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Eur. Pharm. J. 2022, 69(1), 16-26.
ISSN 1338-6786 (online) and ISSN 2453-6725 (print version),
DOI: 10.2478/afpuc-2022-0002

EUROPEAN PHARMACEUTICAL JOURNAL

A Study of Phenolic Bioactive Compounds Of Daucus Carota Subsp. Sativus Fruits of Yaskrava, Nantska Kharkivska and Olenka Species and Of Dauci Carotae Subsp. Sativi Fructuum Extractum Siccum

Original Paper

Kyslychenko V. [1], Protska V[1]✉, Horiacha L[1], Liaudanskas M[2], Zvikas V[2], Trumbeckaite S[2], Ivanauskas L[2], Jakstas V[2], Kyslychenko O[1], Zhuravel I[1], Andriukova L[1], Filyanina N[1]

[1]National University of Pharmacy
[2]Lithuanian University of Health Sciences

Received 31 October, 2021, accepted 8 March, 2022

Abstract The objective of the work was to study the qualitative composition and determine the quantitative content of phenolic compounds of *Daucus carota* subsp. *sativus* fruits of Yaskrava, Nantska Kharkivska and Olenka species and of *Dauci carotae* subsp. *sativi* fructuum extractum siccum. Phenolic compounds were studied by UPLC-ESI-MS/MS method. High content of phenol-origin bioactive substances was fixed in *Daucus carota* subsp. *sativus fruits* of Nantska Kharkivska variety (363.19 μg/g). Dominating compounds were chlorogenic acid, cynaroside, rutin and hyperoside. *Daucus carota* subsp. *sativus fruits* of all study species under question were found to possess permanent qualitative composition of phenolic compounds, whereas their quantitative content in experimental samples differed slightly. The results of analysis confirm promising development of novel antioxidant and cardioprotective drugs on the basis of *Dauci carotae* subsp. *sativi* fructuum extractum siccum.

Keywords *Daucus carota subsp. Sativus – UPLC-ESI-MS/MS – phenolic compounds – antioxidant activity*

INTRODUCTION

As known from The European Heart Network, cardiovascular mortality rate in Europe makes 3.9 million persons annually in Europe, 17.6 million in the USA and 400,000 in Ukraine, which corresponds to about 45%, 30% and circa 55% of all fixed lethal cases, respectively (Balea et al., 2018; Benjamin et al., 2017; Heart disease and stroke statistics-2019 at-a-glance, 2019; Qu et al., 2016; State Register of Medicines of Ukraine, 2018; Townsend et al., 2016). Dynamics of the last 25 years specified rapid growth of cardiovascular diseases among population of European countries, including Ukraine. As per statistics, about 64 million people in Europe and more than 92.1 million Americans were handicapped due to diseases of heart and vascular system (Benjamin et al., 2017; Heart disease and stroke statistics-2019 at-a-glance, 2019; State Register of Medicines of Ukraine, 2018). With due consideration of growing morbidity and prevalence of cardiovascular pathologies, we may conclude that this problem developed from purely medical to medical social level (Balea et al., 2018; Benjamin et al., 2017; Qu et al., 2016; Ravichandra et al., 2014; State Register of Medicines of Ukraine, 2018; Yuanyuan et al., 2017).

The role of oxidative stress in the development of cardiovascular diseases

Numerous studies prove that oxidative stress leads to vessel endothelium fracture and favours development of atherosclerosis, stroke, neurovascular diseases and total vascular dysfunction. Moreover, free radicals provoke cardiac fibroblast proliferation and activate matrix enzymes, leading to cardiac insufficiency development. Free-radical processes destabilize lipoproteins of cardiac muscle cell membranes and limit nitrogen oxide bioavailability, causing ischaemia and eventual lethality (Balea et al., 2018; Halliwell, 2000; Heinecke, 2006; Khurana et al., 2013; Qu et al., 2016; Ravichandra et al., 2014; Suridjan et al., 2017).

Antioxidant properties of phenolic compounds

Phenolic compounds can inhibit oxidative chain reactions in several ways, including direct quenching of reactive oxygen species, inhibition of enzymes and chelation of metal ions

* *E-mail: vvprotskaya@gmail.com*

(Fe^{2+}, Cu^{+}) (Bendary E. et al., 2013). The structure of phenolic compounds is a key determinant of their ability to neutralize free radicals and the formation of chelate complexes with metals (Bendary E. et al., 2013).

One of the protective mechanisms of phenolic compounds works by inhibiting the formation of free radicals, intercepting radical chain reactions, converting existing free radicals into less harmful molecules and repairing oxygen damage of lipid, thus decreasing the formation of volatile decomposition products (e.g. aldehydes, ketones, alcohols and epoxides) (Kumar S. et al., 2014; Thiviya P., et al., 2021). The beneficial effect of phenolic compounds is due to the fact that they donate electrons or hydrogen atoms, absorb free radicals and have reducing power (Zhao H.-X. et al., 2014). It is proved that the activity of these bioactive compounds directly depends on the number of free hydroxyl groups in the chemical structure (Balea et al., 2018; Agunloye et al., 2019; Inbathamizh & Padmini, 2013; Khurana et al., 2013; Qu et al., 2016; Raza et al., 2017), and the aromatic structure of the benzene nucleus is responsible for the stabilization of molecules when interacting with free radicals (Zeb A., 2020). It should be noted that the dissociation energy of hydrogen atoms of hydroxyl groups also affects their reactivity, because the smaller it is, the easier the compound dissociates and reacts faster (Zeb A., 2020).

In the case where a polyphenolic compound neutralizes free radicals by transferring hydrogen ions or electrons, it itself is converted into a radical compound. Stabilization of the formed anion or cation is due to the creation of intramolecular hydrogen bonds with vicinal hydroxyl groups of the aromatic nucleus. Phenolic antioxidants can donate hydrogen atoms to lipid radicals and produce lipid derivatives and antioxidant radicals, which are more stable and less readily available to promote autoxidation (Thiviya P., et al., 2021).

Chelation of variable valence metal ions and free radicals is another important mechanism of antioxidant action of phenolic compounds and depends on their reducing potential (Zeb A., 2020). Phenolic compounds can form complexes with transition metals, such as iron, zinc and copper, and directly inhibit the reduction of the latter, thereby reducing the formation of reactive free radicals in the Fenton reaction (Zeb A., 2020; Perron & Brumaghim, 2009).

On a par with this, as stated in literature, phenolic compounds have versatile therapeutic effects, such as anti-inflammatory, hypotensive, anti-arrhythmic, cardiotonic, antispasmodic, diuretic, capillary restorative and cardiac protective effect (Balea et al., 2018; Agunloye et al., 2019; Inbathamizh & Padmini, 2013; Khurana et al., 2013; Qu et al., 2016; Raza et al., 2017). Quinic acid is a necessary substance for humans because it is a precursor to many biologically active substances, such as hydroxycinnamic acids and amino acids. This compound is characterized as a metabolite of the antioxidants tryptophan and nicotinamide in the gastrointestinal tract, which leads to the induction of DNA repair and inhibition of nuclear factor Kappa B (Pero R. W. et al., 2009). Histopathological studies of the hippocampus and cortex have shown that quinic acid markedly reduces the toxicity of aluminium chloride and preserves the normal architecture of the hippocampus and cerebral cortex. Moreover, quinic acid restores memory deficiency caused by aluminium chloride, increases the activity of antioxidant enzymes (glutathione, catalase and superoxide dismutase) and inhibits the therapeutic target for dementia – the enzyme monoamine oxidase (Liu L. et al., 2020). It is also reported that quinic acid has anti-inflammatory, antitumor, cardio- and hepatoprotective properties (Inbathamizh & Padmini, 2013; Liu L. et al., 2020).

Researchers from Nigeria found that oral daily dosage of 10–15 mg/kg chlorogenic acid to rats with cyclosporine-induced hypertension did normalize arterial pressure, improve nitrogen oxide bioavailability and suppressed activity of key enzymes affecting hypertension development, in particular, angiotensine-1-transforming enzyme, acetylcholine esterase, arginase, etc. (Agunloye et al., 2019). Chinese scientists proved antioxidant, anti-inflammatory and antitumor properties of flavonoid luteolin and its glycosides. In their *in vivo* experiments, these compounds stimulated heart contractility, slowed cardiomyocyte apoptosis, prevent cardiac muscle fibrosis and reduce risks of myocardial infarction (Yuanyuan et al., 2017). Therapeutic dosage of rutin to experimental rats favours total cholesterol and low-density lipoprotein level reduction and inhibits blood plasma activity of serum enzymes – alanine transaminase and aspartate transaminase, which are treated as cardiopathy markers. Besides, this compound stimulates nitrogen oxide production in endothelium cells and favours vascular reactivity restoration under adrenaline hypertension in rats. In *in vitro* experiments, rutin shows anti-aggregant properties by inhibiting platelet activation factors (Ganeshpurkar & Ajay, 2017).

A group of researchers from Iran, Italy and Portugal resuscitated that flavonoid apigenin and its glycoside vitexin exert heart protecting, hypolipidemic, anti-inflammatory, diuretic and hypotensive action; improve blood circulation; reduce cardiac muscle necrosis area and retard cardiomyocyte apoptosis (Salehi et al., 2019; Shahzad et al., 2015). Galangin ability to protect heart and inhibit oxidation was confirmed by Indian scientists on doxorubicin model of rat cardiomyopathy (Ravichandra et al., 2014).

Chinese researchers during rat experiments found that astragalin reduces activity of lactate dehydrogenase, creatine kinase enzymes and intracellular active forms of oxygen; retards heart cell apoptosis; induces superoxide dismutase and glutathione and opposes inflammation [Qu et al., 2016]. In another experiment, scientists resuscitated that hyperoside doses of 25 mg/kg and 50 mg/kg prevent ruin of heart cells, counteract oxidative stress effect on cardiac muscle, oppose coagulation and alleviate vascular endothelium inflammatory process (Raza et al., 2017).

Daucus carota in the treatment of cardiovascular disease

As follows from numerous foreign studies, as well as works performed at M. H. Kholodnyi Institute of Botany, National Academy of Science of Ukraine, Kyiv, Ukraine, *Daucus carota* subsp. *sativus* L. is a cultivated subspecies of *Daucus carota L.* (Arbizu et al., 2014; Kyslychenko et al., 2019; Mezghani et al., 2017).

Folk medicine uses *Daucus carota* subsp. *sativus* fruits as antiseptic, carminative, spasmolytic, anthelmintic, tonic and diuretic for treatment of dysentery, diarrhoea, malaria, cough, renal diseases and dropsy (Al-Snafi, 2017; Pavlyuk et al., 2015; Shakheel et al., 2017).

Judging from literature, doses of 10 mg/kg to 100 mg/kg of *Daucus carota* ethanol extract reduced systolic and diastolic arterial pressure. In guinea pigs, extracts of *Daucus carota* caused dose-dependent reduction of heart contraction frequency and force, exerting vasodilating effect. Iraqi scientists stated that coumarin glycosides extracted from above-ground parts of carrots in *in vivo* experiments reduce arterial pressure due to blockage of calcium channels (Al-Snafi, 2017). Indian researchers found that ethanol extracts and essential oil of *Daucus carota* fruits affect cardiac muscle contraction and restore cardiomyocyte membranes in a rat isoproterenol myocardial infarction model (Pavlyuk et al., 2015; Shakheel et al., 2017). Dosages of 250 mg/kg and 500 mg/kg aqueous extracts of *Daucus carota* exert inotropic effect and reduce levels of serum enzymes – aspartate transaminase, alanine transaminase, peroxidase and lactate dehydrogenase – which are markers of pathological processes in heart. *Daucus carota* addition to animal rations caused changes in blood lipid profile and reduced plasma levels of oxidative stress and inflammatory process markers (Al-Snafi, 2017; Pavlyuk et al., 2015; Shakheel et al., 2017).

As per today, *Martindale: The Complete Drug Reference* specified 10 preparations exist in the world in the form of oral drops, tablets and capsules, including bioactive substances of *Daucus carota*. They are produced in Argentina (Hepatalgina [Altana Pharma SA], Metiogen [Química MedicalArg. SACI] and Palatrobil [Monserrat y Eclair SA]), Chile (Natursel-C and Natur-Zin [Laboratorio Ximena Polanco]), United Kingdom (Sciargo and Watershed [Potter's, Herbal Supplies Ltd]), France (Aroma-detox [Omega Pharma]), Italy (Evamilk [Laboratori Gambar Srl]) and Russia (Urokholesan [Vifitekh]) (Pavlyuk et al., 2015; Sweetman, 2011). These drugs are prescribed as dietary supplements in treatment of radiculitis, lumbago, digestive diseases, for lactation enhancement, detoxification and as antioxidants (Pavlyuk et al., 2015)

Ukrainian State Register of Drugs specified several domestic-produced preparations on *Daucus carota* fruits basis, in particular, 'Urolesan' and 'Holelesan' (Kyivmedpreparat), 'Uroholum' (DKP Pharmaceutical Factory), 'Wild carrot fruit and mangold thick extract' and 'Urolesan thick extract' (Halychfarm) which exert choleretic, diuretic and antiseptic action (Sweetman, 2011). Nevertheless, none *Daucus carota* subsp. *sativus* for prophylaxis and treatment of cardiovascular system exist at the market.

The objective of work was to study the qualitative composition and determine the quantitative content of phenolic compounds of *Daucus carota* subsp. *sativus* fruits of Yaskrava, Nantska Kharkivska and Olenka species and of *Dauci carotae* subsp. *sativi* fructuum extractum siccum.

MATERIALS AND METHODS

Plant material and preparing of extractum siccum

In experimental studies, we used air dried crushed *Daucus carota* subsp. *sativus* fruits of Yaskrava, Nantska Kharkivska and Olenka species, which were harvested at experimental farm of Vegetable and Melon Growing Research Institute, Ukrainian Academy of Agrarian Science at Merefa, Kharkiv Region, Ukraine, in 2016–2018. These varieties are most popular and widely cultivated in Ukraine, and they are entered into The State Register of Plant Cultivars (or Varieties) Suitable for Dissemination in Ukraine.

In the course of laboratory studies, we established optimal terms to obtain *Dauci carotae* subsp. *sativi* fructuum extractum siccum with maximum content of bioactive substances. Extract was obtained from a mixture of Yaskrava, Nantska Kharkivska and Olenka species, as previous studies of the chemical composition of *Daucus carota subsp. sativus* of these species confirmed a slight difference in their qualitative composition and quantitative content of biologically active substances. *Dauci carotae* subsp. *sativi* fructuum extractum siccum was produced by threefold 50% ethanol extraction at solute: solvent relation of 1:5 at the temperature of about 60°C after lipophilic fraction having been totally removed with petroleum ether. Yield of final product is about 15%.

UPLC analysis

Qualitative composition and quantitative content of phenolic compounds in *Daucus carota* subsp. *sativus* fruits of Yaskrava, Nantska Kharkivska and Olenka species and of *Dauci carotae* subsp. *sativi* fructuum extractum siccum were determined by an ultra-performance liquid chromatography-electrospray tandem mass spectrometry (UPLC-ESI-MS/MS) method (González-Burgos et al., 2018; Marksa et al., 2016).

Sample for analyses was prepared by extraction with 10 ml 50% ethanol of 0.30 g powdered for *Daucus carota* subsp. *sativus* fruits of Yaskrava, Nantska Kharkivska and Olenka species and of 0.10 g for *Dauci carotae* subsp. *sativi* fructuum extractum siccum within 40 min using ultrasonic bath at room temperature (20±2°C). The obtained solution was filtered through a membrane filter (0.45 μm) before usage (Marksa et al., 2016).

Phenolic compounds in *Daucus carota* subsp. *sativus* fruits of Yaskrava, Nantska Kharkivska and Olenka species and of

Dauci carotae subsp. *sativi* fructuum extractum siccum were studied using Acquity H-class UPLC system (Waters, USA), which was equipped with a triple quadrupole tandem mass spectrophotometer (Xevo, Waters, USA) (González-Burgos et al., 2018; Marksa et al., 2016).

Phenolic-type compounds were separated at YMC Triart C18 column from YMC Europe GmbH Dinslaken, Germany, which was 100 mm long and 2 mm in diameter. Granularity was 1.9 µm. In the course of experiment, column temperature was maintained constant at 40˚C (González-Burgos et al., 2018; Marksa et al., 2016). As mobile phase for gradient elution, a binary mixture was used of 0.1% formic acid aqueous solution (component A) and acetonitrile (component B) with initial relation 19:1. Mobile phase was introduced into system at the rate of 0.5 ml/min (González-Burgos et al., 2018; Marksa et al., 2016).

Linear gradient profile for solvent A had the form:

0.1% formic acid aqueous solution in acetonitrile, %	Time, min
95	1
70	4
50	7
95	2

In experiments, we used an electric sputtering ionization source (ESI) to obtain MS/MS data. Instrument capillary voltage was 2 kV. Temperature was within the limits of 150°C; desolvation temperature was below 400°C. Desolvation gas flow rate was 700 lph and cone gas 20 lph. Cone collision and voltage energy were optimized particularly for each compound. The content of phenolic compounds was calculated by comparing peak areas and retention times of standards. The results were presented as µg/g of the dry weight of the sample (González-Burgos et al., 2018; Marksa et al., 2016).

Statistical analysis

Statistical analysis was performed using Statistica 10 software (StatSoft Inc., Tulusa, OK, USA). Data were expressed as the mean of five replicates ± standard error of the mean (SEM). Statistical significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

The results of our experiments showed the most versatile qualitative composition in *Daucus carota* subsp. *sativus* fruits of Yaskrava species – as many as 15 compounds were identified. We found 13 compounds in *Daucus carota* subsp. *sativus* fruits of Nantska Kharkivska species and 12 compounds in *Daucus carota* subsp. *sativus* fruits of Olenka species. In each of objects we found alicyclic quinic acid and 8 in each case flavonoid glycosides. In *Daucus carota* subsp. *sativus* fruits of Yaskrava species, four flavonoid aglycones were detected (apigenin, isorhamnetin, galangin and sinensetin), whereas in *Daucus carota* subsp. *sativus* fruits of Nantska Kharkivska and Olenka species, only two aglycones were detected (galangin and sinensetin). We identified two compounds that may be related to hydroxycinnamic acids (chlorogenic and rosmarinic acids) in *Daucus carota* subsp. *sativus* fruits of Yaskrava and Olenka species, whereas *Daucus carota* subsp. *sativus* fruits of Nantska Kharkivska species contained only chlorogenic acid.

Eleven compounds of phenolic nature were identified in *Dauci carotae* subsp. *sativi* fructuum extractum siccum. The alicyclic quinic acid, two hydroxycinnamic acids (caffeic and chlorogenic acids), flavonoid aglycone – apigenin, and seven compounds related to flavonoid glycosides, in particular, cynaroside, scolymoside, isorhamnetin-3-O-glucoside and tiliroside, were identified in the extract. It was noted that the *Dauci carotae* subsp. *sativi* fructuum extractum siccum contained compounds that were not found in any sample of *Daucus carota* subsp. *sativus* fruits of the studied species. In particular, such compounds are caffeic acid and tiliroside. The presence of these compounds in the extract can be explained by their probable low content in *Daucus carota* subsp. *sativus* fruits of Yaskrava, Olenka and Nantska Kharkivska species, which made it impossible to identify them in the raw material under these conditions. At the same time, no compounds were identified that were identified in *Daucus carota* subsp. *sativus* fruits of the studied species, including flavonoids such as rutin, phloridzin, galangin and sinensetin. This is due to the conditions of extraction in obtaining *Dauci carotae* subsp. *sativi* fructuum extractum siccum.

UPLC-ESI-MS/MS chromatograms of *Daucus carota* subsp. *sativus* fruits of Yaskrava, Olenka and Nantska Kharkivska species are shown in Figures 1–3. Figure 4 shows chromatogram of *Dauci carotae* subsp. *sativi* fructuum extractum siccum.

Total content of the identified compounds turned to be the highest in *Daucus carota* subsp. *sativus* fruits of Yaskrava species – 1447.94 ± 36.20 µg/g. *Daucus carota* subsp. *sativus* fruits of Nantska Kharkivska species contained them 1.4 times less and *Daucus carota* subsp. *sativus* fruits of Olenka species contained them 2.2 times less, namely 1043.39 ± 26.08 µg/g and 671.87 ± 16.80 µg/g, respectively. In quantitative content of identified compounds, 65%–80% related to quinic acid – 1166.43 ± 29.16 µg/g, 502.83 ±12.57 µg/g and 680.20 ± 17.05 µg/g for *Daucus carota* subsp. *sativus* fruits of Yaskrava, Olenka and Nantska Kharkivska species, respectively.

High content of hydroxycinnamic acids was observed in *Daucus carota* subsp. *sativus* fruits of Nantska Kharkivska species – 242.48 ± 6.06 µg/g, which corresponded to 67% of the sum of all phenolics and about 23% of the totality of identified compounds in this material. *Daucus carota* subsp. *sativus* fruits of Yaskrava variety had 1.8 times less of these

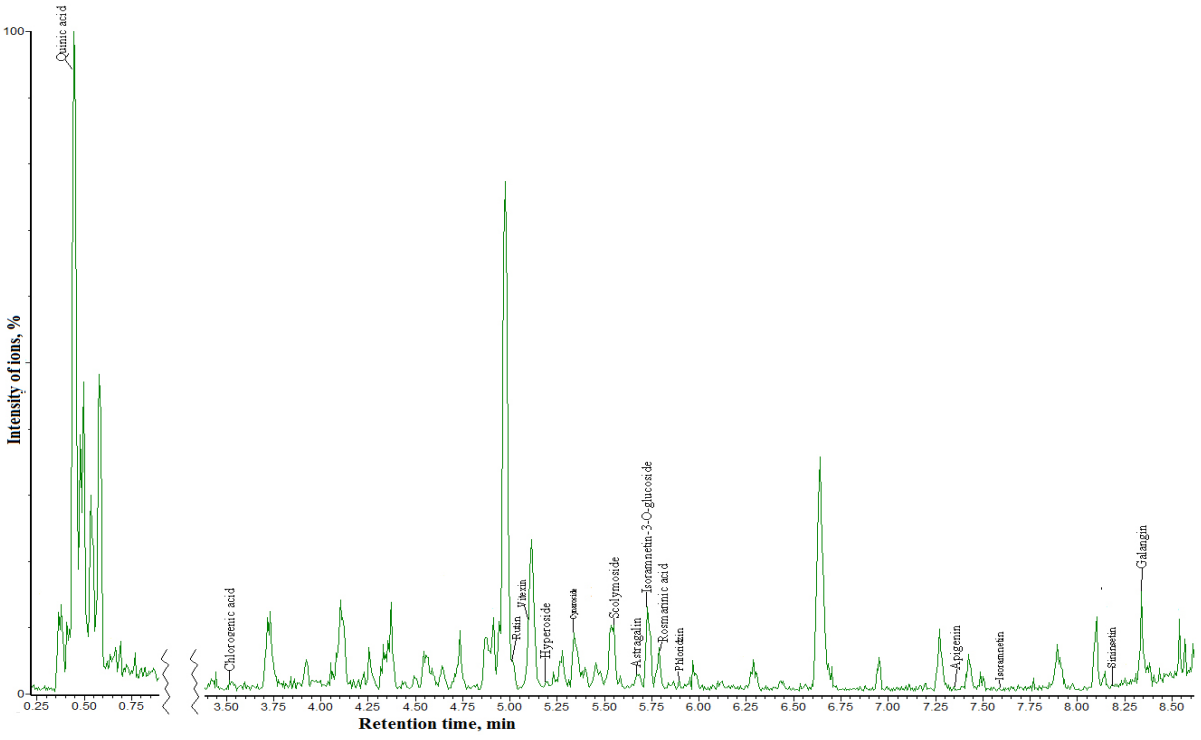

Figure 1. UPLC-ESI-MS/MS chromatogram of Daucus carota subsp. sativus fruits of Yaskrava species.

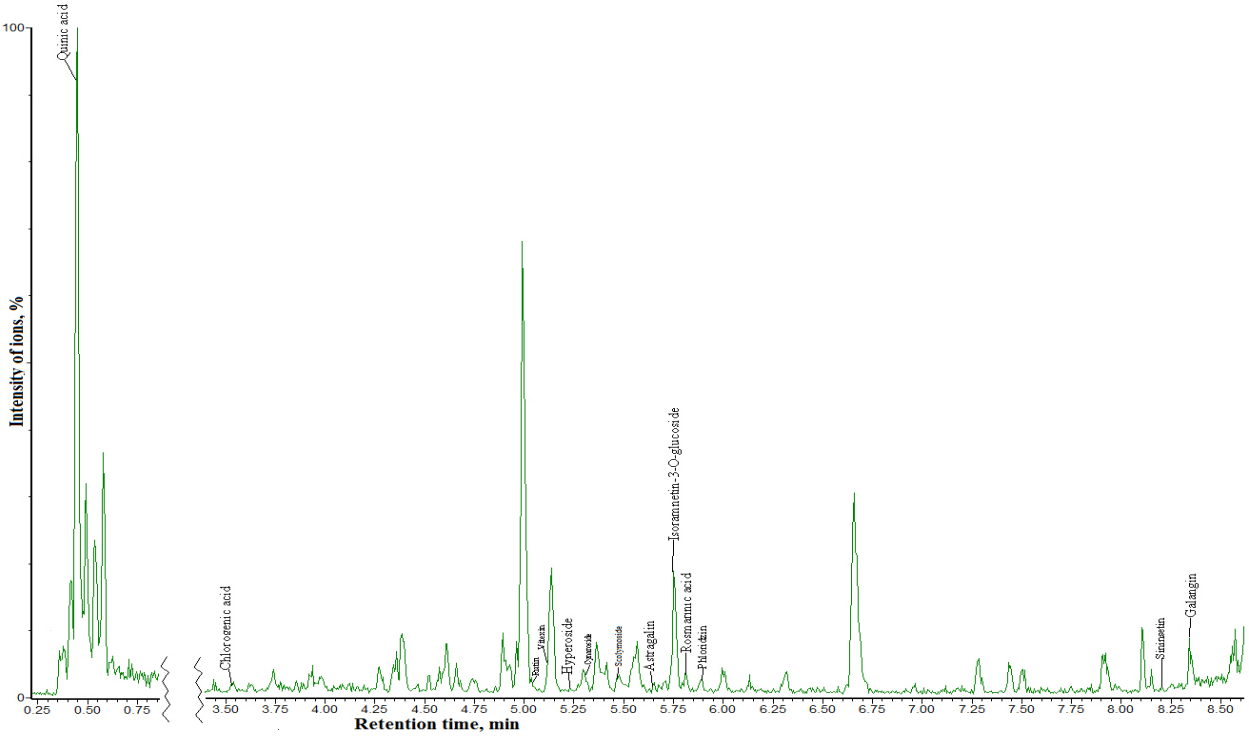

Figure 2. UPLC-ESI-MS/MS chromatogram of Daucus carota subsp. sativus fruits of Olenka species.

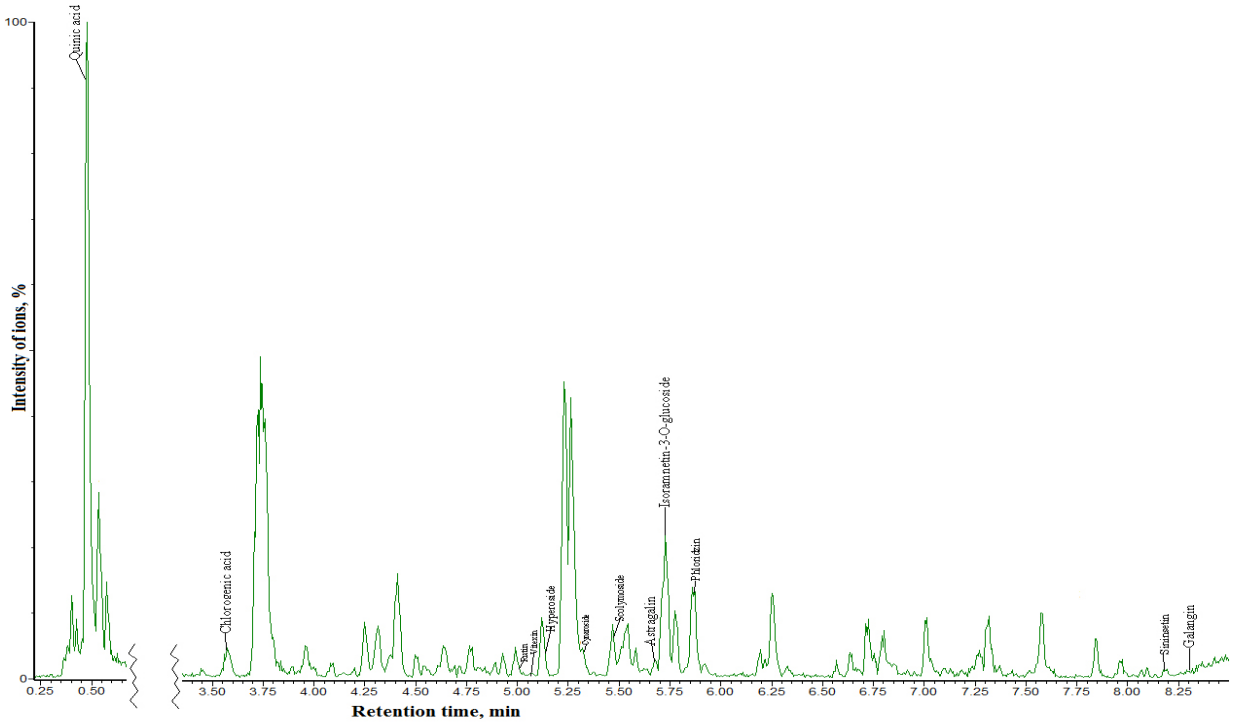

Figure 3. UPLC-ESI-MS/MS chromatogram of Daucus carota subsp. sativus fruits of Nantska Kharkivska species.

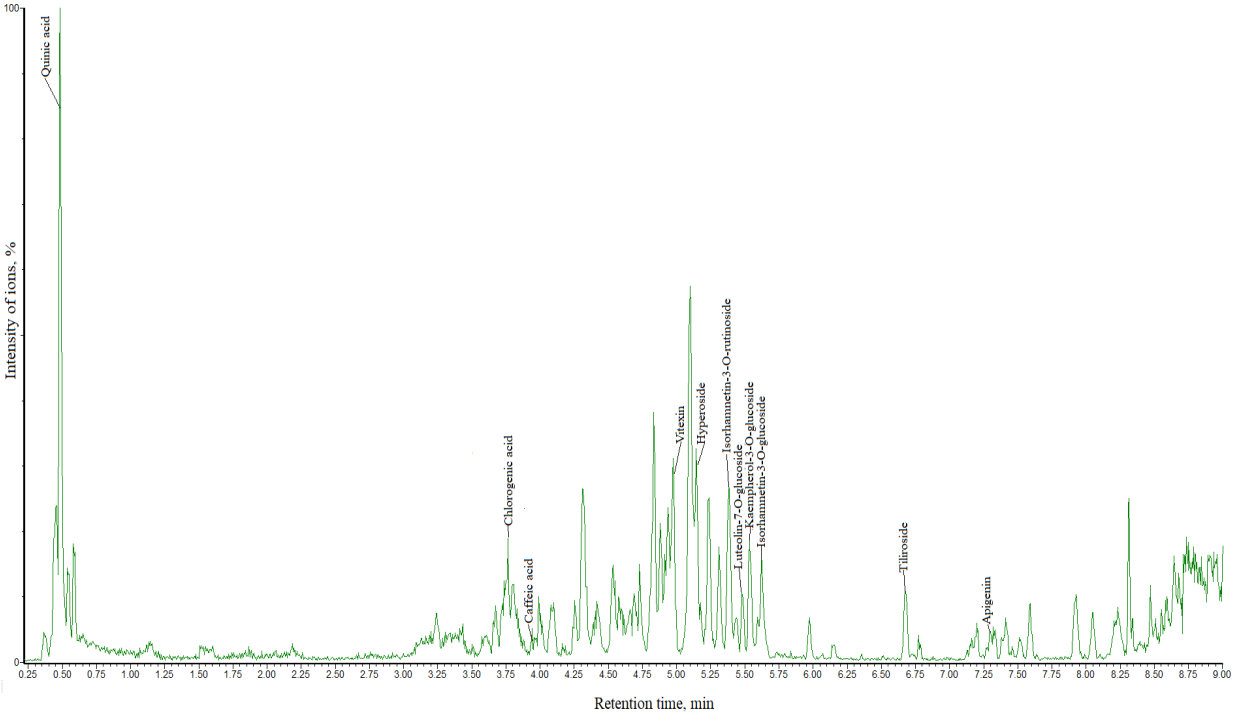

Figure 4. UPLC-ESI-MS/MS chromatogram of Dauci carotae subsp. sativi fructus extractum siccum.

compounds, 130.46 ± 3.26 μg/g, and *Daucus carota* subsp. *sativus* fruits of Olenka species had 6 times less, only 40.44 ± 1.01 μg/g. Share of hydroxycinnamic acids of *Daucus carota* subsp. *sativus* fruits of Yaskrava and Olenka species was 46% and 24%, respectively, of their total phenolic content.

Quantitative content of flavonoids differed insignificantly in all varieties of *Daucus carota* subsp. *sativus* under study. Their maximum content was specified in *Daucus carota* subsp. *sativus* fruits of Yaskrava (151.05 ± 3.78 μg/g). Flavonoids content turned to be almost identical in *Daucus carota* subsp. *sativus* fruits of Olenka and Nantska Kharkivska – 128.58 ± 3.21 μg/g and 120.71 ± 3.02 μg/g, respectively. However, *Daucus carota* subsp. *sativus* fruits of Yaskrava species shared more than 53% of the identified flavonoids from the total amount of phenolics. In *Daucus carota* subsp. *sativus* fruits of Nantska Kharkivska, flavonoids made one third of the totality of phenolics. Daucus carota subsp. sativus fruits of Olenka species accumulated flavonoids 3.8 times more than hydroxycinnamic acids, and they formed 76% of the totality of phenolics in this material.

Comparison of quantitative content of bioactive substances in *Daucus carota* subsp. *sativus* fruits and in *Dauci carotae* subsp. *sativi* fructuum extractum siccum leads to a conclusion that the total content of all identified compounds in this extract was 7446.88 ± 186.17 μg/g – five times more than in most rich in these compounds *Daucus carota* subsp. *sativus* fruits of Yaskrava species. Hydroxycinnamic acids made about 22% of total phenolic content in this extract (762.40 ± 19.06 μg/g), whereas the sum of flavonoids – more than 36% (2686.92 ± 67.17 μg/g), at least 18 times higher than in *Daucus carota* subsp. *sativus* fruits of all study varieties. Total content of phenolic compounds in *Dauci carotae* subsp. *sativi* fructuum extractum siccum was 3449.32 ± 86.23 μg/g – 9.5 times higher than in most rich in these compounds in *Daucus carota* subsp. *sativus* fruits of Nantska Kharkivska.

Qualitative composition and quantitative contents of phenolic compounds of *Daucus carota* subsp. *sativus* fruits of Yaskrava, Nantska Kharkivska and Olenka species and of *Dauci carotae* subsp. *sativi* fructuum extractum siccum are specified in Table 1.

Among all identified phenolic compounds, chlorogenic acid, cynaroside and hyperoside accumulated in largest amounts within all objects of study. On a par with us, substantial amount of rutin was observed in all samples of *Daucus carota* subsp. *sativus* fruits. But this compound was not recorded in *Dauci carotae* subsp. *sativi* fructus extractum siccum. It should be noted that the highest content of all identified compounds was observed in *Daucus carota* subsp. *sativus* fruits of Yaskrava species except chlorogenic acid whose highest content was determined in *Daucus carota* subsp. *sativus* fruits of Nantska Kharkivska.

Among *Daucus carota* subsp. *sativus* fruit samples under study, the highest accumulation of alicyclic quinic acid was found in *Daucus carota* subsp. *sativus* fruits of Yaskrava species – 1166.43 ± 29.16 μg/g, making about 80% of the total amount of identified compounds and being four times higher than the sum of phenolic content in fruits of this species. In *Daucus carota* subsp. *sativus* fruits of Nantska Kharkivska species, quinic acid content was 1.7 times less than that in *Daucus carota* subsp. *sativus* fruits of Yaskrava species. At the same time, quinic acid accumulation in *Daucus carota* subsp. *sativus* fruits of Olenka species was 2.3 times less and 1.3 times less than that in *Daucus carota* subsp. *sativus* fruits of Yaskrava and Nantska Kharkivska species, respectively. *Dauci carotae* subsp. *sativi* fructuum extractum siccum contained 3997.56 ± 99.94 μg/g quinic acid, which made almost 54% of the amount of total identified compounds in the extract, and it had 3.5 times more quinic acid content than *Daucus carota* subsp. *sativus* fruits of Yaskrava species had.

The smallest accumulation of chlorogenic acid was observed in *Daucus carota* subsp. *sativus* fruits of Olenka species – 39.87 ±1.00 μg/g. Its content in *Daucus carota* subsp. *sativus* fruits of Yaskrava and Nantska Kharkivska species was 3.2 times and almost 6 times higher, respectively. *Dauci carotae* subsp. *sativi* fructuum extractum siccum contained chlorogenic acid 2.5 times more than *Daucus carota* subsp. *sativus* fruits of Nantska Kharkivska species and equalled 603.49 ± 15.09 μg/g – 79% hydroxycinnamic acid content in the extract and more than 17% of total sum of identified phenolic compounds.

Caffeic acid content in *Dauci carotae* subsp. *sativi* fructuum extractum siccum was 3.8 times less than that of chlorogenic acid and made more than 20% of total content of hydroxycinnamic acids and 4.6% of phenolic compounds in this object. It is interesting that this compound was not observed in *Daucus carota* subsp. *sativus* fruits of all study species.

Prevailing flavonoid in *Daucus carota* subsp. *sativus* fruits of all study species and in *Dauci carotae* subsp. *sativi* fructuum extractum siccum was cynaroside. Differences of its content in *Daucus carota* subsp. *sativus* fruits of Yaskrava, Nantska Kharkivska and Olenka species were insignificant. Its maximum content (99.57 ± 2.49 μg/g), making about 66% of total flavonoids and about 35% of the sum of identified phenolics, was found in *Daucus carota* subsp. *sativus* fruits of Yaskrava species. Somewhat less amounts of cynaroside was accumulated in *Daucus carota* subsp. *sativus* fruits of Olenka and Nantska Kharkivska species – 87.48 ± 2.19 μg/g and 80.45 ± 2.01 μg/g, respectively. Cynaroside content in *Dauci carotae* subsp. *sativi* fructuum extractum siccum was 20 times more than that in *Daucus carota* subsp. *sativus* fruits of all study species – 1997.36 ± 49.93 μg/g. Cynaroside took one quarter of total content of all identified bioactive substances in *Dauci carotae* subsp. *sativi* fructuum extractum siccum.

Rutin and hyperoside contents were almost identical in *Daucus carota* subsp. *sativus* fruits of Yaskrava, Nantska Kharkivska and Olenka species. Highest rutin concentration was found in *Daucus carota* subsp. *sativus* fruits of Yaskrava species – 14.91 ± 0.37 μg/g. In *Daucus carota* subsp. *sativus* fruits of Olenka species (11.61 ± 0.14 μg/g) and *Daucus carota* subsp. *sativus* fruits of Nantska Kharkivska species (11.50

Table 1. Bioactive substances of Daucus carota subsp. sativus fruits of Yaskrava, Nantska Kharkivska and Olenka species and of Dauci carotae subsp. sativi fructuum extractum siccum.

Compound	*m/z* of $[M-H]^-$	Daughter ion *m/z*	Content of phenolic compounds as absolutely dry matter, μg/g			
			Daucus carota subsp. *sativus* fruits			*Dauci carotae* subsp. *sativi* fructuum extractum siccum
			Yaskrava species	Olenka species	Nantska Kharkivska species	
Quinic acid	191	85	1166.43 ± 29.16	502.83 ±12.57	680.20 ± 17.05	3997.56 ± 99.94
3-(3,4-Dihydroxycinnamoyl) quinic acid (chlorogenic acid)	353	191	129.75 ± 3.24	39.87 ±1.00	242.48 ± 6.62	603.49 ± 15.09
3,4-Dihydroxycinnamic acid (R)-1-carboxy-2-(3,4-dihydroxyphenyl)ethyl ester (rosmarinic acid)	359	161	0.71 ± 0.02	0.57 ± 0.01	—	—
3,4-Dihydroxybenzeneacrylic acid (caffeic acid)	392	174	—	—	—	158.91 ± 3.97
Luteolin-7-O-glucoside (cynaroside)	447	285	99.57 ± 2.49	87.48 ± 2.19	80.45 ± 2.01	1997.36 ± 49.93
Quercetin-3-O-rutinoside (rutin)	609	300	14.91 ± 0.37	11.61 ± 0.27	11.50 ± 0.28	—
Isorhamnetin-3-O-rutinoside (scolymoside)	563	291	6.22 ± 0.16	5.48 ± 0.14	5.10 ± 0.12	124.77 ± 3.12
4′,5,7-Trihydroxyflavone (apigenin)	269	117	0.25 ± 0.01	—	—	30.29 ± 0.76
3′-Methylquercetin (isorhamnetin)	315	300	0.06 ± 0.01	—	—	—
Apigenin 8-C-glucoside (vitexin)	431.1	311	0.15 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	4.17 ± 0.10
Phloretin 2′-glucoside (phloridzin)	435	273	0.09 ± 0.01	—	0.04 ± 0.01	—
Kaempherol-3-O-glucoside (astragalin)	447	284	3.15 ± 0.08	2.65 ± 0.07	2.68 ± 0.07	67.47 ± 1.69
Quercetin 3-D-galactoside (hyperoside)	451	344	15.73 ± 0.39	11.79 ± 0.29	12.23 ± 0.35	273.92 ± 6.85
Isorhamnetin-3-O-glucoside	477	314	6.86 ± 0.17	6.40 ± 0.16	5.75 ± 0.14	155.89 ± 3.90
3,5,7-Trihydroxyflavone (galangin)	486	195	3.96 ± 0.10	2.99 ± 0.07	2.80 ± 0.07	—
3′,4′,5,6,7-Pentamethoxyflavone (sinensetin)	414	272	0.10 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	—
Kaempferol 3-O-(6″-O-p-coumaroyl)glucopyranoside (tiliroside)	528	233	—	—	—	33.05 ± 0.83
Content of alicyclic acids			1166.43 ± 29.16	502.83 ± 12.57	680.20 ± 17.05	3997.56 ± 99.94
Total content of hydroxycinnamic acids			130.46 ± 3.26	40.44 ± 1.01	242.48 ± 6.06	762.40 ± 19.06
Total content of flavonoids			151.05 ± 3.78	128.58 ± 3.21	120.71 ± 3.02	2686.92 ± 67.17
Total content of phenolic compounds			281.51 ± 7.04	169.02 ± 4.23	363.19 ± 9.09	3449.32 ± 86.23
Total content of identified bioactive substances			1447.94 ± 36.20	671.87 ± 16.80	1043.39 ± 26.08	7446.88 ± 186.17

Notation: '—' compound was not present

± 0.28 μg/g), this compound was observed 1.3 times less, which, in turn, made a bit more than 1% of the content of all identified compounds. Rutin was not detected in *Dauci carotae* subsp. *sativi* fructuum extractum siccum.

Hyperoside accumulated a bit more than rutin in carrot fruits, making a bit more than 9% of flavonoids content in all objects. Hyperoside concentration in *Daucus carota* subsp. *sativus* fruits of Yaskrava species was 15.73 ± 0.39 μg/g and that in *Daucus carota* subsp. *sativus* fruits of Olenka and Nantska Kharkivska species were 11.79 ± 0.29 μg/g and 12.23 ± 0.35 μg/g, respectively. Hyperoside content in *Dauci carotae* subsp. *sativi* fructuum extractum siccum was 273.92 ± 6.85 μg/g, which exceeded 17–23 times its amount in *Daucus carota* subsp. *sativus* fruits of all study species. Hyperoside share was 10% of total flavonoids and about 85 of the sum of phenolics in the extract.

Difference in the content of isorhamnetin glycosides (isorhamnetin-3-O-rutinoside and isorhamnetin-3-O-glucoside) in *Daucus carota* subsp. *sativus* fruits of all study species was negligible.

Methylated flavone sinensetin and glycosides of apigenin (vitexin) and of phloretin (phlorizin) accumulated in *Daucus carota* subsp. *sativus* fruits of all studied species at a negligible rate (less than 1 μg/g). These compounds were not detected in *Dauci carotae* subsp. *sativi* fructuum extractum siccum. The only one of the above substances found in *Dauci carotae* subsp. *sativi* fructuum extractum siccum was vitexin (4.17 ± 0.10 μg/g). Its content in extract was 28 times higher than that in *Daucus carota* subsp. *sativus* fruits of Yaskrava species.

HPLC analysis conducted by Polish scientists showed that 40% and 70% of ethanolic extracts from *Daucus carota* fruits contained 12 compounds of phenolic nature. Our experiments allowed us to identify only 10 phenolic compounds in 50% ethanol extract from *Daucus carota subsp. sativus* fruits. Comparison of the results showed that the chemical composition of Polish phenolic compounds and the extract obtained by us differed slightly. In particular, chlorogenic, caffeic acid and apigenin were present in all extracts. At the same time, 40% and 70% of ethanolic extracts from Polish *Daucus carota* fruits contained ferulic, *p*-coumaric, 3-hydroxybenzoic and cinnamic acids, flavonoids catechin, myricetin, luteolin and rutin, which were absent in our extract. However, seven flavonoids (cynaroside, scolymoside, vitexin, astragalin, hyperoside, isorhamnetin-3-O-glucoside and tiliroside) were found only in *Daucus carota subsp. sativus* fruits extract obtained by us. Rutin and *p*-coumaric acid predominated in 70% of Polish ethanol extracts, and chlorogenic acid and cynaroside in our extracts (Pavlyuk I. et al., 2015; Al-Snafi A. E., 2017).

A group of scientists from France and Sri Lanka found kaempferol, quercetin, luteolin and myricetin in *Daucus carota* subsp. *sativus* fruits. Kaempferol (24 mg/g) and quercetin (21 mg/g) dominated in this plant raw material (Thiviya P. et al., 2021). None of these compounds was found in Ukrainian species of *Daucus carota* subsp. *sativus*.

British and Lebanese scientists have identified five compounds of phenolic nature in *Daucus carota* fruits by HPLC, including caffeic acid, quercetin, luteolin, kaempferol and apigenin. Only apigenin was identified in *Daucus carota* subsp. *sativus* samples we studied among all those substances. It was also quantitatively predominant in plant raw materials of British origin. Its content in *Daucus carota* fruits ranged from 50.75 to 118.00 μg/mg, which is much higher than that in *Daucus carota* subsp. *sativus* fruits of Yaskrava species, where this compound was identified (Shebaby W. N. et al., 2015).

Researchers from Serbia using HPLC in *Daucus carota* subsp. *sativus* 70% ethanol extract have identified only phenolic carboxylic and hydroxycinnamic acids (gallic, protocatechuic, caffeic, vanillic, chlorogenic, ferulic, sinapic and rosemary acids), according to which chlorogenic acid (0.80 mg/g) and rosemary (0.65 mg/g) acid dominated (Mladenović J. et al., 2015). The content of chlorogenic acid in the extract obtained by us was almost at the same level. At the same time, the caffeic acid content in the extract obtained by us was 2.6 times higher. Rosemary, gallic, protocatechuic, ferulic, sinapic and vanillic acids were not found in our extract. However, our extract contained seven flavonoids.

The analysis and comparison of the HPLC results of phenolic compounds obtained by us and presented in the literature showed that *Daucus carota* fruits and *Daucus carota* subsp. *sativus* fruits of different species and origins can differ significantly in qualitative composition and quantitative content of hydroxycinnamic acids and flavonoids. Such differences can be explained by the growing conditions of these plants. It was noted that in the samples of fruits of cultivated sown *Daucus carota* subsp. *sativus* forms, the qualitative composition of phenolic compounds is more diverse than that studied in wild *Daucus carota* samples.

The information found in the literature on the chemical composition of extracts from *Daucus carota* and *Daucus carota* subsp. *sativus* indicates that the choice of extractant significantly affects the qualitative composition and quantitative content of dosage forms, each of which is a unique complex of biologically active compounds with potentially different vector activities that requires a further research to be conducted.

CONCLUSIONS

Our research ascertained that qualitative composition of phenolic compounds in *Daucus carota* subsp. *sativus* fruits of Yaskrava, Nantska Kharkivska and Olenka species was almost identical. The main difference in phenolic composition was the fact that flavonoid aglycones – apigenin and isorhamnetin – were found only in *Daucus carota* subsp. *sativus* fruits of Yaskrava species, whereas rosmarinic acid was absent in *Daucus carota* subsp. *sativus* fruits of Nantska Kharkivska species.

The content of the sum of phenolic compounds was from 169.02 ± 4.23 to 363.19 ± 9.09 μg/g. Chlorogenic acid and

flavonoids cynaroside, rutin and hyperoside dominated among phenolics in all study varieties.

In *Dauci carotae* subsp. *sativi* fructuum extractum siccum, we identified 11 compounds; of these, two referred to hydroxycinnamic acids, one to aglycones and seven to flavonoid glycosides. Among hydroxycinnamic acids, chlorogenic acid dominated (603.49 ± 15.09 µg/g), whereas among flavonoids, this role belonged to cynaroside (1997.36 ± 49.93 µg/g) and hyperoside (273.92 ± 6.85 µg/g).

Comparison of the obtained results with the literature data confirmed the variability of the chemical composition of *Daucus carota* and *Daucus carota* subsp. *sativus* of different species and different origins. It is established that samples of *Daucus carota* subsp. *sativus* fruit of Ukrainian species had a unique chemical composition of flavonoids, which has not been previously recorded in the literature. The obtained 50% ethanol *Dauci carotae subsp. sativi fructuum extractum siccum* was also characterized by an excellent chemical composition from similar, proposed by other researchers, extracts from wild *Daucus carota* and *Daucus carota* subsp. *sativus*, which, respectively, affects the spectrum of its biological activity.

The obtained results are indicative of stable phenolic composition in *Daucus carota* subsp. *sativus* fruits of all studied varieties. Experimental results confirmed feasibility and prospects of novel drugs development on the basis of phenolic compounds of *Daucus carota* subsp. *sativus* fruits and of *Dauci carotae* subsp. sativi fructuum extractum siccum.

CONFLICT OF INTEREST

There are no conflicts of interest declared by the authors.

ACKNOWLEDGEMENT

Scientific research was funded by the Ministry of Health of Ukraine from the state budget.

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