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Investigation of chemical components extracted from the aerial parts of *Lactuca serriola* L. by liquid chromatography – mass spectrometric method

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Abstract: This article is dedicated to the research of the chemical compounds of aerial parts (stems and leaves) of *Lactuca serriola* L. The phytochemical investigation of the aerial parts of the *Lactuca serriola* L. (320 g) revealed the presence of flavonoid derivatives. In the acetonic extraction of raw plant material the total extractive contents is researched by the Liquid chromatography–mass spectrometric (LC-MS) method and flavonoid derivatives have been identified. Totally 20 compounds obtained, 11 were identified and they were found belong to the carboxylic acid, monosaccharide, phenolic compounds and flavonoids. LC-MS experiments were realised by a Shimadzu 20 A HPLC system and identification of the substances were performed by Analyst 1.6 software. Gluconic acid, glucose, quercetin are amongst those which were identified. Gluconic acid itself is used in pharmaceutical industry and recognized safe to be used as a food additive by FDA and EU. *L. serriola* has been used in traditional folkloric medicine for the treatment of gastrointestinal, respiratory and numerous other diseases. It also has a high antioxidant capacity, sedative, hypnotic, diuretic, antioxidant, anesthetic, antispasmodic, anticancer, antibacterial, bronchodilator, vasorelaxant activities. Availability, affordability, and medicinal importance draws the attention for investigation of various bioactive substances.

Key Words: *apigenin, Asteraceae, flavonoids, phenolic compounds, quercetin, gluconic acid*

INTRODUCTION

The discovery of the plant containing biological active compounds play an indispensable role in healthcare. World Health Organization declares that, 60% of the world's population relies on herbal remedies and around 80% of the population in developing countries depends almost entirely on it for their fundamental health care needs. In the treatment of chronic and acute maladies phytocompounds and also their chemical analogs have provided plenty of clinically useful medicines [Ahmad, Ahmad, 2019]. Asteraceae family is well known for its number of medicinal plants. Phytochemical components like essential oils, lignans, saponins, polyphenolic compounds, phenolic acids, sterols and polysaccharides that are found in many species of Asteraceae show various pharmacological activities [Rolnik, Olas, 2021]. The economic importance of the Asteraceae family has been described and, for centuries, several species of this family have been used for medicinal and food purposes [Sülsen et al., 2017]. This family is known to include species that are rich in flavonoids. Over the last decades, various species from this family were studied ed because of the great range and quantity of bioactive compounds they synthesize. Among them, flavonoids and terpenoids draw the attention because of their biological activities alongside with potential health benefits [Kim et al., 2007]. Appearing in various plant parts both in free state and as glycosides, flavonoids are the biggest group of naturally occurring phenolic compounds. Plenty of biological activities are found to have including antiangiogenic, antimicrobial, mitochondrial adhesion inhibition, antiulcer, antiarthritic, protein kinase inhibition, anticancer etc. [Sulaiman, Balachandran, 2012]. Being a necessary ingredient in a variety of pharmaceutical, medicinal, nutraceutical and cosmetic applications they are associated with a wide range of health-promoting effects. Now there are around 6000 flavonoids and they contribute to the pigments of fruits, vegetables and medicinal plants. [Panche et al., 2016]. In terms of the glycoside form of flavonoids the most encountering ones are D-glucose, D-galactose, D-xylose, L-rhamnose, L-arabinose, less often D-glucuronic acid, very rare D-fructose. Flavonoids are often form chelated compounds with metals, such as Al, Cu, Mo, Fe, Mg, lignins, polysaccharides and etc. More than 5000 plant species with flavonoid substances are investigated in Azerbaijan, Georgia, Russia, Ukraine and middle Asian countries in XX century. Flavonoids

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have been discovered in more than 100 plant families. Among them Combretaceae, Fabaceae, Berberidaceae, Rosaceae, Rubiaceae, Veronicaceae, Hypericaceae, Elaeagnaceae and Asteraceae are most abundant with flavonoids have been studied. Flavonoids of 19 species of Asteraceae have been determined by E.N. Novruzov [Novruzov, 2010].

According to the literature the separation of the methanolic extract of the aerial parts of *Lactuca indica* L. resulted in the isolation of five flavonoids. These are: quercetin 3-O-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside; uercetin 5-O-β-D-glucopyranoside; luteolin 7-O-β-D-glucuronide; 5,2'-dihydroxy-7-O-β-D-glucuronylflavone; 5,2'-dihydroxy-7-O-β-D-glucuronylflavone; quercetin 3-O-β-D-glucopyranoside [Kim et al., 2007].

Flavonoid components were isolated from the leaves of the *Lactuca sativa* L. the following: quercetin 7-glucoside 3-(6″-malonylglucoside), quercetin 3-glucuronide, quercetin 3-glucoside, quercetin 3-malonylglucoside, and as an anthocyanin cyanidin 3-malonylglucoside [Ferreres et al., 1997].

From *Lactuca dregeana* DC. flavonoid component luteolin-7-O-β-glucopyranoside was extracted from the roots and aerial parts, while quercetin-3-O-β-glucopyranoside was extracted only from the aerial parts [Michalska et al., 2015]. With regards to the phenolic compounds O-caffeoylmalic acid, di-O-caffeoyltartaric acid, O-caffeoyltartaric acid, caftaric acid, 5-O-caffeoylquinic acid and 3,5-di-O-caffeoylquinic acid were identified from *Lactuca sativa* L. varieties [Llorach et al., 2008].

Taking into consideration of the richness of chemical substances and their medicinal significance, investigation of *L. serriola* in Azerbaijani flora draws attention. Determination of chemical compounds from the aerial parts of *L. serriola* is the main purpose of this research.

MATERIAL AND METHODS

Plant material. Aerial parts - leaves and stems of the *Lactuca serriola* L. are collected during the blossoming period, from Novkhany village of Absheron district (40°34’27.9”N; 49°46’31.8”E); 20m below sea level. They have been chopped into small pieces then dried at room temperature. Total weight of the raw material ready for analyses was 320 g.

Extraction. Acetone used as an solvent. Dried raw material (320 g) is extracted three times with acetone, once in every three days [Serkerov, Kasumova, 2019]. Acetone is distilled off and expelled by rotary evaporator (evaporator model: ROVA-N2L; model of water bath: WB-2000). Liquid chromatography-mass spectrometry (LC-MS) method followed up for the component analysis of the dried extract.

LC-MS/MS Analysis of the Extract: LC-MS/MS analysis has been performed using an Absciex 3200 Q-Trap MS/MS detector. Experiments were carried out with a Shimadzu 20A HPLC system coupled to an Applied Biosystems 3200 Q-Trap LC-MS/MS instrument equipped with an ESI source operating in negative ion mode. On account of chromatographic separation, a GL Science Intersil ODS 250 × 4.6 mm, i.d., 5 μm particle size, octadecyl silica gel analytical column operating at 40ºC has been used. The solvent flow rate was maintained at 0.5 mL/min. Detection was carried out with PDA detector.

The elution gradient consisted of mobile phases (A) acetonitrile:water:formic acid (10:89:1, v/v/v) and (B) acetonitrile:water:formic acid (89:10:1, v/v/v). The composition of B was increased from 10% to 100% in 40 min.

Data were collected and processed by Analyst 1.6 software [LC-ESI-MS/MS: https://sciex.com/products/software/analyst-software].

RESULTS AND DISCUSSION

Total dried extract mass ready to analyse was 11.44 g, therefore extraction ratio was 3.57 %. LC-MS method is performed for the identification of the qualitative composition of the components acquired from the aerial parts of the *L. serriola*. species. Totally, 20 compounds obtained and 11 were identified (Fig., Tab.). As indicated in the table, the main characteristic substances of the studied species consist of phenolic compounds and flavonoids which are their subcategory. Identified flavonoids are: apigenin pentoside, luteolin glucoside, quercetin glucoside, apigenin glucoside, 300 trimethoxy flovonoid hexoside and quercetin. Amongst phenolic compounds 5-caffeoylquinic acid, caffeic acid and 3,5-Dicaffeoylquinic identified. Gluconic acid and glucose + hexoside are the next identified ones that belong to carboxylic acids and monosaccharides respectively. Previously the availability of flavonoids and total flavonoid content was determined from the methanolic extract of the aerial parts of *L. serriola.* [Bouimeja et al., 2019]. Apigenin, luteolin and isoquercitrin were isolated from the methanolic extract of aerial parts of *Lactuca indica* L. The first two flovonoids are alike with our findings. Apigenin and luteolin showed α-glucosidase

Table. Summary of the components identified by LC-MS/MS method from extract of the aerial parts of *Lactuca serriola.*

Peak	RT, min	M-H	Molecular ion fragments	Identified
1	3.5	195	177, 159, 129	Gluconic acid
2	5.99	198	163	glucose + hexoside
3	8.392	235	189, 167	Unknown
4	11.744	485	439, 421, 215, 197	Unknown
5	13.216	353	191, 179, 161, 135	5-caffeoylquinic acid
6	14.031	277	226, 215, 197, 185, 171	Unknown
7	14.429	401	269, 161, 143	apigenin pentoside
8	15.239	179	135, 117, 109, 107	caffeic acid
9	16.047	277	215, 203, 185, 149, 133, 121	Unknown
10	16.556	471	425, 225, 179, 113	Unknown
11	21.01	515	353, 335, 191, 179, 173, 161, 135	3,5-Dicaffeoylquinic acid
12	21.648	357	-	358 MW compound (not fragmented)
13	22.181	447	285, 284, 256, 151, 133	Luteolin glucoside
14	23.014	463	300, 271, 255, 243, 179, 151	Quercetin glucoside
15	24.594	431	268, 161, 135, 133	Apigenin glucoside
16	25.085	461	446, 297, 283, 269, 255	300 MW trimethoxy flovonoid hexoside
17	25.980	543	409, 313, 175, 151, 133, 107	Unknown
18	26.849	411	277, 233, 215, 199, 151, 107	Unknown
19	27.407	257	242, 213, 198, 185, 169, 161	Unknown
20	29.005	301	227, 183, 163, 151, 121, 107	Quercetin

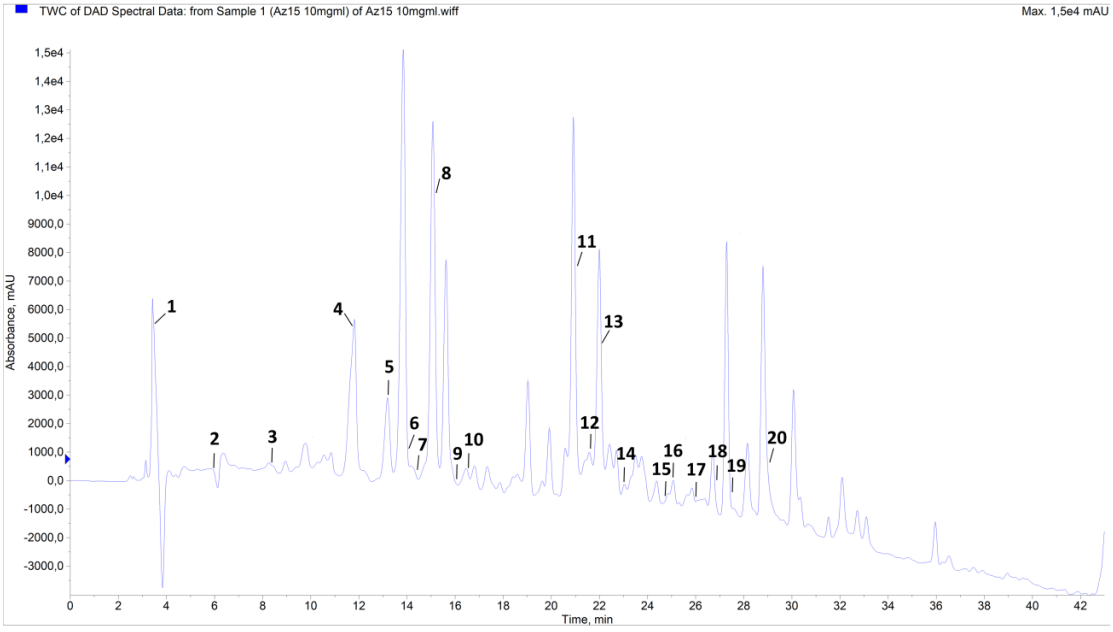

Figure. LC-MS chromotogram of acetonic extract of aerial parts of *Lactuca serriola* L. Substances are listed in the table above.

inhibition activities and this put forth their anti-diabetic activity [Choi et al., 2016].

Also from the fresh herb of *L. indica* was extracted with aqueous acetone and six known flavonoids, quercetin, quercetin 3-O-glucoside, rutin, apigenin, luteolin, and luteolin 7-O-glucuronide were identified [Hou et al., 2003]. With regards to the phenolic compounds 5-Caffeoylquinic acid, 3,5-Dicaffeoylquinic

acid were also extracted from *L. sativa* via UHPLC method [Ribas-Agustí et al., 2011].

Among flavonoids, the flavone quercetin is currently assessed in clinical trials on prostate cancer and its primary prevention [Sülsen et al., 2017]. Another identified compound is a glucose that has been detected in the ethanolic extracts from the roots of *L. serriola* [Petkova, Denev, 2013].

The next compound gluconic acid is an acid sugar belonging to the aldonic acid family. There is a huge interest for the gluconic acid and its derivatives in pharmaceutical, food, textile and construction industries over the past 50 years. Gluconic acid and its derivatives are generally added to dairy products and soft drinks to maintain and boost their sensory properties. The US Food and Drug Administration granted Generally Recognized As Safe status to the GA derivatives, glucono-δ-lactone, sodium gluconate, and authorized their unlimited use as food additive [Cañete-Rodríguez et al., 2016].

In the European Parliament and Council Directive No. 95/2/EC, gluconic acid is listed as a generally permitted food additive (E 574) [EPCD, 2006].

Quercetin has different inhibitory effects on various ways of tumor formation. A plenty of *in vivo* and *in vitro* experiments have shown that it has a strong role in inhibiting metastasis, promoting apoptosis, and its ability for tumor angiogenesis and cell cycle regulation [Tang et al., 2020]. Luteolin has a reducing effect the oxidative stress and neuroinflammation and also prevents apoptosis and enhanced neuronal growth by modulating signaling pathways [Siddique, 2021].

Taking into consideration *L. serriola* is a potential source of natural antioxidants and their usage in pharmaceutical and health functional foods, we recommend further investigations.

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Lactuca serriola L. növü yerüstü hissələrinin maye xromato-mass spektrometriya üsulu ilə kimyəvi tərkibinin tədqiqi

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Tədqiqat işi *Lactuca serriola* L. növünün yerüstü hissələri-nin (gövdə və yarpaqlar) kimyəvi tərkibinin tədqiqinə həsr edilmişdir. Bitkinin (320 q) yerüstü hissələrinin fitokimyəvi tədqiqi zamanı flavonoid törəmələrin mövcudluğu aşkar edilmişdir. Bitki xammalının asetonlu ekstraksiyasında ümumi ekstraktiv tərkib maye xromatoqrafiya-kütlə spektrometrik (LC-MS) üsulu ilə tədqiq edilərək orada flavonoid törəmələrinin olması müəyyən edilmişdir. Ümumilikdə 20 birləşmə əldə edilmiş və bunlardan 11-i identifikasiya edilmişdir. Onların karboksil turşusu törəmələri, monosaxaridlər, fenol birləşmələri və flavonoidlərə aid olduğu müəyyən edilmişdir. LC-MS prosesi isə Shimadzu 20 A HPLC sistemi ilə həyata keçirilib və maddələrin identifikasiyası Analyst 1.6 proqramı ilə yerinə yetirilmişdir. Qlükon turşusu, qlükoza, kversetin identifikasiya edilən birləşmələr arasındadır. Qlükon turşusu əczaçılıq sənayesində istifadə olunur və FDA (Amerikan Qida və Dərman Administrasiyası) və AB tərəfindən qida əlavəsi kimi istifadə olunmaq üçün təhlükəsiz hesab edilmişdir. *Lactuca serriola* ənənəvi xalq təbabəti mədə-bağırsaq, tənəffüs yolları və bir çox digər xəstəliklərin müalicəsi üçün istifadə edilmişdir. Onun həmçinin yüksək antioksidant xassəsi, sakitləşdirici, hipnotik, sidikqovucu, anestezik, antispazmodik, xərçəng əleyhinə, antibakterial, bronxodilatator, damargenəldici xüsusiyyətləri vardır. Mövcudluğu, münasibliyi və tibbi əhəmiyyəti müxtəlif bioaktiv maddələrin tədqiqi baxımından diqqəti cəlb edir.

Açar sözlər: *apigenin, Asteraceae, flavonoidlər, fenol birləşmələri, kversetin, qlükon turşusu*

Исследование химического состава надземных частей *Lactuca serriola* L. с жидкой хромато - масс спектрометрическим методом

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Научная работа посвящена изучению химического состава подземных частей (стеблей и листьев) *Lactuca serriola* L. В результате фитохимического исследования подземных частей *Lactuca serriola* L. (320 г) выявлено наличие производных флавоноидов. При ацетоновой экстракции растительного сырья общее содержание экстракта изучали методом жидкостной хроматографии-масс-спектрометрии (ЖХ-МС) и определяли присутствие производных флавоноидов. Всего получено 20 соединений, 11 которые идентифицированы. Как производные карбоновых кислот, моносахаридов, фенольных соединений и флавоноидов. Процесс ЖХ-МС был реализован с помощью системы ВЭ-ЖХ Shimadzu 20 A, а идентификация предметов выполняласьс помощью программы Analyst 1.6. Глюконовая кислота, глюкоза и кверцетин входят в число идентифицированных соединений. Глюконовая кислота используется

в фармацевтической промышленности и считается безопасной FDA (Американское управление по санитарному надзору за качеством пищевых продуктов и медикаментов) и ЕС для использования в качестве пищевой добавки. *Lactuca serriola* используется в традиционной народной медицине для лечения желудочно-кишечного тракта, дыхательных путей и многих других заболеваний. Вид также обладает высокими антиоксидантными, седативными, снотворными, мочегонными, обезболивающими, спазмолитическими, противораковыми, антибактериальными, бронхолитическими а сосудорасширяющими свойствами. Доступность, пригодность и медицинское значение вызывают интерес с точки зрения исследованияразличных биологически активных веществ полученных из растительного сырья.

Ключевые слова: *апигенин, Asteraceae, флавоноиды, фенольные соединения, кверцетин, глюконовая кислота*