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Study of the Oil Constituents Extracted from Leaf, Flower and Gramineous Stipes of Vitex Pseudo-negundo

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According to experimental findings, limonene (28.8%), myrcene (17.06%) and caryophyllene oxide (27.5%) are the major constituents of oil extracted from leaf, flower and gramineous stipes of Vitex pseudo-negundo, respectively which was collected from Sabzevar province at the east of Iran in August 2004.

Key Words: Vitex pseudo-negundo, Constituents, Oil, Sabzevar, Iran.

INTRODUCTION

Vitex pseudo-negundo is derived from Vitex agnus-castus branch, grows in damp places by streams and on the littoral and mainly found naturally in several regions of Asia. This species is usually propagated around seasonal rivers in Iran. This species has a decidious shrub growing to 3 × 3 m at a medium rate. It is in leaf from June to October and in flower from September to October. The scented flowers are hermaphrodite (have both male and female organs) and are pollinated by insects. The plant prefers light (sandy) and medium (loamy) soils, requires well-drained soil and can grow in nutritionally poor soil. Vitex pseudo-negundo prefers acid, neutral and alkaline soils and it cannot grow in the shade. The plant requires dry or moist soil. The whole plant is aromatic, the leaves and stems are strongly aromatic, the flowers are deliciously scented and the dried seeds have a pungent lemony perfume.

This plant has several edible and medicinal uses[1-3]. The fruit is used as a condiment as a pepper substitute. The aromatic leaves are also used as a spice. This plant forms one of the ingredients of the legendary Moroccan spice mixture. Agnus castus has been used for thousands of years for its beneficial affect on the female hormonal system. Modern research has

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confirmed this use, the seeds being used to restore balanced functioning to the female reproductive system. The seeds and fruits are anaphrodisiac, aphrodisiac, galactogogue, ophthalmic, sedative, stomachic, women's complaints[2-4]. The berries of this plant have a range of medicinal actions but possibly the most important is its ability to rectify hormonal imbalances caused by an excess of oestrogen and an insufficiency of progesterone. It acts upon the pituitary gland, reducing the production of certain hormones and increasing the production of others, shifting the balance in favour of the gestagens. Thus it has a wide application of uses in malfunctions of the feminine reproductive system and has been used with great effect in restoring absent menstruation, regulating heavy periods, restoring fertility when this is caused by hormonal imbalance, relieving pre-menstrual tension and easing the change of the menopause. Some caution is advised since excessive doses can cause a nervous disorder known as formication, which manifests as a sensation of insects crawling over the skin.

The fresh berries are pounded to a pulp and used in the form of a tincture for the relief of paralysis, pains in the limbs, weakness *etc.* Other uses such as making perfume from the flowers and obtaining yellow dye from the leaves, the seeds and the roots are also reported for Vitex pseudo-negundo.

Several pharmaceutical and medicinal properties of this species prompted us to investigate constituents and quantity of essential oils in different parts of this species. Thus, a simple quantitative extraction of oil of this plant is reported. The dried seeds of Vitex pseudo-negundo have a pungent lemon like perfume. The flowers are deliciously scented and the leaves and stems are strongly aromatic.

EXPERIMENTAL

General procedure for extraction of nonvolatile oil from Vitex pseudo-negundo:

Samples of Vitex pseudo-negundo were collected from Sabzevar province situated at the east of Iran in August 2004 during rain-free time.

After addition of an internal standard (methyl decanoate, 10 mg), the air-dried Vitex pseudo-negundo (100 g) was blended with distilled water in Clevenger-type apparatus in a round bottomed (1000 mL) and simultaneously distilled and extracted for 2 h. The light yellow nonvolatile concentrate (0.15-0.52 g) was dried over anhydrous sulfate and concentrated. A Konik 4000A GC (KonikTech S.A., Spain) equipped with a 30 m × 0.25 mm (0.5 μm film thickness) HP-5 fused-silica capillary column or column coated with 0.25 μm 5 % diphenyl dimethyl silicone supplied by J&W (DB-5) equipped with a flame ionization detector (FID) were used.

Injector and detector temperatures were both 250°C. Oven temperature was held at 50°C for 2 min and then raised to 250°C at 4°C/min and held for 10 min. Carrier gas (hydrogen) flow rate was 1 mL/min. Injector and detector temperatures were 250°C. Linear retention indices were calculated against those of *n*-paraffins. Quantitative data were obtained from the electronic integration of the GC peak areas with the use of the internal standard method, neglecting FID response factors.

GC/MS of the volatile concentrates were performed on a Hewlett-Packard series 6890N gas chromatograph equipped with a 5973 mass-selective detector (Agilent Technologies, USA). The chromatographic conditions were the same as those described for GC (FID). Carrier gas (helium) flow rate was 1 mL/min. The detector operated in impact electron mode (70 eV) at 230°C. Detection was performed in the scan mode between 30 and 400 Daltons. Peaks were identified by comparison of their mass spectra with those in NBS, NIST or our IDENT database and confirmed in many compounds by their relative retention indices with literature data[5-7].

RESULTS AND DISCUSSION

Vitex agnus-castus, known as Chaste Tree, is a perennial deciduous shrub to small tree. This plant has long narrrow leaves, about 7 per leaflet, green on top and gray green fuzzy undersides and produces long spikes of lavender flowers, followed by berries which are used medicinally. Both flowers and the leaves produce a rich aroma especially when stroked. There are some named forms of Vitex agnus-castus, but these have been developed for their ornamental value and not for their other uses. This plant involves stable species as trees and shrubs. Three species of this type introduced in Iran. Several pharmaceutical properties and drugs such as antibacterial properties, anti-hormone androgen effects, stimulating of hypophysis exertion, galactopoiesis, improvment of hysterectomy complications and menopause recovery of dyspepsia are known for these types.

Vitex pseudo-negundo grows in different regions of Iran provinces. This species also grow in besides of seasonal rivers as some colonies with two to three meters high in several parts of Sabzevar[1] with the native name of Hendebid. According to the importance of this type in the synthesis of different drugs, quantity and quality of its oil is considerable[8].

The volatile constituents of Vitex pseudo-negundo were obtained by a suitable isolation procedure and analyzed by GC and GC/MS using fused silica or HP-5 capillary columns[9]. Tables 1 and 2 summarize the qualitative and quantitative analyses of the volatiles according to order of elution on the column.

TABLE-1
QUANTITY OF OIL IN DIFFERENT PARTS OF VITEX PSEUDO-NEGUNDO HABITANT OF SABZEVAR

S. No.	Sample	Humidity (%)	Oil (%)
1	Leaf	54.34	0.52
2	Flower	63.70	0.47
3	Gramineous stipes	47.40	0.26
4	Woody stipes	25.60	0.04
5	Root	29.60	0.15

Table-1 describes quantity of oil in different parts of Vitex pseudo-negundo habitant of Sabzevar. We obtained real samples from different parts of the plant in August at the height of 1050 meter in the west of Sabzevar and their oil were extracted using water steam distillation with a Clevenger equipment. The present studies showed 0.52 and 0.47 % of oil in leaf and flower of this type, respectively, based on the weight of the starting dried plant; whereas, gramineous stipes and woody stipes led to 0.26 and 0.04 % of oil, respectively. The root of this plant also resulted in 0.15 % of oil. These findings reveal that quantity of oil in Vitex pseudo-negundo habitant of Sabzevar province in Iran is obviously higher than those reported before[10]. These observations are very crucial for drug synthetic purposes[11].

Table-2 presents the results describing different constituents of oil in leaf, flower and gramineous stipes of Vitex pseudo-negundo habitant of Sabzevar.

TABLE-2
CONSTITUENTS OF OIL IN LEAF OF VITEX PSEUDO-NEGUNDO HABITANT OF SABZEVAR

Entry	Compound	Retention Indices	Leaf (%)	Flower (%)	Gramineous stipes (%)
1	α-Pinene	941	-	7.90	9.80
2	β-Pinene	979	-	8.60	-
3	Myrcene	989	-	17.06	7.80
4	Sabinene	972	-	13.10	-
5	α-Phelandrene	1004	-	4.80	-
6	1,4-Terpineol	1088	-	1.13	-
7	α-Linalool	1092	9.5	0.44	-
8	Terpinolene	1088	16.0	0.94	-
9	β-Caryophyllene	1433	-	4.10	15.80
10	α-Humulene	1462	-	0.68	2.26
11	P-Cymene	1019	4.5	-	-
12	Limonene	1030	28.8	-	16.10
13	*trans*-Ocimene	1045	-	-	6.50
14	Caryophyllene oxide	1590	-	-	27.50

According to these findings, limonene and terpinolene form 28.8 and 16 %, respectively, of oil extracted from leaf as the major constituents of oil. Analysis of oil obtained from flower has led to myrcene, 17.06 % and sabinene, 13.1 %, as the major constituents. Whereas, gramineous stipes of Vitex pseudo-negundo have provided caryophyllene oxide, 27.5 %, limonene, 16.1 % and β-caryophyllene, 15.8 %, as the major constituents[11,12].

The results provided in Tables 1 and 2 have demonstrated that kind and quantity of constituents of the extracted oil are different for each part of the plant. Further more, the oil quantity and its constituents were varied in different seasons were found.

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