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Composition and Anticandidal Activity of the Essential Oil of *Hypericum perforatum* L.

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The essential oil obtained by hydrodistillation of *Hypericum perforatum* L. (Clusiaceae) obtained from Turkey was analyzed by gas chromatography and gas chromatography/mass spectrometry (GC/MS), simultaneously. Main constituents of the oil was found as germacrene D (23 %), β-caryophyllene (14 %), bicyclogermacrene (5 %), caryophyllene oxide (4 %) and spathulenol (4 %). The essential oil was also screened for its anticandidal properties against various *Candida* species.

Key Words: *Hypericum*, St. John's Wort, Clusiaceae, Essential oil composition, Germacrene D, β-Caryophyllene, Antimicrobial activity.

INTRODUCTION

Hypericum perforatum L. (Clusiaceae) is widely distributed in Anatolia, west Europe, west Asia, north Africa and America. It is also cultivated in Germany and some European countries, Australia, China and south America.[1,2] The genus *Hypericum* L. is represented by 70 species in the Flora of Turkey[3,4]. *H. perforatum* is locally used and known as "Binbirdelikotu, Sari kantaron, Kantaron otu, Kantaron cicegi, Ulser otu, Yaki otu, Tenturdiyot cicegi, Kangran otu, Kanotu, Kilicotu, Koyunkiran, Kuzukiran, Mayasilotu, Yaraotu" in various parts of Turkey[2,4,5].

Different parts of *Hypericum* species are used as appetizing, sedative, antispasmodic, antidiareic, antihelmentic and diuretic in Anatolian folk medicine. *H. perforatum* is used as a dye, in flavoring, in food, as a medicine in wound healing, ulcers, the common cold, diabetes mellitus and as an astringent[1,2,4,5].

Phytochemical investigations on *H. perforatum* have shown that it contains flavonols (catechins), naphthodianthrons (hyperisin, pseudohyperisin), xanthones, coumarins, glycosides, anthraquinones, phloroglucinols (hyperforin, adhyperforin), flavonoids (rutin, hyperoside, quercitrin), flavonol glycosides, lactones, pyrones, lipids, triterpenes, tannins, and essential oils. Hypericine and its derivatives have been reported to be responsible for antidepressant activity[6,7].

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Although there are several investigations on the phytochemistry and antimicrobial activity of the extracts of *H. perforatum*, to the best of our knowledge there are only a few studies related to its essential oil[8-11].

The present work reports on the chemical composition of *H. perforatum* essential oil analyzed by gas chromatography and gas chromatography-mass spectroscopy (GC-MS) systems, simultaneously. The essential oil was also investigated for its anticandidal activity by using a microdilution broth assay.

EXPERIMENTAL

Hypericum perforatum was collected during flowering time (16, June, 2008) from the Kirklareli: Kula-Derekoy arasi, Turkey. Voucher specimens were stored in the Herbarium of Anadolu University, Faculty of Pharmacy (ESSE 14452).

Isolation of the essential oil: The plant material was air dried in the shade at room temperature and the aerial parts were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus according to European Pharmacopoeia[11] to produce the essential oils. The yield of oil for *H. perforatum* was 0.18 % (v/w).

GC and GC-MS analysis: GC/MS analysis was carried out using an Agilent 5975 GC-MSD system. Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60 ºC for 10 min and programmed to 220 ºC at a rate of 4 ºC/min and kept constant at 220 ºC for 10 min and then programmed to 240 ºC at a rate of 1 ºC/min. Split ratio was adjusted at 40:1. The injector temperature was at 250 ºC. Mass spectra were obtained at 70 eV. Mass range was from m/z 35 to 450.

The GC analysis was carried out using an Agilent 6890 N GC system. FID detector temperature was set to 300 ºC. In order to obtain same elution order with GC-MS, simultaneous injection was done by using same column and appropriate operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The results of analyses are shown in Table-1.

TABLE-1
COMPOSITION OF THE ESSENTIAL OIL OF
HYPERICUM PERFORATUM

RRI	Compound	(%)
965	3-Methyl nonane	0.4
1032	α-Pinene	4.0
1035	α-Thujene	0.6
1065	2-Methyl decane	0.6
1100	Undecane	0.2
1118	β-Pinene	1.5
1132	Sabinene	1.4
1174	Myrcene	0.9
1176	α-Phellandrene	0.1
1188	α-Terpinene	0.4

1203	Limonene	0.2
1218	β-Phellandrene	0.4
1246	(Z)- β-Ocimene	0.6
1255	γ-Terpinene	0.8
1266	(E)- β-Ocimene	3.4
1280	*p*-Cymene	0.3
1290	Terpinolene	0.1
1300	Tridecane	0.1
1400	Nonanal	tr
1482	(Z)-3-Hexenyl-2-methyl butyrate	0.1
1494	(Z)-3-Hexenyl 3-methylbutyrate	0.1
1495	Bicycloelemene	0.1
1497	α-Copaene	0.1
1506	Decanal	0.2
1535	β-Bourbonene	0.1
1544	α-Gurjunene	0.1
1549	β-Cubebene	0.1
1553	Linalool	0.1
1589	β-Ylangene	0.3
1600	β-Elemene	0.1
1611	Terpinen-4-ol	0.8
1612	β-Caryophyllene	14.4
1628	Aromadendrene	0.2
1639	Cadina-3,5-diene	tr
1661	Alloaromadendrene	0.2
1668	(Z)-β-Farnesene	2.0
1687	α-Humulene	0.8
1704	γ-Muurolene	1.4
1708	Ledene	0.2
1722	Bicyclosesquiphellandrene	0.1
1726	Germacrene D	22.9
1737	(Z, E)-α-Farnesene	1.2
1740	α-Muurolene	0.6
1755	Bicyclogermacrene	5.0
1758	(E, E)-α-Farnesene	0.7
1773	δ-Cadinene	2.1
1776	γ-Cadinene	0.9
1799	Cadina-1,4-diene (=*Cubenene*)	0.1
1807	α-Cadinene	0.2
1849	Calamenene	0.1
1973	Dodecanol	2.2
2008	Caryophyllene oxide	3.7
2050	(E)-Nerolidol	1.7
2057	Ledol	0.1
2071	Humulene epoxide-II	0.3
2077	Tridecanol	2.8
2080	Cubenol	0.2
2088	1-*epi*-Cubenol	0.4
2098	Globulol	0.4

2104	Viridiflorol	0.3
2131	Hexahydrofarnesyl acetone	0.2
2144	Rosifoliol	0.1
2144	Spathulenol	3.9
2173	6-*epi*-Cubenol	0.2
2179	Nor-Copaonone	0.1
2179	Tetradecanol	2.1
2187	T-Cadinol	0.3
2209	T-Muurolol	0.6
2219	δ-Cadinol (=alpha-muurolol)	0.2
2247	*trans*-α-Bergamotol	0.3
2255	α-Cadinol	1.6
2369	Eudesma-4(15),7-dien-4 ß-ol	0.3
2384	Hexadecanol	0.7
2500	Pentacosane	0.1
2503	Dodecanoic acid	0.1
2622	Phytol	0.7
2655	Benzyl benzoate	0.2
2700	Heptacosane	tr
2900	Nonacosane	0.2
2931	Hexadecanoic acid	0.1
	Total	95.4

RRI = Relative retention indices calculated against *n*-alkanes.
%calculated from FID data tr = Trace (< 0.1 %)

Identification of the essential oil components: Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley and MassFinder 3) and in-house "Baser Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data[12-14], was also used for the identification.

Anticandidal activity: The yeasts in 50 % glycerol were refreshed in Mueller Hinton Broth (Merck) at 35-37 ºC and inoculated on Mueller Hinton Agar (Mast Diagnostics, Merseyside, U.K.) media for preparation of *Candida* suspension. Micro-dilution broth susceptibility assay[15,16] was used for the anticandidal evaluation of the essential oil. Stock solution of oil was prepared in DMSO (Carlo-Erba). Dilution series were prepared from 4 mg/mL to 0.001 mg/mL in sterile distilled water in micro-test tubes from where they were transferred to 96-well micro-titer plates. Overnight grown Candida suspensions in Mueller-Hinton broth (Merck) were standardized to McFarland No: 0.5 (*ca.* 10^6 CFU/mL) by using turbidity meter (Biosan) and 100 µL of each bacterial suspension was then added to each well. The last row containing only the serial dilutions of antimicrobial agent without microorganism was used as negative control. Sterile distilled water and medium served as a positive growth control. After incubation at 37 ºC for 24 h the first well without turbidity

was determined as the minimal inhibition concentration (MIC). Ketoconazole (Sigma) was used as standard antimicrobial agent.

RESULTS AND DISCUSSION

Analyses of the hydrodistilled essential oil was performed on GC and GC/MS systems, simultaneously. Composition of the oil of *H. perforatum* is given in Table- 1. Eighty compounds were identified, representing 95.4 % of the total oil components detected.

This oil which was characterized by a high content of germacrene D (23 %), β-caryophyllene (14 %) and bicyclogermacrene (5 %), was dominated by sesquiterpene hydrocarbons.

Micro-dilution broth susceptibility assay was used for the anticandidal evaluation of the *H. perforatum* essential oil. The results are shown in Table-2. *H. perforatum* oil showed moderate inhibitory effects on a panel of *Candida* species (MIC 0.5-0.06 mg/mL). Significantly C. *albicans* (NRRL Y-12983) was inhibited by standard antifungal agent ketoconazole and the essential oil with the same MIC value (0.06 mg/mL).

TABLE-2
ANTICANDIDAL EFFECTS OF *H. perforatum* ESSENTIAL OIL (MIC: mg/mL)

Microorganisms	Sources	EO	St
C. albicans	Clinical isolate	0.5	0.06
C. albicans	ATCC 90028	0.25	0.06
C. albicans	NRRL Y-12983	0.06	0.06
C. utilis	NRRL Y-900	0.5	0.06
C. tropicalis	NRRL Y-12968	0.5	0.03
C. krusei	NRRL Y-7179	0.125	0.01
C. parapsilosis	NRRL Y-12969	0.25	0.06
C. glabrata	Clinical isolate	0.125	0.03

EO: *Hypericum perforatum* Essential oil, St = Ketoconazole.

There are few investigations on the anticandidal activity of *H. perforatum* essential oil. Previous investigations about anticandidal activities of *H. perforatum* essential oil are consistent with our results. It was reported that *C. albicans* was inhibited by the *H. perforatum* oil having a MIC value of 0.05 mg/mL[8]. In another study *H. perforatum* essential oil evaluated against *C. albicans* and showed moderate activity by using a bioautography method[9].

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