Research Article

The determination of qualitative composition and quantitative content of steroidal compounds in *Hosta plantaginea* and *Hosta lancifolia* plant raw material

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ABSTRACT

Introduction: Hosta species are used as antibacterial and anti-inflammatory agents in Far East countries. Many studies conducted by Chinese scientists testify about 50 steroid compounds in these plants composition. These compounds, according to the researchers' opinion, show anti-inflammatory and cytostatic activity. **Materials and Methods:** Qualitative composition and quantitative content of steroidal compounds in *Hosta plantaginea* and *Hosta lancifolia* rhizomes with roots, leaves, and flowers were determined by gas chromatography technique. **Results and Discussions:** As the result of the study, 13 compounds of steroidal structure were found in *H. plantaginea* rhizomes with roots at a total amount of 103 mg/kg. Only five compounds were detected in leaves and six compounds in flowers. The total amount of the analyzed compounds equaled 312 mg/kg and 114 mg/kg, respectively, for the plant raw material listed above. Six compounds of steroidal structure were founds in both rhizomes with roots and flowers of *H. lancifolia*. Their total amount equaled 39 mg/kg and 32 mg/kg for rhizomes with roots and flowers, respectively. *H. lancifolia* leaves contain five identified steroids at the amount of 55 mg/kg. All the analyzed types of plant raw material contained campesterol, stigmasterol, and β-sitosterol at a high amount. The highest content of these compounds was determined in *H. plantaginea* leaves. It equaled 27 mg/kg, 124 mg/kg, and 131 mg/kg, respectively, for each compound mentioned above. Gitogenin was detected only in *H. plantaginea* rhizomes with roots at the amount of 36 mg/kg. **Conclusions:** The obtained data can be used for the development of plant raw material quality control procedures as well as for the development of new drugs on its basis.

KEY WORDS: Gas chromatography, *Hosta lanceolata, Hosta plantaginea*, Steroidal compounds

INTRODUCTION

Plants from Hosta genus are well-known worldwide as ornamental plants. Some of them are used for the creation of flower compositions. Young leaves and plant buds are used as food agents in the East. Water and water-alcohol extracts are used in tobacco industry as flavoring.[1-3] Hosta species were used for inflammatory processes treatment since ancient times in Chinese and Japanese folk medicine. These plants were used as treatment for otitis, pharyngitis, angina, mastopathy, urethritis, as well as women genital organs inflammation.[1,3-6]

A few studies made by foreign scientists determined that Hosta species show anti-inflammatory, antibacterial, antifungal, antiviral, antiacetylcholinesterase, and antitumor effects.[1,2,4-11]

It is known from the literature data that Chinese and Korean scientists isolated circa 50 steroidal compounds from several representatives of Hosta genus. The majority of them are gitogenin, manogenin, and tigogenin derivatives.[1,2,4-11] According to the research data, these compounds show anti-inflammatory and cytostatic activity against leukemia and cervix carcinoma.[1,2,4-11] However, there are no reliable data found regarding steroid compounds qualitative composition and quantitative content in *Hosta plantaginea* and *Hosta lanceolata*.

The aim of our study was the determination of qualitative composition and quantitative content of steroidal compounds in rhizomes with roots, leaves, and flowers of *H. plantaginea* (Lam. Aschers.) and *Hosta lancifolia* Engl.

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MATERIALS AND METHODS OF THE RESEARCH

H. plantaginea and *H. lancifolia* rhizomes with roots, leaves, and flowers were chosen as research objects. Plant raw material was collected in Kharkiv and Khmelnitsky region during 2015–2017.

The qualitative composition and quantitative content of steroidal compounds in both Hosta species was studied using gas chromatography by Agilent Technologies 6890 chromatograph with mass spectra detector 5973. Mass spectra library National Institute of Standards and Technology (NIST) 05 and WILEY 2007 with the total amount of mass spectra over 470,000 in combination with identification programs automated mass spectrometry deconvolution and identification system and NIST were applied.[12]

The sample preparation procedure was carried out by the addition of the inner standard and 0.6 ml of methylene chloride up to the precise sample weight (approximately 0.05 g) which was previously stirred in a 2 ml vial. Tridecane was chosen as an inner standard. Tridecane was taken at the rate of 50 μg per sample weight with further inner standard concentration calculation. This inner standard concentration was used for the further calculations.[12]

The sample was left at 50°°C in an ultrasound desiccator for 3 h or at a room temperature for 24 h. The obtained extract was poured into a 2 ml vial with following concentrating by pure nitrogen purging at a rate of 100 ml/min to a residual volume of 10 μl of extract.[12]

Introduction of a 3 μl of a sample into a chromatographic column was performed in a splitless mode. This mode means injection without flow split during 0.5 min. Such a procedure made it possible to introduce a sample without split loss and increased the chromatographic technique sensitivity by 10–20 times.[12]

A capillary column DB-5 with inner diameter of 0.25 mm and length of 30 m was used for chromatographic analysis. Helium was used as a carrier gas and was supplied at a rate of 1.2 ml/min. The temperature of the sample introductory heater was 350°C and thermostat temperature was programmed from 50°C to 320°C at a rate of 4°/min.[12]

The inner standard technique was applied for quantitative calculations. The component content calculation was done according to the following formula:

$C = K1*K2$, mg/kg,

Where, $K1 = П1/П2$ (П1 - peak square of the analyzed compound, П2 - peak square of the standard compound). $K2 = 50/M$ (50 - inner standard weight [μg], put into the sample, M - sample weight [gram]).[12]

RESULTS AND DISCUSSIONS

The presence of 13 steroidal compounds in total in all types of *H. plantaginea* plant raw material was detected as the result of the study. Only 10 steroidal compounds were in total found in *H. lancifolia* plant raw material. Gas chromatograms of the analyzed types of *H. plantaginea* are shown in Pictures 1 and 2 for *H. lancifolia*, respectively. The results of the experiment are represented in Table 1.

According to Table 1, *H. plantaginea* shows more diverse steroidal compounds content and accumulates them in higher amount than *H. lancifolia.*

A total of 13 steroidal compounds were accumulated in *H. plantaginea* rhizomes with roots and their total content equaled 103 mg/kg. Gitogenin was the dominant compound in this type of a plant raw material (36 mg/kg). Gecogenin and cholest-5-ene-3-ol, 6-methyl-, (3β)- were accumulated in minor amount that equaled just 1 mg/kg. Steroidal compounds dominated in their amount in *H. plantaginea* leaves. They were accumulated in this type of plant raw material at a rate of 312 mg/kg. This plant leaves accumulated stigmasterol and bb-sitosterol the most. Hence, their amount in this plant raw material equaled 121 mg/kg and 131 mg/kg, respectively. *H. plantaginea* flowers accumulated only four steroidal compounds but their total amount was 114 mg/kg. Dominant compounds in this type of plant raw material were stigmasterol and bb-sitosterol at the amount of 57 mg/kg and 37 mg/kg, respectively. *H. plantaginea* flowers accumulated aa-amyrin at the least amount from all other detected steroidal compounds. Moreover, this amount equaled 8 mg/kg. 26-nor-5-cholestene-3β-ol-25-one, stigmasterol, and bb-sitosterol were accumulated in all three types of the studied *H. plantaginea* plant raw material. Six compounds from the detected steroidal compounds were found only in *H. plantaginea* rhizomes with roots. These compounds were gitogenin, gecogenin, cholest-5ene-3-ol 6-methyl (3β)-, and cholesta-3,5-diene-7-one, 4,22-stigmastadiene-3-one as well as stigmast-4-ene-3-one which were accumulated at the amount of 2 mg/kg.

To compare different types of *H. lancifolia*, plant raw material was found the fact that rhizomes with roots and flowers accumulated approximately the same amount of steroidal compounds and it was 39 mg/kg and 32 mg/kg, respectively. A little bit higher amount of these compounds accumulated in laves and equaled 55 mg/kg. According to the study was found out that *H. lancifolia* rhizomes with roots contained five

Table 1: Qualitative composition and quantitative content steroidal compounds in plant raw material of *H. plantaginea* and *H. lancifolia*

Component	Quantitative content of steroidal compounds in plant raw material, mg/kg					
	H. plantaginea			*H. lanceolata*		
	Rhizomes with roots	Leaves	Flowers	Rhizomes with roots	Leaves	Flowers
26-nor-5-cholestene-3β-ol-25-one	19	16	12	7	1	0
Cholest-5-ene-3-ol, 6-methyl-, (3β)-	1	0	0	0	0	0
Cholesta-3,5-diene-7-one	2	0	0	0	0	0
Campesterol	5	27	0	4	8	0
Stigmasterol	11	124	57	11	12	6
β-Sitosterol	16	131	37	11	10	3
Stigmasta-5,24 (28)-diene-3-ol, (3β,24Z)-	0	0	0	0	0	7
Cholesta-9 (11),20 (22)-diene-23-one, 3,6-dihydroxy-, (3β,5α,6α)-	0	0	0	0	0	2
α-Amyrin (4,4,6a, 6b, 8a, 11,12,14b-octamethyl-1,4,4a, 5,6,6a, 6b, 7,8,8a, 9,10,11,12,12a, 14,14a, 14b-octadecahydro-2H-picene-3-on)	3	0	8	4	0	0
4,22-Stigmastadiene-3-one	2	0	0	0	0	0
9,19-Cyclolanost-24-ene-3-ol, (3β)-	5	14	0	0	24	6
Stigmast-4-ene-3-one	2	0	0	2	0	0
24-Methylenecycloartanol (9,19-cyclolanostane-3-ol, 24-methylene-, (3β)-)	0	0	0	0	0	8
Gitogenin ((25R)-5α-spirostan-2α,3β-diol)	36	0	0	0	0	0
Gecogenin (spirostan-12-on, 3-dihydroxy-, (3β,5α,25R)-)	1	0	0	0	0	0
Total steroids content in the sample	103	312	114	39	55	32

H. plantaginea: Hosta plantaginea, H. lancifolia: Hosta lancifolia

steroidal compounds and six steroidal compounds were identified in leaves.

Accumulation of stigmasterol and β-sitosterol is characteristic for all three types of plant raw material. The highest amount of stigmasterol (12 mg/kg) was found in *H. lancifolia* leaves. The highest amount of β-sitosterol (11 mg/kg) was found in *H. lancifolia* rhizomes with roots.

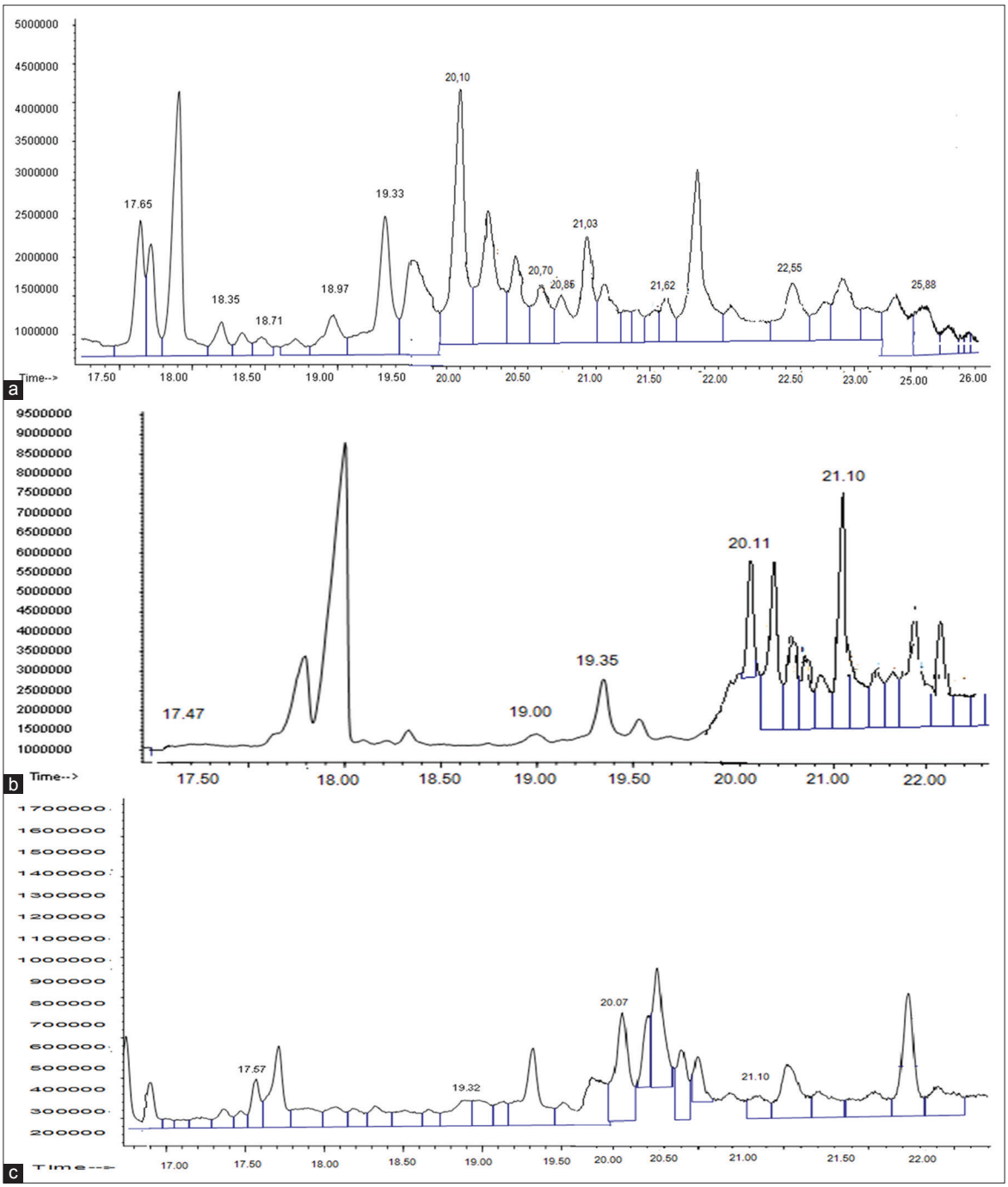
It was found that aa-amyrin and stigmast-4-ene-3-one was accumulated only in rhizomes with roots of the studied plant at the amount of 4 mg/kg and 2 mg/kg, respectively. Only *H. lancifolia* flowers accumulated 7 mg/kg of stigmasta-5,24(28)-diene-3-ol, 2 mg/kg of (3β,24Z)-,cholesta-9(11),20(22)-diene-23-one 3,6-dihydroxy-(3β,5α,6α)-, and 8 mg/kg of 24-methylenecycloartanol.

Rhizomes with roots accumulated stigmasterol and β-sitosterol at the highest amount. Their quantitative content was 11 mg/kg. Stigmast-4-ene-3-one accumulated at the minimum amount (2 mg/kg) in this type of plant raw material. 24-Methylenecycloartanol was dominant compound in leaves and its amount was 24 mg/kg. 26-nor-5-cholestene-3β-ol-25-one was found in *H. lancifolia* leaves in minor amount that equaled 1 mg/kg. 24-methylenecycloartanol was detected in the highest amount (8 mg/kg) in flowers. The least amount of steroidal compounds was determined in flowers for cholesta-9(11),20(22)-diene-23-one 3,6-dihydroxy-(3β,5α,6α) and equaled only 2 mg/kg.

Among all determined compounds, campesterol, stigmasterol, and β-sitosterol were accumulated at the highest amount in the same types of the plant raw material of both Hosta species. They were mostly located in leaves but *H. plantaginea* plant raw material contained it 3–10 times more than *H. lancifolia* plant raw material. The high content of gitogenin (36 mg/kg) was detected in *H. plantaginea* rhizomes with roots. Other plant raw material sample did not contain it at all.

CONCLUSIONS

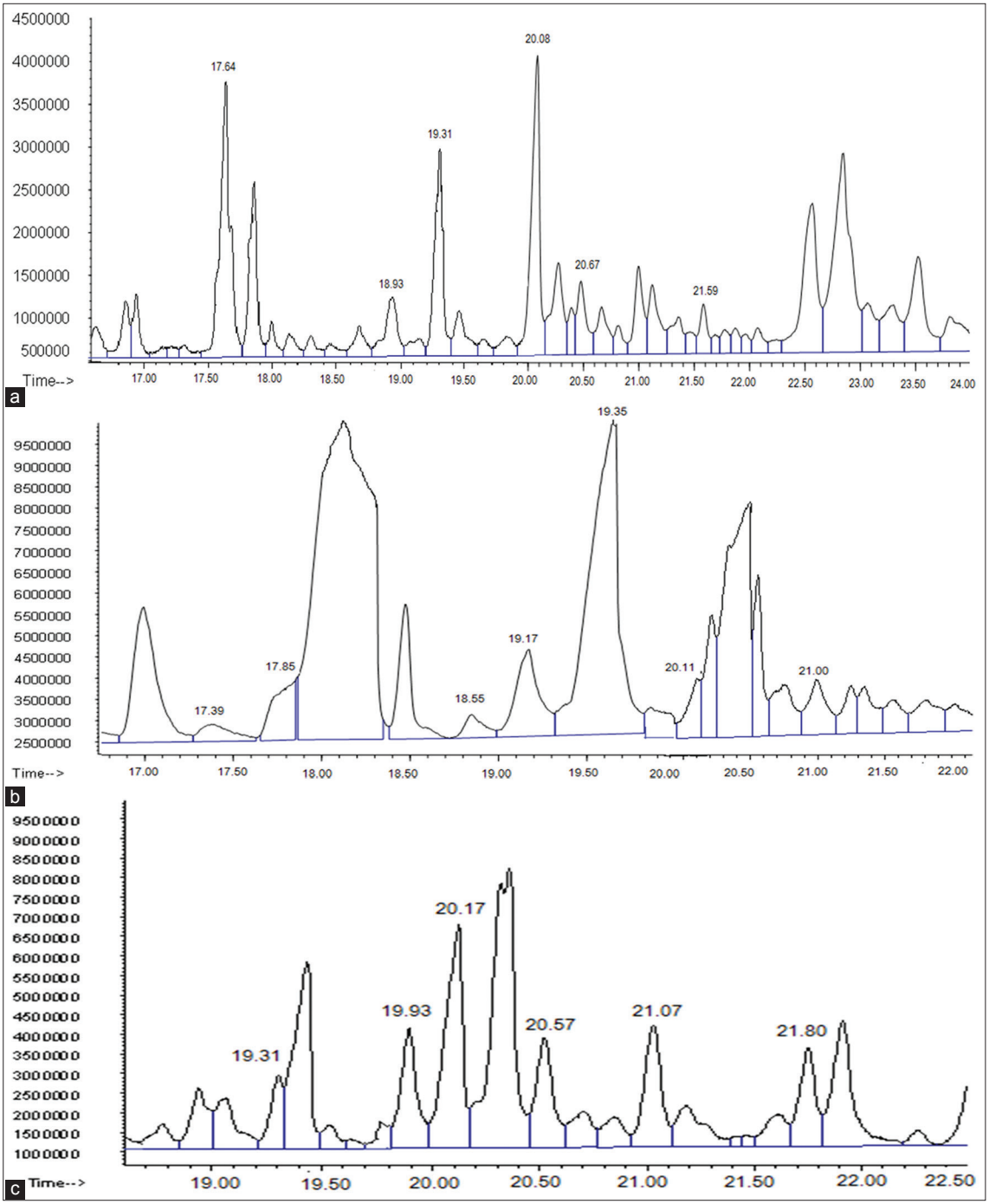
1. Qualitative composition and quantitative content of steroidal compounds in *H. plantaginea* and *H. lancifolia* rhizomes with roots, leaves, and flowers was determined by gas chromatography technique.
2. 13 steroidal compounds were detected in total in all the types of plant raw material of *H. plantaginea*. *H. lancifolia* plant raw material contained only 10 compounds.
3. 13 steroidal compounds at the total amount of 103 mg/kg were determined in *H. plantaginea*



Picture 1: Chromatograms of *Hosta plantaginea* steroidal compounds: (a) Rhizomes with roots (phase of the aboveground part extinction), (b) leaves (fruiting phase), (c) flowers (mass blossoming phase)

rhizomes with roots. This plant leaves contain five such compounds and flowers - only 4. The total amount of steroidal compounds in H. plantaginea leaves equaled 312 mg/kg and 114 mg/kg in *H. plantaginea* flowers.

4. *H. lancifolia* rhizomes with roots contained six steroidal compounds and leaves contained five of them. The total amount of steroidal compounds in this plant rhizome with roots was 39 mg/kg and 32 mg/kg for flowers. The highest amount of steroids (55 mg/kg) was determined in *H. lancifolia* leaves.
5. Steroidal compounds content in leaves of both Hosta species highly dominated steroidal compounds content in all other analyzed samples.
6. Campesterol, stigmasterol, and β-sitosterol were accumulated in high amount in all studied types of plant raw material.
7. Gitogenin was found at the amount of 36 mg/kg only in *H. plantaginea* rhizomes with roots. Cholesta-3,5-diene-7-one and 4,22-stigmastadiene-3-one at the amount of 2 mg/kg and cholest-5-ene-3-ol, 6-methyl-, (3β)-, and gecogenin at the amount of



Picture 2: Chromatograms of *Hosta lancifolia* steroidal compounds: (a) Rhizomes with roots (phase of the aboveground part extinction), (b) leaves (fruiting phase), (c) flowers (mass blossoming phase)

1 mg/kg were also detected only in this type of plant raw material.

8. Such steroidal compounds as stigmasta-5,24(28)-diene-3-ol, (3β,24Z)- at the amount of 7 mg/kg, 2 mg/kg of cholesta-9(11),20(22)-DIENE-23-one, 3,6-dihydroxy-, (3β,5α,6α)-, and 8 mg/kg of 24-methylenecycloartanol were found only in *H. lancifolia* flowers.
9. The obtained data can be used for the development of plant raw material quality control procedures as well as for the development of new drugs on its basis.

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