Digest Journal of Nanomaterials and Biostructures Vol. 5, No 1, March 2010, p. 207 – 213

# NANO SCALE INJECTION FOR THE DETERMINATION OF VOLATILE ORGANIC COMPONENTS OF *VITEX PSEUDO-NEGUNDO* USING VARIOUS EXTRACTION TECHNIQUES

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The chemical composition of Vitex pseudo-negundo essential oil was determined by three different methods: hydrodistillation (HD), microwave-assisted hydrodistillation (MAHD) and Ultrasonic assist with head space solid phase microextraction (UA-HS-SPME). The highest extraction efficiency was achieved with a 100 μm polydimethylsiloxane (PDMS) fiber. Different experimental parameters such as the type of coating used for the fibers, sonication time, extraction time, temperature, and desorption time were optimized. The essential oil extracted from the aerial parts of the plant was analyzed by nano scale injection to a GC/MS system. As a result, 32 constituents, representing 95.67 to 96.65% of the oil, were identified. Comparison of the UA-HS-SPME, MAHD and the commonly used HD method showed that the UA-HS-SPME and MAHD methods are simpler and require smaller samples, shorter extraction times and controlled temperatures in addition to the greater ease of trapping and extracting the volatile and thermo-sensitive compounds. The major components obtained by the three methods HD, MAHD and UA-HS-SPME were α-terpinyl acetate (23.25, 26.96, 29.27%), trans-β-farnesene (1.59, 21.40, 1.78%), α-pinene (20.18, 12.29, 6.15%), limonene (13.52, 10.87, 11.70%), β-caryophyllene (12.45, 0.0, 28.37%) and bicyclogermacrene (5.61, 7.80, 0.0%) respectively.

(Received February 28, 2010; accepted March 20, 2010)

*Keywords: Vitex pseudo-negundo,* Essential oil, Ultrasonic extraction, Microwave-assisted hydrodistillation

## 1. Introduction

The main traditional methods for the isolation of essential oils from plant materials are hydrodistillation (HD), steam distillation, steam and water distillation, and simultaneous distillation extraction [1]. These methods require lengthy extraction times and give a lower isolation yield and are also associated with the decomposition of the essential oil as a result of the thermal extraction process or hydrolytic effects, leading to a complex isolation mixture. In order to reduce these problems, new methods such as microwave-assisted extraction (MAE), supercritical fluid extraction, and ultrasound-assisted extraction have recently been developed [2,3]. Microwave assisted hydrodistillation extraction (MAHD) is based on the combination of microwave heating and dry distillation, and is performed at atmospheric pressure without adding any solvent or water [4-6]. MAHD extracts the volatile component from fresh plant materials or moistened dried materials, and is more simple, rapid and economic. This method involves placing vegetable material in a microwave reactor. The internal heating of the *in situ* water within the plant material causes distention which leads to the disruption of the glands and oleiferous receptacles. This process thus frees the essential oil which is entrained by the *in situ* water in the plant material by azeotropic distillation. The vapor then passes through a condenser outside the microwave cavity

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where it condenses. The distillate is collected continuously in the receiving flask. The excess water is refluxed and recycled to the extraction vessel by cohobating in order to restore the moisture content of the plant material. The essential oil is collected directly and dried without any additional solvent extraction. The UA-HS-SPME technique is based on the employment of the energy derived from ultrasound to enhance the extraction of analytes from the solid sample by the organic solvents [7-9].

The genus *vitex* from the *Verbenaceae* family is represented by 250 species and distributed throughout Asia and South Europe [10]. *Vitex agnus-castus* Linn., commonly known as the chaste tree, is one of this species, and is narrowly distributed in different regions of the Mediterranean, Central Asia and Southern Europe [11] *V. pseudo-negundo* which is a commonly used drug as are other species of the *Vitex* genus, grows naturally around seasonal rivers in Iran [12]. It is commonly used in traditional medicine and reported to have variety of biological activities [13, 14]. It is used in the treatment of premenstrual problems and hyperprolactinemia, and has diuretic, digestive and antifungal properties and is also used to treat anxiety, early birth and stomachache [15-19]. In addition, this plant is known to contain glucosides [13], phenolic compounds [20], triterpenoids [21], flavonoids [21, 22], and iridoids [23].

Our investigation deals with the determination of the chemical composition of the essential oils isolated from the aerial part of *Vitex agnus-castus* L. Var. *pseudo-negundo* Hausskn (syn: *Vitex pseudo-negundo*) using three extraction methods: Ultrasonic assisted with headspace solid phase microextraction (UA-HS-SPME), Microwave assisted hydrodistillation extraction (MAHD) and hydrodistillation (HD).

## 2. Experimental

### 2.1 Chemicals and Reagents

Helium, 99.999%, used as carrier gas, was purchased from Roham Gas Company (Tehran, Iran). The alkane mixture consisting of the $C_8$-$C_{26}$ alkanes (concentration of 40 mg/mL in hexane) was purchased from Fluka. All other chemicals were of the highest purity available from Merck or Fluka. Doubly distilled deionized water was used.

### 2.2 Plant material

The aerial parts of *Vitex pseudo-negundo* were collected from samples cultivated in the Kashan Botanical Garden (Isfahan Province, central area of Iran) at an altitude of ~ 1000 m in November 2008. The voucher specimens of the plants have been placed in the herbarium of the Research Kashan Botanical Garden, Kashan, Iran.

### 2.3 MAHD apparatus and procedure

Microwave assisted hydrodistillation extraction (MAHD) was carried out using a Samsung microwave apparatus. The multimode microwave reactor has a twin magnetron (1000 W, 2455 MHz) with a maximum delivered power of 1000 W variable in 10 W increments. A rotating microwave diffuser (35 cm × 35 cm × 35 cm) ensures homogeneous microwave distribution throughout the plasma-coated cavity. The temperature was controlled by feedback to the microwave power regulator. In a typical MAHD procedure performed at atmospheric pressure, 60 g of fresh plant material was heated using a fixed power of 600 W for 25 min without the addition of solvent or water. A cooling system outside the microwave cavity condensed the distillate continuously. Condensed water was refluxed into the extraction vessel in order to provide uniform conditions of temperature and humidity for the extraction. The extraction was continued at 100 °C until no more essential oil was obtained.

### 2.4 Ultrasonic-Assisted Head Space Solid-Phase Microextraction

The aerial parts of the plant were dried at room temperature by spreading them on clean aluminum foil in the laboratory. 10-g portions of air-dried sample were ground to a coarse powder using a household coffee grinder. The ground samples were stored in nylon bags and placed in a refrigerator prior to analysis. Extraction of the volatiles from the plant sample using SPME fibers was achieved by placing 0.5 g of ground sample into a 40-ml vial to which 500 µl double-distilled water was added as a matrix modifier, the vial was then vigorously shaken by hand to ensure homogeneous dispersal of the spiked water. The sample vial was then placed into an ultra-sonicator and incubated for 15 min to allow the volatiles to equilibrate between the headspace and sample matrix, during which time the sample was heated to 70 ºC. The actual SPME extraction of volatile compounds was accomplished by incubation with a polydimethylsiloxane (PDMS) fiber at 70 ºC for 40 min. Ultrasonic irradiation (18 kHz, 450 W) was applied by means of a PFO100 5RS Series ultrasonicator (Italy) equipped with a water bath in which the extraction vials were placed. The samples were sonicated to create stress in the sample matrix to facilitate the release of the analytes, and control the temperature during the extraction process.

The alkane mixture ($C_8$-$C_{26}$), 40 mg/mL in hexane) was used for the calculation of retention indices (RIs). Loading the alkane mixture onto the fiber was carried out using a 5-min head space extraction from the 10-ml SPME vial including 1 ml double-distilled water spiked with 10 µl of the above-mentioned mixture.

### 2.5 GC–MS analysis

Gas chromatography was performed with a Shimadzu model GC-17A (Kyoto, Japan) instrument equipped with a Shimadzu Quadropole-MS (QMS) model QP5050 detector. Separation was achieved using a 30 m × 0.25 mm I.D capillary fused silica column 6 coated with a 0.25 µm film of DB5-MS (5% Phenyl-95% Polydimethyl Siloxane), and a split/splitless injector with a 1 mm internal diameter glass liner. Ten-, 20- and 40-milliliter sample vials sealed with PTFE coated silicone septa (Supelco) were used for extraction. SPME fibers with PDMS (100 µm, non-bonded) coating, were used as commercial fibers for the extraction of analytes. The fibers were handled using a manual SPME fiber holder provided by Supelco (Bellefonte, PA, USA). Analytes extracted onto the fiber were injected into the injection port of the GC system. A 1.0-nL (1.0 µL of sample diluted in 1000 ml of n-pentane, v/v) volume of sample was injected onto the column in the splitless mode [24-27]. The fiber was kept in the injection port for additional 2 min after injection to ensure the complete desorption of the compounds from the fiber. Every 10 analyses on a GC run were carried out in the presence of the fiber but without sampling to assure complete desorption. The injector was set at 220 ºC. The carrier gas was helium and flowed at a rate of 1 ml/min. The GC was operated in a splitless mode. The column temperature was initially set at 40 ºC and increased to 200 ºC at a rate of 4 ºC/min, and remain at 200 ºC for 1 min, resulting in a total GC run time of 41 min. The temperature of the ion source was kept at 220 ºC, and the transfer line temperature at 250 ºC. The mass fragments were collected in the range from m/z 35 to 450 with an acquisition rate of 1000 to provide a satisfactory number of points per peak for effective spectral resolution. The ionization energy of 70 eV and the detector voltage of 1700 V were applied to the QMS detector.

### 2.6 Qualitative and quantitative analyses

Most constituents were identified by gas chromatography by comparison of their retention indices (RIs) with those reported in the literature [28] and [29] or with those of authentic compounds available in our laboratories. The retention indices (RIs) were determined in relation to a homologous series of n-alkanes (C8–C24) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST 98 and Wiley 5 Libraries or with mass spectra available in the literature [29] and [30]. The relative concentrations of the components were calculated based on GC peak areas without using correction factors.

## 3. Results and discussion

### 3.1 GC–MS analysis resolution

The yellow essential oil from the aerial parts of *Vitex pseudo-negundo* was obtained by hydrodistillation (HD) using a Clevenger apparatus, ultrasonic assisted with headspace solid phase microextraction (UA-HS-SPME) and Microwave assisted hydrodistillation extraction (MAHD) in yields of 0.7%, 0.8% and 1.0% (v/w), respectively. The essential oils were analyzed by GC/MS using an HP-5MS column and components were identified by kovates indices and compared with mass spectra stored in the Wiley 275.L GC/MS library. Thirty-two components have been identified from essential oils by different methods, constituting 95.67% to 96.65% of the total oil (Table 1). The main constituents of the oil from the aerial parts of *V. pseudo-negundo* were α-terpinyl acetate (3.25%), α-pinene (20.18%), limonene (13.52%), *β*-caryophyllene (12.45%), and bicyclogermacrene (5.61%) using the HD method, while α-terpinyl acetate (26.96%), α-pinene (12.29%), limonene (10.87%), and bicyclogermacrene (7.80%) were the major components produced by the MAHD method, and the main constituents in the UA-HS-SPME method were α-terpinyl acetate (29.27%), α-pinene (6.15%), limonene (11.70%), and *β*-caryophyllene (28.37%), respectively. *β*-Caryophyllene which was present in abundance in the HD and SPME extracts, was not detectable in the MAHD isolate. On the other hand, *trans-β*-Farnesene comprised a large proportion of the extract obtained by MAHD, but was detectable at lower levels in the HD and SPME extracts. Also bicyclogermacrene which was observed as the major component in MAHD and HD extracts was not found in the SPME extraction.

*Table 1. The percentage composition of the oil of the aerial parts of Vitex pseudo-negundo from the central Iran (Kashan area) by three methods (HD, MAHD and UA-HS-SPME)*

| Compound | %[a] HD | %[a] UA-HS-SPME | %[a] MAHD | RI[b] |
|---|---|---|---|---|
| *α*-Pinene | 20.18 | 6.15 | 12.29 | 947 |
| Camphene | 0.11 | - | - | 959 |
| Sabinene | - | - | 2.94 | 981 |
| *β*-Pinene | 4.52 | - | 0.27 | 982 |
| Myrcene | 3.12 | - | 3.12 | 996 |
| *α*-Phellandrene | 0.59 | - | 0.40 | 1016 |
| Limonene | 13.52 | 11.70 | 10.87 | 1043 |
| Terpinolene | 0.27 | 1.64 | - | 1094 |
| Linalool | 0.75 | - | 0.71 | 1109 |
| *α*-Terpineol | 0.64 | 0.55 | 0.57 | 1213 |
| Bornyl acetate | 0.30 | - | 0.29 | 1298 |
| *Z*-Dimethoxy-citral | 0.24 | - | 0.24 | 1332 |
| δ-Elemene | 0.16 | - | 0.24 | 1342 |
| Neryl acetate | - | 0.34 | - | 1366 |
| *α*-Terpinyl acetate | 23.25 | 29.27 | 26.96 | 1371 |
| *α*-Gurjunene | 0.33 | - | 0.55 | 1424 |
| α-Bergamotene | - | 0.65 | - | 1443 |
| *β*-Caryophyllene | 12.45 | 28.37 | - | 1446 |
| *trans-β*-Farnesene | 1.59 | 1.78 | 21.40 | 1453 |
| *α*-Humulene | - | - | 0.48 | 1475 |
| *β*-Selinene | 1.41 | - | 2.22 | 1477 |
| Aromadenderene | - | 2.42 | - | 1480 |
| Valencene | - | - | 0.81 | 1483 |
| *β*-Bisabolene | - | 4.63 | - | 1519 |
| Bicyclogermacrene | 5.61 | - | 7.80 | 1521 |

| Compound | %[a] HD | %[a] UA-HS-SPME | %[a] MAHD | RI[b] |
|---|---|---|---|---|
| Hexadecane | - | 3.95 | - | 1602 |
| Spathulenol | 2.02 | - | - | 1607 |
| Caryophyllene oxide | - | 5.20 | 2.57 | 1608 |
| Globulol | 1.81 | - | - | 1610 |
| viridiflorol | 0.65 | - | - | 1620 |
| Torreyol | 1.17 | - | 0.47 | 1669 |
| *β*-Eudesmol | 1.52 | - | 0.47 | 1668 |
| Total | 96.21 | 96.65 | 95.67 | |

[a]Compounds listed in order of elution from DB-5MS column.
[b]Relative retention indices to C8-C26 *n*-alkanes on DB-5MS column.

These data are in agreement with those obtained from different parts of *V. pseudo-negundo* collected from the National Botanical Garden in Tehran, which were found to contain α-pinene (14.7%-35.9%), limonene (5.8%-12.2%) and bicyclogermacrene (8.3%-14.5%) [31]. Limonene which was one of our major compounds, was also extracted by hydrodistillation from *V. pseudo-negundo* obtained from Sabzevar province at the east of Iran in a yield of about 28.8% [32]. Bicyclogermacrene which was found in the sizeable concentrations in the former sample comprised only a minor component of the essential oils that we investigated. The predominance of *α*-terpinyl acetate, which was the first major component in our oil, has also been found as the major component in the oils of *V. agnus-castus* L. which grows in the Amazon region [33] and *V. trifolia* from Thailand [34].

**Comparison of UA-HS-SPME, SFME and HD methods**

Comparison of the UA-HS-SPME and SFME methods with the commonly used distillation method (HD) showed that these methods are simpler and require much smaller samples, shorter extraction times and controlled temperatures in addition to their greater ability to trap and extract the volatile and thermo-sensitive compounds. Analysis of the extracts highlighted the differences in the composition of the essential oil depending on the extraction method (SPME, MAHD and HD) and experimental conditions. For MAHD it was found that the sesquiterpene and oxygenated sesquiterpene fractions decreased with time, power and moisture content. The HD composition confirmed this trend; in the HD extract, the fractions of monoterpene sesquiterpenes and oxygenated sesquiterpenes were greater.

## 4. Conclusion

Using ultrasonic assist with headspace solid phase microextraction, the highest extraction efficiency was achieved with a 100 μm polydimethylsiloxane (PDMS) fiber and it was found that the oxygenated sesquiterpene and oxygenated monoterpenes fractions decreased but the amount of sesquiterpenes and monoterpenes increased. Thus, the experimental parameters such as extraction time, irradiation power and ultra sound effects can be optimized for the particular aim of the MAHD and SPME, either to obtain a high yield of essential oil, or to obtain essential oils of differing composition.

Here are some biological properties and the applications of some major components obtained by the three methods HD, MAHD and UA-HS-SPME from essential oil of *Vitex pseudo-negundo*:

**α-Pinene**: is employed as a fragrance substance to improve the odor of industrial products such as insecticides and antiseptics.

**Limonene:** is used as fragrance material for perfuming household products and as component of artificial essential oils.

**β-Caryophyllene**: is a natural bicyclic sesquiterpene and is notable for having a cyclobutane ring, a rarity in nature. Since the widespread plant natural product beta-caryophyllene is an FDA approved food additive and ingested daily with food it is the first dietary cannabinoid. Whether this compound is able to modulate inflammatory processes in humans via the endocannabinoid system is yet unknown. Beta-caryophyllene does not bind to the centrally expressed cannabinoid receptor type-1 ($CB_1$) and therefore does not exert psychomimetic effects.

**Bicyclogermacrene**: is used in the creation and/or manufacturing of flavor and fragrance agents.

**Acknowledgements**

Financial support made by the Research Affairs Office of the University of Kashan, Kashan, I. R. Iran is gratefully acknowledged.

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