Краткие сообщения

Short communications

УДК 577.11,543.544.3,543.51

ГАЗОВЫЙ ХРОМАТО-МАСС-СПЕКТРОМЕТРИЧЕСКИЙ АНАЛИЗ АЦЕТОНОВОГО ЭКСТРАКТА КОРНЕЙ *LACTUCA TATARICA* (L.) C. A. MEY.

Э. Н. ШУКЮРЛИ[1)]

[1)]*Институт ботаники НАН Азербайджана,*
Бадамдарское шоссе, 40, AZ1004, г. Баку, Азербайджан

Методом газовой хроматографии в сочетании с масс-спектрометрией изучены корни *Lactuca tatarica* (L.) C. A. Mey. В неочищенном экстракте, который получают из корней, обнаружено присутствие природных соединений различных групп. В результате из твердой массы объекта исследования идентифицированы 25 различных веществ, в том числе ациклические соединения (метилмиристат, метилпальмитат, этилпальмитат, метилэлаидат, метилстеарат, этилолеат и др.). Наиболее распространенными эфирами жирных кислот являются метилолеат (6,831 %) и метилпальмитат (2,902 %). Кроме того, выявлены характерные для *L. tatarica* тритерпеноидные соединения (ацетат лупеола, ацетат циклоартенола, ацетат 3β,13β,14β-13,27-циклоурсан-3-ола), среди которых ацетат лупеола является преобладающим компонентом и составляет 56,35 % от общего количества полученных веществ. Также обнаружены элаидиновая кислота, бис(2-этилгексил)фталат и другие соединения. Некоторые идентифицированные вещества ранее были обнаружены у других видов рода *Lactuca* L., однако в *L. tatarica* они (ацетат лупеола, ацетат 3β,13β,14β-13,27-циклоурсан-3-ола, ацетат олеан-12-ен-3-ила) впервые выявлены в рамках настоящего исследования, что делает его значимым с химической точки зрения. Изучаемое растение широко распространено во флоре низменных и приморских районов Азербайджана. Таким образом, полученные результаты открывают новые горизонты для будущих исследований в различных областях, в том числе и в фармацевтике.

Ключевые слова: эфиры жирных кислот; метод ГХ-МС; *Lactuca tatarica*; ацетат лупеола; тритерпеноид.

Образец цитирования:
Шукюрли ЭН. Газовый хромато-масс-спектрометрический анализ ацетонового экстракта корней *Lactuca tatarica* (L.) C. A. Mey. *Экспериментальная биология и биотехнология.* 2022;2:82–87 (на англ.).
https://doi.org/10.33581/2957-5060-2022-2-82-87

For citation:
Shukurlu EN. Gas chromatography – mass spectrometry analysis of acetone extract of *Lactuca tatarica* (L.) C. A. Mey. roots. *Experimental Biology and Biotechnology.* 2022;2:82–87.
https://doi.org/10.33581/2957-5060-2022-2-82-87

Автор:
Эмиль Намик Шукюрли – младший научный сотрудник отдела растительных ресурсов.

Author:
Emil Namik Shukurlu, junior researcher at the department of plant resources.
geneticsster@gmail.com
https://orcid.org/0000-0002-2805-6298

GAS CHROMATOGRAPHY – MASS SPECTROMETRY ANALYSIS OF ACETONE EXTRACT OF *LACTUCA TATARICA* (L.) C. A. MEY. ROOTS

E. N. SHUKURLU[a]

[a]*Institute of Botany, Azerbaijan National Academy of Sciences, 40 Badamdar Highway, Baku AZ1004, Azerbaijan*

Research work is devoted to the chemical investigation of the roots of the *Lactuca tatarica* (L.) C. A. Mey. by gas chromatography – mass spectrometry method. From the crude extract that is obtained from the roots, the presence of natural compounds of various groups has been detected. As a result, 25 different substances including acyclic compounds (methyl myristate, palmitic acid methyl ester, ethyl palmitate, methyl elaidate, methyl stearate, ethyl oleate, etc.) have been identified from the solid mass of the research object. The most abundant fatty acid esters are methyl oleate (6.831 %) and palmitic acid methyl ester (2.902 %). In addition, the triterpenoid compounds (lupeol acetate, cycloartenol acetate, 3β,13β,14β-13,27-cycloursan-3-ol acetate) which are characteristic of the *L. tatarica* have been identified. Lupeol acetate is the predominant component which makes up 56.35 % of the whole obtained substances. Elaidic acid which belongs to the fatty acids group, bis(2-ethylhexyl)phthalate which belongs to the aromatic dicarboxylic acid esters and other compounds have also been identified. Among the identified substances, some are found previously from the other species of the genus *Lactuca* L. but with regards to the *L. tatarica* to the best of our knowledge the majority of them are firstly being reported in this study. The investigated plant is widespread in the lowlands and coastal areas of Azerbaijani flora. The availability of some pharmacological effective compounds (lupeol acetate, 3β,13β,14β-13,27-cycloursan-3-ol acetate, olean-12-en-3-yl acetate) makes the research significant in terms of chemical aspect. Therefore, the results of this investigation open up new horizons for future studies in various fields including the pharmaceutical one.

Keywords: fatty acid esters; GC-MS method; *Lactuca tatarica*; lupeol acetate; triterpenoid.

Introduction

Increasing demand of herbal medicines puts chemical investigation of plants forward. The presence of pharmacological active substances in triterpenoid derivatives and their wide distribution in species of Asteraceae makes their research important [1]. The genus *Lactuca* L. (Asteraceae Bercht. & J. Presl) is characterised by the presence of sesquiterpene lactones [2]. Also 15 species in the Caucasus and 10 species in Azerbaijani flora have been found of the genus *Lactuca* L. [3, p. 551–557]. *Lactuca tatarica* is a perennial bare plant, stalk is straight, simple panicle branched inflorescence, 5–50 cm tall. Sessile leaves, lanceolate in outline, notched pinnate into triangular whole-marginal or short-edged, back facing lateral lobes, upper lobe usually elongated. Outer leaves are lanceolate, inner from a triangular base. In Azerbaijan *L. tatarica* is distributed in Caspian seashores, Absheron – Kur – Araz lowlands, Lankaran, in slightly saline grounds, along coastal sands [3, p. 551–557]. In terms of its chemical substances, plants of the genus *Lactuca* L. have been found to produce sesquiterpene lactones, including guaianolides, along with germacranolides and eudesmanolides [4]. Still lack of chemical research of *Lactuca* species performed in Azerbaijan.

Traditionally *L. tatarica* has been used in folk medicine, such as decoction of whole plant was used for joint pains [5]. Its leaves used for headache, internal wounds, fever and vomiting in Indian folk medicine [6]. In China, its whole herb has been used as a folk medicine for the cure of some ailments, including erysipelas, extravasated blood, appendicitis, red leucorrhea properties and abdominal distension [7].

In general, sesquiterpene lactones and their various derivatives (sesquiterpene lactone glycosides contained guaianolide and germacrolide derivatives) have been extracted from *L. tatarica* plant species according to the world literature findings [8]. From the roots of the *L. tatarica* lactucopicrin, 8-deoxylactucin, crepidiaside B, jacquinelin, lactucin, 11β,13-dihydrolactucin, vernoflexuoside (glucozaluzanin C) and lactuside A are obtained [9]. Tataroside, a germacrolide glucoside, was isolated from the roots of *L. tatarica* [10]. 11βH,13-dihydrolactucin-8-O-*p*-methoxyphenylacetate, picriside C, sonchuside A, 11βH,13-dihydroglucozaluzanin C, benzyl-β-glucopyranoside, cichorioside B, macrocliniside A and ixerin F were isolated from the roots of *L. tatarica* [8]. Additionally, deacetylmatricarin, 2-oxo-11β,13-dihydrosantamarin and 11β,13-dihydrosantamarin have been isolated from the whole part of the respective plant [11].

L. tatarica is rich in flavonoids, triterpenoids and sesquiterpene lactones and some of these compounds are medically effective. Therefore, the goal of this research is the determination of chemical components of roots of *L. tatarica* which is omnipresent in Southern Caucasus region. A brief description of the investigation of the issue and justification of the novelty of the questions that the author considers in the article are given.

Materials and research methods

Collection of plant material. As a research object roots of the *L. tatarica* were collected on summer (2017) during the blossoming period, from the Shabran region (41°12′32.9″N; 48°58′08.2″E). They have been chopped into small pieces and dried at room temperature.

Preparation of extracts. A portion of small-scale chopped and dried research object (820 g) has been extracted with acetone at room temperature for nine days [12]. The extract was decanted, filtered and concentrated by rotary evaporator on the water bath (evaporator model: ROVA-N2L (*MRC*, Israel); water bath model: WB-2000 (*ANM Industries*, India)). Also 10.2 g dried extract were obtained and extraction percentage was 1.24 %. Extractive substances have been separated into fractions through the column chromatography.

Column chromatography. As a mobile phase *n*-hexane-benzene in the volume ratio of 1 : 1 was used and the respective extract was separated into fractions by silica gel (mesh 70–230 (i. e., 63–200 μm); pore size 60 Å) which was the stationary phase. The column was eluted with abovementioned solvent, collecting 100 mL fractions, a total of 62 fractions. Each 100 mL fraction (in some cases more than 2 fractions were gathered together) was collected in a 250 mL flask and the solvent was evaporated under reduced pressure using a rotary evaporator. Substances that are obtained from the 13–16th fractions then solidified in *n*-hexane-benzene (in the volume ratio of 1 : 1) solution. Weight of the solid mass was 0.04 g.

Thin layer chromatography (TLC). As a solvent for TLC analysis benzene was used. Visualisation of spots was conducted by chromatoscope. TLC plates (Silufol UV-254, *Kavalier*, Czech) were observed under UV light at 366 nm. Solid mass was found to have a polycomponent nature during the TLC analysis. Therefore, qualitative and quantitative analysis of solid mass precipitated from the 13–16th fractions was performed using gas chromatography – mass spectrometry (GC-MS) method and its spectrum confirmed the presence of different components.

GC-MS analysis. GC-MS analysis has been performed as described previously by Shukurlu [13]. Gas chromatography – mass spectra were obtained using an Agilent Technologies 6890 N (USA) network GC system equipped with an Agilent Technologies 5975 inert mass selective detector. Compound identification has been conducted using the NIST library database (the NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library (version 2.0.g) build 19 May 2011). The statistical analysis has not been carried out in this research.

Results and discussion

The GC-MS spectrum illustrated in figure and totally 25 different compounds were identified. As shown in the table, the main characteristic compounds of the 13–16th fractions are triterpenoids. The triterpenoid profile of *L. tatarica* was characterised by the high concentrations of lupeol acetate (56.35 %), 3β,13β,14β-13,27-cycloursan-3-ol acetate (20.31 %), while the lowest amounts were detected for olean-12-en-3-yl acetate (1.92 %) and cycloartenol acetate (1.11 %). The following fatty acid esters have been obtained: methyl myristate, methyl palmitoleate, palmitic acid methyl ester, ethyl palmitate, methyl linoleate, methyl oleate, methyl elaidate, methyl stearate, methyl linolelaidate, ethyl-9,12-octadecadienoate, ethyl oleate, methyl 9-*cis*,11-*trans*-octadecadienoate, 10,13-eicosadienoic acid methyl ester, 11-eicosenoic acid methyl ester, methyl arachisate, oleic acid 3-hydroxypropyl ester, bis(2-ethylhexyl)sebacate. Among them the highest abundant ones are methyl oleate (6.831 %), palmitic acid methyl ester (2.902 %), methyl linoleate (2.826 %) and the least abundant ones are 10,13-eicosadienoic acid methyl ester (0.075 %) and methyl myristate (0.047 %). Elaidic acid (0.303 %) which is a monounsaturated trans fatty acid is identified. The other identified compounds are phthalic acid butyl hept-4-yl ester (belongs to aromatic dicarboxylic acid esters); bis(2-ethylhexyl)phthalate (belongs to aromatic dicarboxylic acid derivatives) and a diterpene 4-(3-hydroxy-3-methylpentyl)-3,4a,8,8-tetramethyldecahydro-1-naphthalenol are obtained. To the best of our knowledge, the fatty acid profile of the roots *L. tatarica* has never been reported previously.

Compounds identified in the crystalline mass obtained from the 13–16th fractions by column chromatography from the roots of *L. tatarica* by GC-MS method

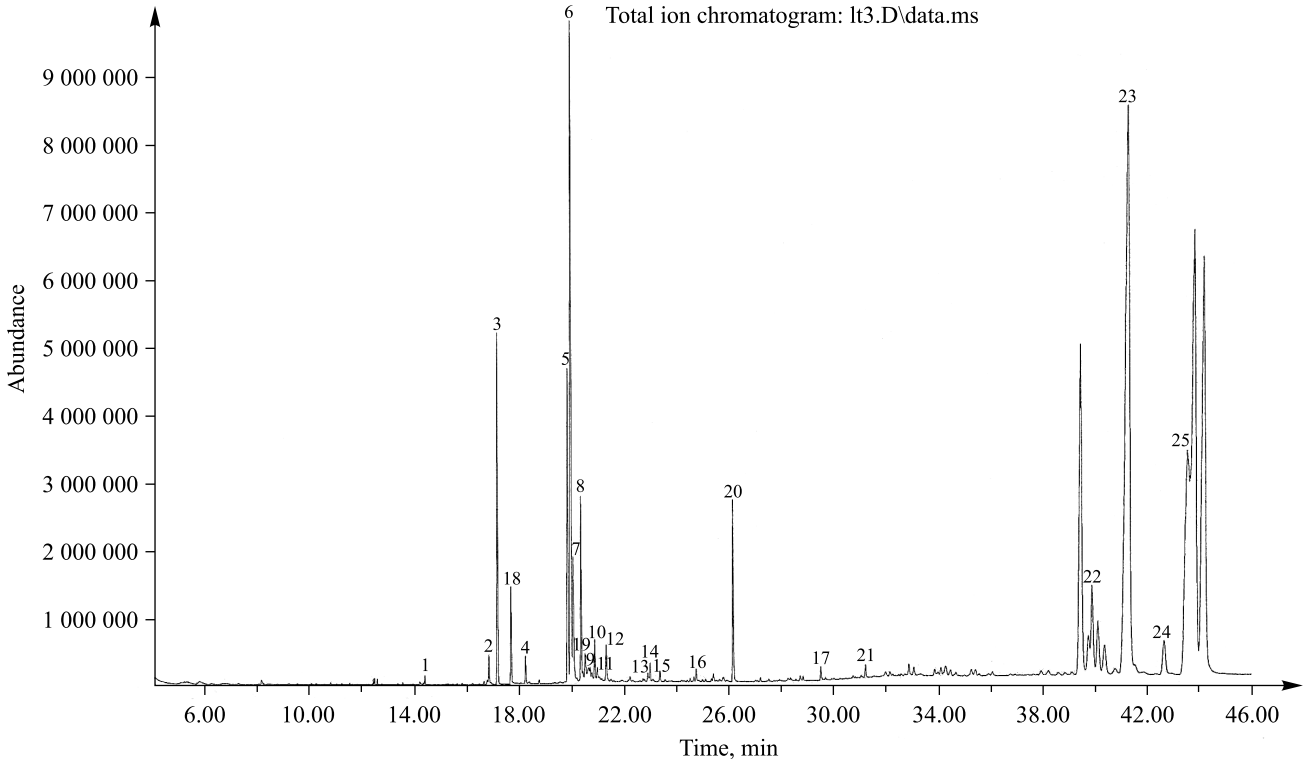
Peak	Retention time, min	Fragments	Molecular weight, g/mol	Name	Percentage of total
Fatty acid esters					
1	14.392	143, 88, 75, 69, 57, 55, 43, 41, 29	242	Methyl myristate	0.047
2	16.824	98, 97, 88, 84, 75, 70, 56, 42	268	Methyl palmitoleate	0.256
3	17.155	227, 143, 88, 75, 69, 57, 55, 43, 41	270	Palmitic acid methyl ester	2.902

Ending table

Peak	Retention time, min	Fragments	Molecular weight, g/mol	Name	Percentage of total
4	18.214	102, 89, 73, 69, 57, 55, 43, 41, 29	284	Ethyl palmitate	0.214
5	19.825	109, 96, 83, 82, 79, 68, 56, 41	294	Methyl linoleate	2.826
6	19.952	98, 96, 87, 84, 75, 70, 56, 43, 41	296	Methyl oleate	6.831
7	20.032	98, 96, 87, 84, 75, 70, 56, 43, 42	296	Methyl elaidate	1.373
8	20.329	298, 143, 88, 75, 69, 57, 55, 43, 41	298	Methyl stearate	1.534
9	20.618	96, 82, 81, 79, 68, 56, 43, 42, 29	294	Methyl linolelaidate	0.297
10	20.852	96, 83, 82, 69, 68, 56, 55, 42	308	Ethyl-9,12-octadecadienoate	0.312
11	20.949	101, 97, 96, 89, 84, 70, 56, 44, 42	310	Ethyl oleate	0.082
12	21.288	109, 96, 82, 79, 69, 68, 55	294	Methyl 9-*cis*,11-*trans*-octadecadienoate	0.375
13	22.887	109, 97, 96, 83, 82, 69, 68, 70, 56, 41	322	10,13-Eicosadienoic acid methyl ester	0.075
14	22.976	292, 98, 96, 84, 74, 70, 67, 56	324	11-Eicosenoic acid methyl ester	0.165
15	23.359	326, 143, 88, 75, 57, 55, 44, 41	326	Methyl arachisate	0.095
16	24.746	265, 99, 81, 70, 67, 60, 57, 44, 42, 31	340	Oleic acid 3-hydroxypropyl ester	0.091
17	29.507	186, 112, 98, 83, 71, 70, 58, 55, 43, 41	426	Bis(2-ethylhexyl)sebacate	0.124
Aromatic dicarboxylic acid ester					
18	17.663	224, 206, 151, 150, 104, 98, 76, 57, 56, 41	320	Phthalic acid butyl hept-4-yl ester	0.765
Monounsaturated trans fatty acid					
19	20.491	98, 96, 84, 70, 57, 56, 43	282	Elaidic acid	0.303
Aromatic dicarboxylic acid derivative					
20	26.163	279, 167, 150, 71, 70, 57, 55, 43, 41	390	Bis(2-ethylhexyl)phthalate	1.505
Diterpenoid					
21	31.210	190, 154, 137, 136, 124, 110, 96, 83, 70, 56	310	4-(3-Hydroxy-3-methylpentyl)-3,4a,8,8-tetramethyldecahydro-1-naphthalenol	0.123
Triterpenoids					
22	39.863	219, 203, 189, 136, 110, 96, 82, 69, 44	468	Olean-12-en-3-yl acetate	1.925
23	41.323	190, 121, 110, 108, 96, 94, 82, 70, 56, 44	468	Lupeol acetate	56.35
24	42.644	121, 109, 107, 95, 93, 81, 70, 55, 44, 42	468	Cycloartenol acetate	1.113
25	43.576	469, 206, 139, 136, 124, 122, 110, 96, 70, 44	468	13,27-cycloursan-3-ol, acetate, (3β,13β,14β)-	20.314

Lupeol acetate has been isolated from the aerial parts of *Lactuca sativa* L. [14] and from *L. denticulata* [15]. Olean-12-en-3-yl acetate was obtained from the leaves of *L. sativa* L. [16].

As a fatty acid ester methyl palmitoleate, methyl linoleate, palmitic acid methyl ester have been isolated from *L. saligna* L. [17]. Methyl oleate and palmitic acid methyl ester are also found in the seeds of *L. tatarica* [18]. Methyl myristate, methyl oleate, palmitic acid methyl ester, methyl linoleate, methyl stearate, methyl arachisate have been obtained from the seeds of *L. sativa* L. [19].



GC-MS chromatogram of crystalline mass obtained from the 13–16th fractions by column chromatography from the roots of *L. tatarica*

Among these components lupeol acetate, 3β,13β,14β-13,27-cycloursan-3-ol acetate and olean-12-en-3-yl acetate are pharmacologically effective substances. Lupeol acetate has an antiarthritic effect [20]. Obtained pentacyclic triterpenoid 3β,13β,14β-13,27-cycloursan-3-ol acetate possesses antibacterial, antioxidant and anticarcinogenic properties [21]. Olean-12-en-3-yl acetate has an antiinflammatory, antidepressant and anticancer activity [22]. Due to the availability of pharmacological active components in *L. tatarica* roots, it can be recommended for further investigations onto the respective area.

Conclusion

In this study, the chemical investigation of *L. tatarica* root extract was performed using column chromatography and GC-MS technique. Briefly 25 components are identified (4 of them belong to triterpenoids, whereas 17 of them are defined as fatty acid esters, 1 of them is aromatic dicarboxylic acid ester, 1 is monounsaturated trans fatty acid, 1 is aromatic dicarboxylic acid derivative and 1 belongs to the diterpenoid group). In accordance with the literature data, there is a lack of information on this species. Acquired outcomes, together with the previous information, could support further investigations, including in the pharmaceutical area.

References

1. Muley BP, Khadabadi SS, Banarase NB. Phytochemical constituents and pharmacological activities of *Calendula officinalis* Linn (*Asteraceae*): a review. *Tropical Journal of Pharmaceutical Research.* 2009;8(5):455–465. DOI: 10.4314/tjpr.v8i5.48090.

2. Khalil AT, Abd El-Fattah H, Mansour ES. Guaianolides from *Lactuca saligna*. *Planta Medica.* 1991;57(2):190–191. DOI: 10.1055/s-2006-960064.

3. Karyagin II, editor. *Flora Azerbaidzhana. Tom 8. Rubiaceae – Compositae* [Flora of Azerbaijan. Volume 8. *Rubiaceae – Compositae*]. Askerova RK, Akhundov GF, Isaev YaM, Karyagin II, Irilipko LI, Sofieva RM, compilers. Baku: Publishing House of the Academy of Sciences of the Azerbaijan SSR; 1961. 690 p. Russian.

4. Beharav A, Ben-David R, Malarz J, Stojakowska A, Michalska K, Doležalová I, et al. Variation of sesquiterpene lactones in *Lactuca aculeata* natural populations from Israel, Jordan and Turkey. *Biochemical Systematics and Ecology.* 2010;38(4):602–611. DOI: 10.1016/j.bse.2010.07.007.

5. Devi U, Seth MK, Sharma P, Rana JC. Study on ethnomedicinal plants of Kibber Wildlife Sanctuary: a cold desert in Trans Himalaya, India. *Journal of Medicinal Plants Research.* 2013;7(47):3400–3419. DOI: 10.5897/JMPR2013.5298.

6. Namtak S, Sharma RC. Medicinal plant resources in Skuru watershed of Karakoram wildlife sanctuary and their uses in traditional medicines system of Ladakh, India. *International Journal of Complementary & Alternative Medicine.* 2018;11(5):294–302. DOI: 10.15406/ijcam.2018.11.00415.

7. Wu ZY. *Xinhua Bencao Gangyao.* Shanghai: Science Technical Publishing Press; 1990. 435 p.

8. Kisiel W, Barszcz B, Szneler E. Sesquiterpene lactones from *Lactuca tatarica*. *Phytochemistry.* 1997;45(2):365–368. DOI: 10.1016/S0031-9422(96)00820-5.

9. Michalska K, Stojakowska A, Malarz J, Doležalová I, Lebeda A, Kisiel W. Systematic implications of sesquiterpene lactones in *Lactuca* species. *Biochemical Systematics and Ecology.* 2009;37(3):174–179. DOI: 10.1016/j.bse.2009.02.001.

10. Kisiel W, Barszcz B. A germacrolide glucoside from *Lactuca tatarica*. *Phytochemistry.* 1998;48(1):205–206. DOI: 10.1016/S0031-9422(97)01106-0.

11. Wang X-X, Gao X, Jia Z-J. Sesquiterpenoids from *Lactuca tatarica. Fitoterapia.* 2010;81(1):42–44. DOI: 10.1016/j.fitote.2009.07.001.

12. Serkerov SV, Kasumova GK, Jahangirova IR. Study of qualitative composition of chemical components of leaves and fruits *Laser trilobum* L. by gas chromatography – mass spectrometry. *Plant & Fungal Research.* 2018;1(1):33–37. DOI: 10.29228/plantfungalres.30.

13. Shukurlu EN. Chemical content of *Lactuca serriola* L.: fatty acid esters and triterpene compounds from the roots. *Plant & Fungal Research.* 2020;3(1):61–65. DOI: 10.29228/plantfungalres.70.

14. Mahmoud ZF, Kassem FF, Abdel-Salam NA, Zdero C. Sesquiterpene lactones from *Lactuca sativa*. *Phytochemistry.* 1986;25(3):747–748. DOI: 10.1016/0031-9422(86)88039-6.

15. Hui WH, Lee WK. Triterpenoid and steroid constituents of some *Lactuca* and *Ageratum* species of Hong Kong. *Phytochemistry.* 1971;10(4):899–901. DOI: 10.1016/S0031-9422(00)97171-1.

16. Mughrbi HN, Auzi AA. *Lactuca sativa* stems as the source of bioactive compounds as well as the leaves. *Journal of Pharmacy and Pharmacology.* 2020;8(5):143–150. DOI: 10.17265/2328-2150/2020.05.003.

17. Abd El-Fattah H, Khalil AT, Mansour ES, Waight ES. Triterpenes and triterpene esters from *Lactuca saligna*. *International Journal of Pharmacognosy.* 1992;30(1):21–26. DOI: 10.3109/13880209209054625.

18. Shukurlu EN, Vitalini S, Iriti M, Garzoli S. Chemical characterization by GC/MS analysis of *Lactuca tatarica* (L.) C. A. Mey. aerial parts and seeds. *Natural Product Research* [Internet]. 2021 November 11 [cited 2021 November 11]. Available from: https://www.tandfonline.com/doi/full/10.1080/14786419.2021.2003356?scroll=top&needAccess=true. DOI: 10.1080/14786419.2021.2003356.

19. Afsharypuor S, Ranjbar M, Mazaheri M, Shakibaei F, Aslani A. Analysis of fatty acid composition of crude seed oil of *Lactuca sativa* L. by GC-MS and GC methods. *Trends in Pharmaceutical Sciences.* 2018;4(2):95–98.

20. Kweifio-Okai G, Carroll AR. Antiarthritic effect of lupeol acetate. *Phytotherapy Research.* 1993;7(2):213–215. DOI: 10.1002/ptr.2650070227.

21. Jacob DR, Vigasini N. Radical scavenging activity and *in vitro* anticarcinogenic potential of dried and powdered mangosteen (*Garcinia mangostana*) Pericarp. *International Journal of Scientific Research.* 2016;5(10):255–258.

22. Eswaraiah G, Peele KA, Krupanidhi S, Indira M, Kumar RB, Venkateswarulu TC. GC-MS analysis for compound identification in leaf extract of *Lumnitzera racemosa* and evaluation of its *in vitro* anticancer effect against MCF7 and HeLa cell lines. *Journal of King Saud University – Science.* 2020;32(1):780–783. DOI: 10.1016/j.jksus.2019.01.014.

Received 09.04.2022 / revised 15.06.2022 / accepted 21.06.2022.