Essential Oil of *Hyptis suaveolens* (L.) Poit. from Tanzania: Composition and Antifungal Activity

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Abstract

The hydrodistilled essential oil (yield 1.2%) of fresh leaves of wild *Hyptis suaveolens* (L.) Poit. was analyzed by GC/MS. Twenty-four compounds representing 90.3% of the oil were identified. The main components of the oil were β-caryophyllene (26.0%), β-elemene (10.4%), *trans*-α-bergamotene (7.7%), spathulenol (7.0%) and bicyclogermacrene (6.5%). The oil exhibited significant antimicrobial activity against *Mucor sp.* when compared to ketoconazole.

Key Word Index

Hyptis suaveolens, Lamiaceae, essential oil composition, β-caryophyllene, β-elemene, antifungal activity.

Introduction

Hyptis suaveolens (L.) Poit (Lamiaceae) is widespread in tropical America (1) and northeast India (2). The plant is used as an appetizing agent, to combat indigestion, stomach pain, nausea, flatulence, colds, and infection of the gall bladder (1-3). The essential oil of *H. suaveolens* has been the subject of previous work. However, the oils from *H. suaveolens* differ in composition according to the geographic origin of the plants (4). Thus, β-caryophyllene (5-9), sabinene (1,5-10), 1,8-cineole (1,3,5,6,10,11), *trans*-α-bergamotene (5,7,9), terpinen-4-ol (5,7), eugenol (4) and fenchone (12) have been described as the main components. Moreover, the literature shows that there are many chemotypes of *H. suaveolens* (1,3-12). The oil has been found to possess antibacterial (13,14) and antifungal (2,15) properties.

This paper reports the chemical composition of the oil of *H. suaveolens* from Tanzania, as well as testing its potential antifungal activity.

Experimental

Plant material and oil isolation: *Hyptis suaveolens* was collected in November 1998 at Kitunda in the Eastern Coast of Tanzania. Local people (Zaramo) call it 'Mhigajini'; in Kiswahili it is 'Kifumbasi kikubwa.' Voucher specimens are kept in the Herbarium of Institute of Traditional Medicine, Muhimbili University College of Health Sciences. Fresh leaves of *H. suaveolens* were hydrodistilled to yield 1.2% oil. The oil obtained was dried over anhydrous sodium sulphate and stored in a refrigerator until analysis.

GC analysis: Analyses were accomplished with use of a Shimadzu GC-R1A (FID) gas chromatograph, fitted with a 30 m x 0.25 mm (0.25 μm film thickness) fused silica capillary column coated with a DB-5 (J&W). The GC operating conditions were as follows: oven temperature programmed from 60°C (3 min) to 240°C at 5°C/min; carrier gas was nitrogen at a flow at 0.9 mL/min. Identification of the components was performed by comparison of

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Table I. Chemical composition *Hyptis suaveolens* oil

Compound	Retention index	Peak area (relative %)	Methods of identification
α-thujene	930	0.5	MS
α-pinene	937	0.2	MS
camphene	953	5.0	MS
sabinene	972	0.5	MS-CO
α-phellandrene	997	1.0	MS
p-cymene	1020	0.3	MS
limonene	1031	3.1	MS-CO
(Z)-β-ocimene	1040	0.4	MS
terpinolene	1088	2.5	MS
linalool	1098	0.4	MS
camphor	1143	0.1	MS
terpinen-4-ol	1177	0.8	MS
α-terpineol	1189	2.2	MS
β-elemene	1391	10.4	MS
β-caryophyllene	1418	26.0	MS-CO
trans-α-bergamotene	1422	7.7	MS
aromadendrene	1439	3.5	MS
α-humulene	1454	3.0	MS
valencene	1481	1.7	MS
bicyclogermacrene	1494	6.5	MS
spathulenol	1576	7.0	MS
globulol	1583	0.9	MS
kaurene	2022	2.7	MS
abietatriene	2054	3.9	MS

Compounds are listed in order of their elution from a DB-5 column; MS: peak identifications based on MS comparison with file spectra; CO: peak identifications based on coinjection

their retention times with those of pure authentic samples. GC/MS analyses were performed with a Perkin Elmer Q-700 equipped with a SE-30 capillary column (30 m x 0.25 mm, coating thickness 0.25 μm). Analytical conditions: oven temperature from 60°C (3 min) to 240°C at 5°C/min; injector and detector temperatures 250°C, carrier gas helium at a flow of 0.9 mL/min, source 70 eV. The oil components were identified by two computer library MS searches using retention indices as a preselection routine, and visual inspection of the mass spectra from literature for confirmation (16,17).

Antimicrobial activity: Antifungal activity was determined according to Provine and Hadley (18). Five-milliliter aliquots of semisolid heart infusion broth containing 0.5% agar, pH of approximately 7.4 (without dextrose, buffer, or indicator) were prepared under sterile conditions with and without an antifungal drug in 16 mm x 125 mm glass tubes. Prepared medium was stored at 4°C and used within one week. The antifungal reference agent, ketoconazole was dissolved in dimethyl sulfoxide according to the manufacturer directions. A suspension that was just turbid (~0.5 McFarland standard) by visual inspection was prepared by suspending the selected yeast or mold in sterile water. Mycelial growth of filamentous fungi was preferred. A standard platinum loopful (~0.001 mL) of the inoculum suspension was inserted deep into each tube of medium containing a known concentration of drug, as well as a drug free control. For filamentous fungi, sterile mineral oil (~0.5 mL) was layered on the inoculated medium to inhibit sporulation. All cultures were incubated for 48 h at 35°C or until good growth was apparent in the drug-free control.

Determination of in-vitro susceptibility: When, by visual inspection, good growth of the yeast or filamentous fungus in the drug-free medium was detected (within 48 h), the growth in all tubes was compared with that of the drug-free control in order to determine inhibition. The growth was scored in the following manner: 4, growth comparable to that of the drug-free control; 3 growth approximately 75% that of the control; 2, growth approximately 50% that of the control,;1, growth 25% or less that of the control; and 0, no visible growth. A collection of three organisms were used, *Saccharomyces cerevisiae*, *Fusarium monoliforme* 7075 and *Mucor sp.*

Results and Discussion

The compounds identified in the oil of *H. suaveolens* from Tanzania are listed in Table I. The sesquiterpene hydrocarbons β-caryophyllene (26.0%), β-elemene (10.4%), *trans*-α-bergamotene (7.7%) and bicyclogermacrene (6.5%), together with the sesquiterpene alcohol spathulenol (7.0%) represented the most abundant components. Of these, only β-caryophyllene (5-9) and *trans*-α-bergamotene (5,7,9) have been reported to occur abundantly. Among the monoterpenes, camphene (5.0%), limonene (3.1%), terpinolene (2.5%) and α-terpineol (2.2%) were found. Unlike previous reports (5-10), sabinene was only found in very small amounts. We did

Table II. Antifungal activity of the oil of *Hyptis suaveolens*

Organism	Essential oil (µg/mL)			Ketokonazol (µg/mL)		
	500	1000	1500	500	1000	1500
Saccharomyces cerevisiae	3	2	2	0	0	0
Fusarium moniliforme 7075	1	1	1	3	2	1
Mucor sp.	1	0	0	4	3	1

4: growth comparable to that of the drug-free control, 3: growth approximately 75% that of the control, 2: growth approximately 50% that of the control, 1: growth 25% or less that of the control, and 0: no visible growth

not find 1,8-cineole (1,3,5,6,10,11), germacrene D (1,12,19) and germacrene B (3). We concluded that *H. suaveolens* under study could be a new chemotype.

The oil showed a strong antifungal activity against *Mucor sp.* and *Fusarium moniliforme*, as shown in Table II.

Acknowledgments

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