

ORIGINAL ARTICLE

Evaluation of Medical Metabolites in Boraginaceae Family

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KEYWORDS

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ABSTRACT: *Boraginaceae* family is known as a medicinal plant classified in dicotyledons. It is originated from Asia (Middle East). The aim of this study was to evaluate ingredient between 4 species of *Boraginaceae* family based on physiological & phytochemical traits as well as seed fatty acid contents. 4 species (*E. russicum*, *E. italicum*, *E. amoenum*, and *B. officinalis*) were evaluated carefully. All seeds were cultivated in identical conditions in a greenhouse in Tehran to assesse parameters such as tannins, phenols, anthocyanin, total protein, seed oil contents, Superoxide Dismutase (SOD), and Catalase (CAT) activity. Analysis of oil from seeds of *Echium*L. determined 7 different fatty acids including Linolenic acid (35.1%), Linoleic acid (16.8%), Oleic acid (16.6%) and Arachidonic acid (15.5%) as major fatty acids, while stearic acid (4.42%), Palmitic acid (6.22%), Gama-Linolenic acid (6.04%) were the minor fatty acids extracted from seeds. Low protein content was observed in *E. russicum*(70 mg/g) and maximum level of protein was in *B. officinalis*(91mg/g). *E. amoenum* had maximum phenols (38mg/g) whereas *E. russicum* had minimum (26 mg/g). For total phenol, *B. officinalis* had maxium phenols (8.1mg/g) whereas *E. italicum* had minimum (3.9mg/g). Anthocyanins: *E. russicum* had maximum anthocyanins (65 mg/g) whereas *B. officinalis* had minimum (41 mg/g). In conclusion, it can be said that different species had different amounts of secondary metabolites so that no regular relation would be detected among plant species that we studied.

INTRODUCTION

In traditional medicine, *Echium L.*, including *E. vulgare L.*, is utilized as exhilarant and mood stimulant. Therefore, finding effective and safe treatments is a hotly contested area in the present time [1]. In the study that antidepressant effects of aqueous and alcoholic extracts of *E.vulgare L.* aerial parts were investigated on

mice, it was observed that the aqueous extract at low doses and ethanolic extract at high doses had significant antidepressant effects [2]. The effects of extracts were similar to imipramine and they may affect neurotransmitters, norepinephrine and serotonin. This herb might be considered as a useful drug in the management of depression [25]. *E. amoenumis* herbal

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plant belongs to different part of Iran and Caucasus, where it grows at an altitude ranging from 60-2200 m [3, 4 and 5]. The phytochemical study on *Echium* L. has demonstrated various chemicals such as anthocyanin, tannins and alkaloids [9, 16, and 19]. As yet more than 140 types of anthocyanin have found. The amount of anthocyanin is affected by light, heat, stress and so that high level of light is favorable condition for anthocyanin production [1].

Polyphenols are characterized by antioxidant; antimicrobial activity [18]. While tannins could be aggregated by albumins to produce protective layers [7]. The amount of gamma-linolenic acid has reported 12% in *Echium humile* [6, 8, and 39]. In another study on *Echium* L. the gamma-linolenic acid contents observed about 20%, so *Echium stenosisiphon* had the high level and 18.8% in *E. pitardii* and 14.4% in *Echium boissieri* [8]. In a research on 16 different fatty acids of Boraginaceae family in Turkey the maximum levels of gamma-linoleic acid reported in *Symphytum tuberosum* (24%) and maximum levels of alpha-linoleic acid reported in *Echium italicum* 43% [15]. Also, the amount of gamma-linolenic acid in *E. pitardii* var. *pitardii* 18.7% and in *E. gentianoides* 27.4%; and the amount of stearidonic acid were 3.8% in *E. pitardii* var. *pitardii* and 8.8% in *E. pininana* [6]. In the study that assessed the efficacy and tolerability of the aqueous extract of *Echium amoenum* in combination with SSRIs in patients with General Anxiety Disorder (GAD), we found that *E. amoenum* is effective on anxiety disorder, especially in higher dosage, without any serious side effects [22]. Medicinal plants have been used for different diseases in the past. There is an increasing need for substances with antiviral activity since the treatment of viral infections with the available antiviral drugs often leads to the problem of viral resistance. In the study on *Echium amoenum* L. plant with ethnomedical background was used for antiviral activity against HSV-1 in different times. *Echium amoenum* L. plant had not toxic effect at highest

concentrations to the cell lines used and showed the most antiviral activity when it was used an hour after virus inoculation [23]. At a study performed on Phenolics contents of *Borago officinalis* L. seeds, three compounds (rosmarinic acid, syringic and sinapic acids) contributed to 3.9% of the dry mass of the crude extract whereas their total contribution in the meal was 0.6% (w/w) [24]. Therefore, finding effective and safe treatments is a hotly contested area in the present time. In the study that antidepressant effects of aqueous and alcoholic extracts of *Echium vulgare* L. aerial parts were investigated on mice, it was observed that the aqueous extract at low doses and ethanolic extract at high doses had significant antidepressant effects. The effects of extracts were similar to imipramine and they may affect neurotransmitters, norepinephrine and serotonin. This herb might be considered as a useful drug in the management of depression [25]. *E. amoenum* herbal plant belongs to different part of Iran and Caucasus, where it grows at an altitude ranging from 60-2200 m (3). *Echium* genus has 4 species in Iran and only *E. amoenum* has medicinal uses. During years the petals of *Echium amoenum* has been used as medicinal part for remedy about different diseases, is none a traditional medicine. It could treat some diseases including diaphoretic, tranquillizer, pneumonia, throat and tranquillizer. Mush study indicated that *E. amoenum* riched in some volatile oil, flavonoids, anthocyanidine, alkaloids. It has been shown that *E. amoenum* is able to improve the immune system as well as anxiolytic effect. Since the *Echium* species have significant effect on health, we decided to study the secondary metabolite existed in this plant [26]. Dried violet-blue petals of *E. amoenum* have been recognized as an important source of phenolic compounds like rosmarinic acid, cyaniding, and delphinidin [27]. Cyanidin 3-glucoside, the most common anthocyanin, which was presented in petals of *E. amoenum* attenuates PGE2 production and cyclooxygenase-2 expressions by inhibiting activation

and translocation of c-Jun and NF- κ B factors into nucleus [28]. Also the neuroprotective activity of cyanidin 3-glucoside has been investigated by Min and colleagues; they suggested that the beneficial effect was related to attenuation of brain superoxide levels resulted from blocking apoptosis-inducing factor release in mitochondria [29]. It is approved that *Echium* species have potentially immunomodulatory, antibacterial, antidepressant, antioxidant, anxiolytic and analgesic effects [30] as well as treatment of obsessive-compulsive disorder [31]. The phenolic compounds like rosmarinic acid, cyaniding, and delphinidin are accumulated in petals of *E. amoenum*[32]. The known anthocyanin existence in *E. amoenum* is Cyanidin 3-glucoside [28]. Beside this, neuroprotective activity of cyanidin 3-glucoside has been related to blocking apoptosis-inducing factor release in mitochondria [29]. So, it has been demonstrated that *E. amoenum* hydroalcoholic extract possessed protective activity against cerulein-induced acute pancreatitis in mice and may suggest a therapeutic potential for therapy or prevention in this inflammatory disease condition [34]. We know that Echium oil (EO) contains stearidonic acid (18:4), a n-3 polyunsaturated fatty acids (PUFAs), and gamma-linolenic acids (18:3), a n-6 PUFA that can be converted to long chain (LC)-PUFAs. In a study that compared a safflower oil (SO)-enriched diet to EO- and fish oil (FO)-enriched diets on circulating and tissue PUFAs levels and glycemic, inflammatory, and cardiovascular health biomarkers in insulin resistant African green monkeys, it was observed that Glucose disposal was improved after EO consumption, that it suggested that PUFAs in EO supplementation have the capacity to alter circulating, RBC and muscle LC-PUFA levels and improve glucose tolerance in insulin-resistant monkeys [35]. Also, the fatty acid profile of vegetable oils (VOs), together with the poor ability of marine fish to convert polyunsaturated fatty acids (PUFA) to highly unsaturated fatty acids (HUFA), lead to important

changes in the nutritional value of farmed fish fed VO, which include increased fat and 18:2n-6 and reduced n-3 HUFA. *Echium* oil (EO) has a good n-3/n-6 balance as well as an interesting profile with its high content of unusual fatty acids (SDA, 18:4n-3 and GLA, 18:3n-6) that are of increasing pharmacological interest. Lipid contents and lipid class compositions were not affected by EO [36]. Pathogen infection stimulates the fatty acid (FA) metabolism and the production of pro-inflammatory derivatives of FA. EO, containing SDA and GLA and with a comparatively higher n3:n6 PUFA ratio, proved more effective than RO in compensating for immunity stress [37]. High amounts of γ -linolenic acid (GLA, 18:3 ω 6) were found in fourteen species of the genus *Echium*, ranging from 18.85% (*E. pitardii* var. *pitardii*) to 27.42% (*E. gentianoides*) on total seed fatty acids. The GLA content related to total seed weight was also significant, ranging from 1.26% (*E. handiense*) to 8.22% (*E. gentianoides*). In addition, considerable amounts of stearidonic acid (SA, 18:4 ω 3) were detected, ranging from 3.78% (*E. bonnetii* var. *bonnetii*) to 8.81% (*E. pininana*) on total fatty acids. Besides all the perennial species, the four herbaceous *Echium* taxa endemic to the Macaronesia also showed high GLA percentages. This is in contrast to the low GLA level found in continental *Echium* species, all of them bearing an herbaceous habit. These results are in good agreement with the available genetic data and show the ability of GLA to discriminate between Macaronesian and continental *Echium* species [38]. Macaronesian *Echium* (*Boraginaceae*) species that were previously reported as the major sources of γ -linolenic acid have been surveyed for hydrocarbon compounds. In addition, six European *Echium* species and the common *Boragoofficinalis* have been analysed for comparative purposes. High squalene amounts were found in all *Echium* plants from the Macaronesia, ranging from 3.73% in *E. simplex* to 20.11% in *E. fastuosum*. Squalene was almost absent from all European *Echium*

species, and the same is true for *B. officinalis*. The relatively high oil content (2.27%) in leaves of *E. fastuosum* raises the total squalene amount to about 0.46% within this tissue. The main fatty acid component in the leaf was α -linolenic acid (ALA, 18:3 ω 3), ranging in the Macaronesian *Echium* from 9.32% in *E. acanthocarpum* to 54.45% in *E. simplex* [7]. The ethanolic extract of *Echium amoenum* flowers at the dose of 50 mg/kg increased the percentage of time-spent and the percentage of arm entries in the open arms of the elevated plus-maze (EPM) and decreased the percentage of time-spent in the closed arms of EPM. These results suggested that the extract of *E. amoenum* seems to possess anxiolytic effect with lower sedative activity than that of diazepam [40]. ‘Front-end’ desaturases of higher plants catalyse the desaturation of either fatty acids attached to phospholipids (Δ^6 -desaturases), or the long-chain base of sphingolipids (Δ^8 -desaturases). In a few plant families like the Boraginaceae, a Δ^6 -desaturase activity is responsible for the synthesis of unusual fatty acids like the γ -linolenic acid (18:3 $n-6$) or the octadecatetraenoic acid (18:4 $n-3$). A Δ^6 -desaturase from *Echium* (Boraginaceae), which likely represents a pseudogene recently originated in this evolutionary lineage [41]. Solvent of seed oil and free fatty acids (FFAs) was employed to obtain γ -linolenic acid (GLA; 18:3 ω 6) concentrates from seed oils of two Boraginaceae species, *Echium fastuosum* and *Borago officinalis* [42].

MATERIALS AND METHODS

Plant Material

The seeds of plant species were prepared from seed and seedling institute in Karaj and then cultivated in identical conditions in a greenhouse in Tehran.

Extraction and isolation

Dried plants of all species were exhaustively extracted with methanol in a Soxhlet apparatus under reduced pressure at 30 °C. The resulting methanolic extract was

filtrated and concentrated in vacuum and after water addition; it was washed successively with hexane. After vaporation of the solvent, the residues were subjected to spectroscopy and GC-Mass.

The sample solution was injected into a GC–MS system (Varian model CP-3800 GC and Saturn 2000 GC/MS).

Methods

Total protein assay

0.05 g Comasi Brilliant Blue G250 was solved in 50 ml ethanol 95% in 2 hours and then 100 ml phosphoric acid 85% added to reach 1000ml. The solution was filtrated by Watman paper. 0.1 ml. Plant extract was added to each solution. Finally, after 2 min absorbance spectra was recorded at 595 nm. Total phenol and tannins assay: There are various methods to assess total phenol and tannins [12, 20]; the following method has been used in this research [13, 14].

Total extractable phenols assay

0.1 ml extract was added to 0.9 ml water and 0.5 ml Folin Ciocalteu (1M) and 2.5 ml NaCO₃ (20%). Solution was shaken and maintained at 35°C. Total extractable tannins assay: 2.0 ml extract was added to 100 mg polyvinyl poly pyrrolydin (PVP) and centrifuged at 3000 rpm at 5 min. Then we added 0.9 ml water and 0.5 fullinshikaltu and 2.5 ml NaCO₃ (20%). After it was maintained for 35 min undershaking at room temperature, the absorbance was measured at 725 nm.

Seed fatty acids assay

Fatty acids were measured by Gas chromatography (GC) characterized by Unikam model 4600 made in England were equipped by flame ionization detector (FID) and column (BPX70, SGE, Melborn, Australia), with helium gas.

RESULTS AND DISCUSSION

After plant harvesting, total biochemical and physiological tests were measured, they included seeds weight (g), saturated and unsaturated fatty acids of seeds (%), total proteins (mg/g), Anthocyanin (mg/g),

Phenols (mg/g), Tannins (mg/g) and activity of Catalase CAT (A240/mg.min FW) and Superoxide Dismutase SOD (U/mg FW). The following results obtained. Seeds weight: Different species with regard to seed weight were significant ($P < 0.05$), so that *E. russicum* had maximum weight (9.6 g), while *E. amoenum* had minimum seed weight (7.9 g). Analysis of oil from seeds of *Echium L.* determined 7 different fatty acids including Linolenic acid (35.1%), Linoleic acid (16.8%), Oleic acid (16.6%) and Arachidonic acid (15.5%) as major fatty acids, while stearic acid (4.42%), Palmitic acid (6.22%), Gama-Linolenic acid (6.04%) were the minor fatty acids extracted from seeds (Figure 1). It can be thought that the presence of Gama-linolenic acid is related to minimum of temperature during seed growth. Regard to Palmitic acid, ecological changes had no significant effects on these fatty acids. The contents of Palmitic acid in arid conditions are more than cold condition. As weather was cold, content of Palmitic acid decreased, whereas warming led to increase it. The high level of Palmitic acid in species was a result of decreasing of temperature and high level of height. Also, soil moisture and temperature were effective parameters on quality and quantity of oil. Plants growth in cold regions had more oils and Linolenic acids [4]. There was relation between seed oils and cold-humidity weather, so there were maximum oils in these regions, whereas in warm regions contents of oils decreased. It has been proved that low temperature increased the contents of Linolenic acid in seeds [2].

Soluble proteins: Content of soluble protein in different species was different significantly. Low protein content was observed in *E. russicum* (70 mg/g) and maximum level of protein was in *B. officinalis* (91mg/g). Accumulation of protein was a plant strategy to face arid condition to balance the osmotic properties. It has been reported that the levels of soluble proteins in species resistant to water-stress conditions were more than other

species. Secondary metabolites: The level of secondary metabolites (Phenols, Tannins and Anthocyanins) in tissues of different species of *Echium L.* growth varied at different regions, so cloudy regions had more secondary metabolites; and contents of tannins varied to environmental stresses. *E. amoenum* had maximum phenols (38mg/g) whereas *E. russicum* had minimum (26 mg/g).

For total phenol, *B. officinalis* had maximum phenols (8.1mg/g) whereas *E. italicum* had minimum (3.9mg/g) (Figure 2). Anthocyanins: *E. russicum* had maximum anthocyanins (65 mg/g) whereas *B. officinalis* had minimum (41 mg/g). The contents of anthocyanins were dependent on leaf structure and UV-C. Production of anthocyanins in plant was a protective mechanism to solar radiation. Anthocyanins were responsible for biological membrane stability. This compound had protective effects to free radical produced by radiation [10]. CAT and SOD activity: The activity of CAT and SOD enzymes varied in leaf of different species of *Echium L.* *B. officinalis* had maximum activity (45.6 A240/mg FW for CAT and 89.3 U/mg FW for SOD). *E. Russicum* had minimum activity (39.8 A240/mg FW for CAT and 81.2 U/mg FW for SOD). Different conditions had different effects on activity of antioxidant enzymes. Water depletion led to increase activity of these enzymes significantly. A consequence of warming was production of more reactive oxygen [31]. In hot-stress condition accumulation of oxygen reactive species damage to plants and led to increase antioxidant system activity. Deficiency in antioxidant system led to disorder in lipid and fatty acids metabolism and will decrease fatty acid production [5]. Also, activity of electron transfer in mitochondria was deficient. There was direct relation between antioxidant system and hot-stress [5]. So, it can be concluded that activity of antioxidant enzymes in *Echium L.* was important factor in biological stability and adaption in ecological conditions.

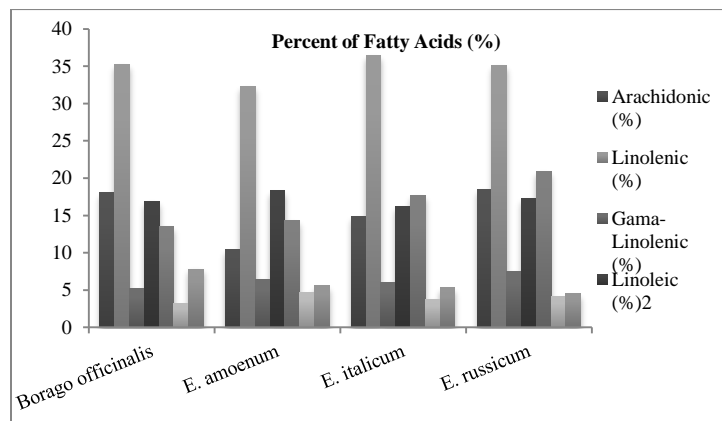


Figure 1. Percent of Fatty Acids

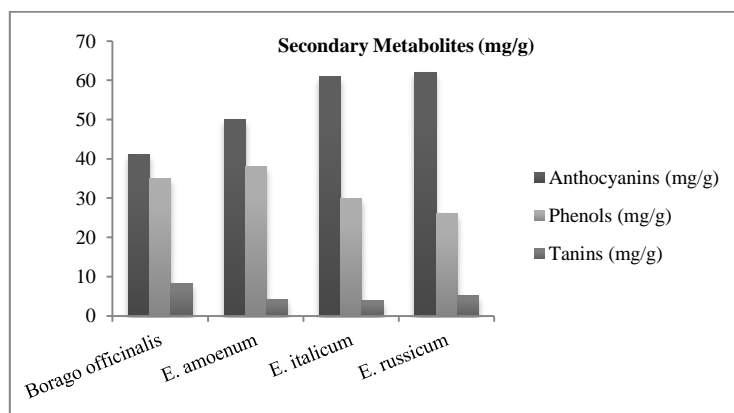


Figure 2. Contents of Medical

CONCLUSION

Analysis of oil from seeds of *Echium* L. determined 7 different fatty acids including Linolenic acid (35.1%), Linoleic acid (16.8%), Oleic acid (16.6%) and Arachidonic acid (15.5%) as major fatty acids, while stearic acid (4.42%), Palmitic acid (6.22%), Gamma-Linolenic acid (6.04%) were the minor fatty acids extracted from seeds. Also miscellaneous amount of tannins, phenols and anthocyanins was observed in studied species. In conclusion it can be said that different species had different amounts of secondary metabolites so that no regular relation would be detected among plant species that we studied.

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