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Research Article

The Study of External Signs, Microscopy and Chemical Composition of Medicinal Plant Materials of *Veronica beccabunga* L. Herb

Angelina V Strelyaeva, Olga A Larina*, Alla M Antsyshkina, Roman M Kuznetsov, Alina A Bondar, Vladimir A Sorokin

ABSTRACT

Introduction: *Veronica beccabunga L.* belongs to the class *dicotyledons*, order *Lamiáles,* family *Scrophulariaceae*. Representatives of the genus *Veronica* have long been used in folk medicine as antiinflammatory, antibacterial, antiseptic, wound healing, hemostatic, choleretic and antispasmodic drugs. Widely studied species are *Veronica officinalis* and *Veronica chamaedrys*. *Veronica beccabunga L.*, which is the object of our study, remains a poorly studied plant. **Aim:** The study of external signs, microscopy and chemical composition of medicinal plant materials of *Veronica beccabunga* L. herb. **Materials and Methods:** Chromato-mass spectrometry was used in the work. **Results and Discussion:** When describing external signs and microscopy, diagnostic signs of Veronica beccabunga were revealed. 27 compounds were identified by chromatography-mass spectrometry. The maximum content falls on: Citronellol epoxide (R or S) (30.5 %), Linolenic acid, ethyl ester (15.18), Diethyl succinate (12.17%), Ethyl palmitate (6.43%), Phytol (4.89%), Acetaldehyde ethyl amyl acetal (3.94%), Dibenzylamine (3.01%), Oleamide (2.77%), 2-(1-Methylbutyl)oxirane (2.7%), Butyl octyl phthalate(1.7%), Ethyl 10-bromodecanoate (1.68), Valeric acid, 4-methyl-, ethyl ester (1.58). Glycoside detected : 1-Benzyl-1H-benzimidazole 3-oxide (0.76%). **Conclusion:** The revealed morphological and anatomical signs of *Veronica beccabunga* herb can be used to diagnose this species and develop authenticity indicators for promising medicinal herbs. 27 compounds were identified by chromatography-mass spectrometry. Using the method of simple normalization, the relative percentage of identified compounds was determined.

Key words: *Veronica beccabunga* L., 1-Benzyl-1H-benzimidazole 3-oxide.

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INTRODUCTION

Veronica beccabunga L. belongs to the class dicotyledons, order Lamiáles, family Scrophulariaceae. The scientific name of the genus *Veronica* dates back to antiquity and is already found in Roman and ancient Greek authors. B. N. Golovkin believes that the name *Veronica* was given in 1542 by the German botanist Leonart Fuchs (1501-1566) in honor of St. Veronica.[1-19] Species numbers vary between two and fifteen with many subspecies. *Veronica beccabunga L.* is widespread. Western Europe: all countries south of 65 ° north latitude. Asia: Turkey, China. North America: USA, Canada. On the territory of the former USSR it is found from the western border to the southern part of the Ural Range, in the Caucasus, in Siberia. In the north reaches the Karelian Isthmus, the southern coasts of Lake Ladoga and Onega, the confluence of Sukhona and Vychegda, in the region of Pechora, Arkhangelsk. It is found in Crimea.[1]

It grows along the banks of reservoirs, in streams, near springs, on key swamps, in places where groundwater emerges, i.e. is a hydrophyte (Figure 1 A, B).[6,8,20] Representatives of the genus *Veronica* have long been used in folk medicine as antiinflammatory, antibacterial, antiseptic, wound healing, hemostatic, choleretic and antispasmodic drugs.[14,17] *Veronica beccabunga L.* is a member of the semi-aquatic plants. Researchers mentioned this medicinal plant in their scientific work, conducting morphometric and molecular studies based on plastid and nuclear ribosomal DNA groups.

Further, flow cytometry has been used to reveal the ploidy level, especially of the Egyptian endemic taxa.[9,13,15,16,18] In the medicinal herb *Veronica*, a number of authors have identified aucubin,catalpol, salidroside, gardoside, mussaenosidic acid, 8-epiloganic acid, arborescosidic acid and alpinoside.[7,10,11,21,22] Widely studied species are *Veronica officinalis* and *Veronica chamaedrys*. *Veronica beccabunga L.*, which is the object of our study, remains a poorly studied plant.

There is no description in the literature of the anatomical structure of autonomic organs and the chemical composition of this species has not been well studied. Therefore, the study of raw materials *Veronica beccabunga L.* with the aim of standardization is relevant.

MATERIALS AND METHODS

Several series of thin sections from the axial organs of the plant were made. Endoderm was also removed

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on both sides of the leaf. To avoid beveled sections, it is necessary to first align the surface to be cut by placing the razor strictly perpendicular to the longitudinal axis of the organ. All subsequent sections are made in the same direction. Slices and endoderm of the leaf were placed in Petri dish with water and selected three of the best, the thinnest, (transparent), and mowed (floating horizontally). Selected sections were placed on a glass slide in a drop of water and their suitability for work was checked. Suitable sections for work were treated with reagents. To do this, a drop of water is wet with filter paper from a glass slide, one of the sections is pushed to the side. Two sections were treated with phloroglucinol and concentrated sulfuric acid (a reagent for lignin), to identify cells with lignified walls, they turned red; one remaining section is chloro-zinc-iodine to detect starch in the cells. After staining, the reagents are wetted with filter paper, a drop of water is applied to the sections, covered with a coverslip and placed under a microscope for a small increase.[3] The preparations were carefully examined both at a small and at a large magnification of the microscope, tissues were recognized. These preparations were sketched, photographed and described.

The component composition of the samples was studied by gas chromatography-mass spectrometry. The study was carried out on an Agilent Technologies instrument, consisting of: 1) a 7890 gas chromatograph (HP-5 column, 50 m × 320 × 1.05 μm) and 2) a 5975 C mass selective detector with a quadrupole mass analyzer.

Chromatography temperature program: at 40 ° C - isotherm 2 min; further programmable heating to 250 ° C at a speed of 5 ° C / min; at 250 ° C - isotherm for 15 min; further programmable heating to 320 ° C at a rate of 25 ° C / min; at 320 ° C - isotherm 5 min. Flow divider 1:50. The temperature of the injector is 250 ° C. The temperature of the interface is 280 ° C. The carrier gas is helium; flow rate - 1 ml / min. Chromatogram of samples - total ion current. Mass spectrometric analysis conditions: energy of ionizing electrons 70 eV; registration of mass spectra in positive ions in the range (m / z) from 20 to 450 at a speed of 2.5 scans / sec. Software - ChemStation E 02.00., licensed. The identification of the component composition (qualitative analysis) was carried out using the library of complete mass spectra NIST-05 and the corresponding values of the chromatographic linear retention indices. The relative content (%) of the components of the mixture (quantitative analysis) was calculated from the ratio of the areas of chromatographic peaks (by simple normalization).

RESULTS AND DISCUSSION

External signs

Whole raw materials. Whole or partially crushed leafy stems with or without flowers, less often with buds or fruits of various degrees of development, sometimes partially showered. Stems cut above brown bottom leaves, 10-50 cm long, up to 0.4 cm thick, ascending, rooting at the base, branched, usually bare, but sometimes slightly pubescent at the top. The leaves are opposite, rounded or oval, fleshy, with a blunt apex, finely serrated or with smooth edges, dark green, shiny, with short petioles. Flowers are collected 10-30 in loose sinus brushes, twice the size of the leaves and greatly elongated with the fruits. Calyx deeply four-divided into oblong-lanceolate acute lobes. The corolla is usually blue with blue stripes, 4–9 mm in diameter, with a very short tube, with a broad-ovoid upper lobe, ovate lateral and narrow-ovoid lower.

Stamens shorter than the corolla, with curved threads and large anthers. The fruits are almost spherical capsules, 3-4 mm long, strong, swollen, glabrous, with a very small notch or without a notch. Seeds are elliptical, about 0.5 mm long, including 20-30 in the nest. The color of the stems and leaves is green, the flowers are blue with blue stripes, the fruits are grayish-green. The smell is faint. The taste is bitter (Figure 2 A, B).

Ground raw materials

Pieces of stems, leaves, parts of flowers and fruits passing through a sieve with holes with a diameter of 7 mm. The color of the pieces of stems and leaves is green, the flowers are blue. The smell is faint. The taste is bitter (Figure 1C).

Microscopic analysis

When studying the anatomical structure of leaves, it was found that leaf blades have a dorsoventral structure. The cells of the upper epidermis of the leaf are less sinuous and larger than the cells of the lower epidermis. The stomata are amphistomatic; numerous, anomocytic (disordered), surrounded by 3-5 cells of the epidermis. The epidermis of the leaf is pubescent with capitate hairs with a bicellular head on a unicellular pedicle (Figure 3 A, B).

No trichomes were found in the epidermis of the stem. In the primary cortex, aerenchyma (assimilation parenchyma) is well developed, which indicates the development of the plant under conditions of excessive moisture. A layer of large elongated cells of starchy endoderm is developed. Sclerenchyma is not expressed. Non-bunch type conductive system. Xylem vessels located in rows are clearly distinguishable (on average 5 vessels in a row, decreasing in diameter to the center). In the parenchyma of the core with a large increase, the presence of cells with brown contents was noted (Figure 3 C, D, E).

Rhizome also has a typical structure for rhizomes of dicotyledonous herbaceous plants. The integumentary tissue is represented by a cork (i.e., the rhizome is perennial). The primary cortex consists of a storage parenchyma represented by an aerenchyma and an endoderm layer. In aquatic plants, the aerenchyma performs not only the function of gas exchange. Air cavities in the stems and rhizome reduce the specific gravity of plants, allowing them to freely float in water. In the central axial cylinder, the conductive fabrics have a non-bundle structure. In the center is the core of the storage parenchyma. The presence of a core is a hallmark of the root (Figure 2 F).

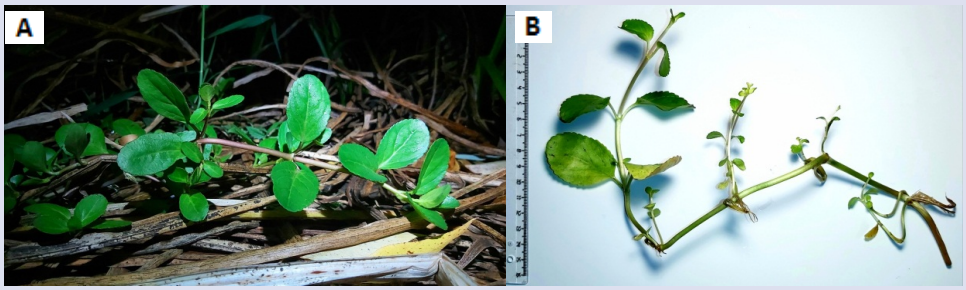


Figure 1: (A) Appearance of *Veronica beccabunga L.* in natural conditions. (B) Fresh herb *Veronica beccabunga L.*

The method of chromatography-mass spectrometry was used to analyze the alcohol extraction obtained from the medicinal herb of *Veronica beccabunga L.* (Figure 4 A, B). The alcoholic extract was prepared from the fresh herb Verónica beccabunga. The grass was crushed and filled with 95% alcohol in a ratio of 1: 5. Insisted for 14 days and then filtered. A sample was injected into an Agilent Technologies instrument. 27 compounds were identified (Table 1).

The relative percentage of each component was calculated taking into account unidentified peaks (Diagram 1).

The maximum content falls on: Citronellol epoxide (R or S) (30.5 %), Linolenic acid, ethyl ester (15.18), Diethyl succinate (12.17%), Ethyl palmitate (6.43%), Phytol (4.89%), Acetaldehyde ethyl amyl acetal (3.94%), Dibenzylamine (3.01%), Oleamide (2.77%), 2-(1-Methylbutyl) oxirane (2.7%), Butyl octyl phthalate(1.7%), Ethyl 10-bromodecanoate (1.68), Valeric acid, 4-methyl-, ethyl ester (1.58). Glycoside detected: 1-Benzyl-1H-benzimidazole 3-oxide (0.76%) (Diagram 2). Other compounds also found :1-Methylbutyl acetate (1.2%), Valeraldehyde, diethyl aceta (1.2%), 2-Myristynoyl-glycinamide (1.09%), Stearoylhydrazine (1.06%), Butylaldehyde diethyl acetal (1.04%), 3-Hydroxypropanoic acid hydrazide (0.91%), Ethyl 5-[(methylamino) acetyl]-10,11-dihydro-5H-dibenzo[b,f]azepin-3-ylcarbamate (0.84%), Hexadecanohydrazide (0.83%), Benzyl carbazate (0.79%), Propanoic acid, 3-hydroxy-, hydrazide (0.79%), 5-Hydroxynorvaline (0.76%), Oxamic acid hydrazide (0.74%), Aminomocovina (0.70%).

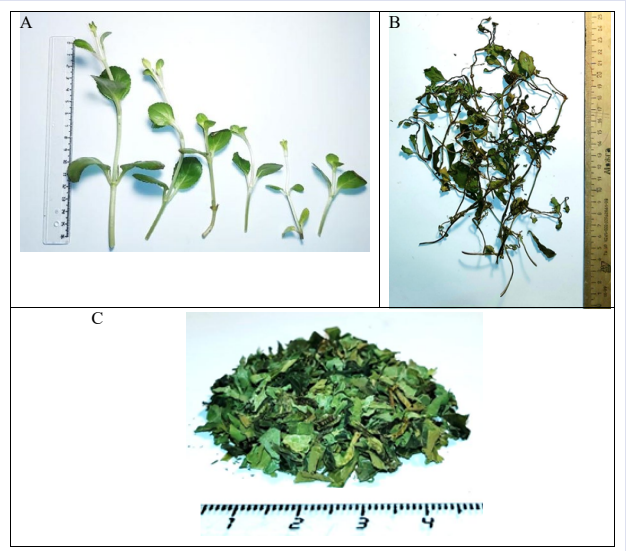


Figure 2: (A) Fresh of V. beccabunga, (B) dried of V. beccabunga, (C) ground raw materials.

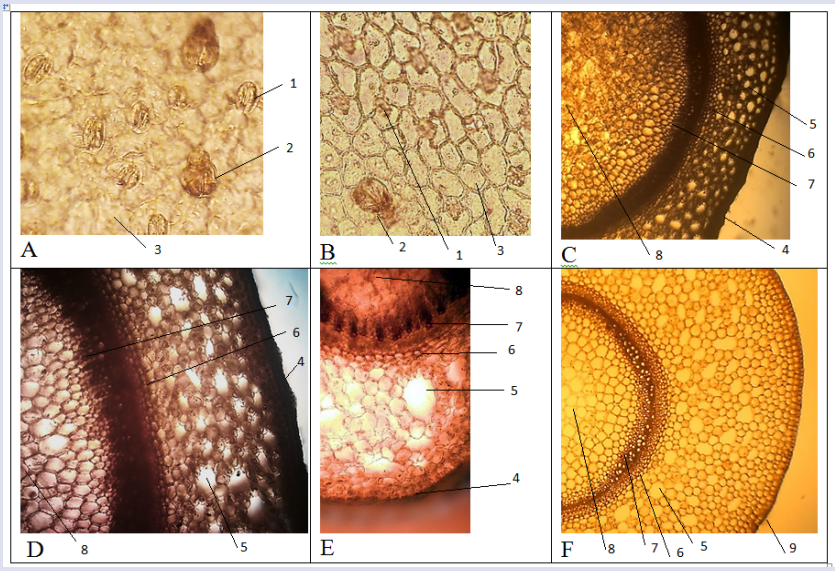


Figure 3: (A). The drug from the surface of the upper epidermis of the leaf (40x / 0.65); (B) The drug from the surface of the lower epidermis of the leaf (40x / 0.65); (C) Cross section of the stem (4x / 0.10); (D) Cross section of the stem (10x / 0.25); (E) Cross section of the stem (40x / 0.65); (F) Cross section of rhizome (4x / 0.10); 1- stomata, 2- trichomes, 3- cells of integumentary tissue, 4-epidermis, 5-aerenchyma, 6- endoderm, 7- xylem, 8-core parenchyma, 9-cork.

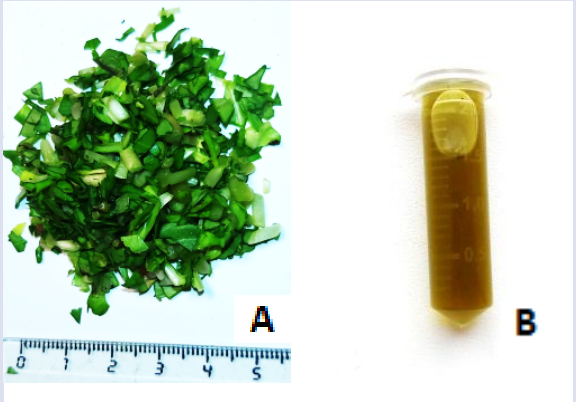
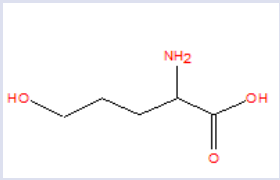
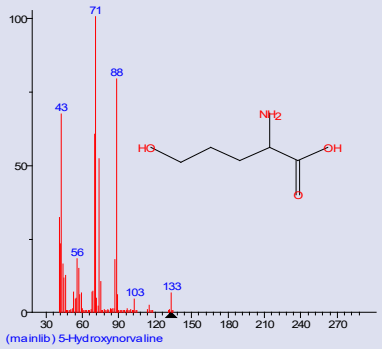
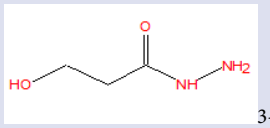
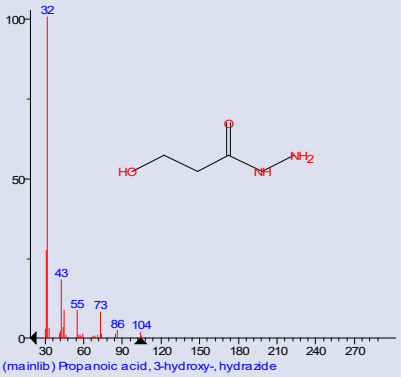
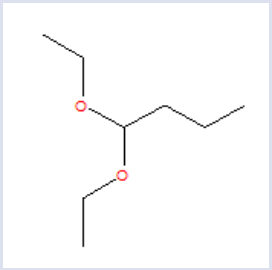
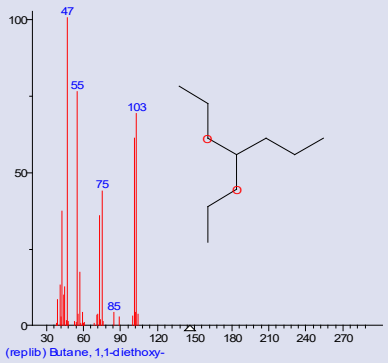
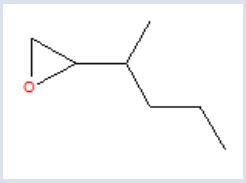
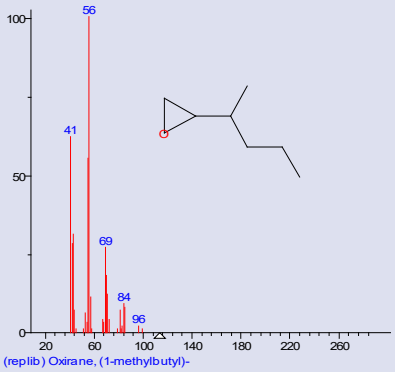
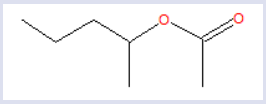
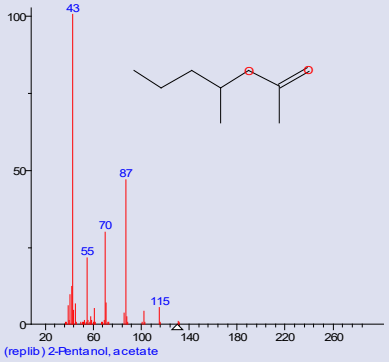
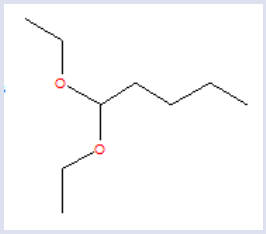
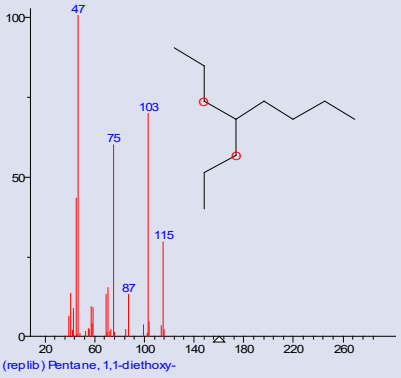
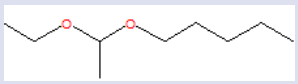
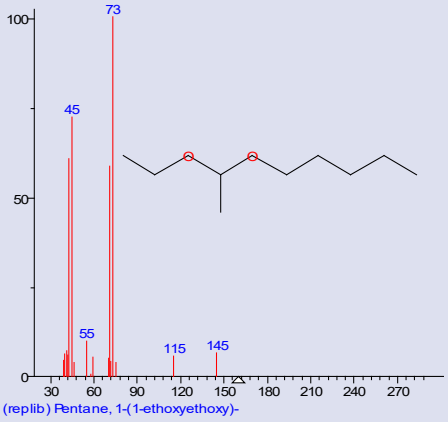
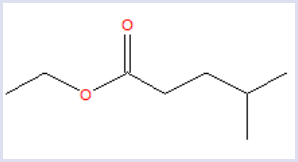
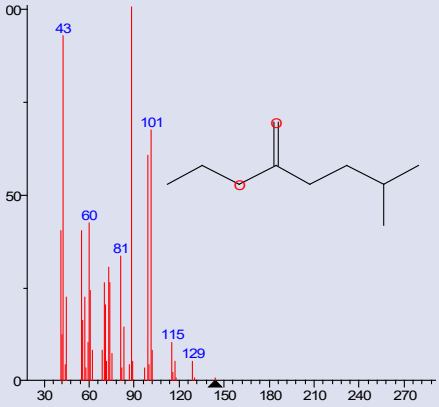
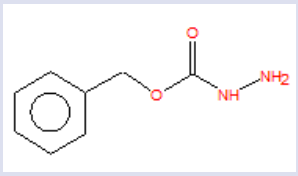
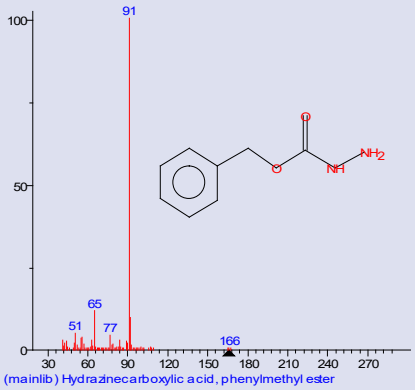
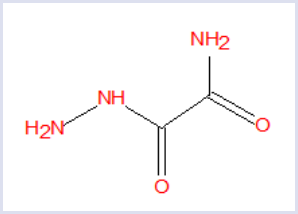
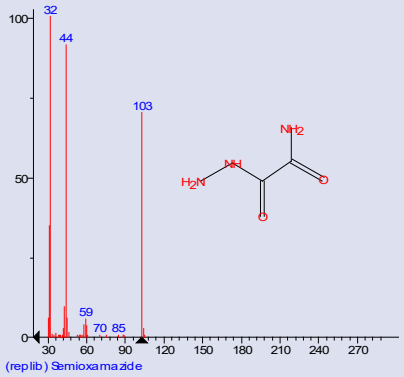
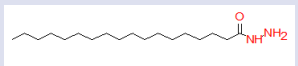
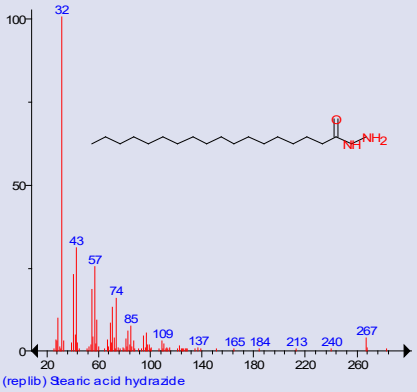


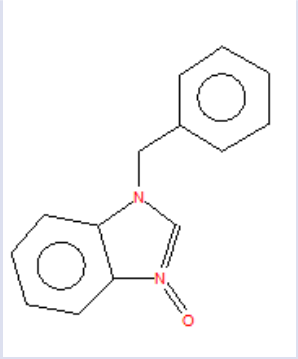
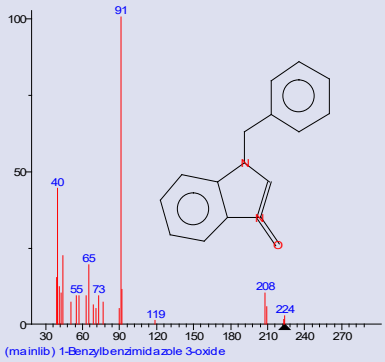
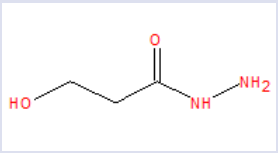
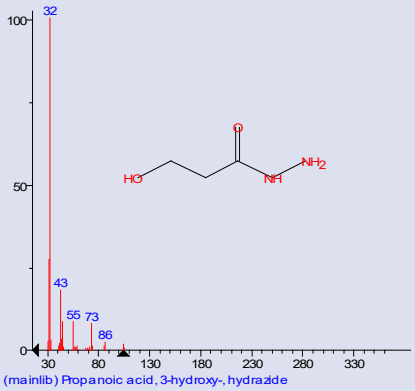
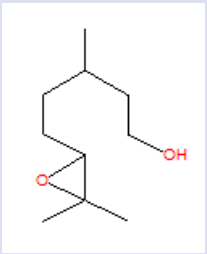
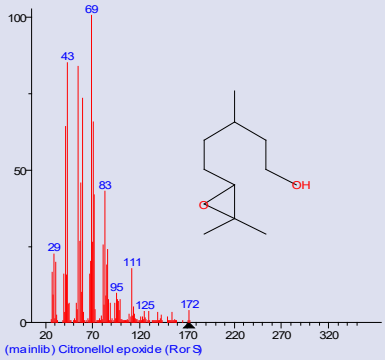
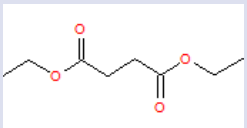
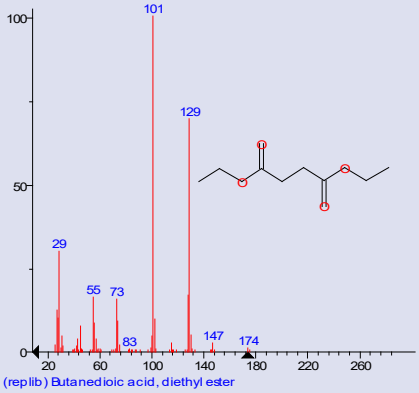
Figure 4: (A) Crushed fresh raw materials, (B) Alcohol extraction.

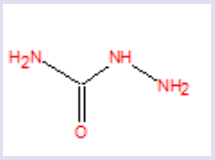
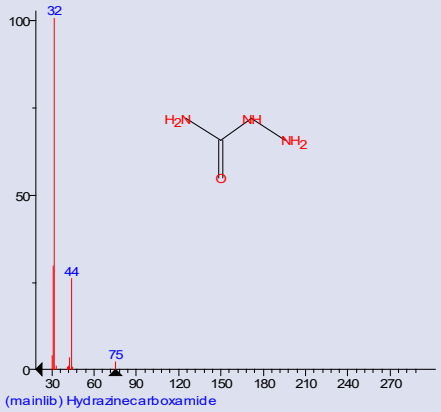
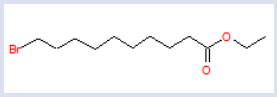
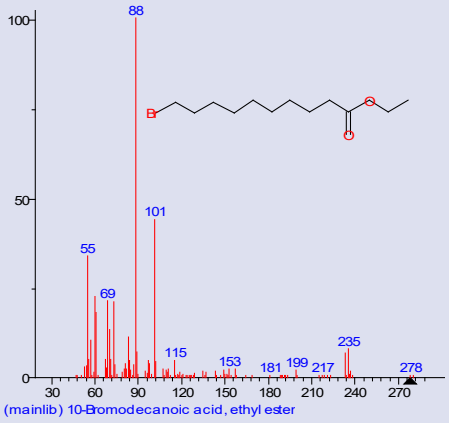
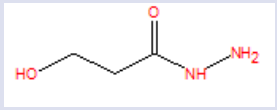
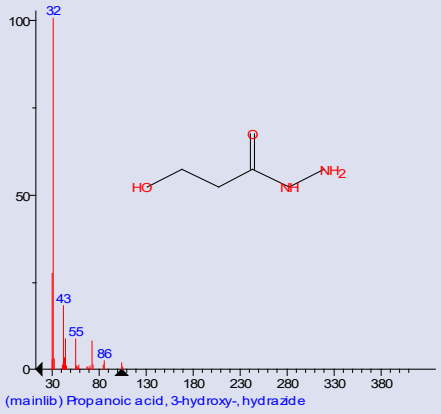
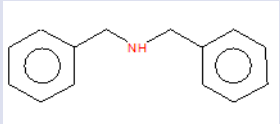
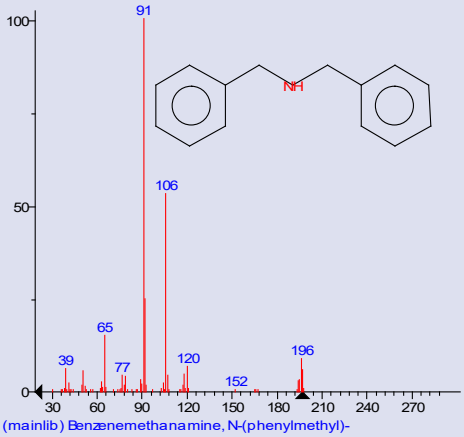
Table 1: The compounds identified in alcohol extraction from the *Veronica beccabunga L.*

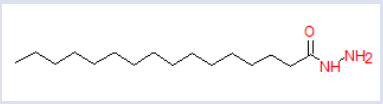
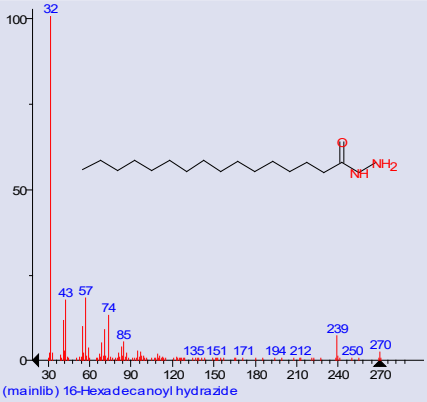
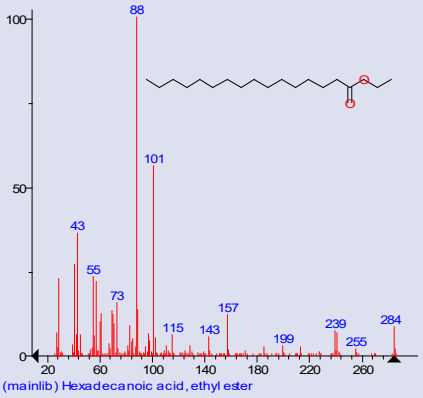
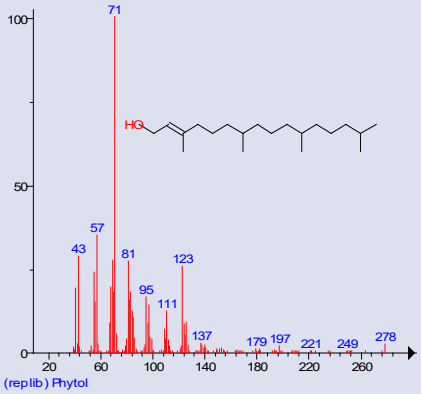
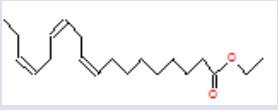
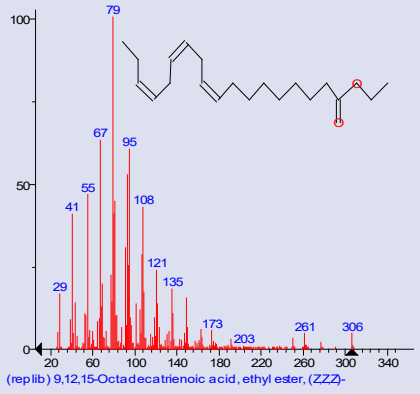
№	Name	Ret Time	Mass spectrum	Area	%
1	 5-Hydroxynorvaline	3.889	 (mainlib) 5-Hydroxynorvaline	33477	0.76
2	 3-Hydroxypropanoic acid hydrazide	4.198	 (mainlib) Propanoic acid, 3-hydroxy-, hydrazide	39998	0.91
3	 Butylaldehyde diethyl acetal	4.828	 (replib) Butane, 1,1-diethoxy-	45522	1.04

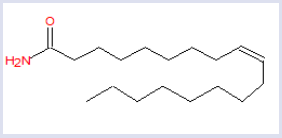
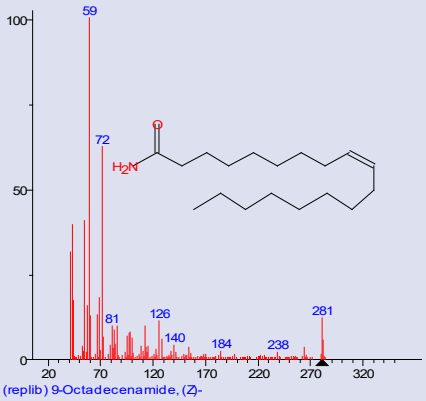
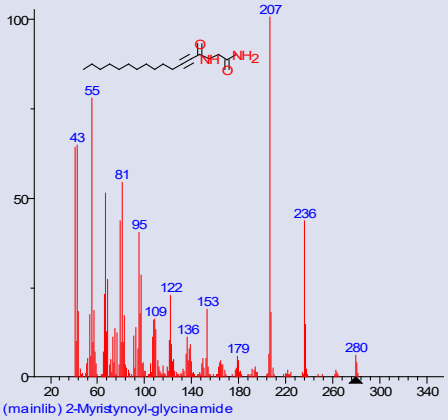
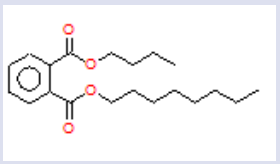
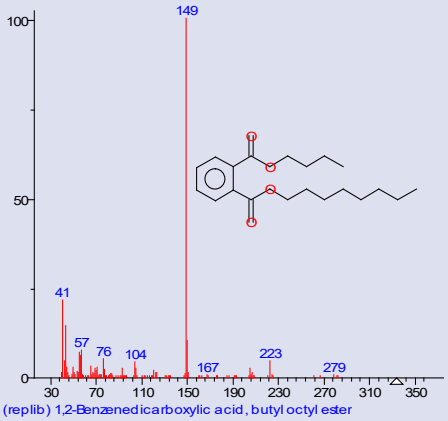
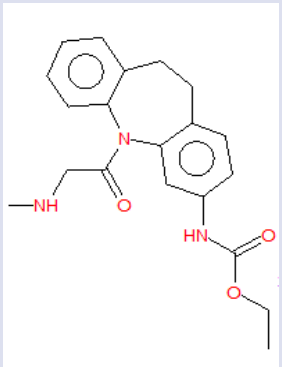
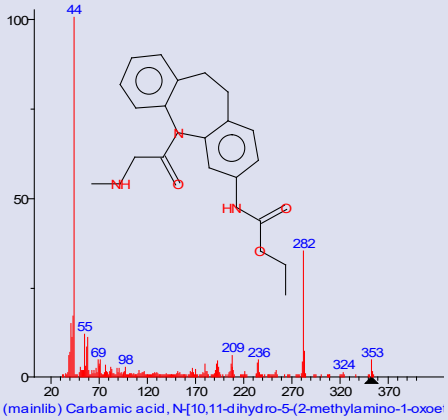
4	 2-(1-Methylbutyl)oxirane	4.953	 (replib) Oxirane, (1-methylbutyl)-	118598	2.70
5	 1-Methylbutyl acetate	5.102	 (replib) 2-Pentanol, acetate	52850	1.20
6	 Valeraldehyde, diethyl aceta	6.422	 (replib) Pentane, 1,1-diethoxy-	52658	1.20
7	 Acetaldehyde ethyl amyl acetal	6.743	 (replib) Pentane, 1-(1-ethoxyethoxy)-	173324	3.94

8	 Valeric acid, 4-methyl-, ethyl ester	7.183		69293	1.58
9	 Benzyl carbazate	7.980	 (mainlib) Hydrazinecarboxylic acid, phenylmethyl ester	34620	0.79
10	 Oxamic acid hydrazide	8.485	 (replib) Semioxamazide	32685	0.74
11	 Stearoylhydrazine	8.883	 (replib) Stearic acid hydrazide	46761	1.06

12	 1-Benzyl-1H-benzimidazole 3-oxide	9.109	 (mainlib) 1-Benzylbenzimidazole 3-oxide	33472	0.76
13	 Propanoic acid, 3-hydroxy-, hydrazide	9.621	 (mainlib) Propanoic acid, 3-hydroxy-, hydrazide	34513	0.79
14	 Citronellol epoxide (R or S)	9.954	 (mainlib) Citronellol epoxide (R or S)	1340250	30.50
15	 Diethyl succinate	10.073	 (replib) Butanedioic acid, diethyl ester	534847	12.17

16	 Aminomocovina	11.030		30657	0.70
17	 Ethyl 10-bromodecanoate	13.052		73701	1.68
18	 3-Hydroxypropanoic acid hydrazide	15.745		32378	0.74
19	 Dibenzylamine	16.340		132422	3.01

20	 Hexadecanohydrazide	16.756	 (mainlib) 16-Hexadecanoyl hydrazide	36570	0.83
21	 Ethyl palmitate	18.177	 (mainlib) Hexadecanoic acid, ethyl ester	282577	6.43
22	 Phytol	18.974	 (replib) Phytol	214789	4.89
23	 Linolenic acid, ethyl ester	19.325	 (replib) 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	666991	15.18

24	 Oleamide	20.466	 (replib) 9-Octadecenamide, (Z)-	121634	2.77
25	 2-Myristynoyl-glycinamide	20.591	 (mainlib) 2-Myristynoyl-glycinamide	47924	1.09
26	 Butyl octyl phthalate	21.578	 (replib) 1,2-Benzenedicarboxylic acid, butyl octyl ester	74845	1.70
27	 Ethyl 5-[(methylamino)acetyl]-10,11-dihydro-5H-dibenzo[b,f]azepin-3-ylcarbamate	23.308	 (mainlib) Carbamic acid, N-[10,11-dihydro-5-(2-methylamino-1-oxoe	37007	0.84

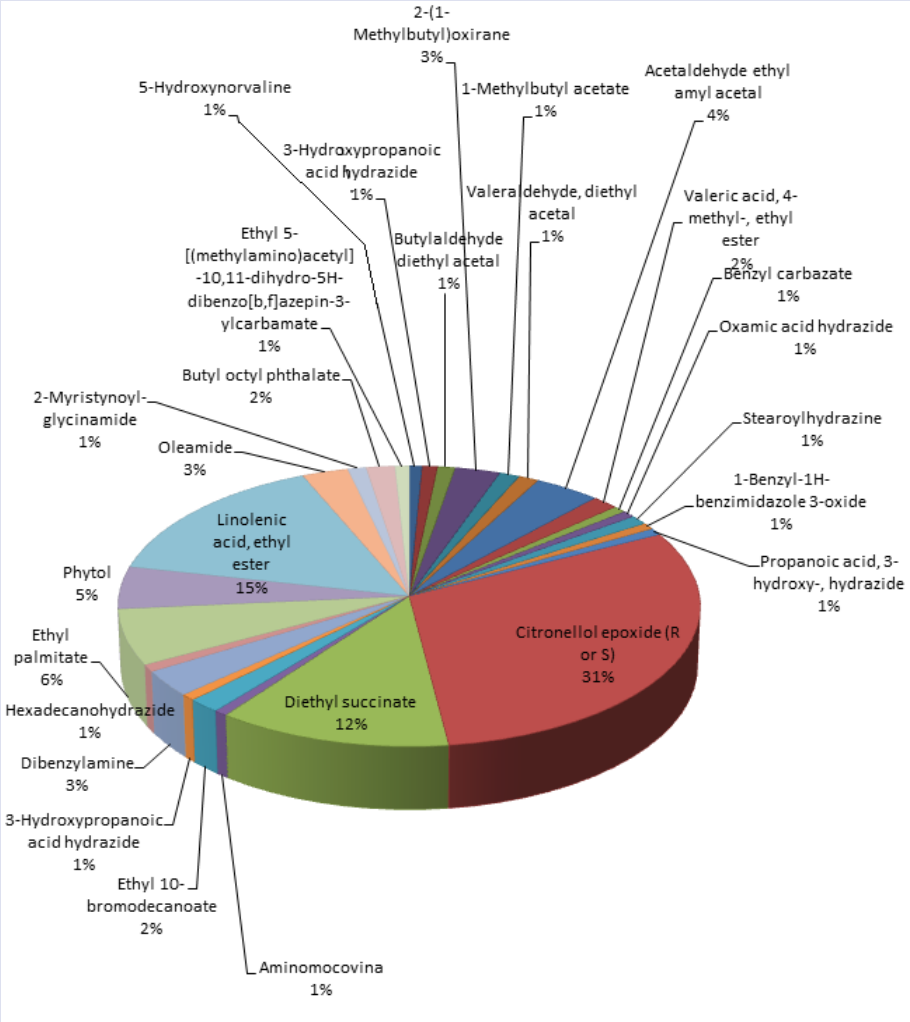


Diagram 1: The compounds identified in alcohol extraction from the *Veronica beccabunga L*.

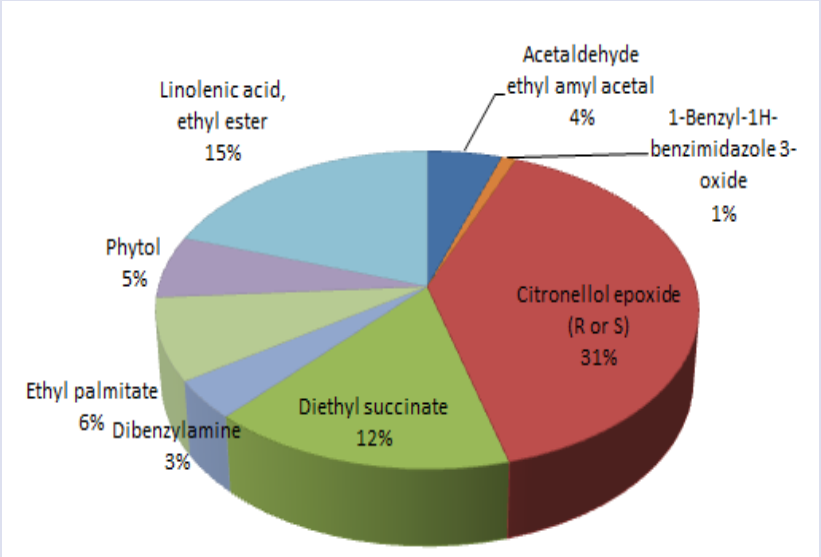


Diagram 2: The main compounds identified in alcohol extraction from the *Veronica beccabunga L*.

CONCLUSION

1. The identified morphological and anatomical features of the medicinal herb *Veronica beccabunga L.* can be used to diagnose this species and develop authenticity indicators for promising medicinal herbs.

2. 27 compounds were identified by chromatography-mass spectrometry. The maximum content falls on: Citronellol epoxide (R or S) (30.5 %), Linolenic acid, ethyl ester (15.18), Diethyl succinate (12.17%), Ethyl palmitate (6.43%), Phytol (4.89%), Acetaldehyde ethyl amyl acetal (3.94%), Dibenzylamine (3.01%), Oleamide (2.77%), 2-(1-Methylbutyl)oxirane (2.7%), Butyl octyl phthalate(1.7%), Ethyl 10-bromodecanoate (1.68), Valeric acid, 4-methyl-, ethyl ester (1.58). Glycoside detected: 1-Benzyl-1H-benzimidazole 3-oxide (0.76%)

3. Using the method of simple normalization, the relative percentage of identified compounds was determined.

ACKNOWLEDGMENTS

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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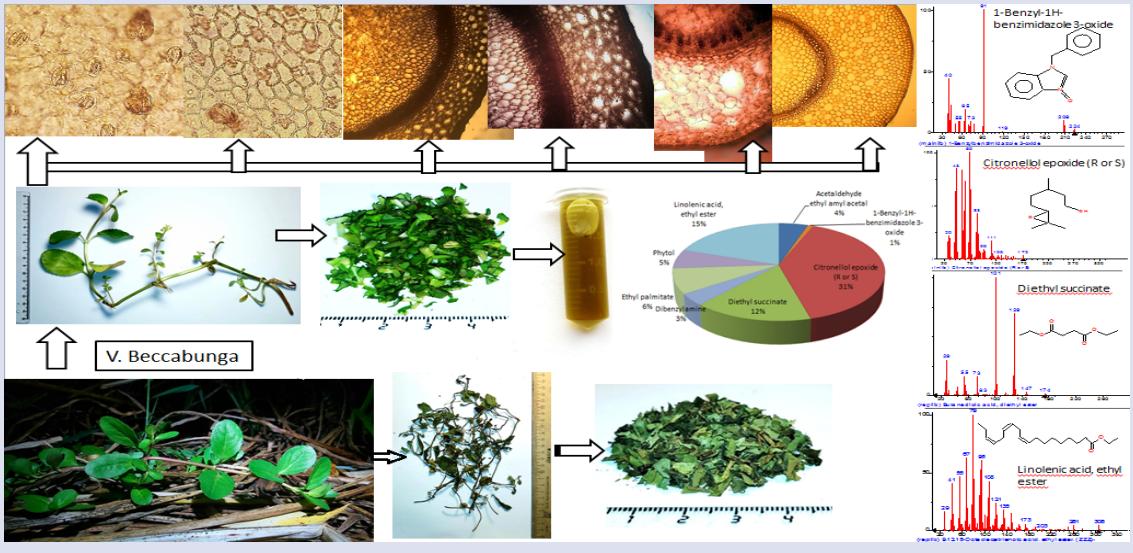
GRAPHICAL ABSTRACT



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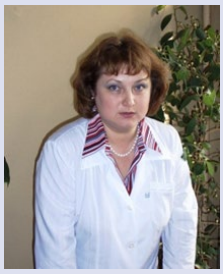

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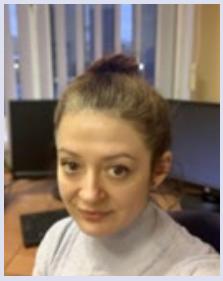

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