

Determination of the Chemical Composition of Lady's Bedstraw (*Galium verum* L.) Herb Extract by GC-MS

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History

- Submission Date: 27-03-2020;
- Review completed: 14-04-2020;
- Accepted Date: 04-05-2020;

DOI : 10.5530/pj.2020.12.123

Article Available online

<http://www.phcogj.com/v12/i4>

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ABSTRACT

Background: The research is devoted to the study of the Lady's bedstraw (*Galium verum* L.) herb ethanol extract composition using the method of gas chromatography with mass spectrometric detection. **Materials and Methods:** Air-dried *G. verum* L. herb was used for ethanol extract preparation. Hewlett Packard 6890, 5973A GC/MSD System equipped with an HP-5MS quartz capillary column with geometric dimensions of 30 m×0.25 mm×0.25 μm was used for GC-MS analysis. **Results:** It was established that the Lady's bedstraw ethanol extract is rich in biologically active substances that determine its wide spectrum of pharmacological action. 15 compounds were identified by comparing with library mass spectra. **Conclusions:** *G. verum* L. is a promising source of crude herbal drugs. In the future, after additional pharmacognostic studies, *G. verum* L. herb can be recommended for implementation in the State Pharmacopeia of the Russian Federation.

Key words: Lady's bedstraw, *Galium verum* L., Ethanol extract, Gas chromatography-mass spectrometry.

INTRODUCTION

The search for new sources of plant materials and the creation of herbal medicine products based on them with high therapeutic efficacy and low toxicity is currently demanded. Lady's bedstraw (*Galium verum* L., Figure 1.1.¹), the *Rubiaceae* family, is of considerable interest for study. It has a wide range of pharmacological effects and is used only in traditional medicine. *G. verum* L. crude herbal drugs (CHD) have anti-inflammatory, antioxidant, antiseptic, restorative, choleric, analgesic, diuretic, antispasmodic, estrogenic effects. Also, this plant is used in the prevention and treatment of cancer.²⁻⁵ The chemical composition of the *G. verum* L is very rich and diverse; it contains tannins⁶, triterpene compounds⁷, essential oil⁸, organic acids⁹, carotenoids¹⁰, flavonoids^{11,12}, iridoid glycosides¹³, anthracene derivatives¹⁴ and other biologically active compounds (BAC). Earlier, we studied the toxicity of Lady's bedstraw aqueous extract in experiments on laboratory animals. We concluded that the Lady's bedstraw liquid extract refers to IV class, known as slightly hazardous substances according to the classification of chemicals by hazard.¹⁵

As *G. verum* L. herb possesses a wide spectrum of biological activity (including cancer prevention and treatment) our research aimed to carry out an in-depth study of the plant's ethanol extract chemical composition by gas chromatography with mass spectrometric detection.

MATERIALS AND METHODS

Plant material

G. verum L. herb was collected in July 2018 in the

southwest of the Moscow region (Russia), dried in the air-shade.

Sample preparation

Sample of the raw material was ground to a particle size passing through a sieve with a hole size of 2 mm. Lady's bedstraw ethanol extract (1:10) was prepared using 70 % ethanol. Then it was evaporated in a nitrogen stream at room temperature to 0.1 cm³ residual volume. The prepared sample was analyzed by gas chromatography with a mass spectrometric detector in total ion current mode.

Instrumentals

Hewlett Packard 6890, 5973A GC/MSD System equipped with an HP-5MS quartz capillary column with geometric dimensions of 30 m×0.25 mm×0.25 μm was used. The stationary phase is 5 % diphenyl-95 % dimethylpolysiloxane.

Gas chromatography conditions

The temperature program was applied as follows. Thermostat: initial – 36±1°C, hold 36 °C – 1 min, final – 260±1, hold 260 °C – 5 min, injector temperature – 150 °C. Injector split ratio was 10:1, the temperature programming rate of the column thermostat is 5 °C/min from 36 to 60 °C, then 15 °C/min from 60 to 260 °C. The carrier gas (helium) flow rate is 1±0.1 ml/min. The injected sample volume is 1 μl.

Mass-spectrometry conditions

Ionization voltage: – 70 eV, ion source temperature – 230 °C, quadrupole temperature – 150 °C, intermediate line temperature – 280 °C, mass scanning range of 15–550 m/z, gas hold-up time – 2.0 min.

Cite this article: Zaichikova SG, Bokov DO, Kiselevskii MV, Antsyshkina AM, Bondar AA, Prostodusheva TV, et al. Determination of the Chemical Composition of Lady's Bedstraw (*Galium verum* L.) Herb Extract by GC-MS. Pharmacogn J. 2020;12(4):857-63.

RESULTS AND DISCUSSION

The result of *G. verum* L. herb ethanol extract qualitative analysis is presented in Table 1.

G. verum L. herb ethanol extract chromatogram is presented in Figure 2.

The mass spectra of the identified compounds (compared to library mass spectra) are shown in Figures 3-10.

The identified compounds, for the most part, are fatty acids and their esters in Lady's bedstraw herb ethanol extract. Phytol – an acyclic

aliphatic organic chemical compound, refers to diterpenes, that have monounsaturated isoprene residues as a base. Phytol is part of chlorophyll, vitamin E, vitamin K₁ as a phytyl substitute.

CONCLUSION

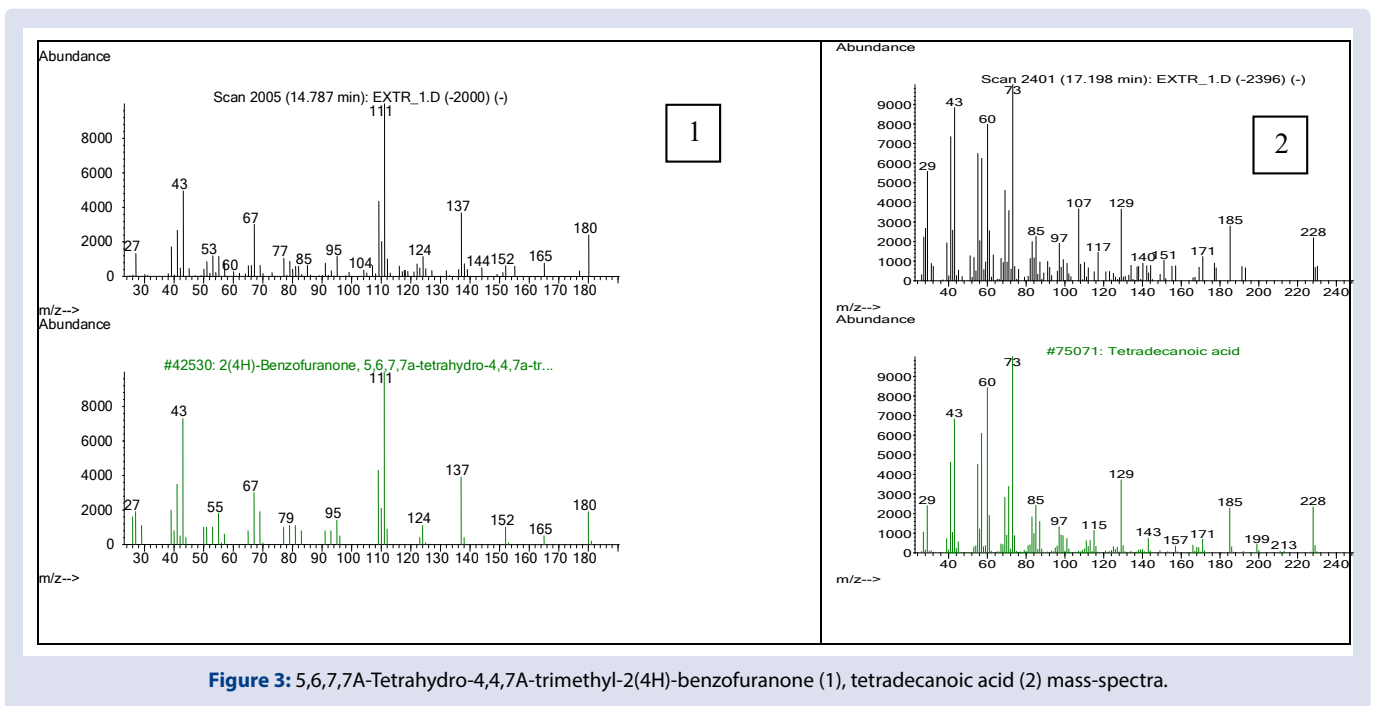
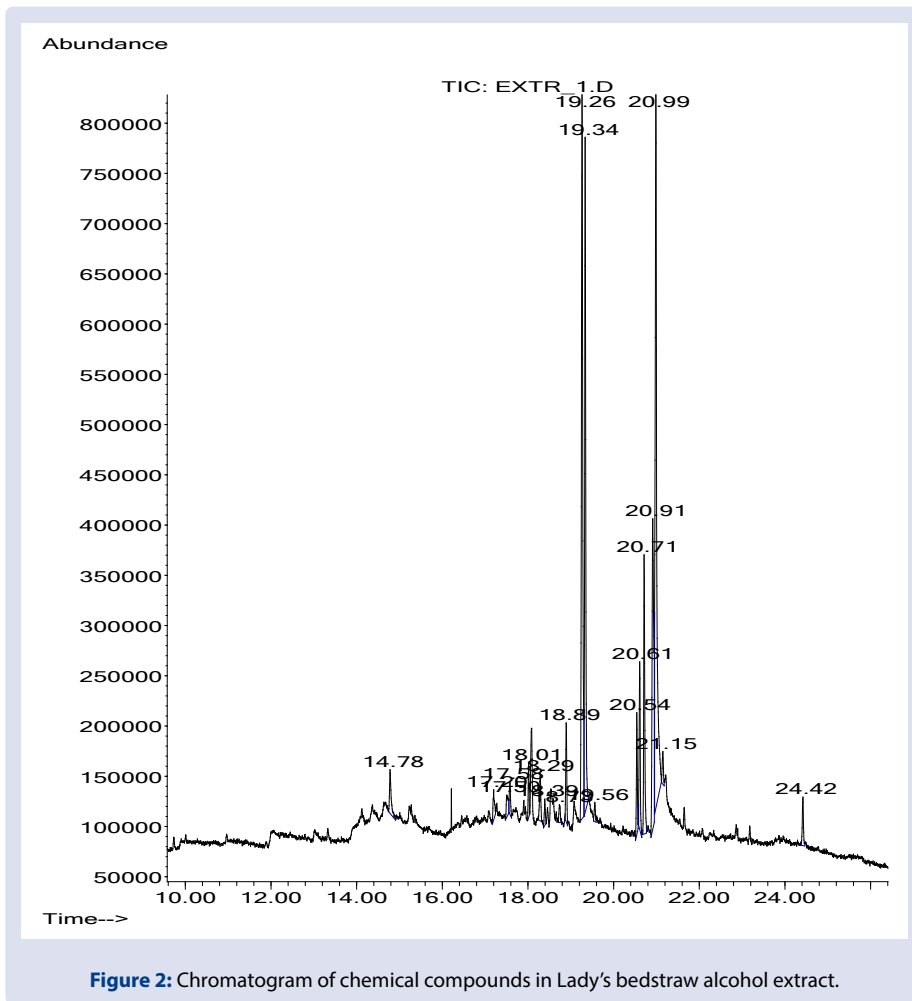
We identified 15 compounds not previously described in the literature. Identification is based on the Lady's bedstraw herb ethanol extract mass spectrometric studies. The obtained data can be used in further study of Lady's bedstraw and the creation of new herbal medicine products and regulatory documentation on its basis.

Table 1: Identified compounds in Lady's bedstraw herb ethanol extract.

No	Retention time, min	Compound name	Chemical formula	M. W. A. W. U.
1	2	3	4	5
1	14,78	5,6,7,7A-tetrahydro-4,4,7A-trimethyl-2(4H)-benzofuranone	C ₁₁ H ₁₆ O ₂	180
2	17,19	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228
3	17,58	3-methyl-1-butanolbenzoate	C ₁₂ H ₁₆ O ₂	192
4	18,01	Bicyclo[3.1.1]heptane	C ₁₀ H ₁₈	138
5	18,28	Benzoic acid butyl ester	C ₁₂ H ₁₆ O ₂	178
6	18,89	Hexadecanoic acid methyl ester	C ₁₇ H ₃₄ O ₂	270
7	19,26	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256
8	19,33	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	278
9	19,56	Hexadecanoic acid ethyl ester	C ₁₈ H ₃₆ O ₂	284
10	20,53	[Z,Z]-9,15-Octadecadienoic acid methyl ester	C ₁₉ H ₃₄ O ₂	294
11	20,60	[Z,Z,Z]-9,12,15-Octadecatrienoic acid methyl ester	C ₁₉ H ₃₂ O ₂	292
12	20,71	Phytol	C ₂₀ H ₄₀ O	296
13	20,91	[Z,Z]-9,12-Octadecadienoic acid	C ₁₈ H ₃₂ O ₂	280
14	20,98	[Z,Z,Z]-9,12,15-Octadecatrienoic acid	C ₁₈ H ₃₂ O	264
15	21,14	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284



Figure 1: Lady's bedstraw (*Galium verum* L.): flowering plant (1), crude herbal drug (2).



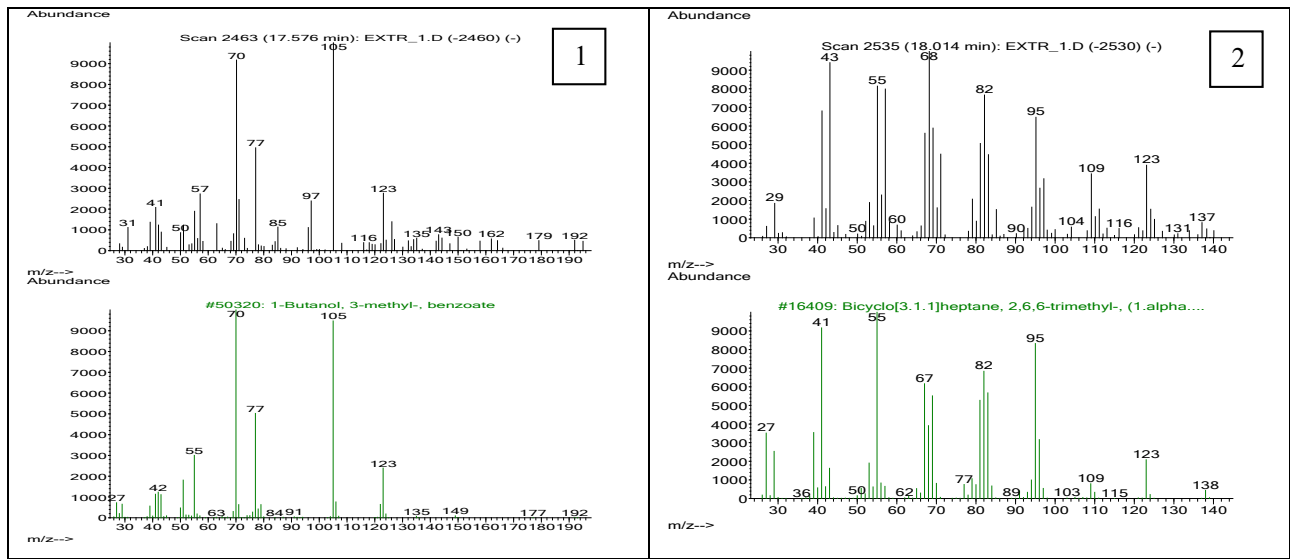


Figure 4: 3-Methyl-1-butanolbenzoate (1), bicycle[3.1.1]heptanes (2) mass-spectra.

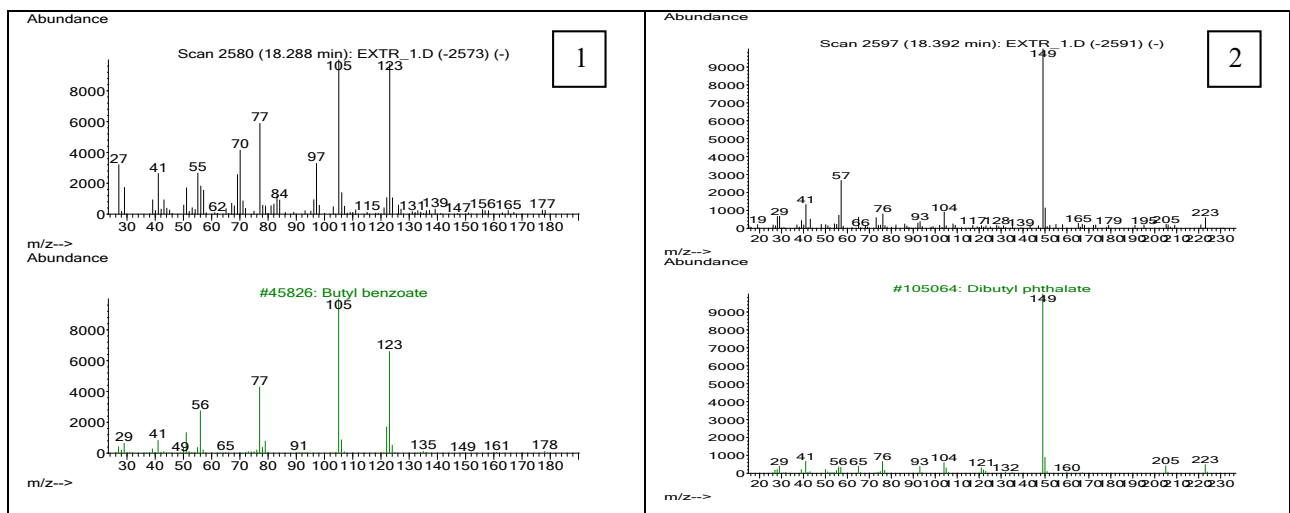


Figure 5: butylbenzoate (1), dibutyl phthalate (2) mass-spectra.

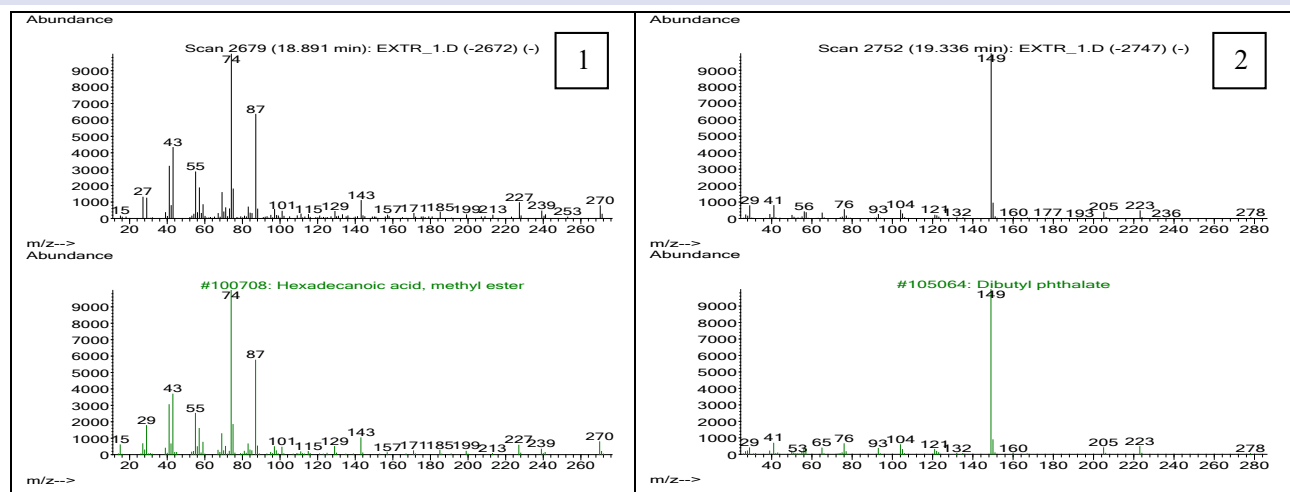


Figure 6: Hexadecanoic acid methyl ester (1), dibutyl phthalate (2) mass-spectra.

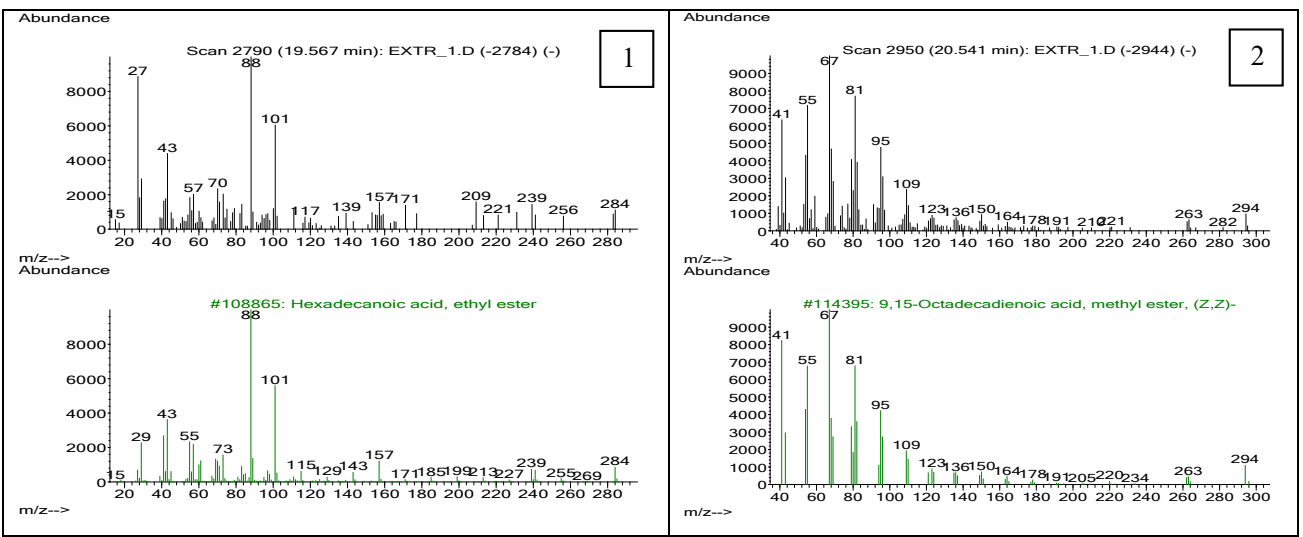


Figure 7: Hexadecanoic acid ethyl ester (1), [Z,Z]-9,15-Octadecadienoic acid methyl ester (2) mass-spectra.

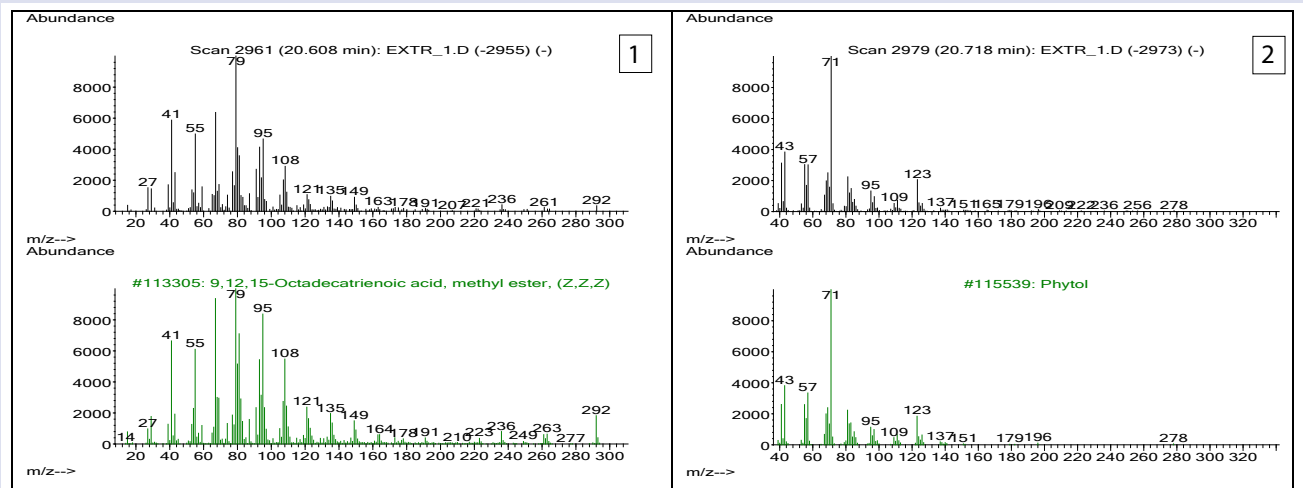


Figure 8: [Z,Z,Z]-9,12,15-Octadecatrienoic acid methyl ester (1), phytol (2) mass-spectra.

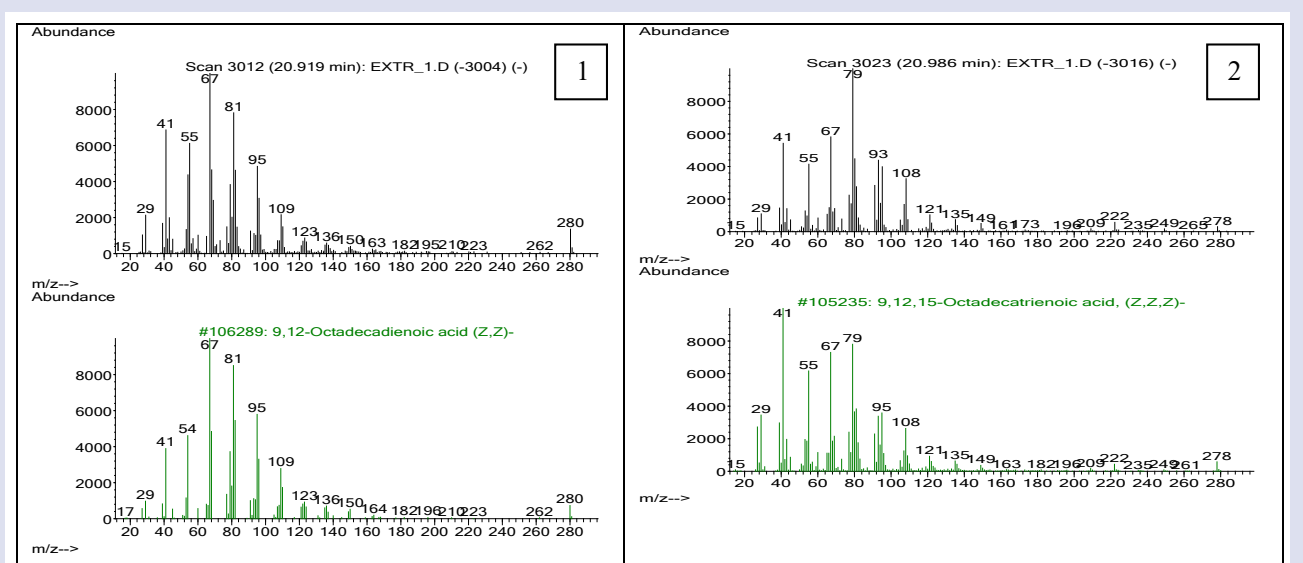


Figure 9: [Z,Z]-9,12-Octadecadienoic acid (1), [Z,Z,Z]-9,12,15-Octadecatrienoic acid (2) mass-spectra.

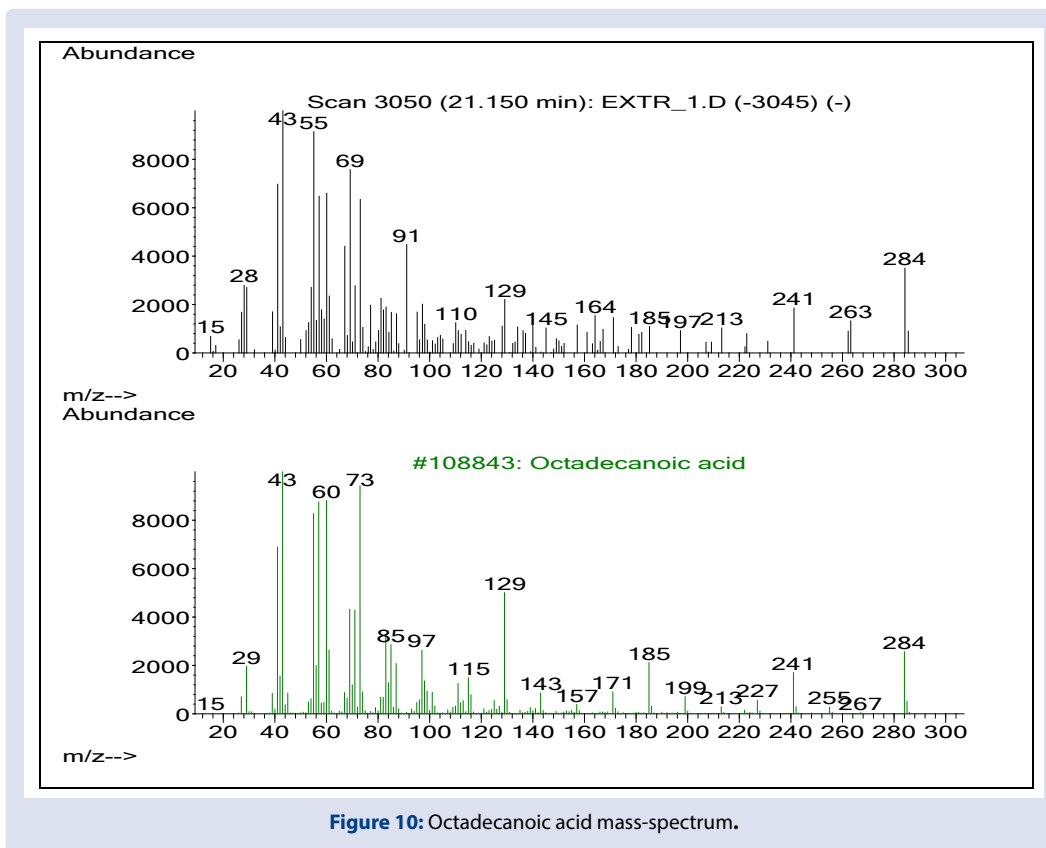


Figure 10: Octadecanoic acid mass-spectrum.

ACKNOWLEDGEMENT

This paper was financially supported by “Russian Academic Excellence Project 5-100”.

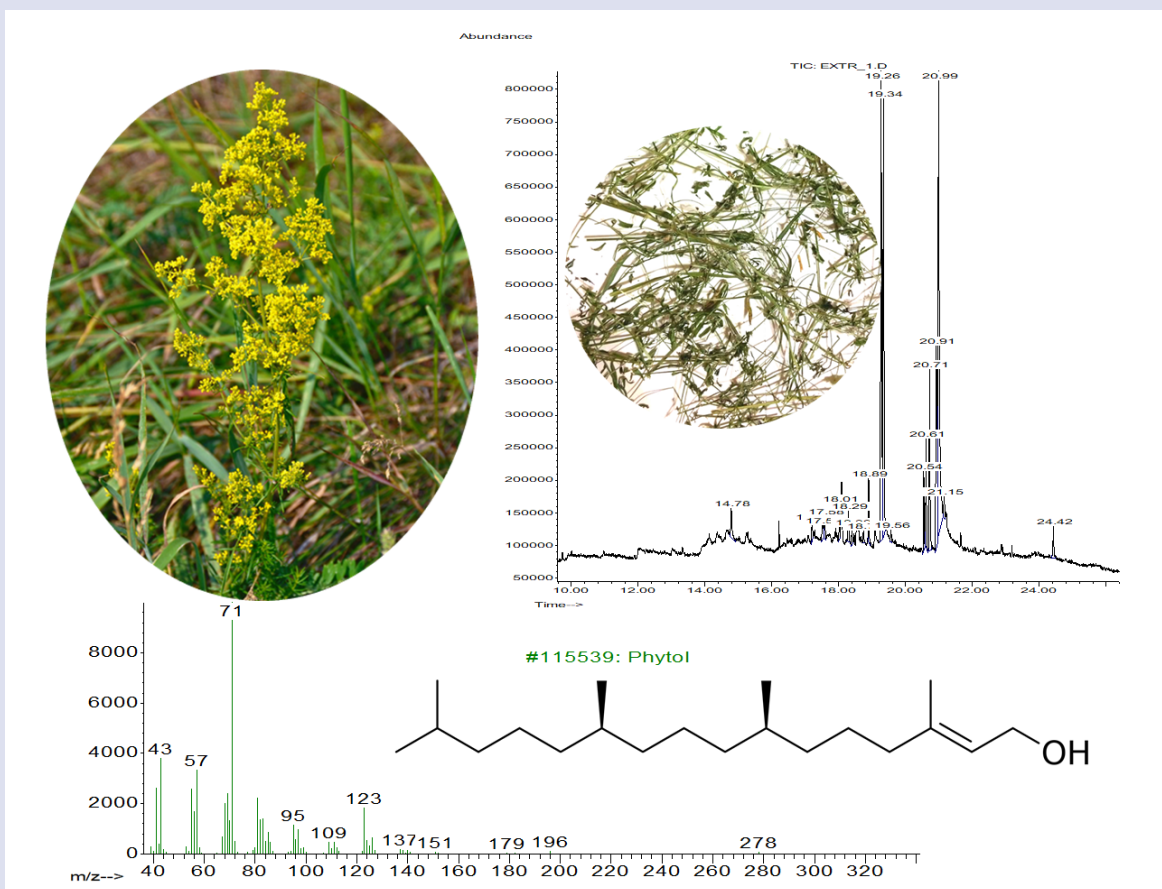
CONFLICTS OF INTEREST

None.

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GRAPHICAL ABSTRACT



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Cite this article: Zaichikova SG, Bokov DO, Kiselevskii MV, Antsyshkina AM, Bondar AA, Prostodusheva TV, *et al.* Determination of the Chemical Composition of Lady's Bedstraw (*Galium verum* L.) Herb Extract by GC-MS. *Pharmacogn J.* 2020;12(4):857-63.