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RESEARCH OF SOPHORA JAPONICA L. FLOWER BUDS VOLATILE COMPOUNDS WITH GAS-CHROMATOGRAPHY/MASS-SPECTROMETRY METHOD

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Sophora japonica L. is a deciduous tree with the height to 30 m. Crown is branchy, almost spherical. Old stem bark is dark-grey with deep cracks. Draws and branches are greenly-grey, without burrs, short-tomentous. Leaves are odd-pinnate with 9-17 oblong-ovoid or oblong- elliptic, sharp, shining, dark green from the upper side, blue-grey green from the lower side leaves. Stipules are linear, caducous. Flowers are with nice smell, 1,0-1,5 cm length, yellow-white, cowered with end-point large lax panicles. Fruit is carnous bear bean with the length of 5-8 cm, filled with yellow-green adhesive sap, with deep incisuras between seeds. Seeds are oval, smooth, opaque, dark brown. Sophora japonica L. root system is very extensional, deep, highly branched [1].

Sophora japonica L. is thermophilic and heliophilous, fast-growing plant. It is well adapted during drought periods as it survives lack of moisture in soil better than excess water. It is quite a frost-resistant crop. Only if temperature goes down lower than – 20°C 1-3-year seedlings freeze a bit. It is connected with late sprouts lignification at the end of vegetation. 30-40-year summer trees are more frost-resistant.

It prefers clay-loam and clay sand. Ability to fix the air nitrogen gives the opportunity to Sophora japonica L. to develop normally on soils which are poor with nitrogen compounds. It survives soil salification to a certain degree.

Crude drug is flower buttons (Alabastrae Sophorae japonicae) and Sophora fruit (Fructus Sophorae japonicae).

Main Sophora japonica L. active substance is rutin which is used for preventive measures and cure for hypovitaminosis and vitamin deficiency P and also during hemorrhagic diathesis, eye retinal hemorrhage, acute vascular purpura, radiation disease, bacterial endocarditis, rheumatism, allergic disease, ischaemic stroke, hypertensive disease [2-6]. Main world manufacturers of rutin are China, Japan, USA.

Apart from rutin other flavonoids were found in Sophora japonica L. buds: quercetine, kaempferol, isorhamnetin, narcissine, genistein, daidzein, biochanin A, formononetin; and also hydroxycinnamic acids, aminoacids, fatty acids, macroelements, microelements, polysaccharides, volatile substances [2-7,11].

Because of Sophora japonica L. buds volatile substances composition data which grows in Ukraine territory the aim of our research was to study Sophora japonica L. buds volatile compounds collected in Bakhchisarai area, Autonomous Republic of Crimea on the next stages of their development: green flower buds, formed flower buds and the beginning of flower buds opening.

Materials and methods

Essential oil assay content in Sophora japonica L. buds on different stages of their development was determined by hydrodistillation.

Qualitative composition and assay content of Sophora japonica L. buds essential oil were determined by gas-chromatography/mass-spectrometry method. Raw material weighed amount (0,5-5g) was placed in a vial of 20 ml, internal standard was added. Tridecane was used as internal standard at the rate of 50 mcg on weighed amount. 10 ml of water was added in the sample and sample volatile compounds with steam vapor during two hours were flapped away using backflow condenser with air-cooling.

During flapping away volatile compounds are adsorbed on the inner surface of backflow condenser. Adsorbed compounds after system cooling were washed down into a dry vial on 10 ml with a slow addition of 3 ml especially pure pentane. Washdown was concentrated with a blowdown (100 ml/min) of nitrogen purge to the final volume of extract of µl which was selected with chromatographic syringe. Next sample concentration was carried out in the syringe itself to the volume of 2 µl.

Sample input in a chromatographic column was carried out in splitless mode, which allows to input a sample without losses and significantly (in 10-20 times) increase response of a chromatographic method.

Essential oils compounds were indentified according to the results of the comparison of received chemical substances mass-spectrum which are in essential oils composition and according to mass-spectrum library NIST 05 and WILLEY-2007 data [8-12].

Apparatus and Conditions. Chromatograph Agilent Technologies 6890 with a mass-spectrum detector 5973. Analysis conditions: capillary (HP-35) with the length of 30 m, inner diameter – 0,25 mm; thermostat temperature – from 50^{o}C to 300^{o}C; carrier gas – helium, carrier gas velocity – 1,2 ml/min.; evaporator temperature – 250^{o}C. Sample input speed - 1,2 ml/min., during 0,2 min.

Results and discussion

In the result Sophora japonica L. buds essential oils assay content was detected. Its content in raw material is less than 0,1%.

In the result of Sophora japonica L. buds volatile compounds research with gas-chromatography/mass-spectrometry method 80 constituents were identified out of which 61 substances are during the green flower buds and beginning of flower buds opening stages, 66 substances are during formed flower buds stage. Substances are represented

by aliphatic and cyclic terpenoids, their alcohols and ketones (Table 1).

Chromatograph schemes of Sophora japonica L. buds volatile compounds collected on different stages are represented in Figure 1, 2 and 3.

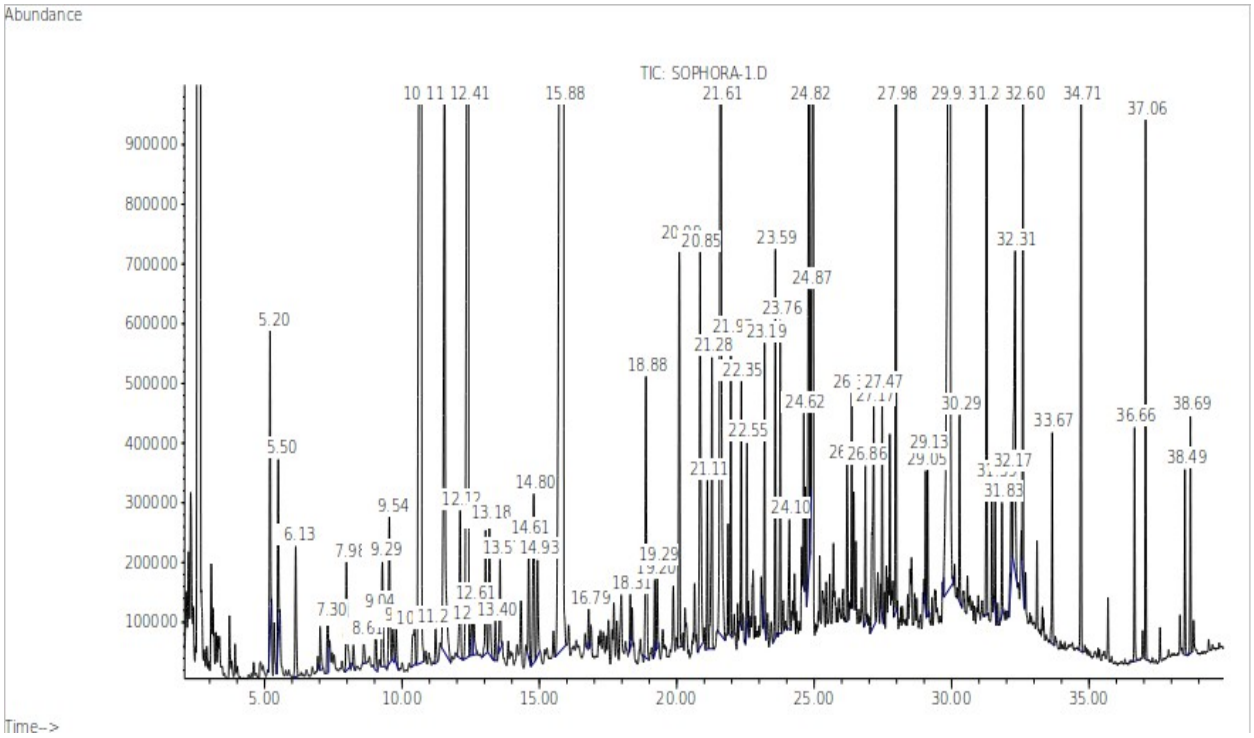

Figure 1. Chromatograph scheme of Sophora japonica L. buds volatile compounds (green buds)

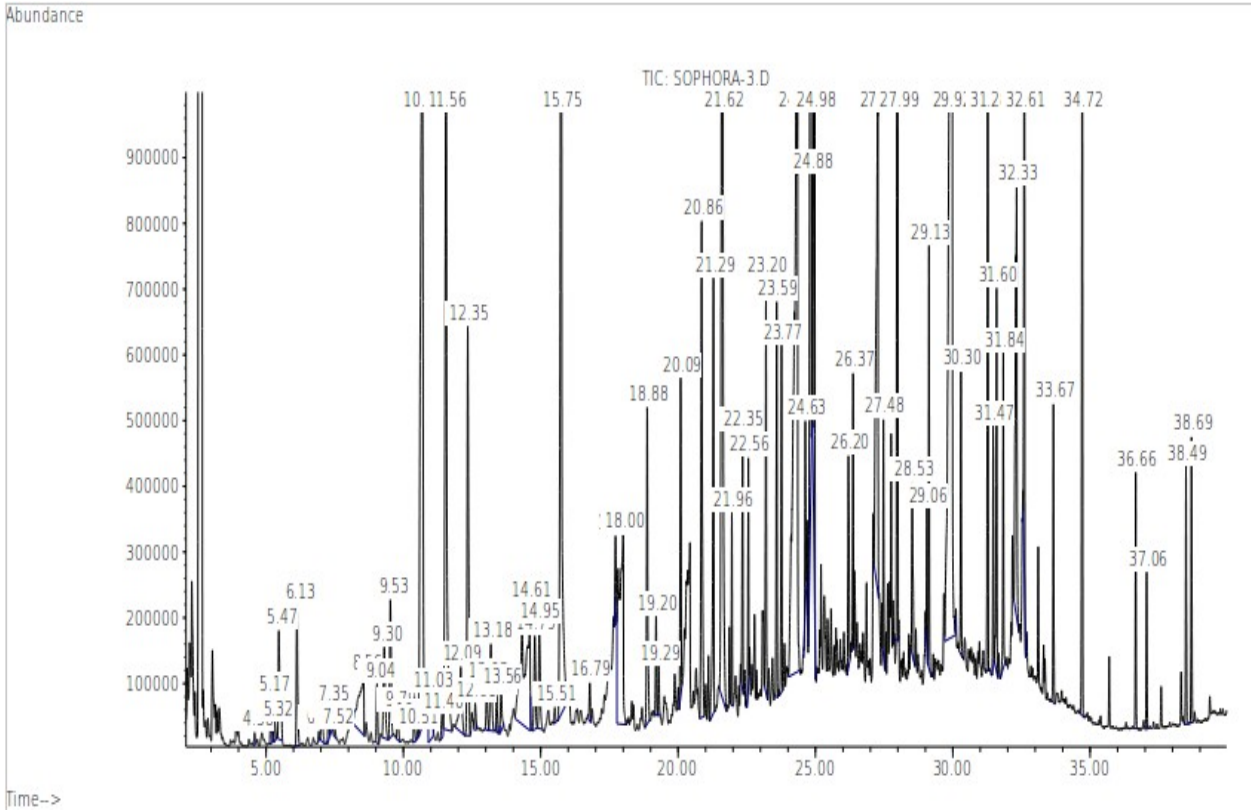

Figure 2. Chromatograph scheme of Sophora japonica L. buds volatile compounds (formed buds)

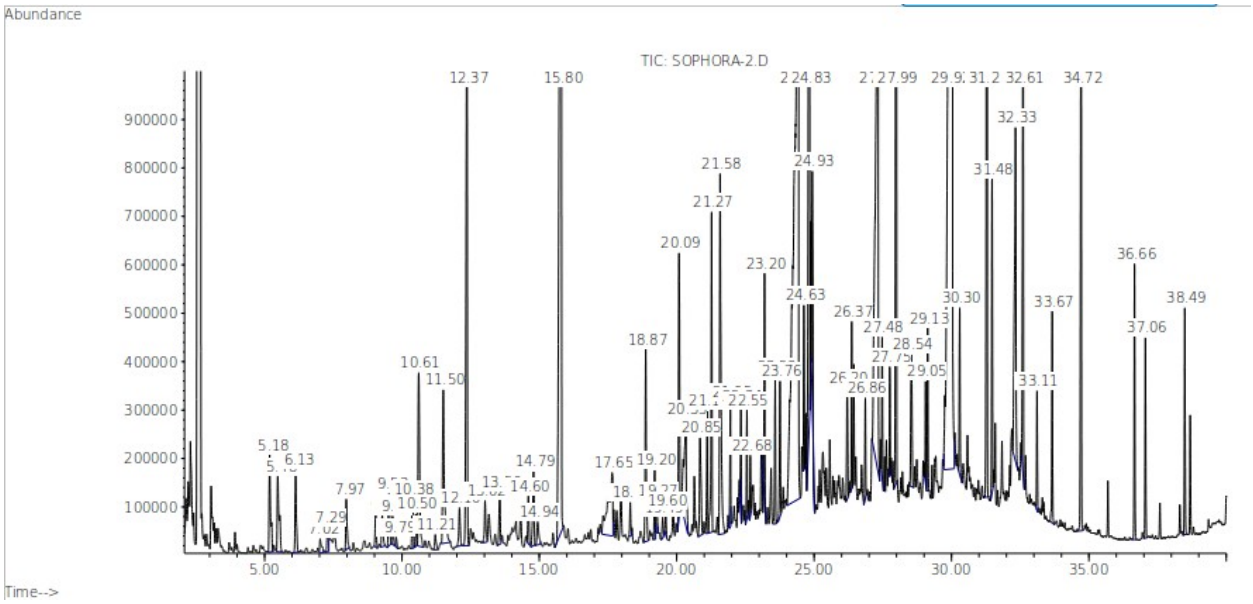

Figure 3. Chromatograph scheme of Sophora japonica L. buds volatile compounds (beginning of buds opening)

Most substances are extracted on the stage of formed buds. At the same time it should be admitted that on this stage substances with potential pharmacological properties are in the biggest proportion: linalool – 38,1 mg/kg, hexahydrofarnesylacetone – 40,5 mg/kg, eugenol – 61,9 mg/kg, nonanoic acid – 26,1 mg/kg, decanoic acid – 18,6 mg/kg, dodecanoic acid – 68,4 mg/kg, tetradecanoic acid – 29,0 mg/kg, linoleic acid – 21,3 mg/kg.

It is known that linalool has antibacterial, wound-healing and antiinflammatory properties; eugenol has cardioprotective, hypolipidemic, hepatoprotective and antibacterial properties; lauric acid, pelargonic acid and myristic acid has antibacterial and antimicrobic properties; linoleic acid takes part in arachidonic acid and prostaglandin synthesis, supports cellular membrane structure which is necessary for visual apparatus and nervous system functioning.

On the stages of green buds and formed buds squalen 1,1 mg/kg and 6,8 mg/kg respectively was identified. Squalen is unsaturated fluidal triterpene hydrocarbon which lowers blood cholesterol level and has anti-inflammatory and antioxidative properties.

Chinese scientists think that volatile substances together with rutin and quercetine influence antioxidative action of Sophora japonica L. buds [8].

Conclusions

1. Sophora japonica L. flower buds were collected during the stages of green flower buds, formed flower buds and the beginning of flower buds opening.
2. Most volatile substances and essential oils were extracted on the stage of formed buds.
3. Component analysis of essential oil of Sophora japonica L. buds was determined with chromate-mass spectrometry method. Its content in raw material was less than 0,1%. Substances are represented by aliphatic and cyclic terpenoids, their alcohols and ketones.

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This work represents the results of the research of essential oil contained in Sophora japonica L. flower buds volatile compounds collected during the next stages of their development: green flower buds, formed flower buds and the beginning of flower buds opening. Essential oil assay content in Sophora japonica L. flower buds was determined with hydrodistillation method. Content of essential oil in the raw material is less than 0,1%. Qualitative composition and assay content of Sophora japonica L. flower buds essential oil constituents were determined with chromato-mass spectrometry method. In consequence of the research 80 constituents were identified in Sophora japonica L. flower buds out of which 61 substances are during the green flower buds and beginning of flower buds opening stages, 66 substances are during formed flower buds stage. Substances are represented by aliphatic and cyclic terpenoids, their alcohols and ketones. Most volatile substances were extracted on the stage of formed buds.

Keywords: Sophora japonica L., volatile compounds, gas-chromatography/mass-spectrometry

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ДОСЛІДЖЕННЯ ЛЕТКИХ СПОЛУК ПУП'ЯНКІВ СОФОРИ ЯПОНСЬКОЇ ХРОМАТО-МАСС-СПЕКТРОСКОПІЧНИХ МЕТОДОМ

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У роботі представлені результати досліджень летких сполук пуп'янків софори японською, зібраних на наступних стадіях їхнього розвитку: зелені пуп'янки, сформовані пуп'янки і початок розкриття пуп'янків. Кількісний вміст ефірної олії в пуп'янках софори японською визначали методом гідродистиляції. Вміст ефірної олії в сировині складає – менше 0,1%. Якісний склад та кількісний вміст компонентів ефірної олії пуп'янків софори японською визначали хромато-мас-спектрометричним методом. В результаті дослідження в пуп'янках софори японською ідентифіковано 80 компонентів: з них 61 речовина на стадії зелених бутонів і початку їх розкриття, 66 речовин на стадії сформованих бутонів. Більшість речовин представлені аліфатичними і циклічними терпеноїдами, їх спиртами і кетонами. Найбільша кількість речовин, що відносяться до летких, була знайдена у сформованих пуп'янках софорі японської.

Ключові слова: софора японська, леткі речовини, газова хроматографія/мас-спектроскопія

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ИССЛЕДОВАНИЕ ЛЕТУЧИХ ВЕЩЕСТВ БУТОНОВ СОФОРЫ ЯПОНСКОЙ ХРОМАТО-МАСС-СПЕКТРОСКОПИЧЕСКИМ МЕТОДОМ

Максютина Н.П., Чолак И.С., Карпюк У.В.

В работе представлены результаты исследований летучих соединений бутонов софоры японской, собранных на следующих стадиях их развития: зеленые бутоны, сформированные бутоны и начало раскрывания бутонов. Количественное содержание эфирного масла в бутонах софоры японской определяли методом гидродистиляции. Содержание эфирного масла в сырье составляет – менее 0,1%. Качественный состав и количественное содержание компонентов эфирного масла бутонов софоры японской определяли хромато-масс-спектрометрическим методом. В результате исследования в бутонах софоры японской идентифицировано 80 компонентов, из них 61 вещество на стадии зеленых бутонов и начала их раскрытия, 66 веществ на стадии сформированных бутонов. Большинство веществ представлены алифатическими и циклиескими терпеноидами, их спиртами и кетонами. Наибольшее количество веществ, относящихся к летучим, было найдено в сформированных бутонах софоры японской.

Ключевые слова: софора японская, летучие вещества, газовая хроматография/масс-спектроскопия

Table 1. Assay content of Sophora japonica L. buds volatile compounds on different stages of their

development

Time	Substance name	Content, mg/kg of dry raw material		
		Green buds	Formed buds	Beginning of buds opening
1	2	3	4	5
Saturated hydrocarbons				
6.13	Decane	1,0	5,1	2,5
9.04	Undecane	0,5	2,1	1,0
18.87	Tetradecane	2,3	10,7	5,4
20.09	2,6,10- trimethyldodecane	2,8	8,8	6,8
21.28	Pentadecane	2,0	13,5	7,4
23.19	Hexadecane	1,3	10,4	3,2
24.87	Heptadecane	0,8	3,4	5,3
26.36	Octadecane	0,9	5,5	2,6
27.75	Nonadecane	-	-	1,8
29.05	Eicosane	0,6	2,7	1,5
30.29	Henicosane	0,9	6,3	3,3
32.59	Tricosane	2,3	17,3	11,5
33.66	Tetracosane	1,1	6,4	3,9
34.71	Pentacosane	3,5	19,9	13,4
36.66	Heptacosane	1,1	5,7	5,4
38.49	Nonacosane	0,9	6,0	4,3
Aldehydes, ketones, alcohols				
4.58	Dihydro -2- methylfuranone -3	-	0,5	-
5.20	Trans-2-hexanal	1,8	1,6	3,5
5.31	2,5- furandion	-	0,7	-
5.49	Furfurol	1,1	6,2	3,4
7.35	1,3- cyclopentanedione	-	0,9	-
7.98	Trans -2-heptenal	1,0	-	1,7
8.23	2,3-octanedion	0,2	-	-
8.61	6-methyl -5-гептен -2-он	0,2	-	-
9.28	Benzaldehyde	0,9	5,2	1,2
9.54	5- methylfurfurol	1,2	6,2	1,9
9.66	Cis-2,4-heptadienal	0,3	-	0,8
10.46	Trans-2,4-heptadien	0,6	0,8	1,5
10.51	3,5-octadiene-2-ol	-	0,2	0,8
11.22	Trans-2-octenal	0,3	-	0,5
12.11	Cis-3,5-octadiene -2-on	1,2	4,8	1,3
13.04	Trans-3,5-octadiene -2-on	0,9	1,7	1,4
13.18	Dimethylcyclohexanol	1,2	4,3	-
13.56	6- methyl-3,5-heptadien-2-on	0,6	1,8	1,6
14.61	Trans-2-nonenal	0,8	23,6	1,3
15.50	Decanal	-	0,5	-
18.30	3,5,7- nonatriene -2-on	0,3	-	0,8
19.29	2,4-decadienal	0,5	1,4	0,9
27.98	Hexahydrofarnesylacetone	6,9	40,5	24,6
Heterocyclic compounds				
7.01	2,6-dimethylpyrazine	0,3	1,6	0,6
7.29	2,3-dimethylpyrazine	0,5	1,0	0,9
7.51	Acetylfuran	-	0,2	-
9.78	2,3,5-trimethylpyrazine	0,3	1,1	0,3

10.70	3-methoxypyridine	19,3	54,6	6,5
12.53	3-ethyl -2,5- dimethylpyrazine	0,3	-	-
12.61	2,3,5,6-tetramethylpyrazine	0,5	1,0	-
19.19	3-pentyl-furan -2-on	0,4	2,8	1,6
21.97	5-pentyl-furanone -2	1,6	4,4	2,4
22.35	Dihydro -5-pentyl-furanone-2	1,3	6,4	2,0
Fatty acids and their ethers				
8.55	Hexanoic acid	-	8,9	-
11.02	Heptanoic acid	-	5,1	-
17.99	Nonanoic acid	-	26,1	7,2
20.43	Decanoic acid	-	18,6	6,6
24.10	Dodecanoic acid	0,8	68,4	63,8
27.16	Tetradecanoic acid	2,3	29,0	38,2
28.52	Pentadecanoic acid	-	5,8	4,1
29.12	Methyl palmitate	0,7	9,1	2,9
31.47	Methyl oleate	-	5,2	5,9
31.58	Methyl linoleate	0,5	9,6	-
31.83	Methyl linolenate	0,6	7,1	-
32.17	Oleinic acid	0,8	-	-
32.31	Linolic acid	3,3	21,3	11,9
33.11	Isolinoloic acid	-	-	2,2
Terpenoids				
11.40	Trans-linalool oxide	-	1,0	-
11.55	Linalool	6,8	38,1	6,6
14.79	Camphor	1,7	4,2	2,6
14.93	Terpinene -4-ol	0,8	4,7	1,0
17.72	Verbenone	-	2,7	-
19.59	α-copaene	-	-	0,5
21.11	Piperitone	-	-	2,7
22.55	Geranylacetone	0,8	4,6	1,9
22.67	Germacrene D	-	-	1,3
Aromatic compounds				
13.39	2-methoxyphenol	0,3	1,4	-
20.85	2-methoxy-4-vinylphenol	3,5	19,4	3,7
21.60	Eugenol	10,4	61,9	12,2
27.47	Benzophenone	1,1	5,2	2,8
Unsaturated hydrocarbons				
23.58	β-ionone	1,9	9,5	3,0
23.76	β-ionone-5,6-epoxide	1,8	8,9	3,0
24.81	Heptadecene -8	6,5	57,2	30,6
38.69	Squalen	1,1	6,8	-

Note: «–» – substance's absence.