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GAS-CHROMATOGRAPHIC DETERMINATION OF VOLATILE COMPOUNDS FROM THE FLOWERING SHOOTS OF SOME *VITEX* L. REPRESENTATIVES

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Abstract

Essential oils isolated from medicinal plants are the rich source for developing new herbal medicinal products. The genus *Vitex* L. (*Lamiaceae* Martinov family) contains more than 200 species of essential-oil bearing plants. The gas-chromatographic determination of volatiles from the flowering shoots of three *Vitex* representatives (*V. agnus-castus, V. negundo* L. and *V. negundo var. cannabifolia* (Siebold & Zucc.) Hand.-Mazz.) was conducted using chromatograph Agilent Technologies 6890 with mass spectrometric detector 5973. The predominant components of *V. agnus-castus* essential oil were eucalyptol (17.36%), sabinene (7.74%), *β*-farnesene (7.17%), *α*-terpineole (6.94%), bicyclogermacrene (5.04%) and *α*-pinene (4.75%). *β*-Caryophyllene (38.84%), germacrene D (7.25%), *β*-bisabolene (5.25%) and *α*-caryophyllene (3.20%) were the major compounds of *V. negundo* essential oil. *β*-Caryophyllene (36.33%), caryophyllene oxide (5.19%) and *α*-caryophyllene (3.07%) dominated in the *V. negundo var. cannabifolia*. Due to the analysis of literature data regarding the properties of predominant components of the studied essential oils, it was supposed that t

hey are promising sources of new medicines with antimicrobial, antifungal, anti-inflammatory, analgesic, antioxidant, insecticidal, and immunomodulating properties.

Keywords: genus *Vitex*, chaste-tree, flowering shoots, essential oil, gas chromatography mass spectrometry

Introduction

Essential oils isolated from medicinal plants are the rich sources for developing new herbal medicinal products which could be alternatives to synthetic drugs for treating many ailments such as infectious diseases, chronic inflammation, cancer, anxious, etc. [1–4]. As resistance to antibiotics is a serious problem to human health worldwide, essential oils of plants are regarded as promising antimicrobial agents [4–6].

The genus *Vitex* L. (*Lamiaceae* Martinov family) contains more than 200 species of essential-oil bearing plants which are spread mainly in the Mediterranean region and Asia [7, 8]. They belong to the subfamily *Premnoideae* Bo Li, Olmstead & P.D.Cantino of the *Lamiaceae* family [9]. *Vitex* is one of several genera that were transferred from *Verbenaceae* J.St.-Hil. to *Lamiaceae* in the 1990s [9].

The species of this genus are deciduous shrubs with opposite palmate-compound leaves. They accumulate the diverse active constituents and have valuable medicinal properties. The fruits of *V. agnus-castus* L. are used in the officinal medicine for treating disorders of female reproductive systems such as menstrual irregularities, menopausal symptoms, cyclic breast pain, etc. [8, 10]. Common name 'chaste tree' traditionally referring to *V. agnus-castus* but is also applied to other species as well.

Besides fruits, scientists investigated the leaves, flowers, shoots of the *Vitex* species growing in the different regions of the world [11–14]. We supposed that phytochemical evaluation of essential oils from the flowering shoots of three *Vitex* representatives (*V. agnus-castus, V. negundo* L. and *V. negundo var. cannabifolia* (Siebold & Zucc.) Hand.-Mazz.) is a quite perspective.

Methods

Plant material and sample preparation

The plant materials were harvested from the shrubs growing on the experimental plots of M.M. Gryshko National Botanical Garden (Ukraine). The essential oils were obtained from the dried flowering shoots by the hydrodistillation.

Chromatographic analysis

Gas chromatograph Agilent Technologies 6890 equipped with mass spectrometric detector 5973 was used for the study of volatile compounds. The chromatographic column was DB-5 capillary (0.25 mm x 30 m). The injection was carried out in the splitless mode (rate 1.2 mL/min during 0.2 min). The flow rate of helium was 1.2 mL/min. The temperature of thermostat was programmed up to 320°C (with a speed of 4 °C/min).

The mass spectra libraries (NIST05 and WILEY 2007) used for the identification of components. The method of internal standard was applied for the quantitative analysis of the contents of volatile compounds.

Results and Discussion

The chemical compositions of the essential oils from the flowering shoots of three *Vitex* representatives were determined by GC-MS (Tables 1, Figures 1-6). The components which content exceeded 0.3% (at minimum in one species) are shown in Table 1.

The predominant components of *V. agnus-castus* essential oil were eucalyptol (17.36%), sabinene (7.74%), β-farnesene (7.17%), α-terpineole (6.94%), bicyclogermacrene (5.04%) and α-pinene (4.75%) (Figures 1, 2). β-Caryophyllene (38.84%), germacrene D (7.25%), β-bisabolene (5.25%) and α-caryophyllene (3.20%) were the major compounds of *V. negundo* essential oil (Figures 3, 4). β-Caryophyllene (36.33%), caryophyllene oxide (5.19%) and α-caryophyllene (humulene) (3.07%) dominated in the *V. negundo var. cannabifolia* (Figures 5, 6). Regarding the specific marker components of each essential oil, ledol and epi-α-cadinol were revealed only in *V. agnus-castus,* and β-bisabolene only in *V. negundo.* Nerolidole, α-selinene and β-selinene were the chemical markers of *V. negundo var. cannabifolia.*

Ulukanli et al. [15] found that essential oil of East-Mediterranean *V. agnus-castus* was also rich in 1,8-cineole (24.38%) and sabinene (22.77%), similarly to the studied *V. agnus-castus* which was cultivated in Ukraine. The main compounds in the essential oil of Serbian *V. agnus-castus* fruits were also 1,8-cineole (16.3%) and sabinene (13.4%) [12]. The quantitative significant components of the essential oil obtained from the Turkish *V. agnus-castus* fruits were 1,8-cineole (24.98%), sabinene (13.45%), and α-pinene (10.60%) [13]. The main volatiles from the Italian *V. agnus-castus* leaves, flowers and fruits obtained using supercritical extraction with CO_2 were α-pinene, sabinene, 1,8-cineole, α-terpinyl acetate, (E)-

caryophyllene, (E)-beta-farnesene, and bicyclogermacrene [16]. Riham et al. [17] found that oxygenated monoterpenes (44.98%) were the major class of the volatiles presented in the Egyptian *V. agnus-castus* essential oil. Yushchyshena et al. [14] found that sesquiterpenoids dominated in the essential oils obtained from the stems, leaves and flowers of *V. agnus-castus* and *V. cannabifolia* harvested in Ukrain.

As it is known, essential oils of plants are very complex mixtures of volatiles with broad-spectrum pharmacological properties [4, 18–20]. It should be mentioned that predominant compounds found in the investigated *Vitex* species possess the proven therapeutic properties. Thus, oxygenated monoterpenoid 1,8-cineole (also known as eucalyptol), the predominant component of studied *V. agnus-castus* essential oil, showed the noticeable pharmacological potential including antibacterial, anti-inflammatory and antioxidant effect, and used mainly for the treatment of respiratory diseases [21]. Fidyt et al. [22] reported that bicyclic sesquiterpene *β*-caryophyllene possesses the antimicrobial, anticancer and analgesic properties. Molecular docking study found that caryophyllene found in the *V. agnus-cactus* essential oil has a powerful anticandidal property [17]. It was found that *β*-caryophyllene is a ligand of the cannabinoid receptor 2 which activates the decrease of pain, a main signal for inflammatory responses. *β*-Caryophyllene enhanced the wound healing through multiple pathways [23, 24]. Woo et al. [25] found the inhibitory effect of *β*-caryophyllene on *Helicobacter pylori* growth. *β*-Caryophyllene can also potentiate the anticancer activity of some drugs [26]. Humulene, also known as *α*-humulene or *α*-caryophyllene, is a naturally occurring monocyclic sesquiterpene with anti-inflammatory effects [27].

The major constituents of the essential oil obtained from the Brazilian *V. agnus-castus* leaves which displayed the activity against some oral bcterial pathogens (*Streptococcus mutans, Lactobacillus casei* and *Streptococcus mitis*), were 1,8-cineole (23.8%), (E)-β-farnesene (14.6%), (E)-caryophyllene (12.5%), and sabinene (11.4%) [28]. Essential oil extracted from the fruits of *V. agnus-castus* contained 1,8-cineole (19.61%), sabinene (14.57%), and *α*-pinene (9.76%) as the major components [29]. This essential oil showed the antioxidant and the anti-*Candid*a actions [29]. Researchers suppose that antimicrobial properties of essential oils are due the synergistic effects of their different components [4, 30, 31]. Pereira t al. [31].revealed that essential oil from the *V. gardneriana* leaves possessed the synergistic effects with the antibiotics (ampicillin, gentamicin, and ofloxacin) against *Escherichia coli* and *Staphylococcus aureus* bacterial resistance.

The essential oil of Brazilian *V. agnus-castus* leaves containing 1,8-cineole (17.6%) and *β*-(E)-farnesene (13.6%) as the major components possessed the significant acaricidal effect against *Tetranychus urticae* [32]. The essential oil of Greek *V. agnus-castus* demonstrated the moderate nematicidal activity [33]. The larvicidal effect against *Aedes aegypti* and cytotoxic activite against some carcinoma cell demonstrated the essential oils obtained from the fresh leaves of *V. agnus-castus* [11]. It contained 1,8-cineole (47.9%), terpinyl α-acetate (11.6%), and sabinene (11.2%) as the major compounds [11]. The antioxidant, antimicrobial and larvicidal activities against *Aedes aegypti* were found for the nanoemulsion from *V. negundo* essential oil [34].

Essential oil from the Turkish *V. agnus-castus* leaves was evaluated as promising candidate in the development of new medicines for the treatment of multi-drug resistant lung cancer [20]. Scientists are intensively searching for the plants' insecticides and herbicides which can control of agricultural pests because the considerable amount of grain is lost because of insects. The essential oil of Brazilian *V. agnus-castus* containing 1,8-cineol (23.8%) as a major component demonstrated the significant fumigant potential [35].

Schepetkin et al. [36] revealed that essential oil isolated from the *Hypericum perforatum* leaves contained germacrene D (25.7%) and *β*-caryophyllene (9.5%) as predominant compounds, similarly to the studied *V. negundo* essential oil. Schepetkin et al. [36] found the significant immunotherapeutic properties of the *Hypericum perforatum* essential oils.

Regarding the properties of the other predominant components of the studied *Vitex* species, bicyclic monoterpene sabinene is used mainly as flavoring and perfume additive [37]. Acyclic sesquiterpene farnesene is the natural pharmaceutic agent, fragrance and solvent [38]. Other terpenoids from the plant raw materials of the *Lamiaceae* representatives are also very perspective for further study [39]. Beside it, using the cultivated medicinal plants wahich are grown under the controlled conditions is a very promising direction of pharmacogostical investigations [40].

Thus, the studied raw materials and essential oils from the *Vitex* species could be regarded as promising sources of new medicines with antibacterial, antifungal, analgesic, anti-inflammatory, antioxidant, insecticidal, and immunomodulating properties.

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Table 1. The chemical compositions of main volatile compounds from the flowering shoots of the *Vitex* representatives determined by GC-MS, %

RT, min	Compound	*Vitex agnus-castus*	*Vitex negundo*	*Vitex negundo var. cannabifolia*
4.66	α-Pinene	4.75	0.20	0.16
6.08	Sabinene	7.74	2.12	2.91
6.36	Myrcene	2.34	0.17	0.21
7.62	Limonene	3.67	0.48	0.40
7.92	β-Phellandrene	3.53	0.92	0.92
8.52	1,8-Cineole (Eucalyptol)	17.36	0.91	0.65
9.53	*trans*-Sabinene hydrate	0.92	1.26	0.89
10.6	Linalool	2.53	1.20	3.09
13.92	Terpinene-4-ol	1.85	2.84	1.42
16.75	δ-elemene	-	0.83	1.67
14.86	α-Terpineole	6.94	0.40	1.42
18.92	β-Elemene	-	-	2.48
19.27	α-Terpinil acetate	3.32	0.88	0.33
20.15	β-Caryophyllene	3.12	38.84	36.33
20.6	β-Farnesene	7.17	0.30	-
20.9	α-Caryophyllene (Humulene)	-	3.20	3.07
21.04	Aromadendrene	1.88	-	0.25
21.56	Germacrene D	0.36	7.25	2.95
21.68	β-selinene	-	-	1.07
21.8	α-selinene	-	-	1.29
21.77	β-Bisabolene	-	5.25	-
22.14	Bicyclogermacrene	5.04	2.20	1.54
24.41	Caryophyllene oxide	-	3.83	5.19
24.5	Ledol	2.00	-	-
24.67	Nerolidol	-	-	1.36
25.23	*epi*-α-Cadinol	1.31	-	-
25.40	Caryophylla-4(12),8(13)-diene-5-β-ol	-	1.74	2.22

Note: "-" – compound was not detected.

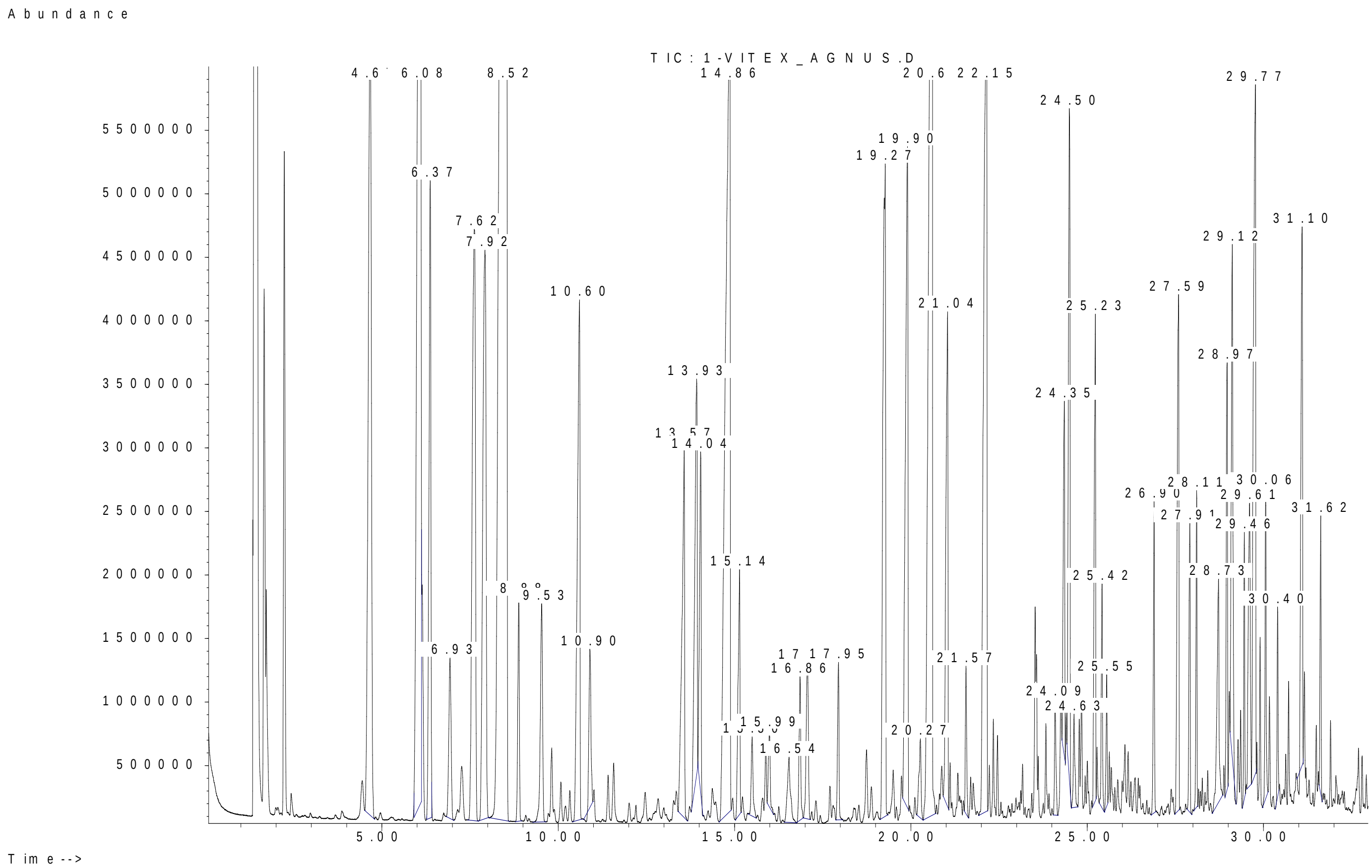
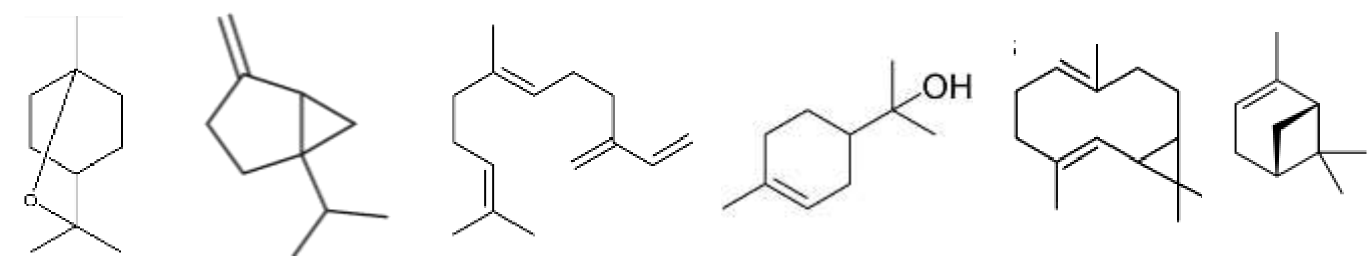


Figure 1. Typical GC-MS chromatogram of the volatiles from the *Vitex agnus-castus* flowering shoots



1,8-Cineole 17.36 %

Sabinene 7.74 %

β-Farnesene 7.17 %

α-Terpineole 6.94 %

Bicyclogerma crene 5.04 %

α-Pinene 4.75 %

Figure 2. Structural formulas and contents of the main volatile terpenoids from the flowering shoots of *Vitex agnus-castus*

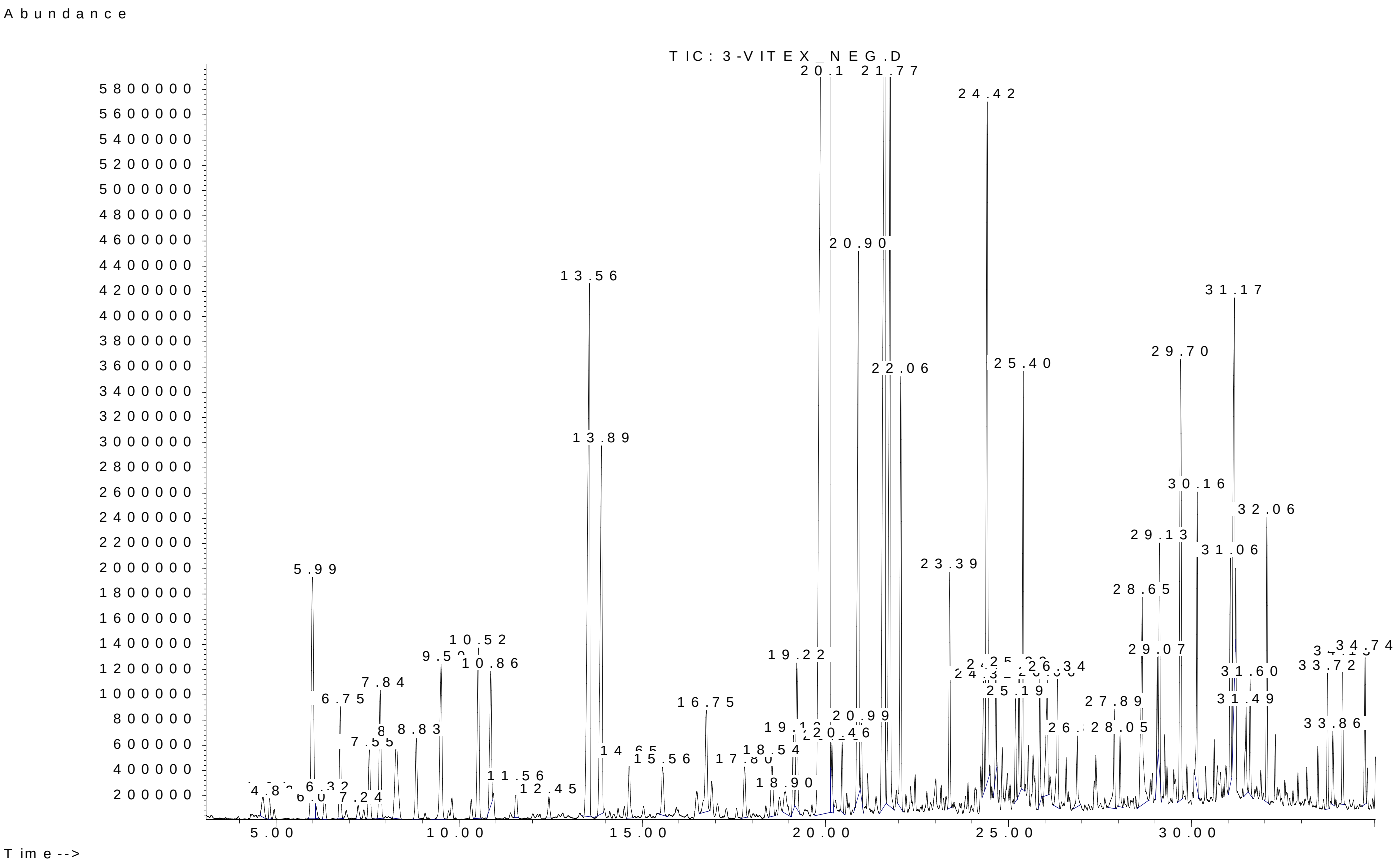
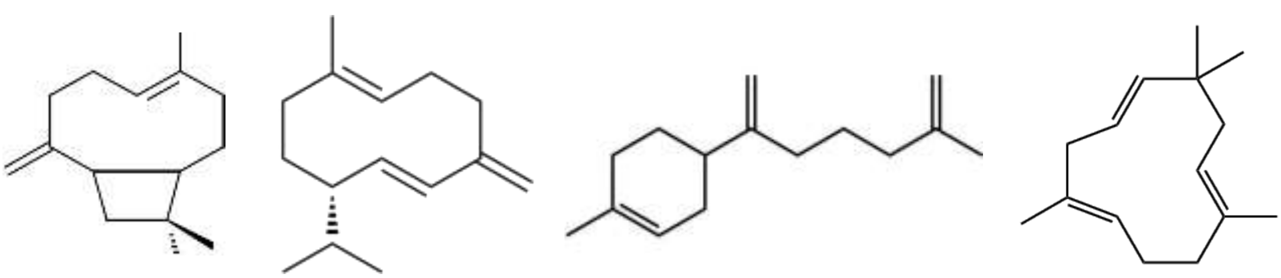


Figure 3. Typical GC-MS chromatogram of the volatiles from the *Vitex negundo* flowering shoots



β-Caryophyllene 38.84 %   Germacrene D 7.25 %   *β*-Bisabolene 5.25 %   *α*-Caryophyllene 3.20 %

Figure 4. Structural formulas and contents of the main volatile terpenoids from the flowering shoots of *Vitex negundo*

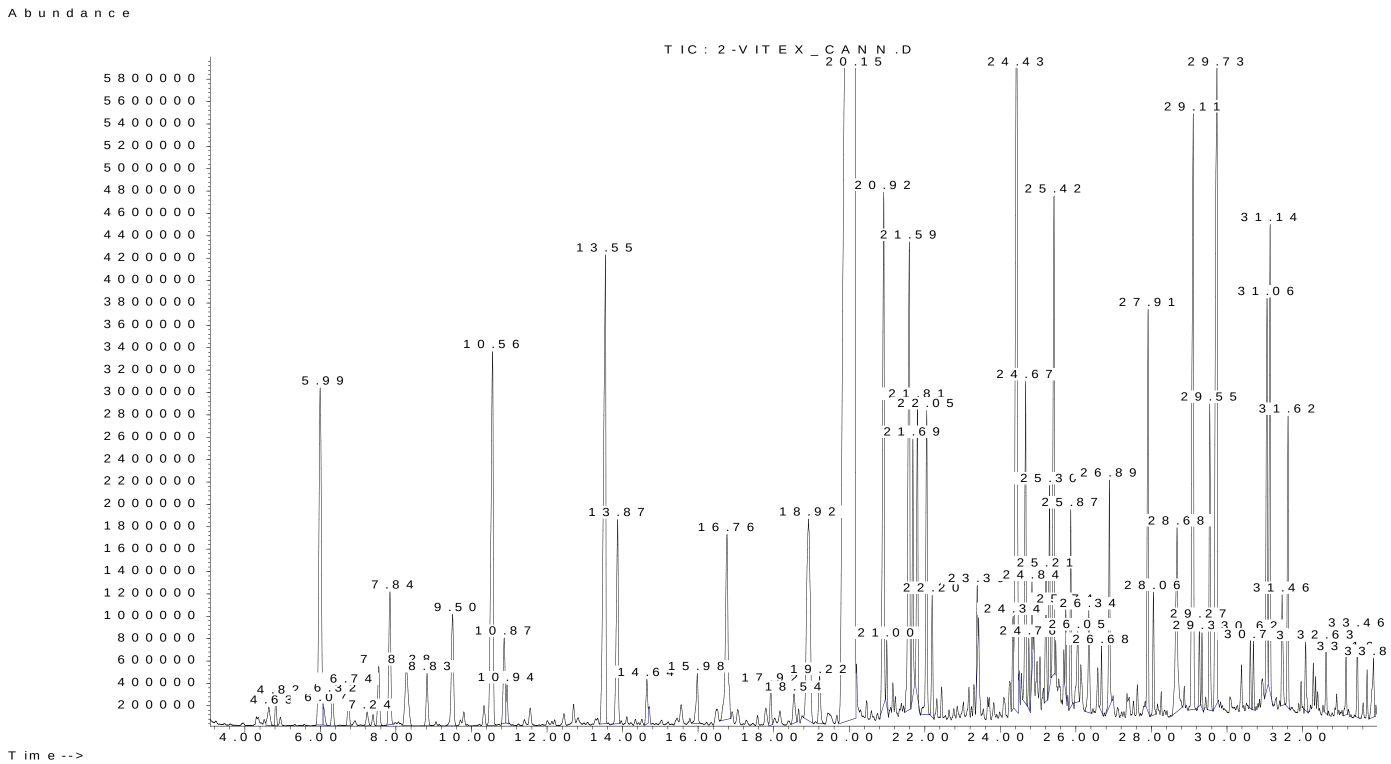
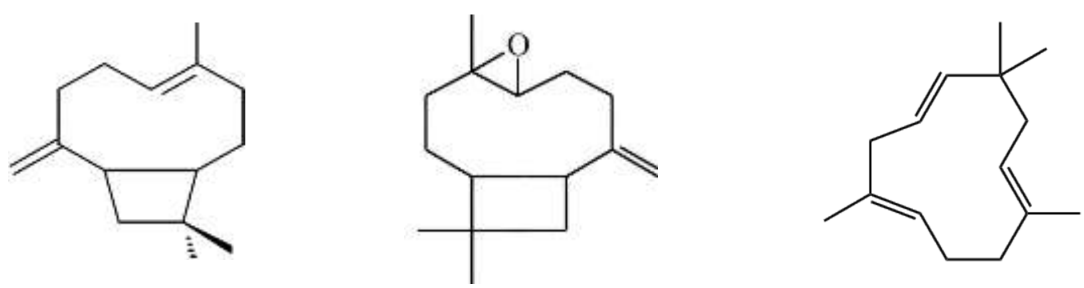


Figure 5. Typical GC-MS chromatogram of the volatiles from the *Vitex negundo var. cannabifolia* flowering shoots



β-Caryophyllene 38.84 %

Caryophyllene oxide 5.19 %

α-Caryophyllene 3.07 %

Figure 6. Structural formulas and contents of the main volatile terpenoids from the flowering shoots of *Vitex negundo var. cannabifolia*