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The determination of phenolic compounds in garlic extracts by HPLC GC/MS technique

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ABSTRACT

Being popular as spices, garlic was used as a treatment agent since ancient times. Its popularity was caused by a wide range of diseases and disorders it could treat. Methanol extracts and methanol extracts after hydrolysis were analyzed by HPLC GC/MS technique. As a result of the research, eight compounds such as luteolin-7-glucoside, luteolin, apigenin-7-glucoside, hyperoside, rutin, caffeic acid, chlorogenic acid and rosmarinic acid were detected in the tested solutions. Luteolin and apigenin-7-glucoside were the only two compounds identified in the studied extract after hydrolysis.

Key words: garlic, extracts, luteolin-7-glucoside, apigenin-7-glucoside, rutin, caffeic acid, chlorogenic acid, HPLC GC/MS.

INTRODUCTION

Garlic is often used as culinary spices. According to the frequency of its usage it is in the same row as turmeric, cayenne pepper, cinnamon and ginger. However, this plant and its plant raw material can be successfully applied for prophylaxis and treatment of different diseases as well as for organ function recovery in case of different disorders [1-5].

Availability and ease of cultivation made garlic one of the most popular means of treatment in the folk medicine since ancient times [1]. Garlic proved to be a strong antibacterial agent as well as good treatment of hypertension, malaria, scurvy, cough, gout and dropsy [2, 3]. Ukrainian folk medicine recommended using garlic for treatment of a large amount of gastro-intestinal tract diseases such as dysentery, flux, colitis and flatulence [1].

Garlic is also applied in homeopathy in D3-D6 dilution. As a component of homeopathic remedies, garlic can be prescribed for treatment of bronchitis, rheumatism, muscle and joint pain relief [1].

Nowadays, it is proved that garlic can stimulate gastro-intestinal tract motoric and inhibits fermentation and putrefaction in the intestine. According to the statistics, people who have garlic in their diet turned out to have cancer much more rarely than those who did not eat garlic [1-5].

Garlic plant raw material is one of the components of some modern drugs which are prescribed for patients suffering from atherosclerosis, hypertension, cholecystitis and constipation. Such an extensive list of useful medicinal properties causes the necessity of working out analytical techniques and precise sample preparation procedures that enable accurate evaluation of qualitative analysis and quantitative content determination for further drug design and standardization [1-5].

MATERIALS AND METHODS

The studied solutions were prepared in the following way [6-8].

Methanol solutions preparation.

25 g of garlic plant raw material was put into a round-bottom flask with further addition of 125.0 ml of methanol and boiling on the water bath with a reflux condenser during 1 hour. Further it was cooled, filtered through "blue stripe" paper filter (solution 1). The solvent was added to 5.0 ml of the obtained solution in the amount enough to get 10.0 ml in total volume.

Extracts preparation after hydrolysis.

5.0 ml of the solution was put into a round-bottom with further addition of 5.0 ml of hydrochloric acid. The obtained solution was boiled on the water bath with a reflux condenser during 30 min. Afterwards it was cooled, put into a 25 ml volumetric flask. The volume was increased with methanol up to the flask mark, then mixed, filtered through "blue stripe" paper filter. The solvent was added to 5.0 ml of the obtained solution in the amount enough to get 10.0 ml in total volume. The next step was conduction of a chromatographic analysis.

The chromatographic study of the tested extracts and standard samples solutions were carried out with the help of Agilent 1200 LC/MSD chromatograph which consisted of a pump unit G1312A, degasator G1322A, column thermostat G1316A, autosampler G1367B, diod-matrix detector G1315D and quadrupole mass spectrometer Agilent 6130. Chromatography was carried out under such conditions: a column made of a stainless steel C18 SunFire (150 x 4,6 mm, 3.5 μ m); the column temperature – 38^oC; diod-matrix detector detection wavelength was 254 nm, 330 nm, 350 nm, 360 nm, 370 nm. Mass detector detection conditions were the following: ionization – electricspray, scanning in the diapason of 150-800 m/z (positive and negative ionization); fragmentor – 70; gain - 1,0; the flow rate of a mobile phase was 1 ml/min; the injected sample volume was 50 μ l [6, 8].

The mobile phase was the following:

Time, minutes	Mobile phase A, % (turnover/turnover)	Mobile phase B, % (turnover/turnover)	Mode
0→1	92	8	isocratic
1→15	92→70	8→30	gradient
15→25	70→0	30→100	gradient
25→33	0	100	isocratic
33→33,5	0→92	100→8	gradient

*Mobile phase A – 0.1% formic acid solution;
Mobile phase B – 0.1% formic acid solution in the mixture (acetonitrile:methanol 1:1).*

Quercetin, hyperoside, rutin, luteolin-7-glucoside and luteolin solutions as well as caffeic acid, rosmarinic acid and chlorogenic acid solutions in methanol were used as standard solutions.

While working, such reagents were used: acetonitrile and methanol for gradient chromatography ("FLUKA" (Germany)); formic acid ("FLUKA" (Germany)); ethanol rectified (according to the Pharmacopoeia requirements), water.

The obtained data were analyzed using Student's t-test.

RESULTS AND DISCUSSION

Table 1 shows the data regarding the retention time of standard substances under the conditions of the studied chromatography technique.

Table 1 The retention time of standard substances

Compound Name	Formula	M.M.	The retention time of the peaks
Quercetin	C ₁₅ H ₁₀ O ₇	302.04	16.886
Hyperoside	C ₂₁ H ₂₀ O ₁₂	464.10	16.920
Rutin	C ₂₇ H ₃₀ O ₁₆	610.15	16.866
Luteolin	C ₁₅ H ₁₀ O ₆	286.05	21.646
Luteolin-7-glucoside	C ₂₁ H ₂₀ O ₁₁	448.10	17.213
Apigenin-7-glucoside	C ₂₁ H ₂₀ O ₁₀	432.11	19.459
Caffeic acid	C ₉ H ₈ O ₄	180.04	9.697
Rosmarinic acid	C ₁₈ H ₁₆ O ₈	360.08	19.954
Chlorogenic acid	C ₁₆ H ₁₈ O ₉	354.10	8.769

The chromatograms of the standard compounds are shown in Fig. 1-8.

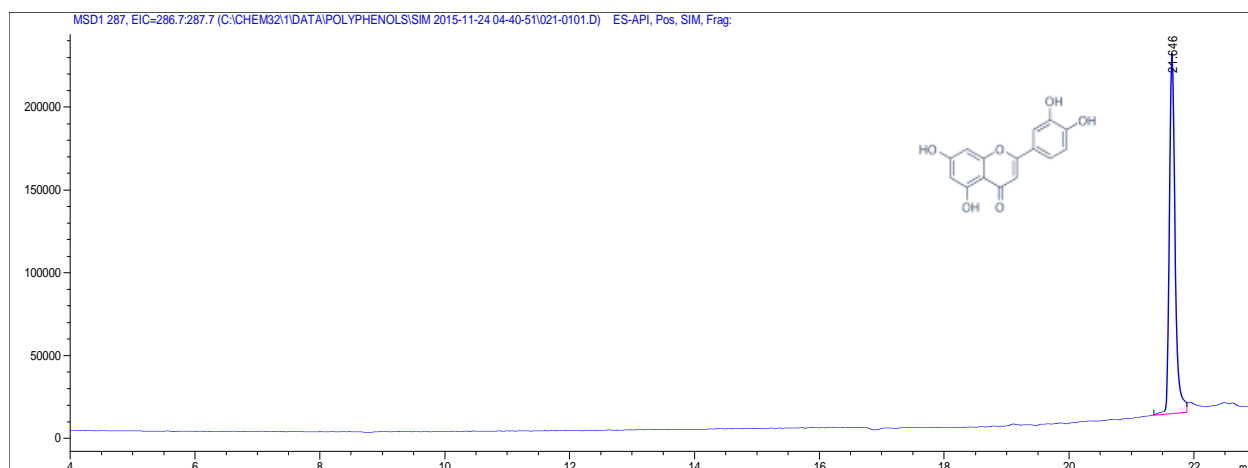


Fig. 1 The chromatogram of luteolin standard solution [+287 u]

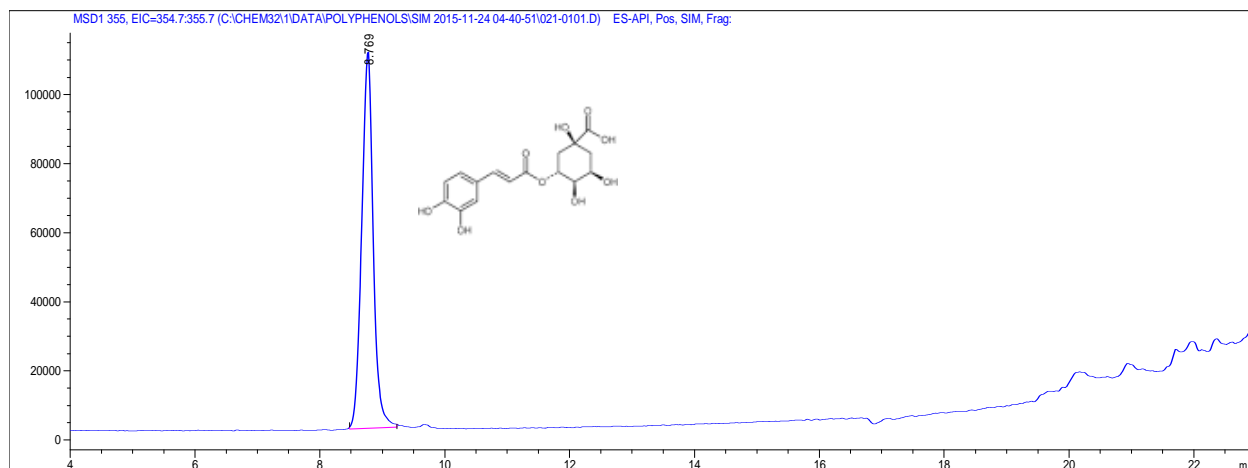


Fig. 2 The chromatogram of chlorogenic acid standard solution [+355 u]

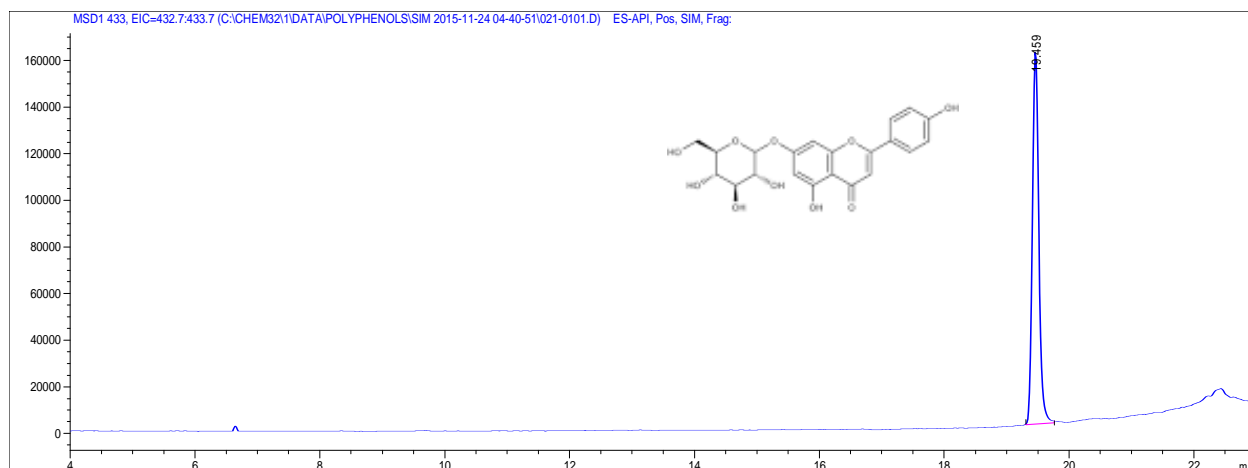
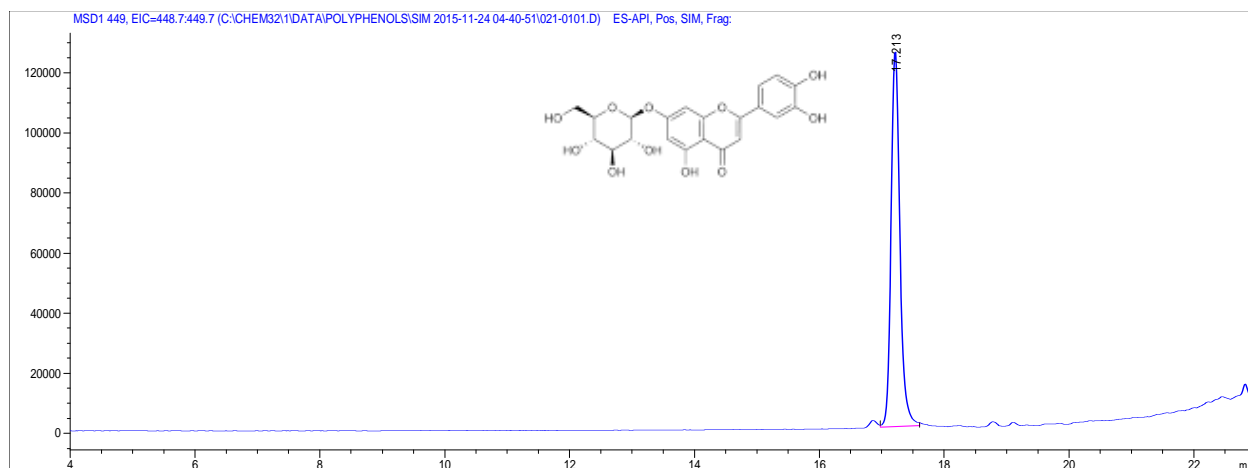
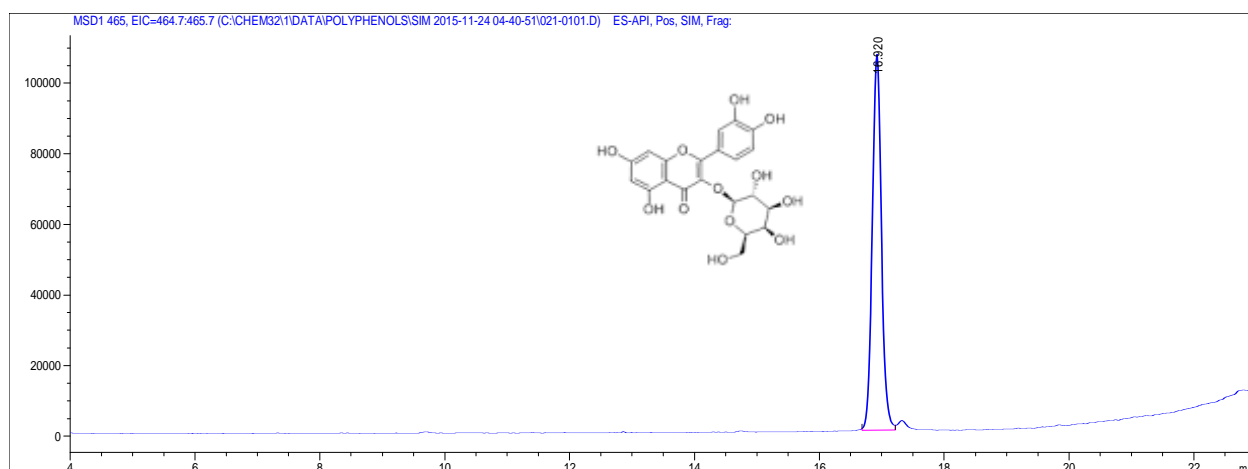
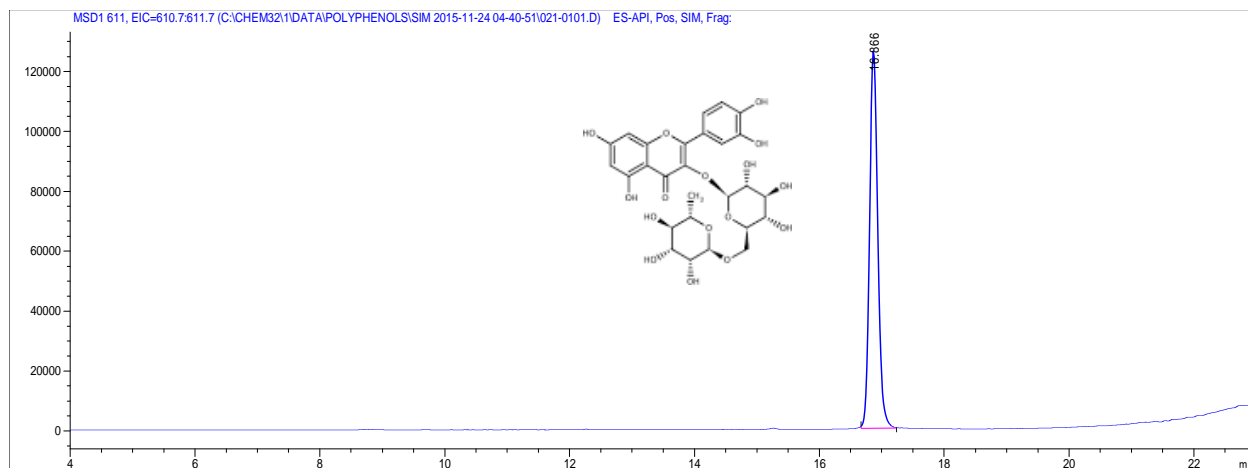


Fig. 3 The chromatogram of apigenin-7-glucoside standard solution [+433 u]

**Fig. 4** The chromatogram of luteolin-7-glucoside standard solution [+449 u]**Fig. 5** The chromatogram of hyperoside standard solution [+465 u]**Fig. 6** The chromatogram of rutin standard solution [+611 u]

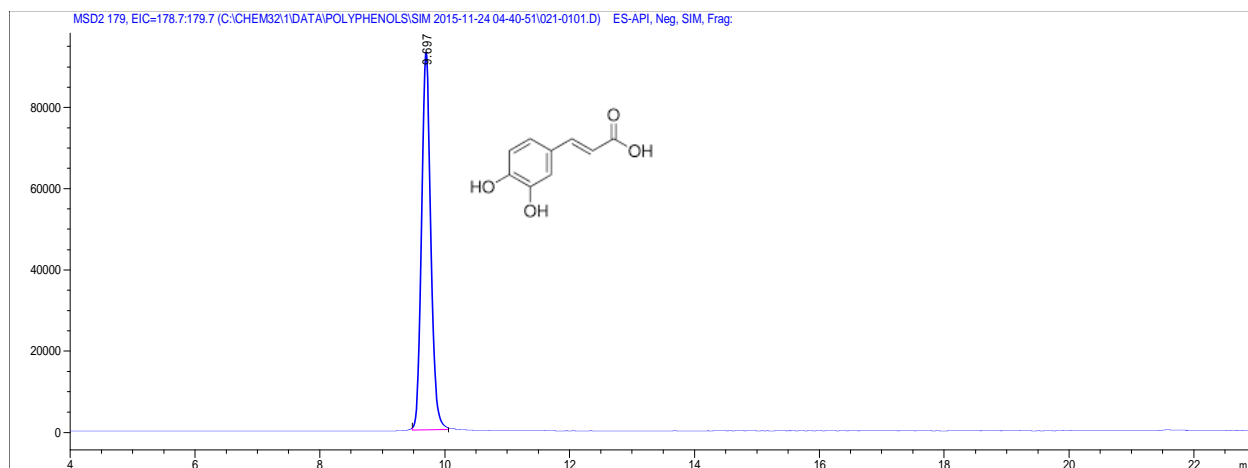
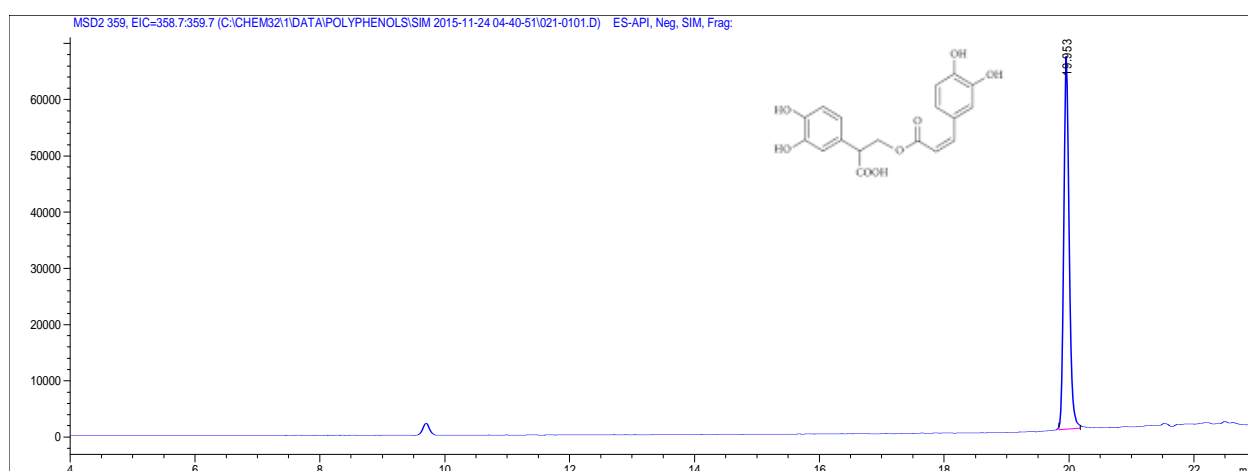
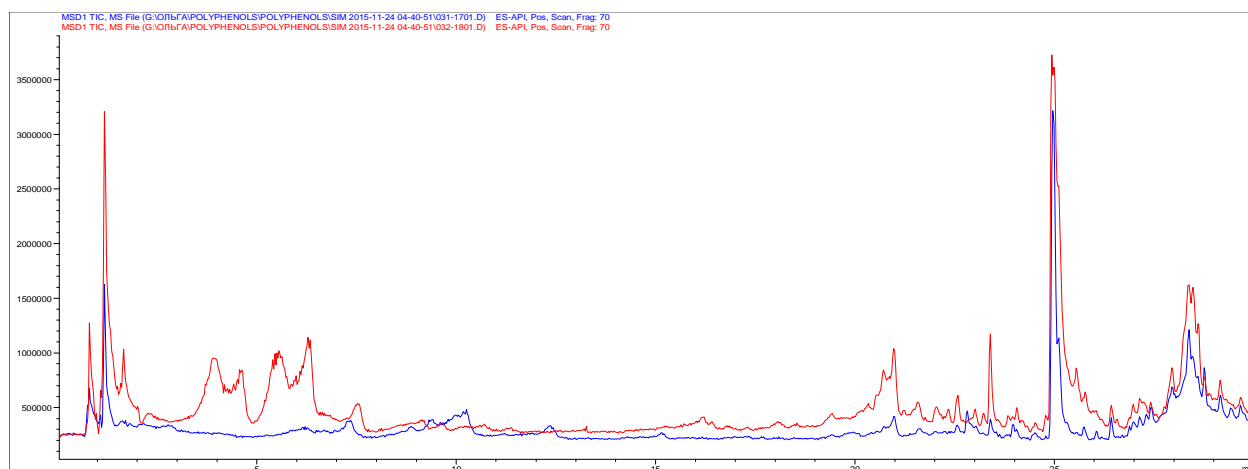
**Fig. 7** The chromatogram of the caffeic acid standard solution [–179 u]**Fig. 8** The chromatogram of the rosmarinic acid standard solution [–359 u]

Fig. 9-10 show chromatograms of the studied solutions. The studied compounds in the solutions tested were identified by retention time as well as by m/z index.

Fig. 9 represents chromatograms of the tested solutions obtained under conditions of positive ionization. Fig. 10 represents chromatograms of the tested solutions obtained under conditions of negative ionization.

**Fig. 9** Chromatograms of the studied extracts, positive ionization

Red line represents the methanol extract; blue line represents the studied extract after hydrolysis

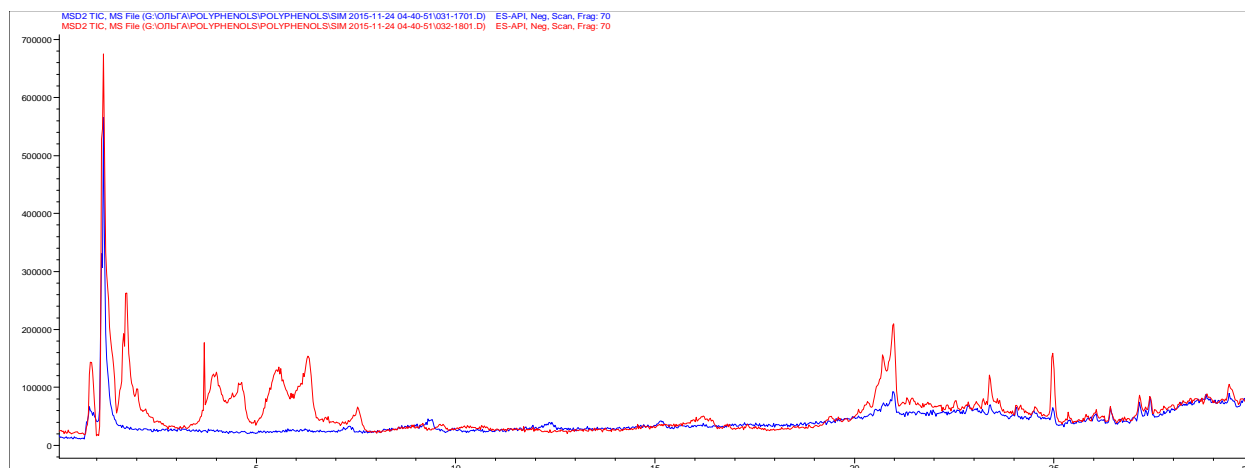


Fig. . 10 Chromatograms of the studied extracts, negative ionization

Red line represents the methanol extract; blue line represents the studied extract after hydrolysis

The data regarding obtained components and their concentrations in the solutions studied are presented in table 2.

Table 2 The results of a quantitative determination of the studied components in methanol and garlic extracts after hydrolysis

The studied component	Garlic extract after hydrolysis	Methanol garlic extract
	Content in %, based on a dry substance	
Luteolin	0.00019	0.2326
Chlorogenic acid	-	trash amount
Apigenin-7-glucoside	0.00068	0.1138
Luteolin-7-glucoside	-	0.28614
Hyperoside	-	0.03756
Rutin	-	0.0319
Caffeic acid	-	0.01258
Rosmarinic acid	-	trash amount

According to the data represented in table 2 all the studied standard compounds were identified in the tested methanol extract. According to the obtained results, luteolin-7-glucoside and luteolin have the highest concentration – 0.28614 and 0.2326 % respectively. Apigenin-7-glucoside, hyperoside, rutin and caffeic acid have less concentration – 0.1138 %, 0.03756 %, 0.0319 % and 0.01258 % respectively. Wherein, chlorogenic and rosmarinic acids were found in the studied solution only in trash amount.

According to the data represented in table 2, only 2 compounds – luteolin-7-glucoside and luteolin were identified in the extract after hydrolysis. The concentration of the compounds mentioned above was 0.00019 % and 0.00068 % respectively.

CONCLUSION

Garlic extracts were studied while conducting the research. The obtained data presented below can be used for the further standardization of special food products and diet supplements.

1. The application of HPLC GC/MS method of analysis helped to work out a technique of detection of 8 biologically active compounds in garlic extracts.
2. Methanol garlic extracts and methanol garlic extracts after hydrolysis were analyzed with the help of the developed technique. As the result of the conducted research luteolin-7-glucoside, luteolin, apigenin-7-glucoside, hyperoside, rutin, caffeic acid, chlorogenic acid and rosmarinic acid were identified in the studied methanol extract. Luteolin-7-glucoside and luteolin had the highest content – 0.28614 % and 0.2326 % based on a dry substance respectively.
3. Only two compounds – luteolin and apigenin-7-glucoside were identified in the studied extract after hydrolysis. The concentration of the compounds mentioned above was 0.00019 % and 0.00068 % respectively.

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