دراسة المكونات الكيميائية للزيت العطري المستخلص من نبتة الريحان Ocimum basilicum من الفصيلة الشفوية Lamiaceae

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الملخص

خلفية البحث وهدفه: هدفت هذه الدراسة إلى تحديد كمية الزيت العطري الموجود في نبتة الريحان المنتشرة في اليمن، وتحديد هوية المركبات الرئيسية المكونة لهذا الزيت المستخلص من نبتة الريحان وكميتها بجهاز الكروماتوغرافيا الغازية المرتبط بجهاز مطياف الكتلة.

مواد البحث وطرائقه: درست المكونات الكيميائية للزيت العطري لنوعي نبتة الريحان Ocimum basilicum (الأخضر والأرجواني) حيث استخلص الزيت العطري للنبتة اليمنية بحسب طريقة دستور الأدوية الأوربي، ثم حللت مكوناته بجهاز الكروماتوغرافيا الغازية المرتبط بجهاز مطياف الكتلة GC-MS وذلك لفصل المركبات و تحديد الكتلة .

النتائج: جرت معرفة وتحليل ستة وعشرين مركبا من نوعي نبتة الريحان (الأخضر و الأرجواني) إِذْ عرف وحلل خمسة عشر مركباً في النوع الفينولي (الأخضر)، وكانت المركبات الرئيسة المعزولة من الزيت المقطر في هذا النوع هي:

لينالوول (46.6%)، فيبرينون (10.0%) ميثايل تشافيكول (8.1%) . جيرانيول (7.3%) ،1و8 سينيول (7.1%) ،بوميول (4.3%). كما وجرت أيضاً معرفة وتحليل أحد عشر مركباً في النوع الفينولي (الأرجواني) من الزيت العطري، وكانت المركبات الرئيسة المعزولة هي:

لينا لوول (41.8%)، فيبرينون، (10.0%) ,ميثايل تشافيكول(.30.9%) . ،1و 8 سينيول(9.7%) ،كا د ينول (4.2%).

الاستنتاج: كشفت الدراسة أنَّ نبتة الريحان تحتوي على زيت عطري طيار يتكون من عدد كبير من المركبات ذات الطبيعة التربينية. كما أنّ الزيوت المستخلصة من نوعي نبتة الريحان (الأخضر والأرجواني) صنفت إلى نوعين :.نوع يسمى لاينولول- مثايل تشافيكول (كيميائي أوروبي) ونوع لاينولول فيبرينون،-مثا يل تشافيكول الذي لم ينشر عنه شيئاً منذ مدة طويلة.

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Study of the Chemical Compositions of the Ocimum basilicum family Lamiaceae

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Abstract

Background & Objective: The aim of this study is to determine the essential oil of Ocimum and to find out the chemical constituents of essential oils of Yemeni Ocimum basilicum Lamiaceae.

Methods & Materials: Two samples of Ocimum basilicum which were collected from Sana'a region, Yemen. The color of leaves was green and purple. Oils were isolated from fresh leaves. The constituents are quantified using Gas Chromatograph equipped with Mass Spectrometry.

Results: The analysis of the oils resulted in the identification of twenty six constituents. Fifteen constituents were identified from green phenotype oil. Linalool (46.6%), verbenone, (10.0%), methyl chavicol (8.1%), geraniol (7.3%), 1, 8-cineole (7.1%) and borneol (4.3%) were found to be the major constituents in the oil distilled from green phenotype. Eleven constituents were identified from purple phenotype oil with linalool (41.8%), methyl chavicol (30.9%), 1, 8-cineole (9.7%) and τ-cadinol (4.2%) as the major constituents.

Conclusion: The study revealed that two chemo-types were identified. The isolated oils could be classified as the linalool – methyl chavicol (European chemotype) and linalool-verbenone –methyl chavicol chemotype, which to the best of our knowledge has not been reported so far.

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Introduction:

Genus *Ocimum* (O) family Lamiaceae (L) comprises herbaceous or shrubby often strongly aromatic plants. Approximately 30 species have been recorded throughout tropical and subtropical regions, seven of which are found in Yemen, and these include *O. basilicum L*, *O. tenuiflorum*, *O. suave Willd*, O. *spicatum Deflers*, *O. gratissimum L*, and *O. forskolei Benth* (1,2).

O. basilicum, sweet basil is used in Yemeni traditional medicine to treat various ailments: abdominal cramps, gastroenteritis, dysentery, and diarrhea. In northern Oman and Saudi Arabia, juice of leaves or crushed leaves is used in the treatment of wounds. It is used also as a deodorant, and considered to be an aphrodisiac, worn by men during visiting their wives[3, 4].

O. basilicum showed a wide range of biological activity such as antimicrobial [5, 6] antileishmanial[7], anti mutagenic[8], antifeedant[9],trypanocidal[10], antigiardial[11] and antioxidant[12]**.** It is well-known that ecological factors such as light, temperature, altitude, latitude, soil, and agricultural practices- fertilization- may modify the oil content and composition[13]. Available literature data indicates that there is a great deal of diversity in the composition of the essential oils of *O. basilicum* growing in different localities[5,7]. The oils were composed mainly of monoterpenes (linalool, 1, 8-cineole, camphor, limonene) and or phenylpropanoids (methyl chavicol, eugenol and methyl-(E)-cinnamate) and on the basis of the chemical composition and geographical origin of *O. basilicum* oils, these species were grouped into four chemotypes. European basil oil-from Italy, France, Bulgaria, Egypt, and South Africa- considered to have finest flavor and has linalool- methyl chavicol as main compounds. Reunion basil-from Comoro Island, Thailand, Malagasy Republic, Vietnam and Seychelles- is characterized by methyl chavicol. Tropical basil oil (Bulgaria, India, Guatemala and Pakistan) is rich in methyl cinnamate. Java basil oil (Indonesia, North-Africa, Russia) is characterized by eugenol as main compound. Besides, other chemotypes of sweet basil contain as main components, linalool, linalool-eugenol, methyl eugenol-linalool, methyl cinnamate-linalool, bergamotene and camphor chemotypes [5, 7, 13 -28]

In earlier work on *O. basilicum* of Yemeni origin, one chemotype was reported with methyl chavicol as main component (83.8%) [14]. The possibility of identifying a different chemotype from that reported earlier prompted us to investigate the chemical composition of two *O. basilicum* samples, green and purple basil collected from the same location in Sana'a region.

Objectives:

To study the chemical compositions of essential oils of Yemeni Ocimum Basilicium Lamiaceae

Materials and Methods:

Plant materials

The leaves of *O. basilicum* (green and purple samples) were collected from Sana'a district (wet and cold atmosphere) in January 2008 (before flowering time). The plants were identified at Botany Department, Faculty of Sciences, Sana'a University. Voucher specimen (*Ocimum basilicum* green and purple (Yemen Medicinal Program) (YMP- La 6, 7) (Voucher Number) of the plant material have been deposited at the Pharmacognosy Department, Sana'a University, Yemen.

Isolation of the essential oils:

Dried leaves of *O. basilicum* (20 g) were hydro distilled (water distillation method) for 3 hours in a Clevenger type apparatus according to European Pharmacopoeia[29] to yield 0.4-0.6 %(v/w) of oil from both samples respectively. The obtained samples were dried over anhydrous Na_2SO_4 and stored at 4 °C before analysis and sent in January 2008 to the Department of Pharmaceutical Biology, Institute of Pharmacy in Germany for analyzing by Gas Chromatography-Mass Spectroscopy.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis:

GC-MS analysis was carried out on a Agilent 6890N Gas Chromatograph and a Mass Selective Detector (Agilent®5973 Network, MSD) was used. Injection was done with Agilent®7683 Series Injector (Split 1:40 at 250 C, 2.0 µl; carrier gas: helium 1.1 mL/min (60 kPa) at 110ºC; pressure rise: 6 kPa/min). The MS operated in the electron impact mode with ionization energy of 70eV. The oven was programmed from 70°C to 220°C at 3°C/min. Full scan mass spectra were acquired from 45-650 m/z at a rate of 4.5 scans/s and with a 5.00 min solvent delay. Chromatography was performed using a 30 m DB-5 column (30 m × 0.25 mm i.d. , film thickness 0.25 µm). (J&W Scientific, Folsom, USA).

The detected compounds were identified by processing of the raw GC-MS data with ChemStation G1701CA software and comparing with National Institute of Standards and Technology, Gaithersburg, USA (NIST) mass spectral database 2.0 d and from retention times and mass spectra of standard compounds. Relative amounts of detected compounds were calculated automatically by the percent report based on the peak areas of the total ion chromatograms (TIC).

Results:

GC-MS-analysis of the essential oil compositions from two samples (green and purple) of *O. basilicum* collected from Sana'a district, Yemen was carried out. The oil yields were calculated on a dry weight basis with 0.4 and 0.6% (v/w) respectively.

The percent composition of the essential oils obtained from the leaves of *O. basilicum* samples is shown in (Table 1).

Table 1. Main Components of the essential oils from: A) *O. basilicum* green and B) *O. basilicum* purple

Compounds	RI	A%	B%
1,8-Cineole	1029	7.1	9.7
Linalool	1086	46.6	41.8
Camphor	1128	2.6	nd
Borneol	1156	4.3	nd
1-Terpinen-4-ol	1169	1.3	1.7
α-Terpineol	1179	3.6	1.8
Methyl chaviacol	1188	8.1	30.9
Verbenone	1195	10.0	nd
Geraniol	1242	7.3	2.2
Borneol acetate	1273	1.7	3.1
Eugenol	1347	1.0	nd
Geranyl acetate	1376	0.8	nd
β-Elemene	1388	1.0	1.2
Germacrene D	1483	1.4	1.6
δ-Cadinene	1518	nd	1.5
τ-Cadinol	1648	2.5	4.2
Total identified		99.3	99.7

Compounds listed in order of their elution on the DB-5 column RI = Retention indices on the DB-5 column relative to C10-C20 n-alkanes

nd = Not detected

DB-5 column: Kind of column, bonded-phase fused silica capillary column relative to C10-C20 n-alkanes for isolating volatile oil

Fifteen compounds from green sample oil were identified representing 99.3% of the oil. Oxygenated monoterpenes predominated in the oil (85.2%), with linalool (46.6%), verbenone (10.0%), geraniol (7.3%), 1, 8-cineole (7.1%), borneol (4.3%) as major compounds. Phenylpropanoids accounted for (9.1%) of the oil, with methyl chavicol (8.1%) as main compound.

Eleven compounds from the oil of purple sample were detected representing 99.7% of the oil. The oil was characterized by substantial amounts of oxygenated monoterpenes (60.3%) with linalool (41.8%) and 1, 8-cineole (9.7%). and phenylpropanoids with methyl chavicol (30.9%). Sesquiterpenoid content of purple sample oil (9.2%) was higher than that of green sample oil (4.9%). The significant sesquiterpenoids were τ-cadinol, germacrene D, β-elemene, and δ-cadinene which was only detected in purple sample oil. Both oils were devoid of monoterpenoid hydrocarbons.

Discussion:

This study reports the Linalool which was analyzed to be as the first major compound present in both oils of sweet basil possessed antimicrobial, antitrypanosomal, hypnotic, anticonvulsant, hypothermic and anti-inflammatory properties [5, 30-33].

Our results appeared to be somewhat different from previous reported data on the chemical composition of *O. basilicum* oil of Yemeni origin, which contained mainly methyl chavicol (83.3%), 1, 8-cineole (4.2%), β-caryophyllene (6.2%) and α-humulene (1.0%). Linalool, eugenol, methyl eugenol were detected in traces (<1.0%)[14].

This earlier reported oil resembles the reunion basil (methyl chavicol chemotype), while the isolated oils from this study could be classified as the linalool – methyl chavicol (European chemotype) and linalool-verbenone –methyl chavicol chemotype, which to the best of our knowledge has not been reported so far [5,13-28]. Methyl chavicol chemotype (Reunion basil) was reported also in *O. basilicum* oils from Brazil, Benin, Nigeria, Germany, Thailand and Togo[14, 16, 19, 20, 23, 27]

Conclusion:

In this study, the isolated oils could be classified as the linalool – methyl chaviacol (European chemo type) and linalool-verbenone –methyl chaviacol chemo type, which to the best of our knowledge has not been reported so far.

Methyl chaviacol chemo type (Reunion basil) was reported also in *O. basilicum* oils from Brazil, Benin, Nigeria, Germany, Thailand and Togo.

Comparison of the compositional data of our samples with those previous obtained for *O. basilicum* from other parts of the world shows that purple basil (European chemotype) is similar to that from USA, Italy, Togo, Turkey, France, Egypt, Hungary, South Africa which had linalool > methyl chavicol as the major constituents.

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