, mentioning that these compounds have higher antioxidant potential than ascorbic acid. Some studies show that in methanolic and hydroalcoholic extracts of *H. purpurascens* L. domina β-ecdizone and cardiac glycosides. Alshatwi (2014) [3a] states that lactones are found in the highest concentration. Among the lactones are mentioned especially protoanemonine (Figure 3) and bufadienolides.

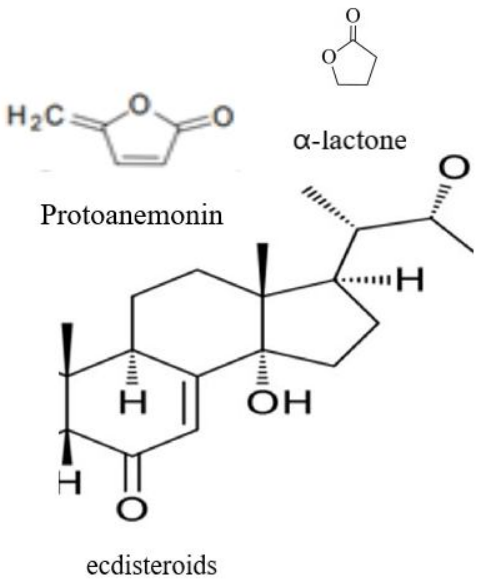

Figure 2. The structures of α-lactone, protoanemonine and ecdisteroids [3, 35, 53]

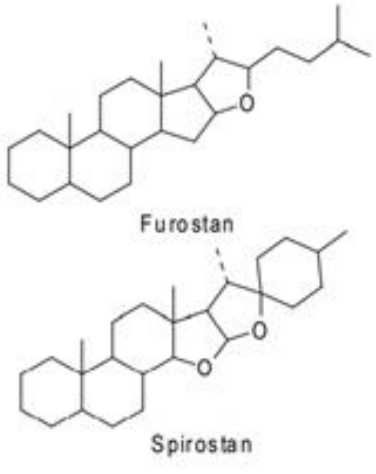

Figure 3. The structure of saponins [35]

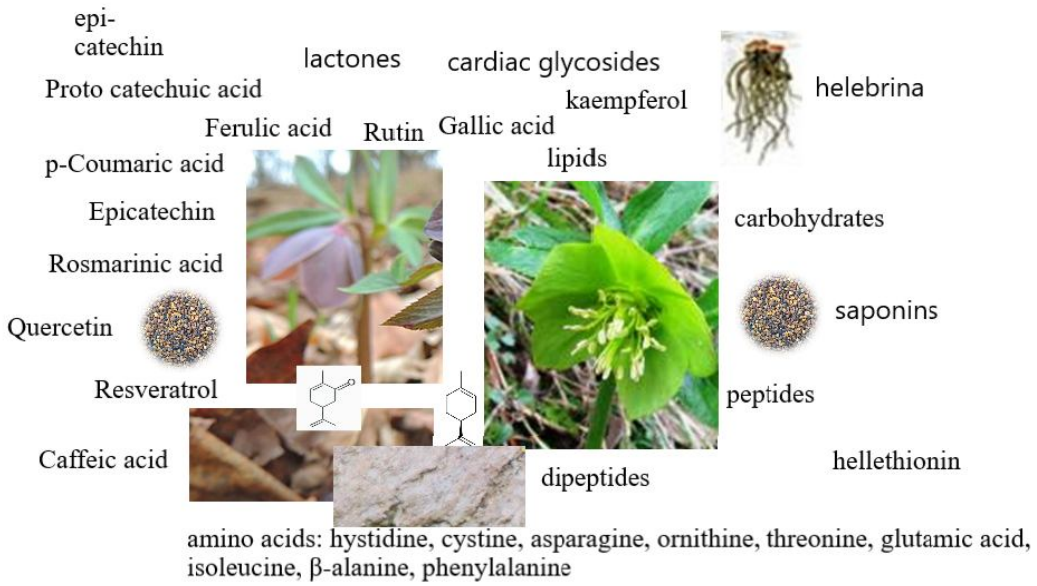

Figure 4. Helleborus - chemical composition [34, 46, 55, 70, 71, 72, 73, 74, 75].

Segneanu et al. (2015) [55] identified a wide range of peptides, dipeptides and amino acids in *H. purpurescens* L extracts (Figure 4). The authors point out that the content of *H. purpurescens* L. extracts in these compounds depends on the solvent. In studies conducted by Franz et al. (2018) [15], a higher concentration of asparagine, γ-amino-butyric acid and pipecolic acid was observed. In most cases the extracts were obtained from the root and rootstock of *H. purpurescens* L.

Glucose, sucrose and raffinose, galactose, fructose have been found in the roots and rhizomes of *Helleborus odorus* L. [6; 7]. Phytochemical screening of extracts from asparagus roots has highlighted other compounds such as phytosterols, tannins and phenolic compounds [30]. *Helleborus odorus* L. has been shown to be a rich source of secondary metabolites, such as bufadenolides, flavonoids, and phenolic heterosides [7]. From the primary metabolites result the secondary metabolites, so that along with the compounds with pharmacological action, in the underground organs of the flake there are also primary metabolites: carbohydrates, lipids, amino acids and peptides [7].

Carbohydrates are sources of energy (Kovacević, 2000) [29] and lipids are energy reserves for plant cells. In the subsoil and Helleborus seeds, unsaturated fats have been observed to dominate.

Živanov-Stakić and Mladenović, (1971) [67] observed an increase of the oil content in the underground parts of *H. odorus*, with the advancement in the vegetation, the maximum value was reached at the formation of the leaves.

Antimicrobial and antiviral activity

Microbiological studies have shown that asparagus has antimicrobial effects. Milbradt et al. (2003) [40] isolated helethionine from *Helleborus purpurascens* L. rhizomes with antimicrobial, antifungal, cytotoxic and antiviral activity. Glycosides are active against riboviruses and adenoviruses (eg influenza, Ebola virus, herpes simplex virus, coronavirus, tick-borne encephalitis virus etc [48]. Caleya et al., (1976) [9] point out that thionines have a high content of cysteine units. Some tests show the antimicrobial effect of Helleborus *bocconei* root extracts against the two species of saprophytic and pathogenic staphylococci, *Staphylococcus epidermalis*, respectively *Staphylococcus aureus* [51].

Extracts from the underground parts of Helleborus showed on the growth of some bacterial species (some involved in respiratory diseases), such as *Streptococcus pneumoniae, Escherichia coli, Staphylococcus aureus, Moraxella catarrhalis, Stenotrophomonas maltophilia, Haemophilus influenzae, Pseudomonas aeruginosa* [47; 51]. Among the lactones is mentioned especially protoanemony, considered a toxic y-lactone [2], volatile, oily and irritating [16] with antimicrobial action [36; 58], fungicidal [41] and antimutagenic [37; 42].

Pharmacological effects

Although *H. purpurescens* L. is considered a toxic plant, its therapeutic value was noticed early, being used to treat rheumatic pain, heart and mental disorders. One of the most widely used and widely used pharmaceuticals was Boicil. This product was obtained by the researcher Boici (1977) from the root and stem of *H. purpurascens* L. In our country it has been used as a natural anti-inflammatory and muscle relaxant analgesic for decades [27a]. The effect of Boicil was undeniable, even though no investigation was conducted into it. Alkaloids, glycosides and saponins found in Helleborus species show activity on the central nervous system [20].

The therapeutic and pharmacological effects of raw extracts of some Helleborus species have been tested "in vitro" and on animal models [12; 35]. The hydrolysis of glycosides and their therapeutic action have been studied. Some authors mention that the therapeutic action of cardioactive glycosides depends on the structure of the aglycone.

According to studies, glycosides have hemolytic, anticancer, antiviral, narcotic (heleborin), but also effect on the cardiovascular system (heleborein), a fact highlighted by Rosselli et al., (2007) [51]. Saponins have an effect on the central nervous system, endocrine system, cardiovascular system [31; 32, 32a], and cancer. In addition, saponins have immunomodulatory [32a] and antihepatotoxic activity. A support in favor of the action of saponins is also the pharmaceutical preparation based on saponins from the roots and rhizomes of *Helleborus spp.,* For the treatment of ulcers obtained and patented in 1976 [10].
There is a possibility that thionines protect plants against phytopathogens [40]. These cysteine-rich peptides are considered to be immunomodulatory [51] and anticancer [47], which is why they have been proposed as immunotoxins in cancer therapy [28]. This proposal is based on research in which the regression of tumor cells was observed [15; 55] have no immunostimulatory effect [21].
Although there are many studies on this plant, the mechanism of action of all compounds is still unknown [19, 40]. According to the WHO, the number of cancer patients is growing and cancer has been the cause of many deaths in 2020 **[**57]. The solution could be herbal phytocomposites, especially Helleoborus, and it is known that the first anti-cancer drugs are of natural origin. Degradation of protoanemonine (lactone) results in anemonine with sedative, antispasmodic and anti-inflammatory properties. There are suggestions that it can be used for the treatment of cardiovascular disease and as a cosmetic agent in case of hypopigmentation [25; 41].

Helleborus odorus L. has various applications in veterinary medicine. It can be used as an antiparasitic and purgative drug [51], but it also has a cardiovascular effect. Regarding toxicity, clinical trials are low, but there are reports that at certain concentrations, some compounds in asparagus are considered toxic. Thus, one of these compounds is heleborein, which is considered toxic at a concentration of 0.176 mg / kg. *Helleborus odorus* L. has also been shown to have an irritating effect on skin and mucous membranes [15; 55; 56].

Conclusions

Helleborus contains promising secondary metabolites in the treatment of diseases and against increasingly common microorganisms in hospitals. The compounds are bioactive against bacteria, fungi and viruses. The increased interest in the genus Helleborus is mainly due to its antiproliferative, antidiabetic, anti-inflammatory and antimicrobial properties, but it should be noted that full clinical investigations are needed to exploit these potentials, especially antidiabetic and antitumor ones. In addition, reports are required on the degree of toxicity of bioactive compounds. The wide range of Helleborus compounds is recommended for use in various pharmaceutical formulations, analgesic preparations, but with caution and with respect to non-toxic doses.

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