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RESEARCH ARTICLE

Investigation of the extract's composition of Viper's bugloss *(Echium vulgare)*

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Abstract: The characteristics of Viper's bugloss (*Echium vulgare*) plant, its pharmacological properties, and extracts' composition are presented in this study. Results of the literature analysis, data on the biologically active compounds and areas of use of this medicinal plant are summarized. Viper's bugloss (*E. vulgare*) is a species of flowering plant in the borage family *Boraginaceae*. It is native to most of Europe as well as western and central Asia. Viper's bugloss (*E. vulgare*) is a plant that has been utilized as food (honey), medicine, a poison, an oil, and as a dye and tannin-producing ornamental plant. Viper's bugloss (*E. vulgare*) is especially rich in pyrrolizidine alkaloids, flavonoids, phenolcarboxylic acids, sterones and naphthoquinones. In traditional medicine, Viper's bugloss (*E. vulgare*) is utilized as exhilarant and a mood stimulant. That is why one of the possible uses of this plant is considered to be treatment of depressive states. Like most representatives of *Boraginaceae* family, it has been insufficiently studied. No previous work quantifying flavonoids content of aerial parts of Viper's bugloss (*E. vulgare*) growing in Ukraine has been presented. Continuing the studies of this species, the aqueous and ethanolic extracts from Viper's bugloss (*E. vulgare*) aerial parts were obtained and their phytochemical composition was investigated. For the first time, the qualitative analysis of biologically active compounds in Viper's bugloss's extract as well as the quantitative analysis of flavonoids by aluminum chloride spectrophotometric method are reported. The experimental results showed that the total concentration of flavonoids was 2.59% in the extract. The maximum yield of extractives was found to be 16%. The obtained research data will be used in future investigations.

Keywords: Viper's bugloss (*Echium vulgare*); biologically active substances; flavonoids; depression.

Introduction

Depressive disorders, including major depression and dysthymia, are serious, disabling, and often difficult-to-treat illnesses. A promising direction in the treatment of depressive disorders is the study of existing and creation of new effective herbal remedies. A large number of herbal antidepressants on the Ukrainian market, such as "Life 900", "Sedariston", "Neuroplant", "Deprivit" and others, based on the extract of St. John's wort (*Hypericum perforatum*).

Among these objects of research, the well-known honeysuckle Viper's bugloss (*Echium vulgare*) attracts special attention as a promising source of biologically active substances (BAS) for the treatment of depression [1]. This paper analyzes the literature sources that contain information on the chemical composition of the plant, its application in folk and traditional medicine and prospects for its use. We also presented our studies of the quantitative content of extractive substances, flavonoids, and qualitative determination of various BAS groups in the plant raw materials [2].

Results and Discussion

Viper's bugloss (*E. vulgare*), or Blueweed, is a species of flowering plant in the borage family of *Boraginaceae*. Viper's bugloss (*E. vulgare*) can be biennial or short-lived perennial. The stems of mature plants are straight and reaching over 70 cm in height. Stem leaves are alternate, linear-lanceolate, sessile, basal leaves narrowed to a petiole,

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dry up during the flowering stage. Both stems and leaves are covered with stout. The stem terminates in a panicle inflorescence, and each branch of the panicle forms a short helicoid cyme subtended by an upper foliage leaf. There can be as many as 50 cymes per stem, and each cyme bears up to 20 flowers on the top. The showy flowers range in size from 1 to 2 cm. The funnel-shaped, five-lobed flowers are typically bright blue, but may also be purple, pink or rarely, white (Figure 1). The seeds of Viper's bugloss (*E. vulgare*) are called nutlets. They are brown or gray with a rounded pyramid shape and are quite small. [3].

Figure 1. Viper's bugloss (*E. Vulgare*).

Viper's bugloss (*E. vulgare*) grows throughout Ukraine. It grows best in sunny areas, such as meadows, overgrazed pastures, poorly drained slopes and roadsides. It usually will not do well in cultivated ground. [4].

E. vulgare belongs to dye and tannin producing, medicinal, ornamental, and bee plants since it is a source of nectar and pollen forage. The flowers of *E. vulgare* are frequently visited by bumblebees and honey bees. Nectar contains 30-40% sugars and is mostly sucrose-dominated. Honey productivity is 300-400 kg/ha. Honey from *E. vulgar*e has a light color, a pleasant smell, and a delicate taste. It contains a number of components that act as conservatives, such as vitamin C, flavonoids, and other phenols, as well as enzymes like glucose oxidase, catalase, and peroxidase, so it can remain preserved in a completely edible form for a long time. Basal leaves and young shoots of *E. vulgare* can be eaten in salads or stirfried.

The decoction of the Blueweed is used in folk medicine as an expectorant and soothing remedy for common or whooping cough, and as anticonvulsant and sedative for epilepsy. It is also used externally against rheumatic pains in joints, tendon sprains etc. [5]. *E. vulgare* contains hepatotoxic pyrrolizidine alkaloids such as cynoglossin and consolidin, which can be toxic to horses and cattle when consumed in large amounts. Its roots yield a water-insoluble carmine-red dye alkannin for wool, and its flowers contain anthocyanin, which appears as a red pigment in acidic and blue in alkaline conditions. Viper's bugloss (*E. vulgare*) has been planted as an ornamental plant. It also has the fatty acid composition of the seed oil (28-32%) and is grown as an oilseed crop. The seed oil from *E. vulgare* contains significant amounts of omega-3 and omega-6 polyunsaturated fatty acids (PUFA) such as g-linolenic acid (GLA) and rare stearidonic acid (SDA) [3].

Based on literature reports on the chemical composition of the Viper's bugloss (*E. vulgare*), we can identify the following groups of BAS (Figure 2):

• flavonoids (derivatives of kaempferol, quercetin, quercitrin, hesperidin, hesperetin, rutin, naringenin, naringin, apigenin);

• alkaloids (echimidine, acetylechimidine, uplandicine, 9-*O*-angelylretronecine, echiuplatine, leptanthine, echimiplatine, echivulgarine, vulgarine, and 7-*O*-acetylvulgarine);

• tannins (in the roots (1.59%), leaves, flowers (0.98%), stems (0.58%));

• dyes (alkannin in the roots and anthocyanin in the flowers);

• fatty acids (saturated palmitic (5.65-17.81%), stearic (1.49-5.08%) fatty acids, mono-unsaturated oleic (8.83-55.32%), eicosenoic (0.22-6.21%), erucic (0.04-8.94%), nervonic (0.08-2.71%) fatty acids, and PUFAs such as linoleic (between 1.41 and 68.44%) and stearidonic acids (between 0.02 and 14.59%);

• polysaccharides (D-galactose, D-glucose, L-arabinose, and L-rhamnose);

• amino acids (16 amino acids, including 7 essential: lysine (247.10 μg/100 mg), tyrosine (236.20 μg/100 mg), glutamic (184.95 μg/kg) and aspartic acids (186.35 μg/100 mg) in the roots; phenylalanine (244.53 μg/100 mg), glutamic acid (488.20 μg/kg), and glycine (283.54 μg/100 mg) in the shoots;

• phenolic acids (gallic, benzoic, isopherulic, chlorogenic, vanillic, salicylic, ferulic, p-hydroxybenzoic, protocatechuic, alpha-coumaric and p-coumaric acids, catechol, catechin);

• microelements (potassium (9.660 μg/kg in the roots, 9.170 μg/kg in the shoots), calcium (2.710 μg/kg in the roots, 2.220 μg/kg in the shoots); the content of silicon in the shoots is 2.580 μg/kg, which is 1.7 times higher than in the roots) [5-7].

The major phytochemicals that were recognized in *E. vulgare* extract are kaempferol 3-*O*-neohesperidoside **1**, which is a derivative of kaempferol **2**, uridine **3**, 3-(3,4-dihydroxyphenyl)lactic acid **4**, and rosmarinic acid **5** (Figure 2) [8].

Iranian scientists at Mashhad University of Medical Sciences have found that the aqueous and ethanolic flavonoids-containing extracts of *E. vulgare* inhibit mono-amino oxidase enzyme (MAO) that can leads to a significant antidepressant effect [9]. The potential antidepressant effects of extracts of *E. vulgare* were investigated on mice using a forced swimming test. This

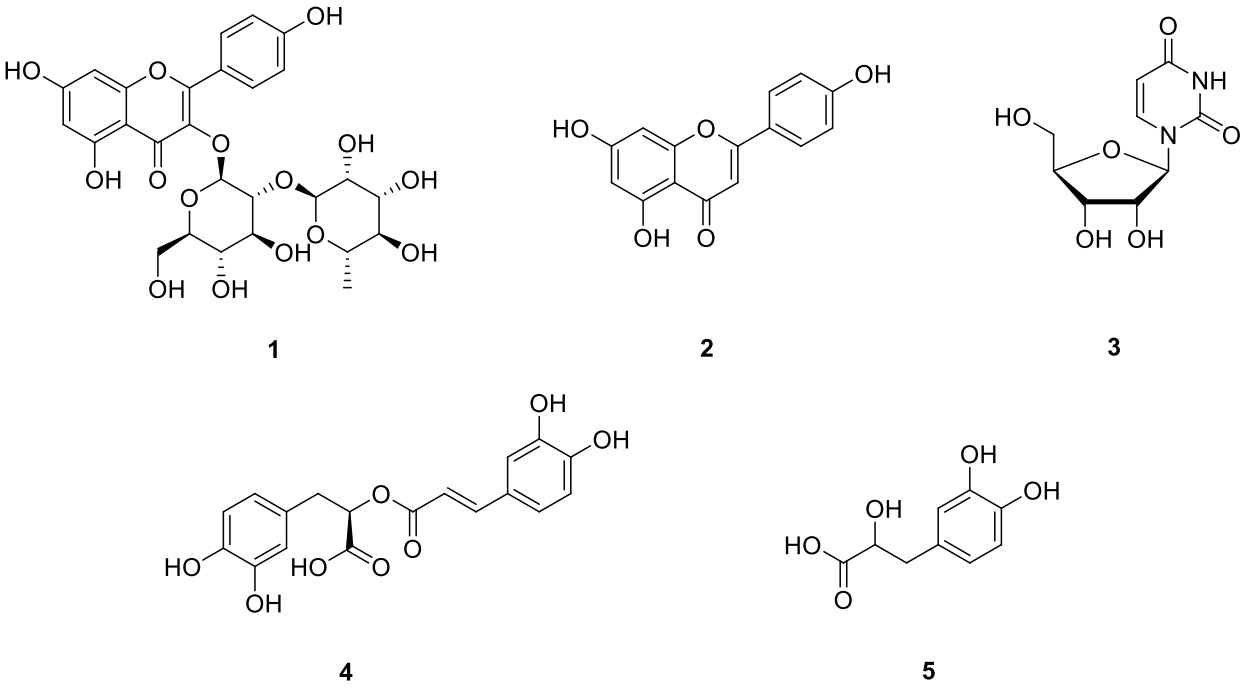

Figure 2. The major components of the Viper's bugloss (*E. vulgare*) alcoholic extract: kaempferol 3-*O*-neohesperidoside **1**, kaempferol **2**, uridine **3**, 3-(3,4-dihydroxyphenyl)lactic acid **4** and rosmarinic acid **5**.

test was performed in two sessions. In the pretest session (24 hours before the main session), each mouse (BALB/c male, weight 25-30 g) was forced to swim in a cylindrical-shaped container (diameter 10 cm, height 25 cm, water height 11 cm, temperature 25±1 °C). This preliminary test is stress-inducing and mice gradually lose their movement behavior. After 15 minutes, animals were removed and dried. Then, 23.5 hours later the relevant extract sample was injected intraperitoneally (IP) into mice. The main test was performed 30 minutes later. In this test, each mouse was left in the same container for 6 minutes and the following behaviors were recorded:

1. Immobility: floating in the water without swimming.
2. Swimming: active movement of extremities and circling in the container.
3. Climbing: active movement of forelimbs on the container wall.

The tests were performed on three groups of mice, eight mice in each. The first group received the drug imipramine, which is a selective inhibitor of monoamine reuptake (antidepressant). Group 2 received aqueous and alcoholic extracts of Viper's bugloss (*E. vulgare*) in various concentrations. The results showed that the extracts have a clear antidepressant activity that is comparable to imipramine. [9].

The pyrrolizidine alkaloids previously identified in floral honey attributed to *E. vulgare* were detected by Boppre *et al.* in Germany. Pyrrolizidine alkaloids were isolated from the aqueous acid extracts of pollen by use of strong cation-exchange, solid-phase extraction and identified by liquid chromatographic/mass spectrometric (LCMS) analysis. The pyrrolizidine alkaloids in the pollen are present mainly as the *N*-oxides. In addition to the previously described pyrrolizidine alkaloids and/or their *N*-oxides (echimidine, acetylechimidine, uplandicine, 9-*O*-angelylretronecine, echiuplatine, leptanthine, and echimiplatine), one unidentified (echivulgarine), but previously found in honey, and two previously undescribed (vulgarine and 7-*O*-acetylvulgarine) pyrrolizidine alkloids and/or their *N*-oxides were identified in the pollen [10]. Pyrrolizidine alkaloid-containing plants are widely distributed throughout the world. The structural types and concentrations of the alkaloids vary among plant species. In addition, within a species of plant, concentrations vary with environment and location. Many pyrrolizidine alkaloids are toxic and cause poisoning in livestock and humans [11-13]. That is why to detect and determine the content of alkaloids in specific plants it is necessary to develop a rapid, sensitive, and specific method, which will be one of our goals of future research.

Experimental section

The source material for phytochemical studies was the stems of Viper's bugloss (*E. vulgare*), which was collected in the Mykolaiv region during the period of maximum accumulation BAR (July 2019). The procurement of raw materials was carried out according to regulatory and analytical documentation [2]. Air-dried raw materials were prepared in an Excelsior-shredding Machine. Studies of the

extracts were performed according to standard methods using reagents that were supplied by Sigma-Aldrich Corp. and Merk. Analytical scales AS 220 from RADWAG, Poland was used to weigh samples.

The amount of extractives was measured by the gravimetric method [2]. The maximum yield of extractives was 16%.

The method of UV spectroscopy (Hitachi U-2810 spectrophotometer) was used to detect the quantitative content of flavonoids. The aluminum chloride method was used to determine the total flavonoid compounds [3]. The content of flavonoids in terms of avicularin and absolutely dry raw material in percent (X) was calculated by the formula:

$$x = \frac{D \times 100 \times 100 \times 25}{330 \times m \times (100 - W)}$$

where D is the optical density of the test solution; 330 – specific absorption rate of avicularin complex with aluminum chloride at 410 nm; m – weight of sample in grams; W – weight loss during drying of raw materials, %.

The powdered plant materials in the amount of 1 g was loaded into a 150 ml flask, 30 ml of 70% alcohol was added and refluxed in a water bath for 30 minutes. The flask was cooled to room temperature, and the mixture were passed through filter paper into a 100 ml volumetric flask. The extraction protocol was repeated 2 times as described above. The combined extracts were filtered, the solids were washed with 70% alcohol and the volume of the filtrate was adjusted to the mark (solution A).

Solution A (4 ml) was loaded into a 25 ml volumetric flask, 2 ml of a 2% solution of aluminum chloride in 95% ethanol was added and brought the volume with a 95% ethanol to the mark; after 20 min, the optical density of the solution was determined using spectrophotometer at a wavelength of 410 nm in a cuvette with a layer thickness of 10 mm. For comparison, the following solution was used: 4 ml of solution A was loaded into a 25 ml volumetric flask, a 1 drop of a dilute hydrochloric acid was added and the volume of the solution was adjusted to the mark with 95% ethanol. The flavonoid content was calculated based on dry raw material and found to be 2.59%.

With the help of qualitative reactions, the analysis for the presence of certain groups of BAS of medicinal plant raw materials was performed. The results of the identified substances are presented in Table 1.

Therefore, the obtained data demonstrated the presence of BAS in the object under study and provide insentives for further study.

It should be noted that the presence echimidine in the extract of the Viper's bugloss (*E. vulgare*) requires additional studies on the toxicity of extracts from this plant. Because this plant is widely used as a honey plant and contains alkaloids, there are several studies on their presence in honey.

Table 1. The results of the qualitative analysis of the Viper's bugloss (*E. vulgare*) shoot extract.

BAS	Reagents	Results
Alkaloids	50% C_2H_5OH, 95% C_2H_5OH, Mg (dust), conc. HCl	+ (red solution)
	Bouchard-Wagner $K[I_3]$	+ (red-brown precipitate)
	Sonnenschein 1% $H_3PO_4 \cdot 12MoO_3 \cdot 2H_2O$	+ (amorphous precipitates with yellowish color)
	0,1% tannic acid soluton	+ (colorless)
Tannins	1% gelatin solution	+ (precipitate → dissolves in excess of reagent)
	$NH_4Fe(SO_4)_2 \cdot 12\ H_2O$	+ (dirty green)
	10% CH_3COOH, 10% $Pb(CH_3COO)_2$	+ (precipitate)
	1% $NH_4Fe(SO_4)_2 \cdot 12\ H_2O$, solid CH_3COONa	+ (dirty green precipitate)
Saponins	Foaming	+
	Salkowsky $CHCl_3$, conc. H_2SO_4	+ (dirty orange solution)
	$FeCl_3$, $K_4[Fe(CN)_6]$	+ (blue solution)
	$CuSO_4$, 1% NH_4SCN	+ (white precipitate)
	$NaHCO_3$, $FeSO_4$, H_2SO_4 aq. sol.	+ (purple → colorless)
Polysaccharides	95% C_2H_5OH	+ (precipitate)

Conclusions

The literature review has shown that Viper's bugloss (*E. vulgare*) as a study object has several advantages and

disadvantages. The positive characteristics of the raw material include its availability and high prevalence. Viper's bugloss (*E. vulgare*) grows throughout Ukraine (rocky slopes, steppes, dry meadows, crops, fields). Significant antidepressant effect is equal in power to the official drugs and confirmed by foreign preclinical studies as well as public sector research of this raw material. The negative characteristics include the presence of pyrolyzidine alkaloids among the plant's secondary metabolites, which are dangerous to human health due to their hepatotoxic effect. On the other hand, this opens new horizons in the study of the psychotropic activity of the Viper's bugloss (*E. vulgare*) along with the development of methods to diagnose the toxicity of raw materials. Studies of Viper's bugloss (*E. vulgare*) indicate the relevance and prospects for further pharmacognostic and pharmacological studies of the plant.

Notes

Author contributions. **A. R. K., A. S. K.**: performed the spectrophotometric analysis. **V. R. H., R. T. K.**: a qualitative and quantitative analysis. **A. S. K.**: wrote the manuscript. **M. S. K.**: writing review. **V. P. N.**: conceptualization, supervision.

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Дослідження компонентного складу екстрактів синяка звичайного (*Echium vulgare*)

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Резюме: Об`єктом наших досліджень було обрано траву синяка звичайного *(Echium vulgare)*. В цій статті надається узагальнена характеристика лікарської рослини синяка звичайного *(E. vulgare)*, його фармакологічних властивостей, вмісту біологічно активних речовин. Синяк звичайний *(E. vulgare)* - це вид квітучої рослини сімейства шорстколистих *(Boraginaceae)*. Поширений синяк по всій Україні. Заготовляють його у районах поширення. Синяк звичайний *(E. vulgare)* - медоносна, харчова, лікарська, отруйна, танідоносна, фарбувальна, олійна і декоративна рослина. Синяк звичайний *(E. vulgare)* особливо багатий алкалоїдами піролізидину, флавоноїдами, фенолкарбоновими кислотами, фітостеронами та нафтохінонами. Ця лікарська рослина, як і більшість представників *Boraginaceae*, недостатньо вивчена. Продовжуючи працювати з дослідженням цього виду, отримали водні та етанольні екстракти з надземної частини синяка звичайного *(E. vulgare)* та визначали їх фітохімічний склад. Вперше наводиться аналіз якісного складу синяка звичайного *(E. vulgare)*, що росте в Україні, щодо вмісту біологічно активних речовин та визначений кількісний вміст флавоноїдів за допомогою спектрофотометричного методу. Експериментальні результати показали, що загальний вміст флавоноїдів становить 2,59%. Максимальний вихід екстрактивних речовин становив 16%. У традиційній медицині синяк звичайний *(E. vulgare)* використовується як кровоочисний та протисудомний засіб,а також як стимулятор настрою. Ось чому рослина розглядається з точки зору можливого використання при лікуванні депресивних станів. Не було зафіксовано жодної попередньої роботи щодо кількісного вмісту флавоноїдів у надземній частині синяка звичайного *(E. vulgare)*, що росте в Україні. Отримані дані будуть використані у майбутніх дослідженнях.

Ключові слова: синяк звичайний *(Echium vulgare)*; біологічно активні речовини; флавоноїди; депресія.