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Gas chromatographic-mass spectral analysis and phytopathogenic activity of theessential oil of *Plectranthus tenuiflorus*

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Abstract
The chemical composition of the hydrodistilled leaf essential oil from *Plectranthus tenuiflorus* growing wild in Yemen was determined by GC-MS-analysis and bioautographic assay was used to evaluate the anti-phytofungal activity of the oil against *Cladosporium cucumerinum.* Thirty-eight components were identified in the essential oil representing 76.2% of the total oil and the major compounds were thymol (47.6.%), τ-cadinol (10.5%), (*E*)-caryophyllene (3.6%), 1,10-di-*epi*-cubenol (3.4%), caryophyllene oxide (3.0%), and β-selinene (1.4%). At the application of 400 µg, PTEO exhibited apotent antifungal activity with inhibition zones of 32 (±1.8). TLC-bioautographic assay was used to identify the acetylcholinesterase inhibitory effect, and thymol was isolated and characterized by LC-MS and analytical TLC as the responsible constituent for anticholinesterase activity.

Keywords: *P. tenuiflorus*; GC-MS, thymol, *C. cucumerinum*, essential oil

1. Introduction
Plectranthus, a large genus containingabout 300 species found in tropical Africa, Asia andAustralia. About 62 species were recordedto be used asornamentals, foods, flavors, fodder, and folk medicines for treating treating skin, digestive and respiratory diseases [1,2], The genus *Plectranthus* (Lamiaceae) includes 12 perennial aromaticherbs and shrubs that grow wild in Yemen [3, 4]. *P.aegyptiacus* (Forssk.) C. Chr.is now considered the correct name of *P.tenuiflorus* (Vatke) Agnew [5]. The oil and extracts of *P. tenuiflorus* were reported to haveanticholinesteraseinhibitory [6], antiprotozoal activiy [7], antimicrobial [8], and wound-healing properties [9, 10]. Literature review revealed that PTEO grown in Nigeria was found to contain mainlygermacrene-D (11.6%), δ-cadinene (8.4%) and α-cadinol (8.4%) [6]. The reported chemical compositions of Saudi PTEO depends mainly on the geographical location. While PTEO harvested from the Taif West Highlands of Saudi Arabia possessed thymol as the major component [11], δ-3-carene was the main compound of Saudi PTEO harvested from Abha South highlands [12]. Mwangi *et al*. [13] found that PTEO from Kenya contained α-terpinene (10.2%), *p*-cymene (10.9%) and carvacrol (14.3%) as its major components. As part of our program to assess essential oils from Lamiaceous plants [14, 15], this work reports,for the first time, the composition andantiphytofungal activity of Yemeni PTEO against the phytopathogenic *Cladosporium cucumerinum* using a semiquantativebioautographic assay. Additionally, a bioautographic TLCwas used to identify thecompound responsible for acetylchlinesterase inhibitory activity.

2. Materials and methods
2.1 Plant material
The leaves of *P. tenuifolia* were collected in the early morning from the Alselw district, Taiz province, Yemen, on August 2012. The plant was identified by Dr. Hassan M. Ibrahim of the Botany Department, Faculty of Sciences, Sana'a University. A voucher specimen (YMP-Plec-4) has been deposited at the Pharmacognosy Department, Sana'a University, Yemen.

2.2 Essential oil extraction
Dried leaves from *P. tenuifolia*were hydrodistilled for 3 h in a Clevenger type apparatus according to the European Pharmacopoeia [16].

The obtained oil was subsequently driedover anhydrous Na_2SO_4 and kept at 4 °C until analysis.

2.3 Gas chromatography–mass spectral analysis

PTEO wasanalyzed by GC-MS using an Agilent 6890 GC with an Agilent 5973 mass selective detector [MSD, operated in the EI mode(electron energy = 70 eV), scan range = 45-400 amu, and scan rate= 3.99 scans/sec], and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a (5% phenyl)-polymethylsiloxane stationary phase, film thickness of 0.25 µm, alength of 30 m, and an internal diameter of 0.25 mm. The carriergas was helium with a column head pressure of 48.7 kPa and a flowrate of 1.0 mL/min. Inlet temperature was 200 °C and interfacetemperature was 280 °C. The GC oven temperature program wasused as follows: 40°C initial temperature, held for 10 min; increasedat 3°C/min to 200 °C; increased 2°/min to 220 °C. A 1% (w/v) solution of the sample in CH_2Cl_2 was prepared and 1 µL wasinjected using a splitless injection technique. Identification of the oilcomponents was based on their retention indices determined byreference to a homologous series of *n*-alkanes (C8-C40), and bycomparison of their mass spectral fragmentation patterns with thosereported in the literature [17], and stored on the MS library [NISTdatabase (G1036A, revision D.01.00)/Chem Station data system (G1701CA, version C.00.01.080]. The percentages of eachcomponent are reported as raw percentages based on total ioncurrent without standardization.

2.4 Anti-phytofungal assay

This semiquantitative test allows a relative estimation of the activity of compounds andextracts with similar diffusion characteristics. The phytopathogenic fungus *Cladosporiumcucumerinum* Ell. et Arth. was used as test organism. Initial tests of fungicidal activity were carried out by the method described previously [18].

2.5 Modified TLC bioautographic assay for acetylcholinesterase inhibition

The test was carried out according to reference [18].PTLC layers were developed with toluene: ethylacetate (93:7, v/v). The positive band was scratched out, eluted with methanol and purified by HPLC using an RP-C18cartridge and subjected to LC-MS measurements and analytical TLC and thymol as the standard compound.

3. Results and discussion

The hydrodistilled essential oil obtained from the dried leaves of *P. tenuiflorus* from Taiz region of Yemen was analyzed using GC-MS. A total of thirty-eight components were identified in the essential oil.

As can be seen in Table 1, the oxygenated monoterpenoids displayed the highest contribution (48.9.1%) among which thymol (47.6.%) was the most abundant, whereas the monoterpene hydrocarbons represented only 1.2 % of the total oil. Compared to sesquiterpene hydrocarbons that constituted 6.2%, with (*E*)-caryophyllene (3.6%) and β-selinene (1.4%), the oxygenated sesquiterpenoids were relatively abundant with 20.3%, including τ-cadinol (10.5%), caryophyllene oxide (3.0%), 1,10-di-*epi*-cubenol (3.4%) and valeranone (1.0%). The fatty acid and phenylpropanoid groups were the poorest fractions. In addition, the oil sample had two unidentified aromatic compounds(11.4% and 6.9%); the total identified compounds accounted to 76.2%. However, while the genus *Plectranthus*has beendeeply investigated for its secondary metabolites and essential oils, there are few reports on the analysis of PTEO. Thymol (85.2%) was reported as major compound in PTEO from the Taif West Highlands of Saudi Arabia [10, 11]. Compared to our oil sample, this oil is devoid of oxygenated sesquiterpenoids and had a lower concentration of sesquiterpene hydrocarbons.It is possible that the analyzed essential oil was from a thymol chemotype of *P. tenuiflorus*. In contrast to our oil composition, the Saudi leaf oil isolated from Abha South highlands showed a different composition with δ-3-carene (52.8%) [12]. Another remarkable variation in GC-MS data was observed between the Nigerian and Kenyan leaf oils and the Yemeni PTEO. While Nigerian leaf oil was found to be composed largely of *p*-cymene (14.2%), germacrene-D (11.6%) and carvacrol (10.0%) [6], the Kenyan oil was represented by carvacrol (14.3%), *p*-cymene (10.9%) and α-terpinene (10.2%) [13] as main components.

The phytofungicidal potential of PTEO was assessed against the phytopathogenic fungus *Cladosporium cucumerinum* using a semiquantative standardized bioautographic TLC technique. At concentration of 400 µg, strong antifungal activity with inhibition zones of 32 (±1.8)mm was observed. The antifungal activity of the essential oil may well be due to the presence of synergy between the oil components because thymol alone showed weak antifungal activity against *C.cucumerinum* [19]. There are few reports on the antiphytofungal activity of Eosand their components against *C. Cucumerinum* [20, 21].

EOs and their components with marked anticholinesterase activity can find practical applications, for example, as natural insecticides, anti-dementia, and for treating head lice [22, 23], and theanticholinesterase activity of some Yemeni essential oils has been reported [21]. The inhibitory effect of PTEO on AChE was detected by bioautographic TLC. The active band was isolated and identified by LC-MS, positive peak with 150.13 (RT 19.56) and 262.07 (RT 25.39) (molecular ion = 236.2) usinganalytical HPTLC and thymol as a reference.

Table 1: Essential oil composition of *Plectranthus tenuiflorus*

RI	Compound	%
982	1-Octen-3-ol	tr
993	Myrcene	tr
1010	δ-3-Carene	tr
1017	α-Terpinene	tr
1025	*p*-Cymene	0.6
1059	γ-Terpinene	0.6
1067	*cis*-Sabinene hydrate	tr
1101	Linalool	0.1
1177	Terpinen-4-ol	0.5
1182	*p*-Cymen-8-ol	tr
1190	α-Terpineol	tr

1219	β-Cyclocitral	tr
1250	Thymoquinone	0.1
1297	Thymol	47.6
1303	Carvacrol	0.4
1355	Thymol acetate	tr
1376	α-Copaene	tr
1413	Unidentified[a]	11.4
1420	(*E*)-Caryophyllene	3.6
1425	2,5-Dimethoxy-*p*-cymene	0.1
1437	α-*trans*-Bergamotene	0.5
1454	α-Humulene	0.4
1478	*trans*-Cadina-1(6),4-diene	tr
1482	Germacrene-D	tr
1488	β-Selinene	1.4
1495	δ-Selinene	0.2
1515	γ-Cadinene	0.2
1525	δ-Cadinene	0.4
1539	Unidentified[b]	6.9
1567	(*E*)-Nerolidol	0.6
1571	Caryolan-8-ol/Caryophyllenyl alcohol	0.2
1579	Spathulenol	0.1
1584	Caryophyllene oxide	3.0
1611	Humulene epoxide II	0.2
1616	1,10-di-*epi*-Cubenol	3.4
1637	Caryophylla-4(12),8(13)-dien-5-ol	0.2
1643	τ-Cadinol	10.5
1656	α-Cadinol	0.9
1673	Valeranone	1.0
1958	Palmitic acid	0.8
	Total Identified (38)	76.2

4. Conclusion

In conclusion, Yemeni PTEO was characterized by a high amount of oxygenated mono- and sesquiterpenoids (48.9.1%, 20.3%), respectively with thymol as main component and responsible for anticholinesterase activity of the oil.The results clearly show that the oil possesses a significantanti-phytopathogenic activity against *C. cucumerinum*. This stron geffect can open the doors for utilizing the plant extracts to control this agricultural pest without harming the environment compared to harmful agricultural chemical fungicides.

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