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Molecular characteristics of drug activities in *Vitex negundo* woody extractives

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The molecular characteristics of stem extractives from *Vitex negundo* were studied to be known and further utilize the drug resources. Fresh *Vitex negundo* stems were pound to pieces and extracted by single extraction and sequential extraction. And then the extractives were obtained and analyzed by GC/MS. The leaching rule of stem extractives from *Vitex negundo* was obvious. What's more, the stem extractives contained drug activities, such as eicosane, 3,3,7,11-tetramethyltricyclo [5.4.0.0(4,11)] undecan-1-ol, etc. The stem extractives of *Vitex negundo* could be used to prepare the rich and rare drug activities.

Keywords: Drug activities, *Vitex negundo*, Stem extractives, Molecular, GC-MS

INTRODUCTION

Vitex negundo, which was a deciduous shrub, was widely distributed in North China. It was often born in the mountain slope to form dense thickets and abundant resources. [1, 2] And *Vitex negundo* was used as drugs for a long time. *Vitex negundo* real was bitter, warm and non-toxic; the leaf was bitter, cold and non-toxic; the root was sweet, bitter and non-toxic; the stem was sweet, flat and non-toxic. *Vitex negundo* as traditional Chinese medicine was recorded in the Pharmacopoeias such as "Herbal classic variorum", "Materials Science", "Qian Jin Fang", and so on. [3] *Vitex negundo* could attend the symptoms including leucorrhea, small intestine hernia, wet phlegm turbid urine and deafness. *Vitex negundo* leaf could be used to heal the nine orifices bleeding, piss hematuria; stems could do burning boil heat and wind toothache. [4, 5]. So *Vitex negundo* had the drug effect.

Vitex negundo had antiasthmatic, antitussive, expectorant effect, on cardiovascular function, blood pressure lowering effect, and other effects [3]. However, the researches on the active ingredients in Vitex negundo were less done, and concentrated in seed. For example, β–caryophyllene, caryophyllene oxide, β-elemene, a-pinene, P-cymene, 1,8-cineole, vanillic acid, alkaloids, and amino acid were found in the volatile oil of Vitex negundo seed. Peng et al. had studied the molecular identification methods, many woody extractives and biomass' chemical composition [7-21]. The researches were very effective and had great reference value. Therefore, the molecular characteristics of the stem extractives in *Vitex negundo* were investigated and analyzed by the optimized extraction techniques so as to further utilize the drug resources.

MATERIALS AND METHODS

Materials

Fresh *Vitex negundo* stems were collected from the Tongbai Mountain, Henan Province, China. The fresh stems were pound to pieces and kept in vacuum. Acetic ether, methanol, benzene, petroleum ether and ethanol were chromatographic grade, preparing for the experiments. Cotton thread and bag were both done in benzene/ethanol extraction for 12 h..

Experimental methods

Single extraction. Weighed 54 pieces of *Vitex negundo* stems, each was about 20 g (0.1 mg accuracy) and finally parceled into the cotton bag and then tied by cotton thread, and signed. Extraction was carried out in 350 ml solvents by the Foss method for 1, 3, 4, 5, 6, 7 hours. Ethanol/ methanol ($V_{ethanol}/V_{methanol}$=2), petroleum ether/acetic ether($V_{petroleum\ ether}/V_{acetic\ ether}$ = 2) , and benzene/ethanol ($V_{ethanol}/V_{benzene}$= 2) extraction were done at the temperature of 75°C, 90°C and 95°C, respectively. After extraction, the one portion was dried in 105°C to oven dry, and weighed. The extractives were obtained by evaporation in 60 - 70°C.

Sequential extraction. Weighed 27 pieces of stems, each was 20 g (1.0 mg accuracy), and finally parceled into cotton bag tied by cotton thread, and signed. Three-step extractions were carried out by large-caliber Soxhletor by ethanol/methanol →

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petroleum ether/acetic ether → benzene/ethanol (JY-YS-BC), petroleum ether/acetic ether → benzene/ethanol → ethanol/ methanol (YS-BC-JY), benzene/ethanol → ethanol/methanol → petroleum ether/acetic ether (BC-JY-YS), respectively. After every step extraction, the one portion was dried in 105°C to oven dry, and weighed. The extractives were obtained by evaporation in 60°C - 70°C.

GC/MS condition. Among the above extractives, the JY extractives in JY-YS-BC extraction, BC extractives in BC-JY-YS extraction, jyYS extractives in JY-YS-BC extraction, and bcJY extractives in BC-JY-YS extraction were analyzed, respectively. Each 0.5 mg extractives was analyzed by online linked GC/MS, respectively. The GC/MS analysis was carried out as the same as the documents [13, 14, 15, 16].

RESULTS AND DISSCUSION

The leaching rates of single extraction and three-step extraction were listed in Table 1 and Table 2, respectively. And the JY, BC, jyYS and bcJY extractives were obtained. The total ion chromatograms of four extractives by GC/MS were shown in Figure 1, respectively.

Leaching rule of stem extractives of Vitex negundo

The trend of leaching rates of stem extractives from *Vitex negundo* in different solvents was described in Table 2. It was observed that during ethanol/methanol extraction, the leaching rate of stem extractives fluctuated, and reached the maximum (9.79%) when extraction time was 5 h.

During petroleum ether/acetic ether extraction, the leaching rate of stem extractives first increased and then decreased, and reached the maximum (3.70%) when extraction time was 4 h. During benzene/alcohol extraction, the leaching rate of stem extractives fluctuated, and reached the maximum (5.19%) when extraction time was 7 h. And the optimal extraction time of ethanol/methanol extraction, petroleum ether/acetic ether extraction, and benzene/alcohol extraction were 5 h, 4 h, and 7 h, respectively.

During the three-step extractions, the ethanol/methanol extraction, petroleum ether/acetic ether extraction, and benzene/alcohol extraction were done for 5 h, 4 h, and 7 h, respectively. The statistical results showed that the leaching rate of *Vitex negundo* stem extractives by JY-YS-BC extraction was 28.42%, 24.23% by YS-BC-JY extraction, and 28.22% by BC-JY-YS extraction. Comparing with table 2, it was observed that the leaching rate of each single extraction was less than that of table 1, suggesting that the solvent effect also existed in the leaching periods of stem extractives. During the three-step extractions, JY-YS-BC extraction was the optimum extraction mode for the leaching rate was 28.42%.

Molecular properties of Vitex negundo stem extractives

Analyzing the MS data, the NIST standard MS map by computer, open-published books and papers, then components and their contents were identified.

According to GC/MS result, 7 components were identified from JY extractives of *Vitex negundo* stem. The result showed that the main components were cyclotrisiloxane, hexamethyl- (36.588%), 3,3,7,11-tetramethyltricyclo [5.4.0.0(4,11)] undecan-1-ol (34.642%), 1,2-benzenedi -carboxylic acid, butyl 2-methylpropyl ester (10.074%), silicic acid, diethyl bis (trimethylsilyl) ester (8.153%), hexanedioic acid, bis (2-ethylhexyl) ester (2.456%), eicosane (2.111%), 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester (5.977%).

Table 1. Leaching rates of each single extraction [%].

Extraction time [h]	ethanol/methanol	petroleum ether/acetic ether	benzene/ethanol
1	5.53	1.20	0.90
3	8.63	1.22	3.21
4	7.16	3.70	4.23
5	9.79	3.44	3.84
6	7.01	3.51	3.48
7	7.22	1.02	5.19

Table 2. Leaching rates of three-step extractions.

Extraction	JY-YS-BC			YS-BC-JY			BC-JY-YS		
Time [h]	4	7	5	7	5	4	5	4	7
Step	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Leaching rate [%]	9.22	7.52	11.68	5.99	7.56	10.68	9.81	9.52	8.89

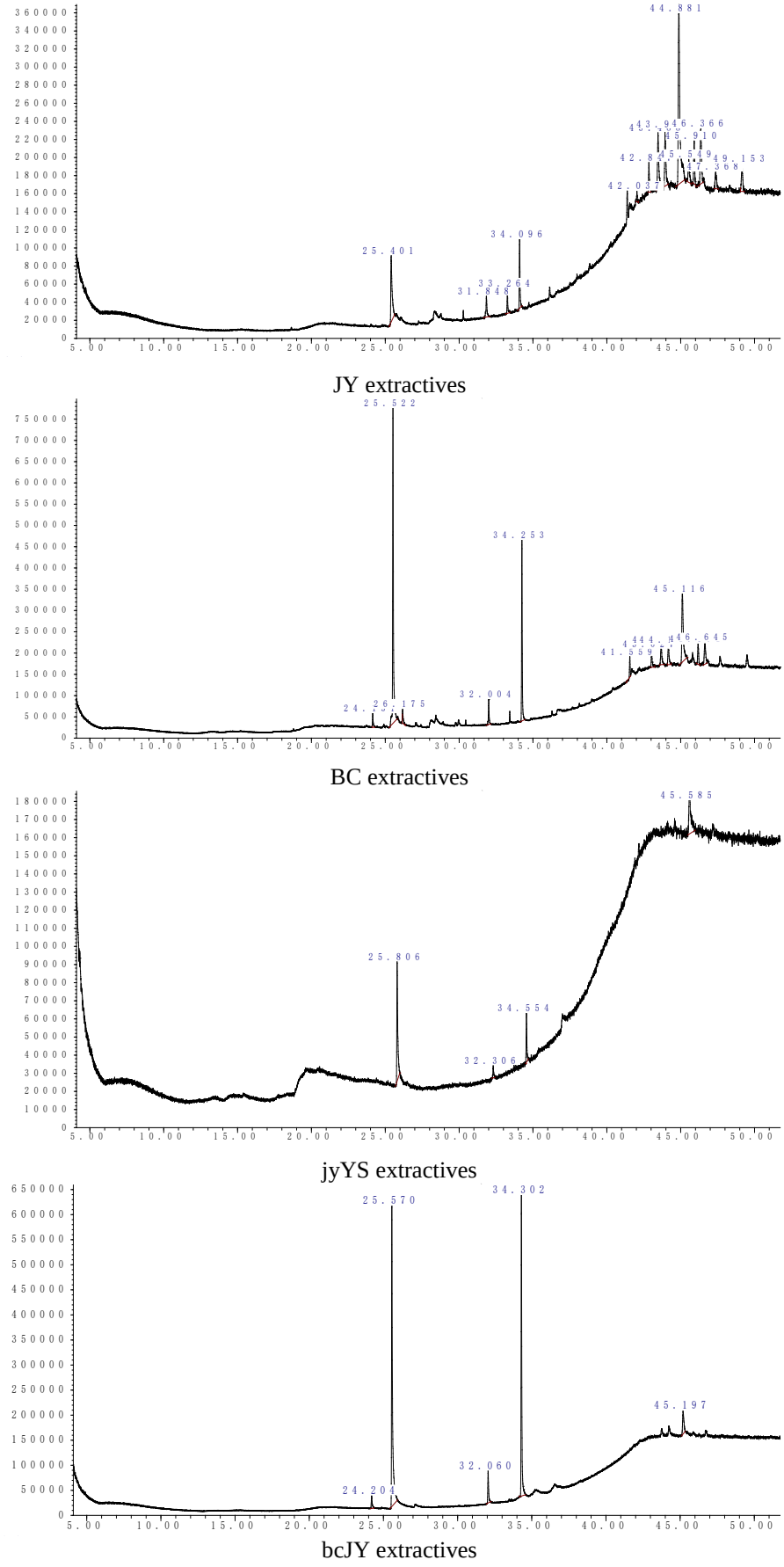

Fig. 1. Total ion chromatogram of four extractives of *Vitex negundo* stems by GC/MS

The 7 components were identified from BC extractives of *Vitex negundo* stem. The result showed that the main components were dibutyl phthalate (40.003%), 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (18.803%), cyclotrisiloxane, hexamethyl- (19.351%), 1,1,1,3,5, 5,5- heptamethyl-trisiloxane (15.402%), hexanedioic acid, bis (2-ethylhexyl) ester (2.965%), phthalic acid, isobutyl nonyl ester (1.58%), benzo [g] pteridine, 2,4-diamino-6,7,8,9-tetrahydro -7-methyl- (1.895%).

The 4 components were identified from jyYS extractives of *Vitex negundo* stem. The result showed that the main components were dibutyl phthalate (45.37%), cyclotrisiloxane, hexamethyl- (35.713%), cyclohexanecarboxylic acid, dodecyl ester (4.003%), 1,2-benzene -dicarboxylic acid, mono (2-ethylhexyl) ester (14.914%).

The 5 components were identified from bcJY extractives of Vitex negundo stem. The result showed that the main components were dibutyl phthalate (49.917%), 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (38.537%), 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester (2.037%), silicic acid, diethyl bis (trimethylsilyl) ester (5.075%), hexanedioic acid, bis (2-ethylhexyl) ester (4.435%).

Resource Properties of Stem Extractives of Vitex Negundo

There were many biomedical components in the stem extractives of *Vitex negundo*. Because of their public role and value, dibutyl phthalate was a pesticide to keep the environment homeostasis. [15, 16] 1, 2-benzenedicarboxylic acid, butyl 2-methylpropyl ester was hydrolyzed to 1,2-benzenedicarboxylic acid which could be used to make some drugs.

Hexanedioic acid, bis (2 -ethylhexyl) ester could be used in various products such as cosmetics, auto interior protectant, and lubricant (www.chemicalsubstances.gc.ca). Eicosane was the main compositions of rose essential oil which had the remarkable beauty effect and health care efficacy [22]. 3,3,7,11-Tetramethyltricyclo[5.4.0.0(4,11)] undecan-1-ol was the significant alcohol of volatile compositions in needle and branch of *Picea crassifolia* which could lure Ips typographus Linnaeus [23]. Phthalic acid, isobutyl nonyl ester was the main small constituents of *Elaeagnaceae* plant which might cure chronic cardiovascular and cerebrovascular diseases and had anti-tumor, anti-inflammatory, antibacterial functions [24]. The components were rare medicinal herds including hexanedioic acid, bis (2-ethylhexyl) ester, cyclohexanecarboxylic acid, dodecyl ester. And there were some drug activities of the stem extractives from *Vitex negundo*.

CONCLUSION

The leaching rule of stem extractives from *Vitex negundo* was obvious. The optimal extraction time of ethanol/methanol, petroleum ether/acetic ether, and benzene/alcohol extraction were 5 h, 4 h, and 7 h, respectively. The leaching rates of each single extraction were less than those of three-step extractions, and JY-YS-BC extraction was the optimum extraction mode with the leaching rate of 28.42%. What's more, the stem extractives of *Vitex negundo*, which was rich in drug activities. For example, hexanedioic acid, bis (2-ethylhexyl) ester was used in Canada in cosmetics, care products, heavy-duty hand cleanser and lubricant; eicosane was the major composition of the rose essential oil which had the remarkable beauty effect and health care efficacy; 3,3,7,11-tetramethyltricyclo [5.4.0.0 (4,11)] undecan-1-ol could lure Ips typographus Linnaeus. And the stem extractives of *Vitex negundo* contained many rare drug activities.

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