Review Article

Eryngium creticum – ethnopharmacology, phytochemistry and pharmacological activity. A review

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A B S T R A C T

Eryngium creticum Lam. (E. cyaneum Sibth. & Sm., E. syriacum Lam.), Saniculoideae, Apiaceae is of great importance in the traditional Greco – Arab medicine. This study was carried out in order to contribute to the ethnopharmacological knowledge of this medicinal species. This review describes the botanical characterization and distribution, as well as critically assesses the phytochemical properties and biological activities of E. creticum, a species that has been used in traditional medicine for many decades. Possible trends and perspectives for future research of this plant are discussed, as well. E. creticum has been found to contain several chemical constituents, mostly sesquiterpenes, monoterpenes, aldehydes, coumarins, sitosterols and sugars. Eryngo with its bioactive compounds possesses a wide range of biological activities. It was reported that in traditional medicine E. creticum was applied mainly as the remedy for snake and scorpion bites. Some published studies have shown a broad spectrum of biological and pharmacological activities, including anti-snake and anti-scorpion venom, as well as antibacterial, antifungal and antileishmanial effects. Other have indicated antihyperglycemic, hypoglycemic and antioxidant activities of this species. The in vitro studies and in vivo models have provided a simple bioscientific explanation for its various ethnopharmacological uses.

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Introduction

To promote traditional medicine and herbal therapeutics, there should be provided the knowledge of plant bioactive compounds playing an important role in human healthcare and the scientific confirmation of their traditional use. Eryngium creticum Lam., Apiaceae is an example of a species that has remained a wild edible plant and it is known mostly to gatherers. This article provides an overview of E. creticum, a traditional herbal remedy used mostly for snake and scorpion bites: the distribution pattern and botanical description of the species, the status of ethnopharmacology, phytochemistry and the laboratory data on the bioactivity. The current status of literature on eryngo has been reviewed.

In brief, this review presents the results of the investigations on E. creticum that have been conducted so far and points out the gaps in knowledge, which disclosure is necessary to understand the mechanism of action of the extracts used in a traditional medicine. Also it indicates the necessity to conduct in-depth examination of the correlation between the pharmacological effect and the presence of the bioactive compounds responsible for the action. The review summarizes the phytochemical analysis and biological studies that may be helpful for researches to undertake further studies supporting the existing knowledge necessary to understand the action and may contribute to the discovery of new uses of this interesting species.

Botanical characteristics and distribution

Genus Eryngium L. comprises about 250 species and is distributed throughout temperate regions of every continent. The Eryngium species grow in Eurasia, North Africa, North and South America, and Australia. It is the most species-rich genus of the Apiaceae (Wolff, 1913; Wörz, 1999, 2004, 2005, 2006; Calvino et al., 2008; Wörz and Diekmann, 2010). Wolff (1913) grouped the species into 34 sections and numerous subsections. He also recognized two major informal groups: Species gerontogae and Species americanae and australiensis, the former representing twelve sections from the Old World, and the latter 22 sections from the Americas and Australia. E. creticum with E. planum and E. caeruleum belongs to Plana section (Wolff, 1913). A new subgeneric classification of the genus was presented by Wörz (2005). It recognizes five subgenera. The classification was based on morphology and did

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not reflect phylogeny or solve problems of infrageneric relationships (Wörz, 2005). The other paper dealt with the Balkan species. The classification of the Balkan species of Eryngium was based on conventional morphological characteristics. The basil leaves provided the main characteristics. Specialization, the high rate of endemism, sectional diversity, and distribution patterns suggest relatively old age of most Balkan Eryngium species. The topographical and climatic diversity resulted in isolation and some ecological adaptations. Exceptions to the old age species are probably the 3 widespread species including E. creticum which are migratory. Within the Circum-Mediterranean Plana section, E. creticum is the only Balkan species. The closest relative is the Caucasian E. caeruleum (Wörz, 2006). The study carried out by Calvino et al. (2008), based on phylogenetic analyses of DNA sequences from three non-coding chloroplast DNA loci and the nuclear ribosomal DNA internal transcribed spacer region, was useful in corroborating the monophyly of Eryngium, dividing it into redefined and mono- phylectic subgenera (E. subgenus Eryngium and E. subgenus Monocotyloidea), and identifying clades that shared several morphological, biogeographical and/or ecological traits (Calvino et al., 2008).

E. creticum Lam. (E. cyanus) Lam., eryngo, flat holly, blue sea holly) was described by Jean-Baptiste Pierre Antoine de Monet de Lamarck in 1798. It is known by several local names, but the most recognizable are: Qors Anneh used in Lebanon and Qarsa‘na (Palestine). Eryngo is a species that belongs to Apiaceae family (Umbelliferae) and it can be found in the Eastern Mediterranean region, mostly in Lebanon, Palestine, Jordan and Syria. It grows at low altitudes, in sunny places. It is commonly found in fallow fields, roadsides, waste places, and, occasionally, even in the understory of olive groves. In Syria it was found in various environments, which indicates its adaptability and abiotic stress tolerance (Jawdat et al., 2010). The species is also found in the Alpine and sub-Alpine calcareous grasslands in the high mountains of northern and central Greece. The soils are usually deep and relatively rich in nutrients (Tutin et al., 1968; Wörz, 2006; Caballero et al., 2009).

The Hebrew name of E. creticum – Charchevina-Makhila, is based on the fact the plant grows in dry weather; the root name is H-R-V which means dry, while the second word means “turning blue”. Despite its name, Charchevina is actually listed in the Mishnah, the 2nd C. AD books of Jewish practices, as one of the Passover bitter herbs (Passachim 2: 6).

E. creticum is a spiny perennial, or sometimes biennial or annual, glaucous and globose herb, reaching up to 50 cm in height and it has erect branched stems. Stem leaves are sessile and palmately divided into 3–8 prickly lobes. Winter rosette leaves are quickly withering, bluish, long petioled, not prickly, entire to dentate, or lobed. Inflorescences are repeatedly forked; umbels of 0.7–1 cm, head-like, with an involucre of 5 long, blue, spiny bracts 2–5 times as long as the head, spreading, linear, boat-shaped, pricky at base or along the long margin. Fruits are scaly-bristly, obscurely ribbed (Konig and Sims, 1806; Don, 1831; Muschler, 1912; Tutin et al., 1968; Mayer-Chissick and Lev, 2014).

It is cultivated for use as a vegetable, mainly in salads. Its young leaves are eaten fresh and thick roots are eaten raw or cooked (Mayer-Chissick and Lev, 2014). An ethnobotanical survey of wild edible plants of Cyprus (Paphos vine zone and Larnaca mixed farming zone) revealed that young stems and leaves of eryngo preserved in vinegar were eaten as appetizers with several kinds of food (Della et al., 2006). The freshly harvested, simple leaves on long stalks of E. creticum and deeply lobed leaves of E. glomeratum are made into pickles by Greek Orthodox monasteries in Cyprus (Lardos, 2012). In Lebanon, eryngo is consumed mainly when freshly gathered and prepared mainly raw as a salad. Most information shows that the plant is appetizing and it is described as bitter in taste. According to an ethnobotanical survey of edible plants in northeast Lebanon, eryngo is consumed about three times a week (Jeambey et al., 2009).

**Phytochemistry**

Unfortunately, the literature on phytochemistry of E. creticum is still scarce. There are some studies on the chemical composition of the essential oil. However, the knowledge about their non-volatile chemical constituents is limited. The phytochemical screenings of aqueous, ethanolic, and methanolic extracts of E. creticum indicated the presence of different bioactive compounds, mainly sesquiterpenes, monoterpenes, aldehydes, coumarins, sitosterols, tannins, resins and sugars. On the other hand, screening for metal by means of spectroscopy showed that this plant contains metals such silver, zirconium, nickel, selenium, niobium and molybdenum, iron, calcium, manganese and copper (Al-Khalil, 1994; Ayoub et al., 2003; Celik et al., 2011; Farhan et al., 2012; Dammous et al., 2014; Dirani et al., 2014; Rammal et al., 2015; Erdem et al., 2015).

One of the first phytochemical investigations of the roots of E. creticum, which grows wildly in Jordan, led to the isolation and characterization of nine compounds: two coumarins – deltion (1) and marmesin (2), cyclic alcohol – quercitol, monoterpene glycoside 3-(β-D-glucopyranosylmethyl)-2,4,4-trimethyl-2,5-cyclohexadien-1-one (3), phloroglucinol glycoside (1-(β-D-glucopyranosyl-3-methoxy-5-hydroxy benzene) (4), δ-sitosterol and its glycoside (β-sitosterol-δ-D glucopyranose), and two sugars – mannitol and dulcitol (Al-Khalil, 1994).

![Diagram](image)

The unique 1-α-propyl-perhydroperpendicular halene 1,2,4a,5,6,7,8,8a-octahydro-4-methyl-1-propyl-naphthalene-7-carbaldehyde (eryng-9-en-15-al) (5), a compound which possesses an unusual sesquiterpene carbon skeleton, was isolated and identified, together with the new natural methyl ketone eicos-8,11-dien-18-ol-2-one (6), from the hexane: ether (1:1) extract of the aerial parts of E. creticum growing in Egypt. The structures of those compounds were established by conventional methods of analysis and confirmed by DEPT, COSY, HMQC and HMBC spectral analysis (Ayoub et al., 2003).
A report by Mahommadhosseini (2013) deals with the chemical composition of the volatile oil from stems of *E. creticum* from Iran. The oil was obtained using a modified Clevenger-type apparatus and the respective analyses were performed by means of GC and GC–MS. It was obtained as a clear yellowish liquid containing 0.18% (w/w). There were identified seventeen components constituting 91.4% of the oil composition. The major components of the oil were found to be bornyl acetate (28.4%), camphor (17.8%), α-pinene (12.1%), germacrene D (9.4%), borneol (8.6%) and e-\thyujene (4.2%). Based upon the chemical profile, the essential oil was mainly characterized by the presence of increased amounts of oxygenated monoterpenes (Mahommadhosseini, 2013).

The chemical composition of the essential oil was determined by direct thermal desorption (DTD)-GC/MS analyses. The result for essential oil yields of *E. creticum* growing in the Aegean region of Turkey was 0.21% (v/w). The oil was colorless, with a weak perfume odor. The essential oil contains a mixture of several compounds, predominantly aldehydes and oxygenated monoterpenes. Hexanal (52.90 ± 2.70%), heptanal (13.90 ± 3.82%) and octane (8.95 ± 2.32%) were the major compounds. The percentage of other compounds ranged from 0.1 to 3.57% (Celik et al., 2011). To the best of our knowledge, it is not possible to calculate RI values lower than 800 using the mixture of C8–C32 alkanes. Moreover, the order of compounds listed in Table 1 is not correct because it is based on RI values and these are unreliable, e.g. neither of the first compounds can have RI below 600; RI of compounds with the same formula, e.g. monoterpeno hydrocarbons (limonene, cine- nes, pinene, camphene), cannot differ as greatly as reported; RI (retention indices) of a pair of homolo- gous compounds such as hexanal (given RI = 816, proper RI = 800) and heptanal (given RI = 1140, proper RI = 900) should differ by ca. 100, and for nonanal (given RI = 1128) RI = 1100 etc. It is not possible to identify enantiomers using GC or GC–MS on non-chiral phase. The methodology and results reported in this article are not reliable.

The total phenolic (TPC; Folin–Ciocalteau method) and total flavonoid (TFC; aluminum chloride method) content in the leaves and stems of *E. creticum* was evaluated and expressed in mg of gallic acid or rutin equivalent per gram of dry weight. The amounts of TPC and TFC in the leaves were higher than in the stems. The ethanolic extracts of both leaves (TPC 253 ± 0.031 mg; TFC 729 ± 0.023 mg) and stems (TPC 230 ± 0.043 mg; TFC 258.9 ± 0.041 mg) of this plant were found to contain higher amounts of TPC and TFC than the aqueous extract (Farhan et al., 2012; Damous et al., 2014). The latest studies this group of authors also showed a high content of phenolics and flavonoids in the methano- lic extracts, which was much greater than in aqueous extracts, and the same of slightly lower than in ethanolic extracts, both in the leaves and stems of *E. creticum* (Rannmal et al., 2015).

As it turns out, *E. creticum* contains various concentrations of elements and heavy metals. It was found that this taxon is a plant which did not show heavy metal tolerance. The results obtained using microwave digestion (X-ray fluorescence spectrometry) demonstrated also that the amount of elements differed between the leaves and stems of the studied plant. The level of these elements was higher in leaves than in stems and calcium was found at higher concentration (321.06 mg kg⁻¹) in the leaves than in stems (46.43 mg kg⁻¹), compared to other elements. These results show that eryngo has good nutritional value (Farhan et al., 2012; Damous et al., 2014).

Many factors such as the geographical origin may be responsible for the variation of the content of bioactive compounds in the plant material, and for this reason the place of *E. creticum* origin was pointed.

**Traditional medicine**

The plant is recognized as one of the varieties which can be used as the bitter herb required for the Passover meal. The Arabs of Israel, who are accustomed to eating various kinds of sea holly, used *E. creticum* in their traditional medicine. Because of its traditional use, the plant is of much scientific interest nowadays. It was reported that eryngo was applied as the remedy for snake and scorpion bites. The Arabs eat the leaves or smear the ground root to cure scorpion stings. Roots and seeds immersed in water have been drunk in order to treat kidney stones and infections, skin diseases and tumors. The seeds serve to cure stomach sores, cataract in the eyes, and to expel worms. The juice of the leaves is also used to treat diabetes. In Islamic medicine, *E. creticum* is used to treat a wide range of ailments; particularly its roots are used against edema, sinusitis, urinary infections, and inflammations. It has also been used to treat liver diseases, poisoning, anaemia and infertility—a standard decoction was prepared from 50 g of whole plant in 1 l water and taken orally, three times per day until improvement occurs (Palevitch et al., 1985; Yaniv et al., 1987; Marles and Farnsworth, 1995; Abu-Rabia, 2005; Thiem and Wiatrowska, 2007; Aburjai et al., 2007; Saad et al., 2008; Jeambeay et al., 2009; Saad and Said, 2011; Mayer-Chissick and Lev, 2014).

These traditional uses of eryngo need scientific *in vitro* and *in vivo* confirmation of its effects.

**Biological and pharmacological activities**

The results of a number of published studies show a broad spectrum of biological and pharmacological activities of *E. creticum* which are attributed to different parts and extracts of this plant, including anti-snake and anti-scorpion venom, antibacterial and antifungal, and antileishmanial effects (Table 1). Others include antihyperglycemic, hypoglycemic and antioxidant activities (Table 1).

**Anti-snake and anti-scorpion venoms properties**

Snake bites and scorpion stings are major health hazards that lead to suffering and high mortality of the victims. Thousands of injuries associated with such bites and stings of venomous animals have been the cause of innumerable cases of deaths worldwide (Oukkache et al., 2014). The plant remedies may be beneficial in the treatment of snake bites and scorpion stings and they may be regarded as an alternative to anti-venom. The plant mentioned in the literature of the Mediterranean region that used to treat snake and scorpion venoms was *E. creticum*. It was reported that an aque- oux extract of the root was applied as the remedy for scorpion stings in the rural areas of Jordan (Yaniv et al., 1987).

When testing the efficiency of *E. creticum* aqueous extract, it was found to significantly prolong the life of guinea pigs from 20 min to 8 h when combined with *Leiurus quinquestriatus* scorpion venom (Jaghabir et al., 1989). The same extract combined with *Cerastes snake* venom prolonged the life span from 12 to 72 h (El-Saket, 1990).

In another study, the anti-venom activity of *E. creticum* aqueous extract was tested using isolated rabbit and guinea pig jejunum and trachea. The extract caused a dose-dependent inhibition of tracheal and jejunum contraction induced by *L. quinquestriatus* scorpion venom and inhibited spontaneous movements of the jejunum (Affifi et al., 1990).

The aqueous and ethanol extracts (1.0 μg ml⁻¹) of fresh and dried leaves and roots of *E. creticum* were tested for their inhibitory properties against snake and scorpion venoms (*Cerastes cerastes, Leiurus quinquestriatus*). The fresh leaf extract gave higher
Table 1
Pharmacological activity of Eryngium creticum.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Part of plant</th>
<th>Type of extract/compound</th>
<th>Experimental model</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-snake and anti-scorpion venoms properties</td>
<td>Roots</td>
<td>Aqueous extract</td>
<td>Guinea pigs treated with extract combined with Leirus quinquestriatius venom</td>
<td>Prolongs the life of animals from 20 min to 8 h</td>
<td>Jaghabir et al. (1989)</td>
</tr>
<tr>
<td></td>
<td>Roots</td>
<td>Aqueous extract</td>
<td>Guinea pigs treated with extract combined with Cerastes cerastes venom</td>
<td>Prolongs the life of animals from 12 to 72 h</td>
<td>El-Sakout (1990)</td>
</tr>
<tr>
<td></td>
<td>Roots and leaves</td>
<td>Aqueous extract</td>
<td>Isolated rabbit and guinea pig jejunum and trachea treated with extract combined with Leirus quinquestriatius venom</td>
<td>Inhibits tracheal and jejunum contraction and inhibits spontaneous movements of the jejunum</td>
<td>Afifi et al. (1990)</td>
</tr>
<tr>
<td></td>
<td>Roots and leaves</td>
<td>Aqueous and methanolic extract</td>
<td>Sheep red blood cells treated with Cerastes cerastes and Leirus quinquestriartus venoms</td>
<td>Inhibits haemolytic activity of snake and scorpion venoms</td>
<td>Alkofahi et al. (1997)</td>
</tr>
<tr>
<td>Antibacterial and antifungal activity</td>
<td>Aerial parts</td>
<td>Aqueous and ethanolic extract</td>
<td>Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Proteus vulgaris and Pseudomonas aeruginosa</td>
<td>Antibacterial activity was low or moderate depending on tested strain</td>
<td>Ali-Shahyeh et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>Leaves, stems and roots</td>
<td>Aqueous and ethanolic extract</td>
<td>Staphylococcus aureus, S. epidermidis, Escherichia coli, Pseudomonas aeruginosa and Enterococcus faecalis</td>
<td>Antibacterial activity was low, moderate or significant depending on tested strain</td>
<td>Makti et al. (2015)</td>
</tr>
<tr>
<td></td>
<td>Flowering plant</td>
<td>Essential oil</td>
<td>Methicillin-resistant Staphylococcus aureus</td>
<td>Inhibits the activity of the reference</td>
<td>Celik et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>Leaves, roots and fruits</td>
<td>Aqueous and ethanolic extract</td>
<td>Candida albicans</td>
<td>Inhibits the activity of the reference</td>
<td>Ali-Shahyeh et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>Petroleum and methanolic extract</td>
<td>Barys zinerea, Alternaria solani, Penicillium sp., Chado sporum sp., Fusarium oxysporum f. sp. melonis, Rhizoctonia solani, Verticillium dahlia and Phytophthora infestans</td>
<td>Inhibits the activity of the reference</td>
<td>Abou-Jawdah et al. (2002)</td>
</tr>
<tr>
<td>Antimalarial and antileishmanial activity</td>
<td>Aerial parts</td>
<td>Methanolic and dichloroethanolic extract</td>
<td>Plasmodium falciparum and Leishmania donovani promastigotes</td>
<td>Activity against promastigote cultures of L. donovani. No antimalarial activity</td>
<td>Fokialakis et al. (2007)</td>
</tr>
<tr>
<td>Antioxidant activity</td>
<td>Leaves and stems</td>
<td>Aqueous and ethanolic extract</td>
<td>DPPH assay</td>
<td>A significant antioxidant activity</td>
<td>Dammous et al. (2014)</td>
</tr>
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<td></td>
<td>Leaves and stems</td>
<td>Aqueous and ethanolic extract</td>
<td>H2O2 test</td>
<td>A significant antioxidant activity</td>
<td>Farhan et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>Leaves and stems</td>
<td>Aqueous and ethanolic extracts</td>
<td>Ferrozine test</td>
<td>A significant antioxidant activity</td>
<td>Dammous et al. (2014)</td>
</tr>
<tr>
<td></td>
<td>Leaves and stems</td>
<td>Aqueous, methanolic and ethyl acetate extract</td>
<td>Ferrozine test</td>
<td>A significant antioxidant activity</td>
<td>Rammal et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>Whole plant</td>
<td>Ethanol extract</td>
<td>Ferrozine test</td>
<td>A significant antioxidant activity</td>
<td>Hijazi et al. (2015)</td>
</tr>
<tr>
<td></td>
<td>Leaves and stems</td>
<td>Aqueous and ethanolic extract</td>
<td>VCEAC</td>
<td>A significant antioxidant activity</td>
<td>Farhan et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>Leaves and stems</td>
<td>Aqueous and ethanolic extract</td>
<td>Iron-induced lipid peroxidation in rat liver homogenates</td>
<td>It moderately suppresses Fe3+ -induced lipid peroxidation</td>
<td>Dammous et al. (2014)</td>
</tr>
<tr>
<td></td>
<td>Aerial parts</td>
<td>Methanolic and dichloroethanolic extract</td>
<td>Monkey kidney fibroblast (Vero) cell line</td>
<td>Not cytotoxic up to conc. of 47.6 μg mL⁻¹</td>
<td>Lubuncic et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>Leaves and stems</td>
<td>Aqueous, methanolic and ethyl acetate extract</td>
<td>Ferrozine test</td>
<td>A significant antioxidant activity</td>
<td>Fokialakis et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Leaves, stems, roots and whole plants</td>
<td>Aqueous and ethanolic extract</td>
<td>MCF7 breast cancer cell line by the XTT</td>
<td>Inhibits growth of cells by 68–72%</td>
<td>Rammal et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>Aerial parts</td>
<td>Ethanol extract</td>
<td>HeLa cancer cells by Natural Red Assay</td>
<td>Inhibits cell viability up to 97%</td>
<td>Dirani et al. (2014)</td>
</tr>
<tr>
<td>Antimutagenic activity</td>
<td>Ethanol extract</td>
<td>Rat hepatocyte primary cultures treated with N-methyl-N′-nitro-N-nitrosoguanidine Streptozotocin-induced diabetic rats</td>
<td>Rat hepatocyte primary cultures treated with N-methyl-N′-nitro-N-nitrosoguanidine Streptozotocin-induced diabetic rats</td>
<td>A direct antimutagenic activity and an increased recovery</td>
<td>Khader et al., 2010</td>
</tr>
<tr>
<td>Antihyperglycemic and hypoglycemic effects</td>
<td>Aerial parts</td>
<td>Aqueous decoction</td>
<td>Streptozotocin-induced diabetic rats</td>
<td>Significant reductions in blood glucose concentration when given orally</td>
<td>Jaghabir (1991)</td>
</tr>
<tr>
<td></td>
<td>Aerial parts</td>
<td>Aqueous extract</td>
<td>In vitro (according to Granfeld method) and in vivo enzymatic starch digestion</td>
<td>Lack of in vitro inhibitory activity</td>
<td>Kasabri et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>Aerial parts</td>
<td>Aqueous extract</td>
<td>Biosynergistic effects of β-cell proliferation and insulin secretion as well as glucose diffusion</td>
<td>Highly significant proliferative efficacies: lack of any statistically substantial glucose diffusion hindrances into external solution across dialysis membrane</td>
<td>Kasabri et al. (2012)</td>
</tr>
</tbody>
</table>
percentage inhibition of the haemolytic activity of the scorpion venom *Leiurus quinquestriatus*, as compared to the dried leaf extract. Extracts of both fresh and dried roots gave 100% inhibition of the snake and scorpion venom. However, ethanol extracts of the leaves and roots enhanced RBC haemolysis rather than inhibit venom activities on red blood cells (Alkofahi et al., 1997).

Unfortunately, the authors do not perform phytotoxic analysis of the tested extracts and did not respond to the question about which group of secondary metabolites or which particular compound is responsible for the activity. However, it is known from the literature that the plant reputed active against snake bite venom is another species belonging to *Eryngium* genus: *E. yuccifolium* Michx., known in North America as ‘rattlesnake master’. Dimethyl ether (veratum aldehyde) present in this species, which was officially published in the US Pharmacopeia of 1820–1860, was indentified to neutralize the effects of snake venoms (Mors et al., 2000).

**Antibacterial and antifungal activities**

There has been launched extensive research aimed at obtaining new antimicrobial medicines from different sources. Despite progress in the development of antibacterial agents and antifungal, the need to find new antimicrobial agents is still particularly urgent due to the development of multiresistant bacteria and fungi. The extracts and essential oil from *E. creticum* have been tested against different strains of bacteria and fungi (Ali-Shtayeh et al., 1998; Ali-Shtayeh and Abu Ghdeib, 1999; Abou-Jawad et al., 2002; Celik et al., 2011; Makki et al., 2015; Erdem et al., 2015).

The *in vitro* antibacterial activity of *E. creticum* was investigated against five bacterial species (Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris and Pseudomonas aeruginosa) by the disc diffusion method. The aqueous extract (inhibition zone with diameter 7.0 mm) proved to be more effective against *K. pneumoniae* than the ethanolic extract (6.0 mm), while the ethanolic extract (8.0 mm) proved to be most effective against *P. vulgaris*. There were no differences between the effect of the aqueous and ethanolic extracts against *S. aureus* (9.1 mm), *E. coli* (6.0 mm) and *P. aeruginosa* (6.0 mm) (Ali-Shtayeh et al., 1998).

The antibacterial activity against these three mentioned species of bacteria (*S. aureus, E. coli, P. aeruginosa*) and the two new species (Staphylococcus epidermidis, Enterococcus faecalis) was also measured by broth microdilution method. According to the research the aqueous extracts were stronger than ethanolic extracts for the growth inhibition of both Gram-positive and Gram-negative strains. Gram-positive strains were generally more sensitive to the tested extracts (MIC = MBC = 5.0 mg mL<sup>-1</sup>). The authors concluded that their studies have demonstrated that the extracts *E. creticum* have a promising antibacterial effect and the further phytochemical analysis on bioactive compounds responsible for this effect will be performed (Makki et al., 2015).

The *in vitro* anti-MRSA activity of the essential oil from *E. creticum* was assessed by the disc diffusion method using a panel of Gram-positive clinical isolates of nine methicillin-resistant *Staphylococcus aureus* strains. The antibacterial activity of the essential oil was lower (inhibition zones with diameters from 0 to 11 ± 1 mm) than that of the reference antibiotic vancomycin (from 15 ± 1 to 16 ± 1 mm) (Celik et al., 2011).

The *in vitro* antifungal activity of plant extracts was investigated against Candida with the disc diffusion method. Aqueous and ethanolic extracts of *E. creticum* did not differ in their activity against the tested microorganism (6.0 mm) (Ali-Shtayeh et al., 1998).

The aqueous extracts reduced colony growth of three dermatophytes: Microsporum canis, Trichophyton mentagrophytes and *T. violaceum* when tested by means of the agar dilution method. However, the inhibitory effect of extracts calculated as the percentage mycelia inhibition of tinea capitis dermatophytes was moderately or low and varied from about 12.4 ± 4.26% (*M. canis*) to 56.6 ± 7.42% (*T. mentagrophytes*) inhibition, in comparison with the control treatment – reference antibiotic – Griseofulvin (Ali-Shtayeh and Abu Ghdeib, 1999).

Moreover, the petroleum and methanolic extracts were tested *in vitro* against eight plant pathogenic fungi: Botrytis cinerea, Alternaria solani, Penicillium spp., Cladosporium sp., Fusarium oxysporum f. sp. melonis, Rhizoctonia solani, Verticillium dahlia and Phytophthora infestans. The extracts had low efficacy of mycelial growth inhibition, whereas they showed inhibition of spore germination. *E. creticum* showed >95% inhibition of spore germination in at least two fungi: *B. cinerea* and *F. oxysporum* f. sp. *melonis*. The level of antymycotic activity determined by mycelia growth inhibition test varied, depending on the fungus and extract tested, from 33 to 98% for petroleum, and from 3% to 75% for methanolic extracts (Abou-Jawad et al., 2002).

To conclude, little is known about the antimicrobial activity of *E. creticum*, but the experiments as referred to above showed a very wide spectrum of activity against many animal, human, and plant pathogens, including fungi, yeast, and bacteria.

**Antimalarial and antileishmanial activities**

Malaria and leishmaniasis are two of the most common parasitic diseases that infect a large human population over five continents. There is a rapid increase in the resistance of malaria parasites to antimalarial drugs. Most of the treatments available for parasitic diseases show limited efficacy and undesirable side effects.

The extracts of aerial parts of *E. creticum* from West Crete have been investigated for *in vitro* antiprotozoal activity. There was performed an evaluation of their activity against chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum* and *Leishmania donovani* promastigotes. The extracts were tested against promastigote cultures of *L. donovani*. IC<sub>50</sub> (the concentration causing 50% of cell deaths) was 35 µg mL<sup>-1</sup> and 38 µg mL<sup>-1</sup> for methanolic extract and dichloromethanolic extract, respectively. Plant extracts were also tested for antimalarial activity. However, the extracts of *E. creticum* (4, 20, and 100 µg mL<sup>-1</sup>), as well as the other species from the same genus tested in the same experiment, namely *Eryngium campestre*, were not active against *P. falciparum* (Fokialakis et al., 2007).

**Antioxidant activity**

The beneficial effect of many herbal plants on human health may be due to their antioxidant properties. The ability of the bioactive compounds present in *E. creticum* (including phenolic acids and flavonoids) to act as efficient free radical scavengers, as well as their natural origin in contrast to synthetic antioxidants, is the main advantage.

There was a number of studies carried out in order to evaluate the antioxidant power of the extracts (aqueous, ethanolic/methanolic and ethyl acetate) from different parts of *E. creticum* (leaves, stems) using several methods (Ljubuncic et al., 2005; Farhan et al., 2012; Rammal et al., 2013; Dammous et al., 2014; Dirani et al., 2014; Hijazi et al., 2015).

Three *in vitro* antioxidant methods, namely DPPH radical scavenging method (DPPH assay), scavenging activity of hydrogen peroxide radical (H<sub>2</sub>O<sub>2</sub> test) and chelating effects on ferrous ions (the Ferrozine test), were used by Farhan et al. (2012) and Dammous et al. (2014) to check the antioxidant activity of fresh leaves and stems of Lebanese *E. creticum*. Moreover, ABTS radical scavenging capacity assay was used to determine their total antioxidant activity (Farhan et al., 2012). The results showed significant antioxidant activity in both aqueous and ethanolic extracts, revealing that
*E. creticum* is a rich source of different antioxidant compounds (Farhan et al., 2012; Dammous et al., 2014). In DPPH assay, ethanolic extracts from both leaves and stems have higher antioxidant activity than aqueous extracts at different concentrations. The leaves possess higher free radical scavenging activity than the stems (Dammous et al., 2014). The free radical scavenging activity (H$_2$O$_2$ test) was enhanced with the increase in the concentration of the extract. The extracts from both the leaves and stems and from both the aqueous and ethanolic extracts of *E. creticum* were able to scavenge H$_2$O$_2$ in a concentration-dependent manner (Farhan et al., 2012; Dammous et al., 2014).

The chelating capacity (Ferrozine-test) of the extracts increases with concentration. Both extracts, the aqueous and ethanolic one, provide almost the same chelating activity (Dammous et al., 2014).

The antioxidant capacity of different extracts of *E. creticum* has been evaluated using two in vitro tests, the DPPH and iron chelating (spectrophotometric analysis). In the first experiment, the methanolic and aqueous extracts from both the leaves and stems have exerted an antioxidant power in DPPH radical scavenging activity, depending on the concentration. To confirm the results from the DPPH method, the Iron-Ferrous method was used. The results showed that the antioxidant activity of leaves was higher than that of stems, regardless of the concentration. The authors suggested that this activity was due to leaves containing more phenolic compounds, as compared to stems (Rammal et al., 2013). In the second experiment, the antioxidant activity was investigated depending on the different percentages of ethanol in the extracts (40%, 80%, 100%), extraction times (2 h, 4 h, 12 h) and extraction methods (maceration, reflux, microwave assisted extraction). The microwave assisted extraction was the most effective extraction technique. Generally, 80% ethanolic extracts possessed the highest DPPH scavenging activity and 40% ethanolic extracts had the highest iron chelating activity (Hijazi et al., 2015).

The total antioxidant capacity, expressed as vitamin C equivalent antioxidant capacity of fresh leaves and stems, was 79.00 ± 0.80 mg and 50.42 ± 0.50 VCE/100 g fresh weight, respectively (Farhan et al., 2012; Dammous et al., 2014).

The antioxidant potential of the extracts at different concentrations was tested by quantifying their ability to suppress the extent of iron-induced lipid peroxidation in rat liver homogenates. The extract efficacy IC$_{50}$ was very low and the value of the maximum inhibition was moderate compared to other extracts. To conclude, the extracts of *E. creticum* can moderately suppress Fe$^{2+}$-induced lipid peroxidation (Ljubuncic et al., 2005).

All of these results showed that although *E. creticum* does not show strong antioxidant activity, it might be considered a good source of natural substances that may be employed in the treatment of different diseases associated with oxidative stress.

**Cytotoxicity**

The cytotoxicity of *E. creticum* extract obtained from a plant from West Crete was tested on a mammalian/monkey kidney fibroblast cell line. The aerial part extract at the concentration of 47.6 μg ml$^{-1}$ was not cytotoxic to mammalian cells (Fokialakis et al., 2007).

The cytotoxicity of three extracts (aqueous, methanolic and ethyl acetate) from leaves and stems of fresh plants on the MCF7 breast cancer cell line by the XTT cell viability technique was studied. The results showed that the aqueous and ethyl acetate extracts of both leaves and stems (at the concentration of 2.5 mg ml$^{-1}$) did not show any cytotoxicity after 48 h of treatment on this cell line, while the methanolic extract of both studied parts inhibited the growth of MCF7 by 72% and 68%, respectively (Rammal et al., 2013).

The MIT and LDH assays are well-established methods (Bunel et al., 2014) to assess mitochondrial competence and cell membrane integrity, respectively. Using these assays, the authors found that the aqueous extract (1.0 μg ml$^{-1}$–1.0 mg ml$^{-1}$) of *E. creticum* did not suppress mitochondrial respiration or increase LDH leakage in PC12 and HepG2 cell cultures (Ljubuncic et al., 2005).

In the recent studies, the cell viability and cytotoxicity effect of aqueous and ethanolic extracts (leaves, stems, roots, and whole plant) of *E. creticum* in HeLa cancer cells were evaluated using the Natural Red Assay. The effect of inhibition was dose and time-dependent. The results showed that the aqueous extracts from leaves (150, 200 and 250 μg ml$^{-1}$ acting for 48 and 72 h (90% inhibition on HeLa cells) and from roots (250 μg ml$^{-1}$, 72 h, 90% inhibition) exerted anti-proliferative activity. On the other hand, the ethanolic extracts showed a significant effect at different concentrations and times of exposition (up to 97%) (Dirani et al., 2014).

**Antimutagenic activity**

In the study, the antimutagenic activity of ethanolic extract (the highest concentration applied was 120 μg ml$^{-1}$) of *E. creticum* was tested in rat hepatocyte primary culture treated with N-methyl-N’-nitro-N-nitrosoguainidine (MNNG), a directly acting mutagen which methylates DNA. It was shown that MNNG induced a massive chromosomal damage to hepatocytes. Antimutagenicity testing was performed in three modes: pre-treatment, combined treatment and post-treatment of the primary cultures with plant extract and MNNG. Therefore, both the induction of metabolizing enzymes, direct interaction of plant constituents with the mutagen and increased recovery, i.e. enhanced repair of induced DNA damage, could be evaluated. The results of the investigation clearly indicated the inhibitory effect of the plant extract on MNNG-dependent mutagenicity, and this effect could be attributed to direct antimutagenic activity and increased recovery (Khadar et al., 2010).

**Antihyperglycemic and hypoglycemic effects**

An aqueous decoction of *E. creticum* leaves is used in traditional medicine in Palestine and Jordan as anti-diabetic therapy (Yaniv et al., 1987; Kasabri et al., 2011, 2012).

Jaghabir et al. (1991) carried out an investigation of the hypoglycemic effect of *E. creticum*. The hypoglycemic activity of the aqueous decoction of aerial parts was tested in normoglycemic and hyperglycemic rats with streptozocin-induced diabetes. Drinking water for the animals was replaced with the plant extract and blood glucose concentration was measured after 1, 4, and 24 h. It was found, admittedly, that the extract of euryngo decreased glucose levels in both diabetic and non-diabetic animals and this change was statistically significant for the hyperglycemic rats only. Nevertheless, it is not known if the rats had free access to food or were fasting within 24 h of the experiment which influences the interpretation of these results. Moreover, replacing water with the tested extract is not a method which gives us certainty that the active substance was really taken in by each animal. The authors suggested that *E. creticum* may have increased the peripheral utilization of glucose, but this interpretation seems to be uncertain regarding the very short period of observation (24 h) and the single dose of the extract used in the study (Jaghabir, 1991). However, we must note that replacing water with the tested extract is questionable, and to obtain reliable results of this experiment the extracts should have been applied intragastrically.

Kasabri et al. (2011) evaluated the effect of the aqueous extract (up to 50 mg ml$^{-1}$) from fresh aerial parts of Jordanian *E. creticum* on *in vitro* (according to Granfeld method) and *in vivo* enzymatic
starch digestion. In vitro enzymatic starch digestion with acarbose, an α-glucosidase-inhibitor, used as a control or with plant aqueous extract was assayed using α-amylase and α-amylglucosidase; while in vivo there were observed changes in glycaemia in rats treated with plant extract or with acarbose that were compared to the effects of starch feeding. The results demonstrated the lack of the effect of E. creticum extract on enzymatic starch digestion in comparison to the effect induced by acarbose, a reducing agent used widely in postprandial hyperglycaemia. Nevertheless, the aqueous extract from E. creticum significantly decreased glucose level during starch tolerance test. Although the E. creticum-treated rats did not demonstrate any decrease in overall glycemic excursion versus the control and the drug-treated animals, substantial improvements of glucose handling were evident at a later time – 90 and 135 min after corn starch ingestion. Unfortunately, oral administration of E. creticum extract had no marked improvement in glucose tolerance in the rats. Moreover, when glucose load was ingested by E. creticum rats, the corresponding glycemic incremental curves were not affected during the 165 min time course of the acute experiment. The authors suggest that the lack of in vitro inhibitory activity does not relate to the in vivo activity, because the intestinal luminal activation of effective entities precursors offers a valid justification for the acute in vivo outcomes. The contrasting effects ascribed to E. creticum do not necessarily role out any other potential pancreatic or extrapancreatic mode of action (Kasabri et al., 2011).

Next year, the same group of authors aimed at investigating the pancreatic and extrapancreatic effects of the aqueous extract from fresh aerial parts of Jordanian E. creticum. They recruited bioassays of β-cell proliferation and insulin secretion, as well as glucose diffusion, as possible models of action. The mouse insulinoma MIN6 β-cell line was the cellular model to examine the in vitro effects of E. creticum extract on pancreatic β-cell proliferation and insulin secretion. E. creticum extract was found to be significantly potentiating MIN6 glucose stimulated insulin secretion (GSIS). E. creticum evoking GSIS in MIN6 cells was shown to take place by modulating Ca²⁺ regulated exocytosis machinery. Moreover, E. creticum substantially augmented the β-cell proliferation in a dose-dependent manner (0.001–1.0 mg ml⁻¹). E. creticum aqueous extracts may offer a promising avenue for the potential treatment of β-cells demise in diabetes. Hence, intensive chronic testing of plants inducing the pancreatic β-cell expansion will allow the emergence of safe and efficient cell replacement therapies. The authors concluded that future directives may assess the use of E. creticum as a new potential source of functional food or nutraceuticals or active leads into diabetes type 2 pharmacotherapy (Kasabri et al., 2012).

Taking into consideration both papers published by Kasabri et al. (2011, 2012), the in vivo hypoglycaemic activity of E. creticum extract is uncertain, but further studies should be focused on anti-hyperglycaemic effect documented in vivo and in vitro by glucose diffusional retardation effects. The results of present investigative study of Twaij and Al-Dujaili (2014) did not ultimately elucidate the main mode of action of E. creticum extract. It was found that the oral administration of aqueous extract from the aerial parts of E. creticum produced significant effect in rats, namely reduced postprandial (post-load) hyperglycaemia. Interestingly, the hypoglycemic effects of the aqueous extracts from E. creticum were confirmed only in normal and not in alloxan-induced diabetic animals which suggests its insulinotropic activity (Twaij and Al-Dujaili, 2014).

The traditional use of E. creticum in the treatment of diabetes is supported by laboratory results from these studies, suggesting the need to isolate and evaluate active constituents responsible for the exhibited biological activity.

Other activities

The methanolic extract of E. creticum was evaluated for their hormone sensitive lipase inhibitory potential, quantified by a colorimetric assay that measured the release of p-nitrophenol as HSL substrate and expressed as % inhibition of XO activity. Hormone-sensitive lipase (HSL), a neutral lipase, is a vital enzyme in lipid metabolism and general energy homeostasis in mammals. HSL is a component of the metabolic switch between glucose and FFAs as energy sources. The pivotal role of elevated plasma FFAs in the development of insulin resistance and type 2 diabetes have rendered HSL as a potential therapeutic target for this disease; lowering plasma FA levels and, thereby, reducing insulin resistance. The percentage of residual activity of HSL was determined for each extract by comparing the lipase activity of HSL with and without the extract. However, the extract showed weak activity (Bustanji et al., 2011).

Conclusion

This present review offers basic information on current knowledge for further studies of E. creticum. The in vitro studies and in vivo models have provided a simple bioscientific explanation for its various ethnopharmaceutical uses. Unfortunately, some of the studies appeared to be only partial and did not answer questions from selected research area. Other studies, particularly in the field of phytochemical analysis, remain unimplemented or their results are unreliable due to improperly constructed research methodology. Besides, the majority of the pharmacological studies were carried out using crude and poorly characterized extracts. Therefore, the authors of this article took in vitro cultures of E. creticum to amplify the plant material needed for in-depth studies of selected phytochemicals and selected biological activities.

Authors’ contributions

MK contributed in the review of the scientific bibliography using the following publishers websites and electronic databases to collect information on the subject: Elsevier, Springer, PubMed, Medline, Google Scholar and Web of Science. Additionally, she analyzed published monographs theses, and proceedings of scientific congresses. JK drew all the chemical formulas. JK, MD and BT participated in the critical reading and final editing of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

References
