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Characterization of the major chemical compounds found in Thymus vulgaris plant grown wildly in Chahar Mahal and Bakhtiari province of Iran

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Abstract: Thyme (thymus vularis) plant is fairly grown wildly throughout Iran, especially in Chahar Mahal and Bakhtiari province. Regarding the various therapeutic effects of this plant in traditional medicine, it was decided to isolate and characterize the major chemical components of the essential oil obtained from the upper parts of this plant by hydrodistillation method. The oil obtained by hydrodistillation, on cooling in liquid nitrogen, afforded a white crystalline material which was then separated. This fraction was further purified by TLC and column chromatography on silica gel with benzene:chloroform (3:1 v/v) as the mobile phase. The remaining oil was separated and put under successive TLC and column chromatography on silica gel with benzene:chloroform (3:1 v/v) as the mobile phase which resulted in the separation of two fractions with R_f =0.52 and R_f =0.36. IR, ^{1}HNMR, and MS (EI) spectra of these fractions were taken and compared with the corresponding standard spectra. It was concluded that the fraction isolated through cooling of the essential oil in liquid nitrogen, was thymol; 2-isopropyl-5-methylphenol **(I)**, the fraction with with R_f =0.52 as Carvacrol, [(2-Methyl-5-isopropylphenol)] **(II)** and the fraction with R_f =0.36 was Linalool [(3,7-dimethyl-1,6-octadien-3-ol)] **(III).**

Keywords: Thymus vulgaris, thymus, thyme, carvacrol, thymol, linalool.

Introduction

Thyme (Thymus vulgaris) is a perennial plant with numerous procumbent stems, 6 to 12 inches high, covered with fine hair and pale brown bark. The leaves are small, opposite, sessile, and gray-green with slightly rolled edges. The small, blue-purple flowers are two-lipped and grow in dense, whorled clusters, blooming from May to September. Thyme is a genus of about 350 species of aromatic perennial herbaceous plants and sub-shrubs to 40 cm tall, in the family Lamiaceae and native to Europe, North Africa and Asia and widely cultivated in Europe and the United States.[1] **[Scientific classification:** Kingdom; Plantae: Division; Magnoliophyta: Class; Magnoliopsida: Order; Lamiales: Family; Lamiaceae: Genus; **Thymus L.**: Species; Thymus vulgaris L.**]**. It is a commonly used culinary herb which is best suited to well drained soils and enjoys full sun. The stems tend to be narrow or even wiry; the leaves are evergreen in most species, arranged in opposite pairs, oval, entire, and small, 4-20

mm long. The flowers are in dense terminal heads, with an uneven calyx, with the upper lip three-lobed, and the lower cleft; the corolla is tubular, 4-10 mm long, and white, pink or purple.[2] Thyme is widely cultivated as a herb grown for its strong flavor which is due to its content of thymol.[3]It retains its flavor on drying better than many other herbs. Thyme is often used to flavor meats, soups and stews. Medicinally thyme is used for respiratory infections in the form of a tincture, tisane, salve, syrup or by steam inhalation. The content of essential oil varies drastically with climate, time of harvest and storage conditions; extreme values are 0.75% and 6.5%. Main components are the phenols thymol (ca. 40%) and carvacrol (ca. 15%). In winter, phenol content is lower (but mostly thymol); in summer, more phenols (up to 70%) are found, with significant amounts of carvacrol. Further components in the essential oil are thymol methyl ether (2%), cineol, cymene, α-pinene, borneol and esters of the latter two.[4]Common thymes bloom in spring and attract early butterflies and many different kinds of beneficial flies and wasps. They are also a favourite of honey bees. The leaves can be used fresh any time; but for drying, it is best to cut fresh growth after the bloom cycle. When three or four inch pieces of new growth can be harvested, cut these in the early morning, after the dew has dried, and make small bundles. Hang these out of direct light and check often for dryness. How long this will take depends on the moisture in the air. It is very important to make sure the thyme is completely dry before storing, because improperly dried herbs can mildew and rot. If the herb is crispy when crushed between the fingers, then it is dry.[5]Thyme is used as antiseptic, tonic, antibacterial and deodorizing. Often used in toothpaste, mouthwashes, perfumes and soap.[6]Thyme has been used in traditional medicine as a general remedy for colds, flu, fevers, cough and bronchitis (to take four to five cups of thyme tea a day) as an antiseptic, antispasmodic and antifungal. It is also an expectorant and vermifuge.[7]

Experimental

NMR spectra were recorded on Bruker NMR spectrophotometer AC 80 MHz (Germany) using TMS as internal standard. Mass spectra were recorded on Mass spectrometer (Vannian Mat, 311-A, Germany) instrument. Infrared spectra were recorded using JASCO, IR700 Infrared spectrophotometer. Refractive indices were masured by using Abbe Refractometer (Model 2W, China). All the chemicals were purchased from Merck.

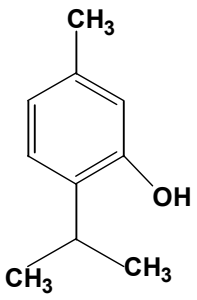
1. **Collecting thymus plant:** The plant was collected in May around the city of Shahre Kord, the capital city of Chahar Mahal and Bakhtiari province, Ian and identified as **Thymus vulgaris** by the department of Pharmacognosy, School of Pharmacy of Tehran University of Medical Sciences, Iran. Sufficient amount of the plant were collected and air dried in the shade then ground by using an electrical mill.
2. **Direct steam ditillation of the ground plant:** Direct steam distillation was performed using 20 g of the dried ground plant material in 300 mL distilled water for about 2 hours. The oil obtained was extracted with dichloromethane and dried over anhydrous calcium chloride. A bright yellow colour oil with a pungent odour was obtained and stored in a dark glass bottle at 4°C. The process was repeated several times to collect enough of the essential oil for further analysis**.**
3. **Hydrodistillation of the ground plant:** The oil of air-dried and finely ground from the whole aerial parts of the thymus plant was obtained by hydrodistillation using a Clevenger-type hydrodistillation apparatus. Distillation was performed using 20 g of the dried ground plant material in 300 mL distilled water for about 2 hours. The oil obtained was extracted with dichloromethane and dried over anhydrous calcium chloride. A bright yellow colour oil with a pungent odour was obtained and stored in a dark glass bottle at 4°C. The process was repeated several times to collect enough of the essential oil for further analysis.
4. **Thin Layer Chromatography analysis of the essential oil:** TLC on silica gel (GF 254) with benzene:chloroform (3:1 V/V) as the mobile phase was carried out. The spots were developed by using a UV lamp. The results showed two spots with R_f =0.52 (as the major spot) and R_f =0.36 (as the minor spot).
5. **Column Chromatography analysis of the essential oil:** 1 mL of the essential oil was column chromatographed on silica gel (35-70 mesh) with benzene:chloroform (3:1 V/V) as the mobile phase. One major fraction with R_f =0.52 and a minor fraction with R_f =0.36 were separated and characterized. The process was repeated several times to collect sufficient amount of each of the fractions for further anyalysis. Successive preparative TLC of each of the fractions on silica gel with benzene:chloroform (3:1 V/V) as the mobile phase was carried out. Finally, IR, ^{1}HNMR and MS spectra of the purified fractions were taken.
6. **Separation of thymol from the essential oil:** The whole crude essential oil was cooled in liquid nitrogen, a crystalline material was separated. TLC of this crystalline material on silica gel with

benzene:chloroform (3:1 V/V) as the mobile phase showed a fraction with R_f =0.52. This fraction was further purified by sublimation method through sing a coldfinger.

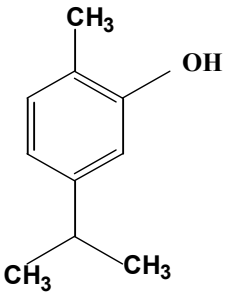
7. **Characterization of the component with R_f =0.52 (separated through cooling the crude essential oil in liquid nitrogen):** It had n_D=1.5223. Its IR (mull in nujol) had ν^-(cm^{-1}): 3226 (OH, s), 2956, 2924, 2866 (C-H, s), 1619 (C=C, m-w), 1243 (C-O, s), 853, 804, 736 (=CH_{oop}, m); its ^{1}HNMR ($CDCl_3$, 80 MHz) hadδ (ppm): 1.50 (d, 6H, 2×CH_3), 2.30 (s, 3H, CH3), 3.03 [sept, 1H, C<u>H</u>(CH_3)$_2$], 4.32 (br, 1H, OH), 6.43 (s, 1 H, aromatic, H-6), 6.65 (d, 1H, aromatic, H-3/H-4), 6.58 (d, 1H, aromatic, H-4/H-3); its MS (EI) showed m/z: 150 [$M^{\cdot+}$, 97.45%], 135 [(M – CH_3)$^+$, 100%], 107[(M – C<u>H</u>(CH_3)$_2^+$, 12.74%]. Based upon these results and comparison of the spectra with the corresponding standard spectra of thymol, this fraction was identified as thymol; 2-isopropyl-5-methylphenol **(I)**.
8. **Characterization of the component with R_f =0.52 (separated by column chromatography):** It had n_D=1.5223. Its IR (neat liquid) had ν^-(cm^{-1}): 3372 (OH, br, s), 3018 (C-H, aromatic, w), 2956, 2924, 2866 (C-H, s), 1586, 1520, 1502 (C=C, m), 1250, 1172 (C-O, s), 937, 864, 811 (=CH_{oop}, m); its ^{1}HNMR ($CDCl_3$, 80 MHz) hadδ (ppm): 1.45 (d, 6H, 2×CH_3), 2.45 (s, 3H, CH3), 2.90 [sept, 1H, C<u>H</u>(CH_3)$_2$], 6.30 (s, 1H, OH), 6.93 (s, 1 H, aromatic, H-6), 7.03 (d, 1H, aromatic, H-3/H-4), 7.28 (d, 1H, aromatic, H-4/H-3); its MS (EI) showed m/z: 150 [$M^{\cdot+}$, 93.63%], 135 [(M – CH_3)$^+$, 100%], 107[(M – C<u>H</u>(CH_3)$_2^+$, 18.47%]. Based upon these results and comparison of the spectra with the corresponding standard spectra of carvacrol, this fraction was identified as carvacrol; 2-methyl -5-isopropylphenol **(II)**.
9. **Characterization of the component with R_f =0.36:** It had n_D=1.4567. Its IR (neat liquid) had ν^- (cm^{-1}): 3390 (OH,, s), 3082 (=C-H, w), 2968, 2922 (C-H, s), 1639 (C=C, m), 1459 (CH_2, m), 1373 (CH_3, m), 1111 (C-O, s), 918, 894 (=CH_{oop}, m-s); its ^{1}HNMR ($CDCl_3$, 80 MHz) hadδ (ppm): 1.30 (d, 3H, CH_3), 1.40 (s, 6H, 2×CH3), 1.2-1.90 [m, 4H, 2×CH_2], 2.0 (s, 1H, OH), 4.7-5.1 (m, 3H, =CH_2, =CH), 5.5-5.9 (dd, 1H, =CH); its MS (EI) showed m/z: 155 [$(M+H)^{\cdot+}$, 1.92%], 154 [$M^{\cdot+}$, 3.29%], 137 {[(M+H) – H_2O]$^{\cdot+}$, 96.15%}, 136 {[(M – H_2O]$^{\cdot+}$, 41.66%}, 81{[136-HC=C(CH_3)$_2$]$^+$, 100%}. Based upon these results and comparison of the spectra with the corresponding standard spectra of linalool, this fraction was identified as linalool; 3,7-dimethyl-1,6-octadien-3-ol **(III)**.

Discussion

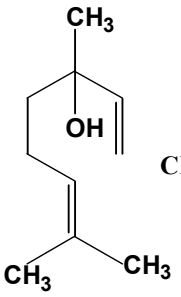
Thyme (thymus vulgaris) plant is fairly grown wildly throughout Iran, especially in Chahar Mahal and Bakhtiari and Fars provinces. It has been used in traditional medicine as a general remedy for colds, flu, fevers, coughs, bronchitis and as an antiseptic, antispasmodic and antifungal. It is also an expectorant and vermifuge.[7] Regarding these therapeutic effects, it was decided to isolate and characterize the major chemical components of the essential oil obtained from the upper parts of the plant by hydrodistillation method. The oil of the air-dried, finely ground whole aerial parts of thymus after flowering season of the plant was obtained by hydrodistillation, first cooled in liquid nitrogen, then a white crystalline material separated. This fraction was further purified by TLC and column chromatography on silica gel with benzene:chloroform (3:1 V/V) as the mobile phase. IR, ^{1}HNMR, and MS (EI) spectra of the purified fraction were taken. Based upon the results obtained and comparison of the spectra with the corresponding standard spectra of thymol, this fraction was identified as thymol; 2-isopropyl-5-methylphenol **(I)**. The remaining oil was isolated and put under successive TLC and column chromatography on silica gel with benzene:chloroform (3:1 V/V) as the mobile phase which resulted in the separation of two fractions with R_f =0.52 and R_f =0.36. IR, ^{1}HNMR, and MS (EI) spectra of these fractions were taken and compared with the corresponding standard spectra. The spectra were carefully analyzed. It was concluded that the fraction with R_f =0.52 was carvacrol [2-methyl -5-isopropylphenol] **(II)** and the fraction with R_f =0.36 was Linalool [3,7-dimethyl-1,6-octadien-3-ol] **(III)**. Therefore, we were able to isolate and characterize the three major components of the essential oil obtained from thymus vulgaris plant grown in Chahar Mahal and Bakhtiari province of Iran; Thymol **(I)**, Carvacrol **(II)** and Linalool **(III)**.



(I)



(II)



(III)

$CH_2=CH-C(OH)(CH_3)-CH_2CH_2CH=C(CH_3)_2$

(III)

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