REVIEW

BRYONIA ALBA L. AND *ECBALLIUM ELATERIUM* (L.) A. RICH. - TWO RELATED SPECIES OF THE *CUCURBITACEAE* FAMILY WITH IMPORTANT PHARMACEUTICAL POTENTIAL

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Abstract

The importance of the *Cucurbitaceae* family consists not only in the species that are widely known for various economically important human uses, but also in the species that have proven an important and promising potential concerning their biological activities. *Bryonia alba* L. and *Ecballium elaterium* (L.) A. Rich. are two species belonging to this family, that are known since ancient times for their homeopathic or traditional use in the treatment of numerous disorders. There is clear evidence that links between the two species are not only related to family morphological characters, but also to a certain degree to the sexual system and, most importantly, to the active principle content or to potential medicinal uses. All these elements helped to include both species in the same tribe and may result in important reasons for heading future studies towards the elucidation of their complete phytochemical composition and mechanisms of the biological activities. The present study aims to review the existing scientific literature on the two species and to offer sufficient evidence in order to justify a most detailed study of their pharmaceutical potential.

Rezumat

Importanţa familiei *Cucurbitaceae* constă nu numai în specii care sunt cunoscute în sens larg pentru utilizările economice, dar şi în specii care şi-au demonstrat un potenţial important şi promiţător ca specii cu activitate biologică. *Bryonia alba* L. şi *Ecballium elaterium* (L.) A. Rich. sunt două specii aparţinând familiei *Cucurbitaceae* care sunt cunoscute din cele mai vechi timpuri pentru utilizările în homeopatie sau în medicina tradiţională pentru tratamentul a numeroase afecţiuni. Există dovezi ştiinţifice clare pentru legăturile dintre cele două specii, care se referă nu numai la caracterele morfologice specifice familiei, dar de asemenea într-o anumită măsură şi la sistemul sexual şi, cel mai important, la conţinutul în principii active sau la potenţialele utilizări medicinale. Toate acestea au favorizat includerea ambelor specii în acelaşi trib şi reprezintă dovezi importante pentru îndreptarea ulterioară a studiilor spre elucidarea completă a compoziţiei fitochimice şi a mecanismelor implicate în activitatea biologică. Studiul de faţă îşi propune aşadar să ofere o trecere în revistă a dovezilor ştiinţifice existente în literatura de specialitate legate de cele două specii şi să ofere argumente suficiente pentru a justifica un viitor studiu amănunţit al potenţialului lor farmaceutic.

Keywords: *Bryonia alba* L., *Ecballium elaterium* (L.) A. Rich., pharmaceutical potential

Introduction

The *Cucurbitaceae* family (*Cucurbitales* order) is one of the most important families known worldwide [41], especially for the species that have economically important uses in many fields, among which food, agriculture or cosmetic industries are the most frequently known [65]. The family comprises 90 genera, with approximately 700 species, which grow especially in the tropical regions [41]. Species as *Citrullus lanatus* (Thunb.) Mansf., *Cucurbita pepo* L. and *Luffa cylindrica* L. are the most popular species of the family [47, 91, 97] and are known for their importance in alimentation [3] or in different other industries [65, 91, 97]. However, some plants belonging to this family have proved to be potentially medicinally valuable, not only for their content in nutrients, but also because of their content in active principles, as for example cucurbitacins, which are the most important compounds found in the composition of these plants [41].

Cucurbitacins are highly oxygenated compounds, belonging to the class of tetracyclic triterpenoids [7, 26, 33, 51, 60, 76]. Currently, there are 12 classes

of cucurbitacins known, which contain the 16 cucurbitacins (A-S), grouped according to their different molecular variations (Figure 1) [76]. Out of these aglycones, hundreds of derivatives have been discovered. Structurally, cucurbitacins are derivatives of the tetracyclic nucleus skeleton cucurbita-5-ene (IUPAC name: 19-(10→9-β)-abeo-5 alpha- lanostane), also known as 9-β-methyl-19-nor-lanosta-5-ene, which can be modified by oxygen-containing groups and by double bonds to produce the 16 cucurbitacins known [7, 51]. Most of the cucurbitacins are found as glycosides, having the glycosidic part linked to the carbon found in the position number 2 (2-O-β-glycosides) [7].

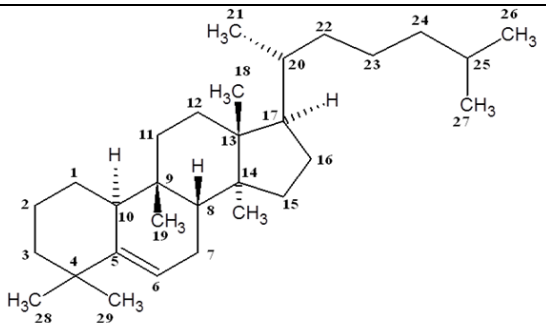
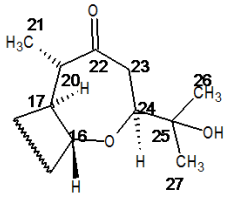


Figure 1.
General structure of the cucurbita-5-ene, basic structure of cucurbitacins

Table I
Structure of the cucurbitacins [7, 51]

Compound	Modification of the basic structure										
	1-2	**2**	**3**	**11**	**16**	**19**	**20**	**22**	**23-24**	**24**	**25**
Cucurbitacin A	-	-OH	=O	=O	-OH	-OH	-OH	=O	=	-	-OAc
Cucurbitacin B	-	-OH	=O	=O	-OH	-	-OH	=O	=	-	-OAc
Cucurbitacin C	-	-	-OH	=O	-OH	-OH	-OH	=O	=	-	-OAc
Cucurbitacin D	-	-OH	=O	=O	-OH	-	-OH	=O	=	-	-OH
Cucurbitacin E	=	-OH	=O	=O	-OH	-	-OH	=O	=	-	-OAc
Cucurbitacin F	-	-OH	-OH	=O	-OH	-	-OH	=O	=	-	-OH
Cucurbitacin G = Cucurbitacin H	-	-OH	=O	=O	-OH	-	-OH	=O	-	-OH	-OH
Cucurbitacin I	=	-OH	=O	=O	-OH	-	-OH	=O	=	-	-OH
Cucurbitacin J	=	-OH	=O	=O	-OH	-	-OH	=O	-	-OH	-OH
Cucurbitacin K	=	-OH	=O	=O	-OH	-	-OH	=O	-	-OH	-OH
Cucurbitacin L	=	-OH	=O	=O	-OH	-	-OH	=O	-	-	-OH
Cucurbitacin O	-	-OH	-OH	=O	-OH	-	-OH	=O	=	-	-OH
Cucurbitacin P	-	-OH	-OH	=O	-OH	-	-OH	=O	-	-	-OH
Cucurbitacin Q	-	-OH	-OH	=O	-OH	-	-OH	=O	=	-	-OAc
Cucurbitacin R	-	-OH	=O	=O	-OH	-	-OH	=O	-	-	-OH
Cucurbitacin S	=	-OH	=O	=O							

Although plants belonging to *Cucurbitaceae* family that contain cucurbitacins are mostly known for their toxicity [51, 60], they are also studied for their large number of potential pharmacological activities [33, 51]. Among these, the most known and considered by the scientific literature are the antioxidant, anti-inflammatory [26], antidiabetic and antiathero-sclerotic activities [60], but particularly the cytotoxic and anticancer ones [7, 33, 76]. In the last years, plants belonging to *Cucurbitaceae* family have experienced an important increase in the attention of many researchers, due to their important content in active principles that have proved significant biological activities and important potential for their introduction in the therapy of numerous pathologies [60].

Bryonia alba L. and *Ecballium elaterium* (L.) A. Rich. are two species of the *Cucurbitaceae* family that have proven important potential as medicinal plants [115]. Phylogenetic studies [65, 102] cite a link between the two species and therefore *Bryonia alba* L. appears to be the elder sister of *Ecballium elaterium* (L.) A. Rich. [102]. The similarities between the two species include morphological characters and help the inclusion of both species in the *Bryonieae* tribe [65, 102]. The two species are included in the genera that present few differences that are mostly related to the sexual system [102]. The shifts that are related to monoecy and dioecy occur in the same genera, as in the case of *Bryonia*, or, as in the case of *Ecballium*, in the same species [114]. Actually, the differences between the two

species are not significant and despite them, there are phylogenetic studies that cite the close relationship between the two species [65, 102, 103, 114, 116], which were also the main reasons for their inclusion in the same tribe, together with an Australian endemic genus, *Austrobryonia* [103].

The present bibliographical study is an overview of the existing data in the scientific literature that aims to bring out the important pharmaceutical potential of these two related species that may provide potent compounds for the treatment of various diseases.

Bryonia alba L.

Bryonia genus, with 10 species [102], comprises monoecious or dioecious, perennial, herbaceous and climbing species, with simple leaves, simple tendrils and small greenish-yellow flowers, 5 stamens, grouped in 3 bundles, 3-lodged globular ovary and a black globular glabrous small-sized indehiscent berry as a fruit [115].

Figure 2.
Left - Spontaneous *Bryonia alba* L. in Cluj-Napoca county (North Western Transilvania, Romania); Right – Macroscopic characters of the *B. alba* species

Bryonia alba L. is a monoecious species, which grows especially in dried soils, preferring rocky slopes in the mountainous areas, dry riverbeds or even sand dunes. Special adaptations help it to resist to external conditions. It has large tubers, which help storing the water underground [115]. Phylogeographic studies cite its presence in the Central European region, with some extensions in Northern, Eastern and Southern parts, reaching even to Kazakhstan, North Africa, Canary Islands or Central Asia [102, 114, 115]. Studies performed by Volz and Renner in 2009 state that there is a high possibility that it is the only *Bryonia* species found in Romania, as the presence of *Bryonia dioica* Jacq. remains uncertain [115].

Figure 3.
Geographic distribution of the *B. alba* L. species, as described by Volz and Renner (2009) [115]

The species is mostly known for its homeopathic uses. The root is used in homeopathic preparations to induce dryness of the mucous membranes [35], for laxative and purgative effects [115] and for its antiinflammatory effect in the inflammation of the serous tissues or for the treatment of cough, pneumonia and rheumatism [78]. It is a species that has also proven its importance in traditional medicine, particularly as purgative, mostly due to its content of cucurbitacins [22, 66, 67, 85, 88, 92]. Besides cucurbitacins, which are the most important compounds found in the composition of the species, there are also other compounds, which can increase or can act synergistically with them for increasing the biological activity [53]. Table II contains the most important compounds that are found in the composition of *B. alba*.

Table II
The most important active principles found in the composition of the *B. alba* species

Compounds	Part of the species	Reference(s)
Cucurbitacins	Root	[22, 66, 67, 85, 88, 92]
Flavonoids	Aerial part	[69, 70]
Sterols	Roots	[87]
Lectins	Roots	[48]
Amino-acids	Roots	[59]
Trihydroxyoctadecadienoic acids	Roots	[58, 84]
Lipids	Roots	[89]

The compounds found in different parts of *B. alba* give an important potential for its use as therapeutic or adjuvant treatment. The most important activity is due to the high content of cucurbitacins in the roots. Because of this reason, this part of the species is the most studied [22, 48, 58, 59, 66, 67, 87]. Cucurbitacins, especially cucurbitacins B, D, E, I and their derivatives, but also cucurbitacins J, K, L are found in important amounts in the roots [22, 66, 67] and provide cytotoxic effects [22] or even antitumor activities. The most cytotoxic cucurbitacins seem to be cucurbitacin B, D, E and I, but the others present nevertheless moderate activities [66]. There is also evidence of the fact

that roots of *B. alba*, containing trihydroxy-octadecadienoic acids (class of trihydroxy fatty acids), can exhibit hypoglycaemic activity [108] or can even correct major abnormalities typical in severe diabetes mellitus [58]. Other studies state that the same compounds may exhibit preventive atherogenic or antiatherosclerotic activities [84]. At the same time, in the context of the protective activities for biological systems, there are studies that prove the hepatoprotective activity of the roots extract [78] or even the protective activity for human cells against endogenous oxidative DNA damage, although the ability of protection towards the exogenous oxidative DNA damage could not be demonstrated [81].

B. alba roots are also widely known for their adaptogen activity, which some sources associate to the content of cucurbitacins (especially cucurbitacin R diglucoside), which enhance the sensitivity level to stress, due to the effects of eicosanoids and corticosteroids [85]. Actually, *B. alba* roots can also increase nitric oxide (NO) in humans [86], which can be responsible for the adaptation of the human organism to heavy physical exercise. On the contrary, the same NO molecule, which is responsible for numerous inflammatory processes [34], is inhibited by *B. alba* roots in its formation process, by inhibition of the activity of its inducible enzyme, iNOS (inducible NO synthase), which can be a possible mechanism of action for the anti-inflammatory effect [92].

All of these findings represent important arguments for the promising phytopharmacological potential of *B. alba* species. At the same time, they are supplementary reasons for a future most detailed investigation, as many details remain to be clarified about the subject.

Ecballium elaterium (L.) A. Rich.

Ecballium genus comprises only one species, which is an annual herb, with no tendrils, small yellow flowers, 5 stamens, grouped by 2 and the fifth being free, a medium-sized oblong hairy berry, watery and ejecting seeds by elastic contraction [4, 20, 32, 52]. This single species is divided in 2 subspecies, with different breeding systems: subspecies *dioicum*, which is dioecious and subspecies *elaterium*, which is monoecious. This intraspecific variation provides a unique opportunity to study the influence of the sexual system on the genetic consequences [36].

It grows mostly on disturbed ground from Northern Spain, through Southern Europe to the Mediterranean, North Africa and Southwest Asia [36, 102]. Because of its habitat, it has developed important morphological and anatomical features, mostly related to the structure of the leaf, that help it to withstand the stressful external conditions [32].

Figure 4.
Left - Spontaneous *Ecballium elaterium* (L.) A. Rich. in Constanța county (South Eastern Dobrogea, Romania); Right – Macroscopic characters of the *E. elaterium* species

Figure 5.
Geographic distribution of the *Ecballium elaterium* (L.) A. Rich. species, as described by Costich and Meagher (1992): in light red distribution of *Ecballium elaterium* ssp. *elaterium* and in dark red distribution of *Ecballium elaterium* ssp. *dioicum* [36]

As in the case of its older relative, *E. elaterium* is mostly known for its important content of cucurbitacins [19], which can provide the species with various biological activities. Actually, the first known cucurbitacin, elaterin (cucurbitacin E) was isolated for the first time from the fruit juice of *E. elaterium* [52]. Because of this reason, the species is considered to be an important "indicator plant" among the species with high content of cucurbitacins [19]. The highest concentration of cucurbitacins was found in fruits, followed by stems and leaves [18]. Besides cucurbitacins, the species contains other important compounds, which can enhance its biological activities [20]. Table III summarizes some of these compounds.

Table III

The most important active principles found in the composition of the *E. elaterium* species

Compounds	Part of the species	Reference(s)
Cucurbitacins	Roots, leaves, stems, flowers, fruits and seeds	[10, 19, 30, 52, 73, 74, 88, 98, 104-106]
Flavonoids and phenolic compounds	Leaves, stems, flowers, fruits and seeds	[10, 55, 56, 95, 110]
Sterols	Roots, leaves, stems, flowers, fruits and seeds	[10, 54, 74, 111]
Fatty acids, lipids and oils	Roots, leaves, stems, flowers, fruits and seeds	[10, 30, 54, 74]
Essential oils	Leaves, fruits and seeds	[46, 96]
Carotenoids	Roots, leaves, stems, flowers, fruits and seeds	[10]
Soluble sugars (e.g. sucrose, raffinose)	Leaves	[6, 10, 30]
Trypsin inhibitors	Seeds	[13]
Tannins	Leaves, flowers	[10]
Alkaloids	Roots, leaves, stems, flowers, fruits and seeds	[10]
Amino-acids and amino-acis decarboxylases	Seeds	[37, 43]

Several biological activities of the species, as anti-microbial [1, 4, 25, 82], anti-inflammatory [90, 107, 112], antioxidant [101] or cytotoxic [14, 15, 17, 28, 74, 98, 101, 111] are the most frequently described in the scientific literature. Studies of the undiluted fruit juice state that there is an important cytotoxic and genotoxic effect on *Allium cepa* root tip cells [30]. The main responsible molecules for these activities seem to be again the cucurbitacins and most specific cucurbitacin E, which is mostly known to provide the cytotoxic activity [15, 17, 28, 98]. Cucurbitacin E is not studied only for its cytotoxic effect, but also for its ability to stimulate the immune system [14, 16], which can be vital for the possible anti-cancer activity. At the same time, it seems to have minor cytotoxic effects on normal cell lines [17]. Not only cucurbitacin E is present in the composition of different part of the species, but also other compounds, as for example cucurbitacin B, D or I [21, 109]. There are a lot of studies that aim to develop specific methods in order to increase the concentration of these compounds [109].

There are also many other activities that *E. elaterium* species is responsible for. Traditional medicine describes mostly the use of the fruit juice as nasal drops for the treatment of jaundice in new-borns [2, 9, 80]. Despite of this fact, there are studies that consider it as a highly toxic plant, especially for its juice, which exhibits its toxic activity on the respiratory, cardiac and gastric systems, but also for the mucous membranes it takes contact with [8, 12, 20, 27, 29, 30, 44, 64, 77, 90, 94, 99, 100, 113]. There are studies which state that the administration of the juice to rats with surgically induced jaundice produced a decrease in serum bilirubin concentration, which apparently is due to its content of cucurbitacin B, D and E and may increase the binding of domain specific ligands, ibuprofen and bilirubin [49, 50].

The species is also traditionally used as a treatment for otitis, sinusitis, malarial fever, headaches or sinusitis and rheumatism [20, 30, 42, 71]. Studies performed by Agil *et al.* [5] and by El Naggar *et al.* [45] suggested a hepatoprotective activity against induced hepatotoxicity in rats. Another study, performed by Dubeau and his co-workers state the neuroprotective activity of cucurbitacin E, isolated from *E. elaterium* [11]. It may also have beneficial effects on sepsis-induced lung injury, which is demonstrated by the research team of Demir in rats [40]. Protective activity of *E. elaterium* may be due to its ability to reduce abdominal adhesion and may be a useful tool for the antiinflammatory activity in the prevention of postoperative peritoneal adhesion [83].

A lot of studies aim to investigate the anti-trypsin activity of *E. elaterium*. Seeds of the species contain a specific polypeptide, actually a protease inhibitor, which specifically acts against trypsin, being one of the most potent trypsin inhibitor [20]. It may also represent an important target for the isolation of small peptide molecules that have affinity for acceptor molecules [31]. The EETI-II (*Ecballium elaterium* trypsin inhibitor) has a rigid molecular scaffold [68] and a structure that can be mutated in its loops in order to bind with high affinity to integrin receptor associated in tumours and a radiolabelled version of this knotting peptide can help to develop the non-invasive imaging of integrin expression in humans [61, 63, 72, 79]. This type of molecule represents a target for protein engineering that can also be useful in cancer therapy [62] or can represent the basis of the synthesis of modified derivatives with potent inhibitory activity towards pancreatic elastase, chymotrypsin and human leucocyte elastase [75].

Cucurbitacins that are present in the composition of the species (mostly cucurbitacin D and cucurbitacin I) may perform an important role in the defence mechanisms of the plants, as suggested by Bar-Nun *et al.* in 1989 and 1990, when the activity against *Botrytis cinerea* Pers., due to the inhibition of laccase formation, was demonstrated [23, 24]. In 1999, Villalobos and co-workers demonstrated that leaves extract of *E. elaterium* can exhibit a potent

anti-insect activity, which can be the result of the internal defence mechanisms of the plant [93]. This is also the main reason for the possibility of using the extracts of the plant to inhibit the germination of growth of some other plant species [96]. However, essential oils obtained from the species could also play a role in the allelopathic effect [57]. In the context of improving the methods of crop maintenance, this appears as an innovative technique, beside methods that already imply growing patterns [39] in order to diminuate the quantity of unwanted compounds, especially in foods [38].

All of these emphasize the reasons to carry on the investigations on the important therapeutically potential of the *E. elaterium*, but also give arguments for a more detailed investigation on the species, as there are a lot of unclear aspects, that need further evidence in order to establish the real pharmaceutical potential of *E. elaterium*.

Conclusions

Bryonia alba L. and *Ecballium elaterium* (L.) A. Rich. are two species belonging to the *Cucurbitaceae* family, that are related from many points of view. Similarities are related to morphological characters (e.g. root or leaf morphology), in part to the sexual system (e.g *B. alba* is monoecious and *E. elaterium* can be both monoecious and dioecious), but also to the content of active compounds (e.g. cucurbitacins, flavonoids, sterols, lipids) and to their biological activities (e.g. cytotoxic, antiinflammatory, hepato-protective). There are also some differences between the two species, which are concerning the same points of view: morphological characters (e.g. flowers, fruits morphology), sexual system (e.g *B. alba* is only monoecious and *E. elaterium* can be both monoecious and dioecious), active compounds content (e.g. trihydroxyoctadecadienoic acids for *B. alba* and trypsin inhibitors for *E. elaterium*) or biological activities (e.g. antidiabetic and anti-atherosclerotic for *B.alba* and anti-trypsin for *E. elaterium*). Nevertheless, phylogenetic studies have confirmed the link between the species and helped including both in the same tribe (*Bryonieae*) of the family *Cucurbitaceae*. There are also many data that prove their important potential as medicinal plants. Despite the existence of many studies that aim to establish links between the content of active compounds and the biological activities, there are yet a lot of unknown or unclear aspects, which however have the necessary background to be clarified. This is the reason why the present study aimed to bring into attention the most important points related to the two species and offer the necessary arguments for a future most detailed study of the two species. It is important to clarify the aspects related to the biological activities of the two plants in order to state their use in the treatment of different disorders. As *Bryonia alba* L. and *Ecballium elaterium* (L.) A. Rich. offer promising pharmaceutical potential, it is essential to further investigate and clarify all the aspects which are related to it.

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