



ХРОМАТОГРАФІЧНИЙ АНАЛІЗ КАРБОНОВИХ КИСЛОТ У КВІТУЮЧИХ ПАГОНАХ ДЕЯКИХ ВИДІВ РОДУ *Vitex* L.

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CHROMATOGRAPHIC ANALYSIS OF CARBOXYLIC ACIDS IN THE FLOWERING SHOOTS OF SOME *Vitex* L. SPECIES

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Реферат

Вступ. Під *Vitex* L. налічує понад 200 видів чагарників і дерев, поширених у субтропічних регіонах земної кулі. В офіційній медицині застосовуються лише плоди вітексу священного (*Vitex agnus-castus* L.)

Метою нашого дослідження був газохроматографічний аналіз карбонових кислот у квітконосних пагонах трьох представників роду *Vitex* при їх культивуванні в Україні.

Матеріали та методи. Для аналізу складу та вмісту карбонових кислот у квітучих пагонах *Vitex agnus-castus* L., *Vitex negundo* L. та *Vitex negundo* var. *cannabifolia* (Siebold & Zucc.) Hand.-Mazz. використовували метод газової хроматографії з мас-спектрометрією (ГХ/МС).

Результати і обговорення. Найбільший вміст коротколанцюгових аліфатичних кислот (2454 мг/кг) виявлено у сировині *Vitex agnus-castus*. У пагонах усіх досліджуваних об'єктів домінували малі, щавлева, лимонна та яблучна кислоти. Найбільший вміст ароматичних кислот виявлено у *Vitex negundo* var. *cannabifolia* (3926 мг/кг). Серед ароматичних кислот у сировині всіх видів переважала *p*-гідроксибензойна кислота. Найбільш вагомий вміст ненасичених жирних кислот був у *Vitex negundo* var. *cannabifolia* (4793 мг/кг).

Abstract

Introduction. The genus *Vitex* L. counts more than 200 species of shrubs and trees common to subtropical areas of the Earth. Only fruits of *Vitex agnus-castus* L. are used in official medicine.

The **aim** of our study was to perform the chromatographic analysis of carboxylic acids in the flowering shoots of three *Vitex* representatives under their cultivation in Ukraine.

Materials and Methods. Gas chromatography with mass spectrometry (GC/MS) was applied for the analysis of composition and contents of carboxylic acids in the *Vitex agnus-castus* L., *Vitex negundo* L. and *Vitex negundo* var. *cannabifolia* (Siebold & Zucc.) Hand.-Mazz. flowering shoots.

Results and Discussion. The *Vitex agnus-castus* raw material contained the most significant amount of short-chained aliphatic acids (2454 mg/kg). Malonic, oxalic, citric, and malic acids noticeably prevailed in all the raw materials. The largest amount of aromatic acids was found in the *Vitex negundo* var. *cannabifolia* (3926 mg/kg) flowering shoots. *p*-Hydroxybenzoic acid dominated among the aromatic acids in the plant raw materials of all species. The most noticeable total content of unsaturated fatty acids was in the *Vitex negundo* var. *cannabifolia* (4793 mg/kg).

Conclusions. To summarize the contents of valuable

Висновки. Отримані результати дають змогу провести обґрунтування подальших фармакологічних досліджень рослинних субстанцій на основі квіткових пагонів трьох представників роду *Vitex*.

Introduction

The genus *Vitex* L. belongs to the Mint (*Lamiaceae* Martinov) family. It counts more than 200 species of woody plants which are common mostly to subtropical regions [1]. Some species of this genus are nowadays spreading in Ukraine due to their ornamental properties [2].

The fruits of *Vitex agnus-castus* L. known as *Agni casti fructus* are widely used in official medicine of many countries mainly for treating disorders of female reproductive system [1, 4]. Such valuable secondary metabolites of fruits as well as leaves, flowers or shoots of the *Vitex* representatives as terpenoids or phenolic compounds were within the scientific scope of many researchers [1, 2, 5, 6]. A lot of compounds of primary biosynthesis including carbohydrates, carboxylic acids, vitamins, amino acids also possess the valuable pharmacological properties [7, 8] and deserve considerable attention. There is a lack of scientific data on contents of carboxylic acids in the flowering shoots of the *Vitex* species.

Carboxylic acids are diverse in their structures as well as in pharmacological effects [7, 10]. Ferulic, benzoic, *p*-hydroxybenzoic, *p*-hydroxycinnamic, citric, vanillic, and succinic acids were recognized by many researchers as pharmacologically active substances [11, 15]. Polyunsaturated fatty acids are very useful for prevention of many health ailments such as atherosclerosis, neurodegeneration, etc. [9, 10]. The amount of them in the different plants can vary dependently on their genetic peculiarities, geographical origin, season of gathering, etc. [8, 16, 17].

The aim of this study was the analysis of carboxylic acids in three *Vitex* species (*Vitex negundo* L., *Vitex negundo* var. *cannabifolia*, and *Vitex agnus-castus*) to reveal the potential for their further use in medical practice.

Materials and Methods

The flowering shoots of *Vitex* sp. were gathered from the experimental plots located in M.

carboxylic acids, the obtained results provide the evidence for the development and further pharmacological study of herbal preparations from the flowering shoots of three *Vitex* species.

Gryshko National Botanical Garden (Kyiv). They were shade dried.

The preparation of raw materials for the (GC/MS analysis included adding the internal standard (50 µg of tridecane in hexane) and 1.0 mL of BC13 (14%) in methanol [18]. This mixture was kept in a vial at 65°C (for 8 h) for the extraction of lipids as well as for their hydrolysis and methylation.

Chromatograph Agilent Technologies 6890 N with 5973 N mass spectrometric detector was used in the study [8].

Results and Discussion

The amounts of carboxylic acids with short-chained molecular structure in the *Vitex* species are represented in Table 1. The GC/MS chromatograms are presented in Figures 1-3.

Analyzing the data in Table 1, it turned out that the total contents of short-chained aliphatic acids decreased in the following sequence: *Vitex*

Table 1
 Composition and contents of the short-chained carboxylic acids in the flowering shoots of some *Vitex* species, mg/kg

Acid	Retention time, min	<i>Vitex negundo</i>	<i>Vitex negundo</i> var. <i>cannabifolia</i>	<i>Vitex agnus-castus</i>
Oxalic	9.30	329	704	1064
3,3-dimethoxypropionic	9.55	16	42	52
Malonic	11.59	658	611	702
Fumaric	12.34	19	19	48
Furfurylic	13.02	30	39	29
Succinic	13.42	66	88	110
Benzoic*	13.91	10	—	—
Malic	21.68	277	349	144
Citric	29.01	289	556	305
Vanillic*	31.97	133	137	86
<i>p</i> -Hydroxybenzoic*	37.07	2706	2620	1217
<i>p</i> -Hydroxycinnamic*	38.94	584	1039	—
Ferulic*	39.80	230	130	64
Total amount of aliphatic acids		1684	2408	2454
Total amount of aromatic acids*		3663	3926	1367
Note: * – aromatic acid; "—" – compound was not detected				

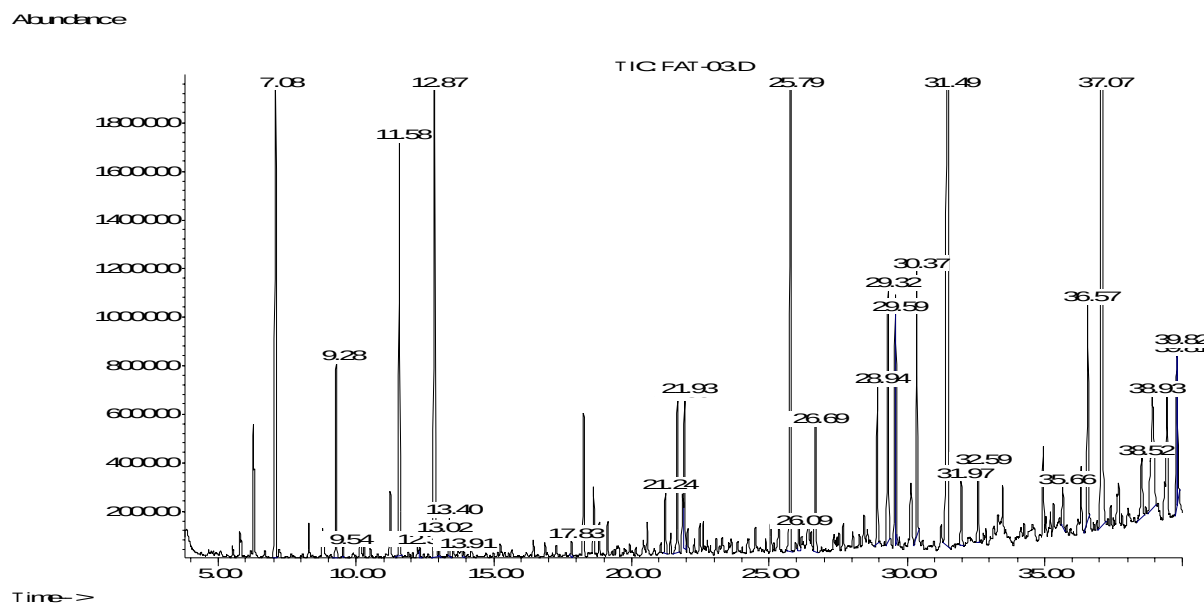


Figure 1
Typical GC/MS chromatogram of the carboxylic acids methyl esters in the *Vitex negundo* shoots

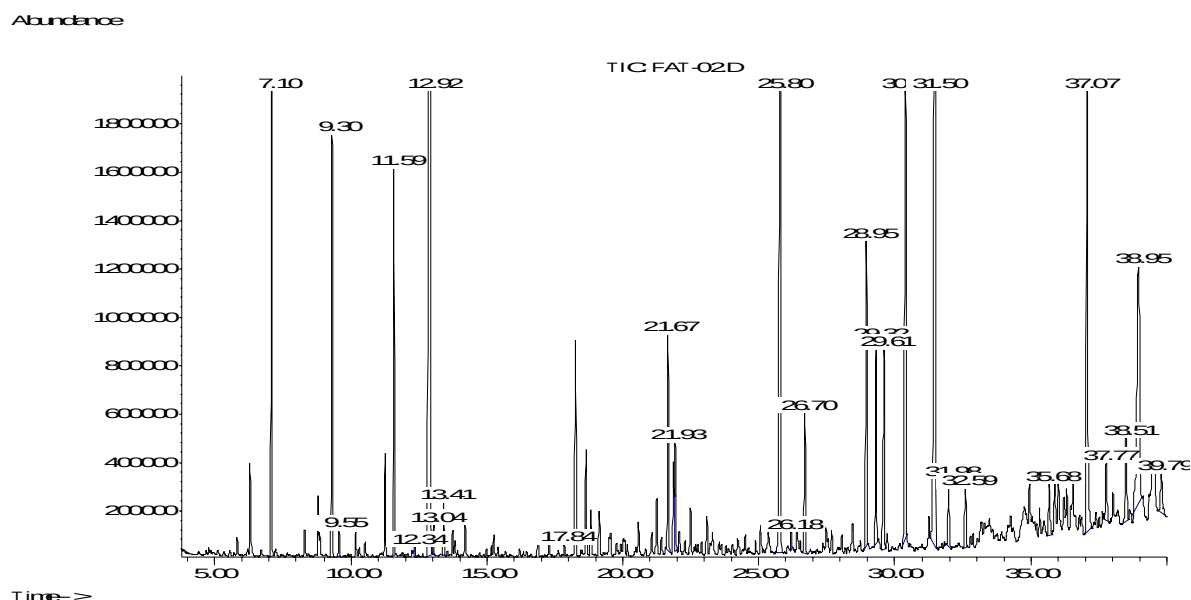


Figure 2
Typical GC/MS chromatogram of the carboxylic acids methyl esters in the *Vitex negundo* var. *cannabifolia* shoots

agnus-castus (2454 mg/kg) > *Vitex negundo* var. *cannabifolia* (2408 mg/kg) > *Vitex negundo* (1684 mg/kg). Such compounds as malonic, oxalic, citric, and malic acids prevailed in all studied species.

Taking into account the high pharmacological potential of major revealed aromatic acids (Figure 4), it should be mentioned that their total contents decreased in the following sequence: *Vitex negundo* var. *cannabifolia* (3926 mg/kg) > *Vitex negundo* (3663 mg/kg) > *Vitex agnus-castus* (1367 mg/kg). The raw material of *Vitex negundo* was

characterized by the largest accumulation of *p*-hydroxybenzoic acid (2706 mg/kg), whilst *p*-hydroxycinnamic acid (1039 mg/kg) dominated in *Vitex negundo* var. *cannabifolia*). The highest content of ferulic acid (230 mg/kg) was found in *Vitex negundo*. In comparison to *Vitex negundo* and *Vitex negundo* var. *cannabifolia* that accumulated 133 mg/kg and 137 mg/kg of vanillic acid, respectively, *Vitex agnus-castus* had 86 mg/kg of this component. Besides it, small amount of benzoic acid (10 mg/kg) was revealed only in the *Vitex negundo* raw material.

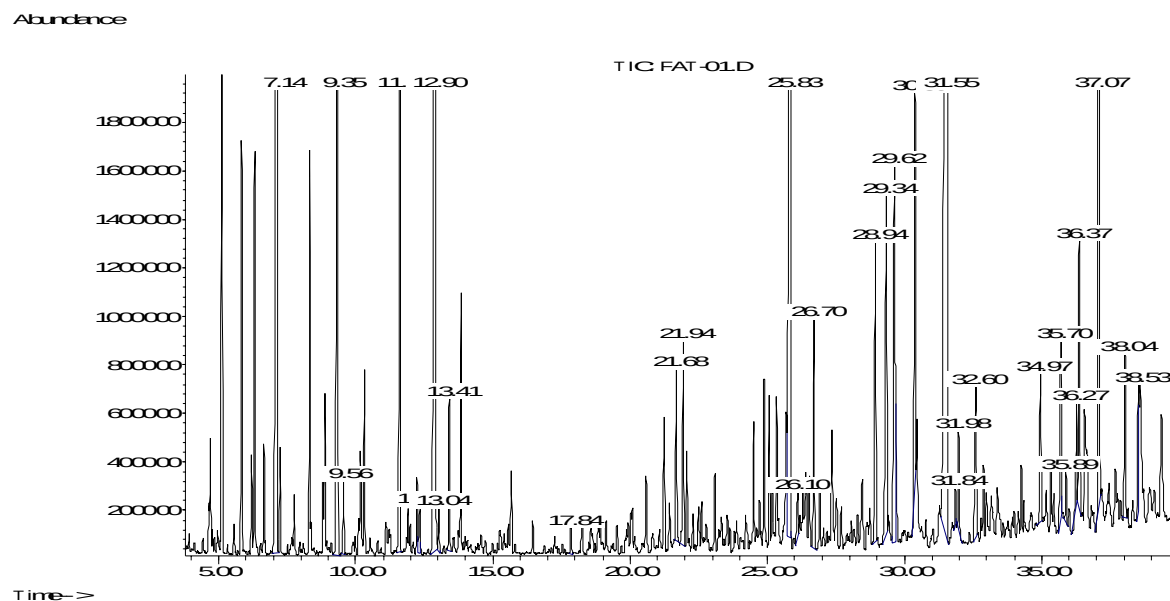


Figure 3

Typical GC/MS chromatogram of the carboxylic acids methyl esters in the *Vitex agnus-castus* shoots

It should be noted that identified aromatic acids are often used in pharmacy as well as in food and perfume manufacturing. For instance, *p*-hydroxycinnamic acid is used due its antioxidant and anticancer effects [14]. Michels et al. [13] described the prominent nootropic activity of ferulic acid. Liu J et al. [11] found that *p*-hydroxybenzoic acid has the valuable antibacterial, antifungal and antioxidant properties [11]. It was revealed by Mao et al. [12] that benzoic acid effectively regulate the functions of intestinal tract and could be used as food and feed additive. Vanillic acid can alleviate the oxidative stress induced by peroxide [15].

As can be concluded from Table 2, the

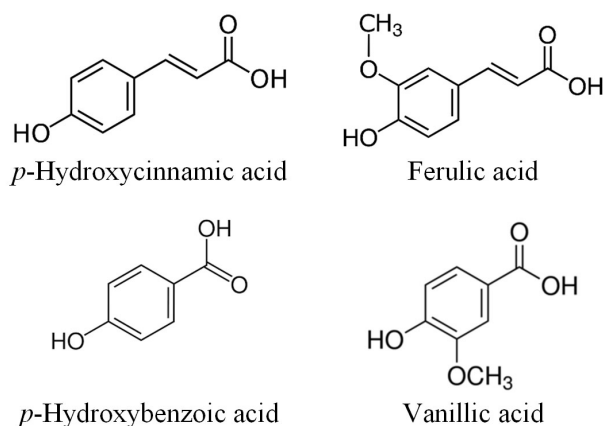


Figure 4

Structural formulas of the main aromatic carboxylic acids detected in the *Vitex* species

most profound content (4793 mg/kg) of unsaturated fatty acids was in *Vitex negundo* var. *cannabifolia*. The unsaturated omega-3 α -linolenic acid prevailed in all investigated species. The most significant amounts of linoleic (973 mg/kg) and oleic (531 mg/kg) acids were in the flowering shoots of *Vitex negundo* var. *cannabifolia*. In brief, the detected polyunsaturated acids may play an important role in the prevention

Table 2

Composition and contents of the long-chained carboxylic (fatty) acids in the flowering shoots of some *Vitex* species, mg/kg

Acid	Retention time, min	<i>Vitex negundo</i>	<i>Vitex negundo</i> var. <i>cannabifolia</i>	<i>Vitex agnus-castus</i>
Laurinic	17.84	27	20	24
Myristic	21.92	282	195	184
Palmitic	25.80	1435	1738	1289
Palmitoleic*	26.18	51	29	54
7-Hexadecenic*	26.69	272	291	228
Stearinic	29.31	550	429	373
Oleic*	29.59	320	531	490
Linoleic*	30.37	523	973	369
α -Linolenic*	31.50	2995	2969	2480
Arachidic	32.59	170	135	187
Behenic	35.66	95	149	234
Tetracosanic	38.52	166	225	142
Total amount of unsaturated fatty acids†		4161	4793	3621
Total amount of saturated fatty acids		2725	2891	2403

Note: * – unsaturated fatty acid

of atherosclerosis due to the ability to normalize the cell membrane structures [19, 20].

Conclusions

The varieties of carboxylic acids in three *Vitex* representatives were studied using GC/MS. The obtained results provide the evidence for further pharmacological studies of herbal preparations on the basis of studied plant raw materials.

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