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Composition and Antibacterial Activity of *Pseudocytisus integrifolius* (Salisb.) Essential Oil from Algeria

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The essential oil composition of an endemic Algerian *Cruciferae*, *Pseudocytisus integrifolius* (Salisb.) Rehder, was analyzed by gas chromatography (GC) and GC−mass spectrometry (MS). Eighty-three components representing more than 96.5% of the oil were identified. The major components were dimethyl disulfide (33.4%), dimethyl trisulfide (24.2%), and an unsaturated nitrile (31.7%). Fractionation on a silica gel column led to the identification of trace-level compounds, in particular, polar compounds such as nitriles and aldehydes, and to the isolation of dimethyl disulfide, dimethyl trisulfide, and an unsaturated nitrile. Structural analysis using high-resolution mass spectrometry (HRMS) and ^{1}H,^{13}C NMR techniques enabled the identification of pent-4-enenitrile. Variation in essential oil composition and yields was studied according to harvesting time, drying, and parts of the plant. The essential oil of aerial parts was tested for its antibacterial activity using a paper disk method. The oil was effective on the inactivation of *Escherichia coli* and *Pseudomonas aeruginosa* and ineffective on the inactivation of *Staphylococcus aureus*.

KEYWORDS: ***Pseudocytisus integrifolius*; *Cruciferae* (*Brassicaceae*); essential oil composition; dimethyl disulfide; dimethyl trisulfide; nitriles; aldehydes; antibacterial activity**

INTRODUCTION

Cruciferae constitutes a systemically difficult taxon that is widely distributed and consists of approximately 340 genera and 3350 species (*1*). This classification has been subject to several modifications since Schulz's system (*2*). Numbers of tribes and genera have led to the fact that this family is often regarded as difficult for taxonomic classification. This classification of genera and systematic relationships of subtribes within *Cruciferae* is yet not resolved and is currently being studied. Chemical composition, in addition to morphology, anatomy, and cytogenetics, is a major tool for botanists in performing plant classification. In fact, certain plant constituents such as fatty acids, sterols, and glucosinolates can be used in taxonomic studies of the *Cruciferae* (*3*). Identification of volatile compounds such as nitriles, isothiocyanates, and thiocyanates is an easy and rapid method for characterizing glucosinolates present in a plant.

Pseudocytisus integrifolius (Salisb.) Rehder subsp. *glabrescens* (Coss.) Lit. Et Maire, called Queçdir or El-Kasdir, is endemic to Algeria (*4*). It is a shrub measuring between 30 and 60 cm in height that grows naturally in the southwest of Algeria (*5*, *6*) with a blossoming period in March and April. It is a member of the *Cruciferae* family and belongs to the subtribe Vellinae (*7*).

This plant is used by local people to nourish the sheep population in this desert region for what they consider to be its antiparasitic properties. This plant is also widely used as an ingredient in numerous local medicines. The essential oil is characterized by a very strong, pungent, and sulfury odor. To our knowledge, there is no report on chemical composition and

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biological activity of *Pseudocytisus integrifolius* essential oil. The aim of this paper is to present qualitative and semiquantitative analysis of the essential oils of this *Cruciferae* species growing in the Atlas, to compare their chemical compositions according to harvesting time, drying, and parts of the plant, and to elucidate its antibacterial effects.

MATERIALS AND METHODS

Plant Material. Plant materials were collected in western Algeria (*8*), in the south of Tlemcen in March 2003. Botanical identification of the plant was conducted by Dr. Noury Benabadji, "Laboratoire d'Ecologie et Gestion des Ecosystèmes", Abou Bekr Belkaid University, Tlemcen (Algeria). Voucher specimens of the plant are deposited in the Herbarium of the "Jardin Botanique de la Ville de Nice", Nice, France (NICE B-3981). Before steam distillation, plants were extended by ground, in one layer, in an open room protected from the sun. During drying time, plants were turned over to allow homogeneous drying.

Essential Oil Isolation. Aerial parts (2000 g) dried 8 days at room temperature were subjected to steam distillation (4 h) to yield 0.034% oil. The oil was dried over anhydrous sodium sulfate and stored at low temperature prior to analysis. The oil obtained was light yellow with a strong sulfury odor. The physicochemical characteristics of the oil were determined according to AFNOR (*9*) standards at 20 °C: d(20;20) = 0.9920, n(D;30) = 1.694.

As in the case of whole plant, the flowers and leaves were manually separated from the whole of material after 8 days of drying and subjected separately to steam distillation. However, essential oils from flowers and foliage give very poor yields (<0.01%); volatile extracts were obtained using chloroform as solvent trapping.

Chemical Analyses. Identification of volatile compounds was performed using gas chromatography−mass spectrometry (GC-MS). The component relative concentrations in each essential oil were calculated based on GC peak areas without using correction factors.

Analytical GC. GC analyses were carried out using a Hewlett-Packard 6890N gas chromatograph, under the following operating conditions: vector gas, He; injector and detector temperatures, 250 °C; injected volume, 0.1 μL, splitless; HP1 column (J&W Scientific), poly(dimethylsiloxane) (50 m × 0.20 mm i.d., film thickness 0.5 μm) and INNOWAX column (J&W Scientific), poly(ethylene glycol) (50 m × 0.20 mm i.d., film thickness 0.33 μm); constant flow 1 mL/min; temperature program 60−250 °C at 2 °C/min and 250 °C for 60 min. Retention indices were determined with C_5 to C_{26} alkane standards as reference. Relative amounts of individual components are based on peak areas obtained without FID response factor correction. Three replicates were performed for each sample. The average of these three values and the standard deviation were determined for each component identified.

GC-MS Analyses. Each oil was analyzed by GC-MS using a Hewlett-Packard 5890/5970A system with a HP1 column (50 m × 0.20 mm fused silica capillary column; film thickness, 0.5 μm). GC oven initial temperature was 60 °C and was programmed to 200 °C at a rate of 2 °C/min and 200 °C during 120 min under the following operation conditions: vector gas, He; injector and detector temperatures, 250 °C; injected volume: 0.2 μL, splitless. Retention indices were determined with C_5 to C_{28} alkane standards as reference. The mass spectra were performed at 70 eV of the mass range of 35−400 amu.

Identification of the constituents was based on comparison of the retention times with those of authentic samples on computer matching against commercial (Wiley, MassFinder 2.1 Library, Nist98) libraries and our homemade library of mass spectra built up from pure substances and MS literature data (*10*−*12*) and confirmed by comparison of retention indices with published index data (*13*, *14*).

Chemicals. The standard compounds were as follows: dimethyl sulfide (Acros, 12776), 3-methylbutanal (Aldrich, 14,645-5), dimethyl disulfide (Acros, 16559), hexanal (Aldrich, 11,560-6), *n*-octane (Aldrich, 41,223-6), hex-2(*E*)-enal (Aldrich, 13,265-9), hexanenitrile (16,665-0), heptan-2-one (Acros, 15400), heptanal (Aldrich, H-212-0), *n*-nonane (Aldrich, N2,940-6), benzaldehyde (Aldrich, B133-4), α-pinene (Aldrich, 14,752-4), heptanenitrile (Aldrich, 40,489-6), dimethyl trisulfide (Acros, 41503), β-pinene (Aldrich, 11,208-9), octanal (Aldrich, O-560-8), myrcene (Fluka, 64643), Δ-3-carene (Fluka, 21986), α-terpinene (Aldrich, 22,318-2), phenylacetaldehyde (Aldrich, 10,739-5), *p*-cymene (Fluka, 30039), limonene (Aldrich, 18,316-4), 1,8-cineole (Aldrich, C8,-060-1), acetophenone (Prolabo), γ-terpinene (Fluka, 86478), octanol (Prolabo), fenchone (Aldrich, 19,643-6), nonanal (Aldrich, N3,080-3), benzyl cyanide (Aldrich, 18,572-8), camphor (Aldrich, 14,807-5), borneol (Aldrich, 42,024-7), terpinen-4-ol (Acros, 36002), indole (Aldrich, I-340-8), *trans*-anethole (Fluka, 10368), thymol (Prolabo), caryophyllene oxide (Fluka, 22076); *p*-cresyl acetate was obtain by reaction between acetyl chloride (Aldrich, 114189) and *p*-cresol (Prolabo) using standard procedure; product was characterized by ^{1}H and ^{13}C NMR before GC analyses.

Chromatography of the *Pseudocytisus integrifolius* Essential Oil. Two grams of the essential oil from plant material was subjected to chromatography over silica gel (70−230 mesh, Merck) and eluted sequentially with pentane, pentane/diethyl gradients, and finally diethyl ether. Eight fractions were obtained that were all analyzed by GC and GC-MS. Three compounds (RI on apolar/polar columns: 731/1095, 731/1275, 957/1380) were isolated from fractions 1 and 7 (eluted with pentane 100% and ether 100%).

Characterization of the Isolated Compounds. The structure of compounds with RI = 731/1095 and 957/1380 were established from ^{1}H,^{13}C NMR as dimethyl disulfide (RI = 731/1095) and dimethyl trisulfide (RI = 957/1380). Identification was confirmed by direct comparison of the ^{1}H,^{13}C NMR and MS data with standards compounds.

Structure of compounds with RI = 731/1275 was established from one- and two-dimensional NMR experiments as pent-4-enenitrile. Its formula was confirmed by high-resolution mass spectrum analysis. To the best of our knowledge, spectral data of this compound are being reported for the first time.

GC−HRMS. GC−HRMS analyses were performed using a Carlo-Erba 8000 gas chromatograph under the following operating conditions: carrier gas, He; detector temperature, 200 °C; split ratio, 1/70; total flow, 1.5 mL/min; HP1 column, poly(dimethylsiloxane) (50 m × 0.32 mm i.d., film thickness, 0.52 μm); temperature program, 40−280 °C at 4 °C/min, and 280 °C for 10 min, coupled to a Fisons VG-Prospec magnetic spectrometer. High-resolution mass spectra were obtained by electron ionization at 70 eV, *m*/*z* 35−400, source temperature 250 °C.

^{1}H,^{13}C NMR and 2D NMR. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200 FT spectrometer at 20 °C, in $CDCl_3$ with TMS as internal standard. Correlation spectroscopy spectra were recorded on a Bruker Avance 500 spectrometer at 25 °C. Multiplicity was indicated as follows: d for doublet, t for triplet, or m for multiplet.

Spectral Data of Pent-4-enenitrile. ^{1}H NMR: δ 2.38 (m, 2H), 2.42 (m, 2H), 5,15 (dm, 1H, J = 10.2 Hz), 5.18 (dm, 1H, J = 17.1 Hz), 5.82 (ddt, 1H, J = 17.1, 10.2, 6.5 Hz). ^{13}C NMR: δ 16.9, 29.2, 117.6, 119.2, 134.2. GC-MS: 81 (M^+, 23.0), 54 (28.4), 53 (8.6), 52 (6.1), 51 (5.5), 50 (3.9), 42 (3.7), 41 (100), 40 (4.7), 39 (38.2), 38 (5.9), 37 (3.9). HRMS calculated for C_5H_7N: 81.057 849. Observed: 81.056 984.

Antibacterial Assays. The paper-disk diffusion method was used (6 mm nonimpregnated disks; Sanofi Diagnostic Pasteur). *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 25923) were the bacteria used. The activation of the bacteria species stressed by cold has been done with Trypton salt solution adjusted at pH = 7. Divided among assay tube, they were put at 120 °C during 20 min in an autoclave. The suspension culture selective media were EMB agar for *E. coli*, Cetrimid agar for *P. aeruginosa*, and Chapman agar for *S. aureus*. For all cultures, incubation temperatures were 37 °C. Incubation time was 48 h for *E. coli* and *P. aeruginosa*; because for *S. aureus* no culture appeared after 48 h, incubation was left 72 h. Seeding was performed on Mueller Hinton. The antibiotics (Pasteur Institute) ampicillin (ref 66126, 10 μg/disk), staphylomycin (ref 67546, 15 μg/disk), and piperacillin (ref 67228, 100 μg/disk) served as positive controls for the bacteria**.** Each paper disk was impregnated with 20 μL of essential oil and the different dilutions, 50, 25, and 12.5 (% v/v), of the previously prepared solution. The reading of the plates, incubated at 37 ± 1 °C, showed after 48 h inhibitory zones around the paper disks. When the inhibitory zone diameter is lower or equal to 6 mm, the sample tested was considered as not active. Two replicates were performed for each analysis.

Table 1. Variation of Water Content and Essential Oil (EO) Yields from Aerial Parts According to Drying Time

	drying time (days)											
	1	2	3	4	5	6	7	8	9	10	11	12
% H_2O	74.8	50	40.2	37	33.8	33.5	33.1	29	27.5	27	26.3	25.8
EO yields[a]	tr[b]	tr[b]	tr[b]	tr[b]	0.005	0.016	0.029	0.034	0.026	0.014	0.005	0.004

[a] Steam distillation for 4 h; 2000 g of aerial parts of the plant. [b] Yield not quantified.

RESULTS AND DISCUSSION

To optimize essential oil yields, several studies were performed on the fresh vegetable, in particular, variation of water content and essential oil yields (Table 1) shows that in a first time, between 1 and 5 days, yields obtained after steam distillation for 4 h were very poor. This can be explained by the high level of water content in the plant. Yields were optimum after 8 days and decreased after this drying time. The decline is certainly due to the evaporation of the volatile compounds during long drying times. So, effective time of drying is 8 days, corresponding to 29% water content.

The essential oil studied in this work was obtained in a yield of 0.034% by steam distillation of aerial parts of *Pseudocytisus integrifolius*. The components of the essential oil, the percentage of each constituent (by direct integration of FID responses), and the retention indices are listed in Table 2 in relation to their elution order on the HP1 column. Identifications have been carried out by means of GC and GC-MS analyses in combination with retention indices and authentic samples injections. Essential oil fractionation on silica gel column and GC-MS analyses of each fraction led to the identification of many compounds present in trace levels, in particular, in apolar (eluted with pentane 100%) and polar (eluted with diethyl ether 100%) solvents.

We isolated from fraction 1 and 7 dimethyl disulfide and dimethyl trisulfide (fraction 1) and an abundant compound (fraction 7, RI = 721/1275) not identified by its mass spectrum and retention indices. Structural analysis (HRMS, ^1H and ^{13}C NMR) enabled us to elucidate the structure as pent-4-enenitrile. This compound has already been identified in *Cruciferae* such as *Farsetia aegyptia* (*15*), *Farsetia ramosissima*, *Brassica oleracea* convar. *acephala* cultivars (*16*), and *Brassica napus* (*17*). In conclusion, chromatographic profiles of the essential oil from aerial parts of the plant revealed 83 identified constituents, which represented 96.5% of the total GC area for the essential oil. These analyses revealed that *Pseudocytisus integrifolius* oil contained nine sulfur-containing compounds (58.5%), nine nitriles (32.7%), 23 aldehydes (2.1%), seven monoterpene hydrocarbons (0.3%), and six oxygenated monoterpenes (0.3%). The major components were dimethyl disulfide (33.4%), pent-4-enenitrile (31.7%), and dimethyl trisulfide (24.2%).

Like many *Cruciferae*, *Pseudocytisus integrifolius* contains glucosinolates, which are precursors of many volatile compounds, in particular, nitriles and isothiocyanates. Isothiocyanates, odorous and strongly pungent compounds, are formed by the action of the enzyme thioglucoside glucohydrolase, EC 3.2.3.1 (myrosinase), on the glucosinolates when the plant tissue is disrupted (*18, 19*). Nitriles are formed by the enzymatic action of the myrosinase in specific conditions or by thermic degradation of glucosinolates. Hydrodistillation gave predominantly nitrile degradation products as observed in the chemical composition of the different essential oils, because it is performed on intact plant material (*20*). According to isothiocyanates and nitriles identified, this study allowed us to infer the presence of seven glucosinolate precursors of many volatile compounds from *Pseudocytisus integrifolius* (Table 3) (*21*−*23*).

It is known that natural products can contribute to the fight against pathogenic microorganisms. *Pseudocytisus integrifolius* is used in sheep food to perform some therapeutic activities, in particular, antibacterial and antiparasitic activities. For evaluation of the antimicrobial activity of the essential oil from aerial parts of plant, three strains of microorganisms were studied in this assay, one Gram-positive (*S. aureus*) and two Gram-negative bacteria (*E. coli* and *P. aeruginosa*). Table 4 presents the antibacterial activity of the essential oil from aerial parts and its different dilutions, 50, 25, and 12.5 (% v/v). *E. coli* and *P. aeruginosa* are affected by the essential oil and the corresponding dilutions; however, *S. aureus* bacteria is resistant and does not show any inhibitory zone. Diameter values of the inhibitory zones are lower for *P. aeruginosa* than for *E. coli* for the essential oil and the different dilutions. Since this *Cruciferae* presents a high percentage of sulfur-containing components, these assays confirm the importance of studying the correlation between its chemical composition and antimicrobial activity (*24*).

Moreover, volatile compounds from flowers and leaves have been studied. Essential oils obtained from leaves and flowers led to very poor yields. This can be explained by the high level of water content from their plant parts: 80% for leaves, 78% for flowers, and 66% for stems. These results obliged us to trap volatile compounds during steam distillation using chloroform. Analyses of volatile extracts obtained were performed using GC and GC-MS (Table 2). Leaf volatile extract is characterized by 39 identified compounds, which correspond to 97.7% of FID total area with four sulfur-containing compounds (63.7%), six nitriles (11.7%), and 11 aldehydes (4.5%).

In the flower volatile extract, we were able to identify 75 compounds (97.8% of the FID total area), of which seven were sulfur-containing compounds (67.3%), four nitriles (4.5%), and 17 aldehydes (1.8%). This extract is characterized by a limited level of nitrile (4.5%) compared with 11.7% for leaf volatile extract, and a high amount of terpenoids, in particular, oxygenated monoterpenes (11 compounds, 2.7%), such as borneol (0.2%), terpinen-4-ol (0.3%), or α-terpenyl acetate (0.2%), and oxygenated sesquiterpenes (seven compounds, 8.0%), such as spathulenol (3.2%), copaborneol (3.0%), or 1-*epi*-cubenol (0.7%).

Chloroform trapping to obtain the oils from leaves and flowers makes it difficult to compare their compositions with that from essential oil of aerial parts. However, qualitative comparison shows that terpenoid compounds resulted from the flower and leaves.

In conclusion, our GC and GC-MS study of *Pseudocytisus integrifolius* essential oil led to the identification of 83 compounds representing 96.5% of the total GC area. The major components were dimethyl disulfide (33.4%), pent-4-enenitrile (31.7%), and dimethyl trisulfide (24.2%). Study of flower volatile extract enabled the identification of numerous terpenoid compounds, such as oxygenated monoterpenes and sesquiterpenes.

The results presented here for the antibacterial activity study demonstrate the activity of *Pseudocytisus integrifolius* and support the use of parts of this plant in traditional livestock herd nourishment.

Table 2. Chemical Composition of *Pseudocytisus integrifolius* Essential Oils

compounds[a]	RI[b]		EO[c]	EO flowers[c]	EO leaves[c]	identification methods[d]
dimethyl sulfide	505	746	0.2	tr[g]		MS, RI, std
3-methylbutanal	640	916	0.2	tr		MS, RI, std
but-2(*E*)-enenitrile[e]	637	1157	tr			MS, RI
dimethyl disulfide	731	1095	33.4 ± 0.6	40.9 ± 2.4	23.8 ± 0.1	MS, RI, ^{1}H, ^{13}C NMR
pent-4-enenitrile	731	1275	31.7 ± 0.4	3.6 ± 1.0	9.9 ± 0.1	MS, RI, ^{1}H, ^{13}C NMR
pent-2-enenitrile[e]	755		tr		0.8	MS, RI
hexanal	776	1098	0.2	tr	0.1	MS, RI, std
n-octane	801	800	tr			MS, RI, std
3-methylbutanenitrile[e]	809	1196	tr			MS, RI
hex-2(*E*)-enal	825	1216	0.1	tr		MS, RI, std
hexanenitrile	850	1303	tr		0.1	MS, RI, std
2,4-dithiapentan[e]	862	1300	tr	tr		MS, RI
heptan-2-one	868	1165	tr			MS, RI, std
heptanal	880	1184	0.1	0.1	0.1	MS, RI, std
n-nonane	901	898	tr			MS, RI, std
5-methylhexanenitrile[e]	917	1350	0.4 ± 0.1	0.3	0.4	MS, RI
α-thujene	922	1020		tr		MS, RI
hept-2(*E*)-enal[e]	931	1280	tr			MS, RI
benzaldehyde	934	1518	tr			MS, RI, std
α-pinene	932	1010	tr	0.1		MS, RI, std
heptanenitrile	945	1396	tr			MS, RI, std
3-butenyl isothiocyanate[e]	959	1489	tr			MS, RI
6-methylhept-5-en-2-one[e]	962	1347	tr			MS, RI
octan-2-one[e]	965	1275	tr			MS, RI
dimethyl trisulfide	957	1380	24.2 ± 0.1	24.8 ± 0.1	35.4 ± 0.3	MS, RI, ^{1}H, ^{13}C NMR
sabinene	968	1120		tr		MS, RI, std
β-pinene	970	1096	tr	tr		MS, RI, std
furfuryl methyl sulfide[e]	975	1425	tr			MS, RI
2-pentyl furan[e]	981	1216	0.1	tr	0.1	MS, RI
octanal[e]	983	1263	0.1	tr	tr	MS, RI, std
myrcene	984	1140	tr	tr		MS, RI, std
Δ-3-carene	1003	1129	tr	tr		MS, RI, std
α-terpinene	1011	1171		tr		MS, RI, std
β-phellandrene	1014	1161	tr	tr		MS, RI, std
phenylacetaldehyde	1013	1626	tr			MS, RI, std
p-cymene	1016	1250	0.1	5.0 ± 0.1	1.8	MS, RI, std
2,2,6-trimethylcyclohexanone[e]	1017	1310		0.2		MS, RI
limonene	1025	1195	0.2 ± 0.1	0.1	tr	MS, RI, std
1,8-cineole	1027	1220	tr	0.1	tr	MS, RI, std
oct-2(*E*)-enal[e]	1033	1429	tr	tr		MS, RI
acetophenone	1035	1552	tr			MS, RI, std
p-methylbenzaldehyde[e]	1042	1605	tr			MS, RI
γ-terpinene	1050	1235		0.1		MS, RI, std
octanol	1051	1294	tr			MS, RI, std
nonan-2-one[e]	1070	1393	0.1	0.2		MS, RI
dehydro *p*-cymene[e]	1074			0.1		MS, RI
fenchone	1075	1410	tr			MS, RI, std
nonanal	1083	1393	0.3	0.1	1.2	MS, RI, std
benzyl cyanide	1095	1896	0.5	0.4	0.2	MS, RI, std
2,3,5-trithiahexane[e]	1097	1632	tr	0.2		MS, RI
undecane[e]	1099	1106	0.1			MS, RI
nona-2(*E*),6(*Z*)-dienal[e]	1127	1587	0.2			MS, RI
camphor	1130	1520	tr	0.2		MS, RI, std
non-2(*E*)-enal[e]	1135	1540	tr			MS, RI
4-methyl-2,3,5-trithiahexane[e]	1144		tr	0.4	0.1	MS, RI
p-cresyl acetate	1146	1684	0.1 ± 0.1			MS, RI, std
2-methoxy-3(1-methylpropyl)-pyrazine[e]	1155		tr			MS, RI
borneol	1150	1723		0.2		MS, RI, std
cryptone[e]	1156	1659	tr	0.6		MS, RI
terpinen-4-ol	1163	1581		0.3		MS, RI, std
decan-2-one[e]	1172	1491	tr			MS, RI
safranal[e]	1175	1614	0.1	0.1	0.5	MS, RI
decanal[e]	1186	1490	0.3	tr	tr	MS, RI
dimethyl tetrasulfide	1190	1438	0.7	1.0 ± 0.1	4.4	MS, RI, std
β-cyclocitral[e]	1203	1600	tr	tr	0.3	MS, RI
cuminaldehyde[e]	1213	1753		0.5		MS, RI
dec-2(*E*)-enal[e]	1238	1628	tr			MS, RI
unknown **1**[f]	1244		tr	1.0	0.7	
2-phenylbut-2-enal[e]	1240	1896	0.3	tr	1.2	MS, RI
phellandral[e]	1251	1720		0.9		MS, RI
indole	1256	2489	0.3	0.1		MS, RI, std
trans-anethole	1264	1798	0.2	tr		MS, RI, std
thymol	1270	2162		0.6		MS, RI, std
undecan-2-one[e]	1272	1590	0.3	tr		MS, RI
carvacrol	1278	2190		0.1		MS, RI, std
undecanal[e]	1274		tr			MS, RI
4-vinyl-guaiacol[e]	1286	2170	tr	0.3		MS, RI
deca-2(*E*),4(*E*)-dienal[e]	1290	1792	tr	tr		MS, RI
tridecane[e]	1297	1290	tr			MS, RI
α-terpinyl acetate	1332	1672		0.2		MS, RI, std
undec-2(*E*)-ena[e]	1340	1740	tr	tr	0.5	MS, RI
(*E*)-β-damascenone[e]	1361	1797	0.1	0.1	0.5	MS, RI
α-copaene[e]	1375	1470	0.1	0.1	1.5	MS, RI
tetradecane[e]	1397	1405	0.1	tr	1.0	MS, RI

Table 2. (Continued)

compounds[a]	RI[b]		EO[c]	EO flowers[c]	EO leaves[c]	identification methods[d]
α-ionone[e]	1402	1805	tr	0.1		MS, RI
geranyl acetone[e]	1428	1850	0.1	tr	0.5	MS, RI
β-ionone[e]	1469	1907	0.4	0.3	1.4	MS, RI
tridecan-2-one[e]	1474	1786	0.1		0.5	MS, RI
tetradecanal[e]	1489	1932	0.1	tr	0.3	MS, RI
pentadecane[e]	1496	1499	0.1			MS, RI
calamenene[e]	1509	1800		0.2	0.4	MS, RI
Δ-cadinene[e]	1514	1755		0.2	0.4	MS, RI
spathulenol[e]	1564	2093		3.2	2.4	MS, RI
caryophyllene oxide	1567	1947		0.4	2.6	MS, RI, std
epiglobulol[e]	1578	2080		0.4	0.3	MS, RI
hexadecane[e]	1586	1600	tr	0.1		MS, RI
copaborneol[e]	1592	2147		3.0 ± 0.3		MS, RI
1-*epi*-cubenol[e]	1612	2031		0.7	0.6	MS, RI
Δ-cadinol[e]	1626	2155		0.1		MS, RI
β-eudesmol[e]	1632	2222		0.2		MS, RI
unknown **2**[f]	1658		1.3	0.1	0.3	MS, RI
unknown **3**[f]	1687	2109	0.1	1.4	0.9	MS, RI
pentadecanal[e]	1693	2020	0.1	0.1	0.3	MS, RI
heptadecane[e]	1696	1700	tr	0.1		MS, RI
3-indolylacetonitrile[e]	1740		0.1	0.2	0.3	MS, RI
octadecane[e]	1804	1789	tr	0.1		MS, RI
6,10,14-trimethylpentadecan-2-one[e]	1830		0.6	1.3	2.4	MS, RI
neophytadiene[e]	1836	1910		0.1	1.4 ± 0.1	MS, RI
palmitic acid[e]	1940		0.1			MS, RI
identified compounds (%)			83(96.5)	75(97.8)	39(97.7)	
nonidentified compounds (%)			3(1.4)	3(2.5)	3(1.9)	
sulfur compounds (%)			9(58.5)	7(67.3)	4(63.7)	
nitriles (%)			9(32.7)	4(4.5)	6(11.7)	
aldehydes (%)			23(1.7)	17(1.7)	11(4.5)	
monoterpene hydrocarbons (%)			7(0.3)	12(5.4)	2(1.8)	
oxygenated monoterpenes (%)			6(0.3)	11(2.7)	3(0.8)	
sesquiterpene hydrocarbons (%)			1(0.1)	3(0.5)	3(2.3)	
oxygenated sesquiterpenes (%)				7(8.0)	4(5.9)	

[a] Compounds are listed in order of their elution from an HP1 column. [b] RI = retention indices as determined on HP1 and INNOWAX column using the homologous series of *n*-alkanes. [c] EO = essential oil (% ± SD with SD = standard deviation). [d] Methods of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those from the literature; std, by injection of an authentic sample; ^{13}C,^{1}H NMR, by isolation of the compound by column chromatography and spectral analysis. [e] Compound tentatively identified according to the mass spectrum (MS) and by comparison of RI with the literature (RI). [f] Unknown **1** (RI = 1244): 174 (0.3), 147 (93.4), 132 (66.2), 118 (20.3), 117 (21.3), 116 (100), 90 (15.3), 89 (59.3), 77 (34.4), 63 (29.4), 62 (12.7), 51 (13.8), 39 (11.2). Unknown **2** (RI = 1658): 234 (8.4), 206 (14.9), 205 (100), 198 (12.9), 190 (9.8), 183 (32.2), 134 (22.0), 105 (9.7), 91 (24.4), 71 (10.1), 43 (13.2), 41 (9.2). Unknown **3** (RI = 1687): 186 (64.4), 171 (24.3), 156 (32.3), 155 (100), 154 (28.8), 146 (17.1), 130 (18.4), 128 (46.6), 127 (30.6), 101 (21.4), 77 (15.7), 40 (16.8). [g] Trace (<0.1%); trace level of unidentified compounds are not mentioned.

Table 3. Glucosinolates Identified in *Pseudocytisus integrifolius*

glucosinolate[a]	glucosinolate hydrolysis products identified
isobutyl GLS	3-methylbutanenitrile
gluconapin	pent-4-enenitrile but-3-enyl isothiocyanate
n-pentyl GLS	*n*-hexanenitrile
4-methylpentyl GLS	5-methylhexanenitrile
n-hexyl GLS	*n*-heptanenitrile
glucotropaeolin	benzyl cyanide
glucobrassicin	3-indolyl acetonitrile

[a] GLS = glucosinolate.

Table 4. Antibacterial Activity of *Pseudocytisus integrifolius* Essential Oil from Aerial Parts[a]

bacterial species	Gram classification	EO	50	25	12.5	ampicillin	staphylomycin	piperacillin
E. coli	–	26	24	18	8	17	13	13
P. aeruginosa	–	22	18	8	*b*	*b*	13	20
S. aureus	+	*b*				17	15	*b*

[a] Values represent diameters of inhibitory zone (mm) at indicated dilutions (% v/v); EO = essential oil from aerial parts (20 μL/disk) [b] Not active.

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