J. *Essent. Oil Res.,* 17,230-231 (MarchiApril2005)

Essential Oil of *Rynchosia minima* DC. from Kenya: Composition and Antibacterial Properties

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Abstract

The hydrodistilled essential oil (yield, 0.1 %) of semi-dried leaves of *Rynchosia minima* DC. was analyzed by GC and *GC/MS.* Twenty-four compounds representing 95.9% of the oil were identified. The major components were found to be ~-caryophyllene (30.4%), germacrene B (17.9%), camphor (7.8%), cc-humulene (7.4%) and y-muurolene (7.3%). The oil was found to exhibit antibacterial activity against *Bacillus cetus, Staphylococcus au reus* and *Micrococcus luteus.*

Key Word Index

Rynchosia minima, Papilionaceae, essential oil composition, ~-caryophyllene, germacrene B, antibacterial activity.

Introduction

Rynchosiaminima DC. (Papilionaceae) can be found growing in its natural habitat on the uplands of Kenya (1). It is a twining or wide, trailing annual plant. Stems are very slender having slight pubescence at the beginning. Leaflets sub-curiaceous or membranous, 0.75-2.5 cm, broad, conspicuously gland-dotted; flowers yellow in short-peduncled racemes; pods 1.25-1.5 em long, glabrescent, mostly two-seeded. Roots have bacterial nodules that enrich the soil (2). The plant thrives well even in

••..... dry weather spells in Kenya. There are no references about the medicinal use of this plant in Kenya but it is used for treatment of bilharzia in other parts of Africa (3). In East Africa, R. *hirta* is used as a purgative and for treatment of retained placenta, bilharzia and stomach problems. *Rynchosia sublobata* is used for treatment of chest diseases, stomach problems and as an antidote to snakebites. *Rynchosia congensis,* R. *usambensis* and R. *viscosa* are used for treatment of chest diseases, abdominal pains and itchy rashes (4).

There are no references about the oil content and chemical composition of R. *minima* oil. We report here the results of our studies on the composition of R. *minima* oil from Kenya.

Experimental

Plant material and oil isolation: Rynchosia minima (local name 'Kahurura' in Kikuyu) flowering plants were collected in Maragwa, in the Central Province of Kenya, in July 2000. Voucher specimens are kept in the Herbarium of Faculty of Pharmacy, University of Nairobi. Semi-driedleavesofR. *minima* were hydrodistilled in Clevenger-like apparatus to yield 0.1 % oil. The oil obtained was dried over anhydrous sodium sulfate 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 urn film thickness fused silica capillary column coated with a DB-5. The GC operating conditions were as follows: oven temperature programmed from 40°-230°C at 2°C/min, injector and detector temperatures 240°C, carrier gas was nitrogen at a constant flow at 0.9 mUmin. Identification of the components was perfonned by comparison of their retention times with those of pure authentic samples. Quantitative data were obtained from electronic integration of area percent data.

GC/MS analyses were perfonned with a Perkin ElmerQ-700 equipped with a SE-30 capillary column (30 m x 0.25 mm, 0.25 urn film thickness). Analytical conditions: oven temperature from 40°-230°C at 2°C/min, carrier gas helium at a constant flow at 0.9 mUmin, 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 (5,6).

Antimicrobial acivity: A collection of eight microrganisms were used, including Gram-positive bacteria *Bacillus cereus* (from rice), *Enterococcus faecalis* (ATCC 29212), *Micrococcus*

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Received: April 2002
Revised: June 2002
Accepted: July 2002

1 041-2905/05/0002-0230$6.00/Q-

Table I. Chemical composition of the oil of *Rynchosia minima*

Compound	Retention index	Peak area (relative %)	Methods of identification	Compound	Retention index	Peak area (relative %)	Methods of identification
~·pinene	980	1.5	MS-CO	a-cube bene	1352	0.8	MS
p-cymene	1025	0.3	MS	a-ylangene	1373	0.2	MS
limonene	1031	0.2	MS-CO	a-copaene	1376	1.3	MS
~-phellandrene	1033	2.2	MS-CO	~-bourbonene	1385	0.5	MS
(Z)-~-ocimene	1040	0.2	MS	~-elemene	1391	1.3	MS
y-terpinene	1062	1.0	MS	~-caryophyllene	1418	30.4	MS-CO
terpinolene	1088	1.9	MS	a-humulene	1454	7.4	MS
camphor	1143	7.8	MS-CO	y-gurjunene	1463	1.5	MS
borneol	1165	2.0	MS	y-muurolene	1476	7.3	MS
terpinen-t-ol	1177	1.5	MS	germacrene 0	1480	4.7	MS
carvone	1242	2.5	MS-CO	y-cadinene	1513	1.3	MS
o-elemene	1339	0.2	MS	germacrene B	1556	17.9	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 coiniection

luteus (ATCC 9341), *Staphylococcus aureus* (ATCC 25212) and *Staphylococcus epulermulis* (from cow milk), Gram-negative strains *Escherichia coli* (from water), *Klebsiella* ssp. (from bird food) and *Proteus mirabilis* (from human urine). All the samples of microorganisms were characterized at the Department of Micro-biology, National UniversityofRioCuarto, Argentina and voucher specimens were preserved. All the strains tested were maintained at 4°C in Tripsein-Soy Agar and were subcultured every month. The paper disc diffusion method was used to test antibacterial activity. It was performed using an IS-h culture, growth at 37°C and adjusted to approximately 106 cfulmL. The inoculum (200 ilL) was spread over plates containing Mueller-Hinton agar and a paper filter disc (4 mm) impregnated with 10 ul., of the oil was placed on the surface of the media. A gentamycin disc (Brittania Co.) containing 10 Ilg was used as a reference. The plates were left for 30 min at room temperature to allow the diffusion of the oil and then incubated at 37°C for 24 h. After this, the inhibition zone around the disc was measured with a calliper.

Results and Discussion

The results of the analysis of the oil ofR. *minima* are presented in Table I. Constituents are listed in order of their elution from a D B-5column. The major components of the oil are ~-caryophyllene (30.4%), gemacrene B (17.9%), camphor (7.S %), a-humulene (7.4 %) and y-muurolene (7.3 %). The oil exhibited significant inhibition against *B. cereus,* S. *aureus* and M. *luteus* (Table 11).

Acknowledgements

The authors thank CONICOR, CONlCET and SECyT-UNC for finanCial support and M r. M *athenge of Botany Department, University of Nairobi for plant identification.*

Table II. Antibacterial activity (inhibition zone in mm) of the oil of (10 Jll) *Rynchosia minima* compared to that of 10 Jlg gentamicin

Organism	Oil inhibition zone (mm)	Gentamicin inhibition zone (mm)
Bacillus cereus 18 Enterococcus		25
faecalis 9 Micrococcus lute us 16		13
Staphylococcus aureus 11.5		20
Staphylococcus epidemidis NI		15
Escherichia coli NI *Klebsiella* ssp. NI		3
Proteus mirabilis NI		
		18
		22
		23

inhibition zone diameter measured in mm, the disc diameter of 4 mm being included; NI: no inhibition zone

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