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Bioactive Phenylpropanoids from *Daucus crinitus* Desf. from Algeria

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The volatile constituents of *Daucus crinitus* Desf. from Algeria were analyzed by GC and GC-MS, The main constituent was isochavicol isobutyrate (39.0%), an uncommon phenylpropanoid. By synthesis of a series of homologous esters, it was also possible to determine the presence of small amounts of isochavicol propionate, which has never been described previously as a natural product. The antibacterial and antifungal activities of the whole essential oil, of these two esters, and of isochavicol itself were investigated against a wide range of bacteria and fungi. Additionally, their antimalarial and antiradical properties were also evaluated, showing an interesting antiplasmodial activity of isochavicol.

KEYWORDS: *Daucus crinitus*; isochavicol esters; antibacterial activity; antifungal activity; antimalarial activity

INTRODUCTION

The phytochemistry of the genus *Daucus* (Apiaceae) has been extensively studied in analytical works on the composition of various extracts of its members. Hence, the presence of flavonoids (*1*−*3*), carotenoids (*4*), polyacetylenes (*5*), anthocyans (*6*), and volatile constituents (*7*−*12*) was reported in studies concentrated mainly on miscellaneous subspecies of *Daucus carota*, but some less common *Daucus* species were also investigated, almost exclusively through the analysis of their essential oils: *Daucus reboudii* Coss. (*7*), *Daucus glaber* Forsk. (*9*), *Daucus gingidium* L. (*11*), *Daucus syrticus* Murb. (*12*), etc. The biological activities of *Daucus* essential oils, mostly *Daucus carota* ssp., have been reported in several studies, which emphasized their antibacterial (*13*−*17*) and antifungal (*13*, *18*−*20*) activities, which were, however, rarely attributed to single constituents (*17*).

Daucus crinitus Desf. (syn. *D. meifolius* Brot.) is one of the 11 *Daucus* species growing in Algeria. It is distributed in Northern Africa as well as in the Iberic peninsula and is a perennial plant with erect stems often longer than 50 cm and leaves (10−20 cm) divided into numerous linear pseudoverticillate segments. Its basal leaves are hairy, numerous, and suberect, and the umbels are convex or flat with unequal rays. It has nontuberous taproots usually 6 cm long, and its fruits are small (average weight = 3.25 g/1000 seeds). In Algeria, it is locally known as "Fassoukh" and as "Eurq Sidi Messaoud" (*21*) and the traditional belief maintains that the ingestion of its raw roots can cure spells.

To the best of our knowledge, no previous phytochemical investigation of *D. crinitus* has been reported in the literature. In the course of our studies of the Algerian species, we noted that the volatiles of *D. crinitus* contained an important part of uncommon phenylpropanoids structurally related to several interesting antimicrobial and antiparasitic compounds recently discovered in the genus *Pimpinella* (*22*, *23*). Accordingly, we undertook an extensive screening of the biological activities of the essential oil of *D. crinitus*, as well as of two of its constituents, isochavicol isobutyrate (**2**) and isochavicol propionate (**3**), the latter being reported here for the first time.

MATERIALS AND METHODS

Chemicals. Standard compounds, solvents, and reagents were purchased from Sigma-Aldrich, except linalool, caryophyllene oxide, and triethylamine, obtained from Fluka. Diethyl ether was dried by refluxing on sodium/benzophenone before use.

Plant Material. Aerial parts (including stems, leaves, and flowers) were collected from wild-growing populations at full flowering stage, in June 2006, at Megress Mountain, Wilaya of Setif, in northeastern Algeria. A voucher specimen was deposited in the Herbarium of the Muséum d'Histoire Naturelle de la ville de Nice (voucher B-8989).

Essential Oil Isolation. The aerial parts were hydrodistilled for 3 h using a Clevenger type apparatus. The essential oil, obtained in 0.3% yield (w/w) from dried material, was stored in hermetically sealed colored glass vials in a refrigerator at 4 °C prior to analysis.

Gas Chromatography (GC) and Gas Chromatography−Mass Spectrometry (GC-MS). GC and GC-MS analyses were carried out using an Agilent 6890N gas chromatograph apparatus equipped with a

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flame ionization detector (FID) and coupled to a quadrupole Agilent 5973 network mass selective detector working in electron impact (EI) mode at 70 eV (scanning over the 35−350 amu range). The gas chromatograph was equipped with two fused silica capillary columns HP-1 (PDMS, 50 m × 0.2 mm i.d., film thickness = 0.33 μm). The analytical parameters (identical for GC and GC-MS analyses unless specified) were the following: The carrier gas was helium at a flow rate of 1 mL/min. The oven temperature was programmed from 60 to 250 at 2 °C/min and held isothermal for 40 min. The injector (split mode, ratio 1/100) temperature was 250 °C. The FID temperature was set at 250 °C, and in the GC-MS analyses, the temperatures of the ion source and transfer line were 170 and 280 °C, respectively. Retention indices (RI) were determined from the retention times of a series of *n*-alkanes with linear interpolation. Quantitative data were obtained from FID area percentages without the use of correction factors. The constituents of the essential oil were identified by comparison of their mass spectral pattern and RI with those of pure compounds registered in commercial libraries and literature data and with a laboratory-made database built from authentic compounds. The identification of isochavicol esters **2**−**4** was carried out by co-injection of the essential oil with ester mixtures synthesized from pure isochavicol (vide infra).

NMR. The NMR spectra were recorded on a Bruker WM 200 MHz spectrometer in $CDCl_3$. The chemical shift values are reported with reference to tetramethylsilane, and the coupling constants are given in hertz.

Essential Oil Fractionation. The essential oil was submitted to flash chromatography on 40−63 μm silica gel, using petroleum ether with increasing amounts of diethyl ether as eluent. The fractions were analyzed by GC-MS, and those containing the isochavicol esters were gathered for the preparation of isochavicol.

Synthesis of Reference Compounds. *Isochavicol* (***1***). *D. crinitus* essential oil fraction rich in isochavicol esters (prepared as described above) (1.01 g) was diluted in 6 mL of anhydrous diethyl ether and added dropwise to a stirred suspension of 362 mg of lithium aluminum hydride (LAH) in diethyl ether (15 mL). The mixture was stirred at room temperature for 24 h, then cooled to 0 °C, and quenched by the sequential addition of water and diluted hydrochloric acid until acidic pH. The mixture was then decanted and the aqueous layer extracted twice with diethyl ether. The combined organic phases were washed with brine, dried on magnesium sulfate, and evaporated to give 907 mg of a slightly yellow oil, which was purified by flash chromatography on silica gel using petroleum ether/diethyl ether 4:1 as eluent, to give finally 324 mg of 4-(prop-(1*E*)-enyl)phenol (isochavicol **1**) as white crystals: ^{1}H NMR δ 1.85 [dd, 3H, J = 1.5, 6.4 Hz; H7], 4.68 [s, 1H, OH], 6.08 [dq, 1H, J = 6.4, 15.7 Hz; H6], 6.33 [br d, 1H, J = 15.7 Hz; H5], 6.76 [d, 1H, J = 8.7 Hz; H2], 7.21 [d, 1H, J = 8.7 Hz; H3]; ^{13}C NMR δ 154.38, 130.86, 130.24, 127.02, 123.42, 115.38, 18.69; EI-MS *m*/*z* (%) 77 (22), 91 (14), 103 (9), 105 (28), 107 (29), 115 (12), 117 (9), 119 (10), 133 (78), 134 (100).

Isochavicol Esters. The typical procedure was performed as follows: To 1 equiv of **1** diluted in dichloromethane (DCM) (ca. 4 mL/100 mg **1**) were added sequentially under stirring 1.2 equiv of triethylamine and 1.3 equiv of acyl chloride (either as a single compound or in mixture). The solution was stirred for 24−72 h and then poured into water. After decantation, the aqueous phase was extracted twice with DCM, and the combined organic phases were washed with brine, dried on magnesium sulfate, and evaporated to give an oily product, which was purified by flash chromatography on silica gel using petroleum ether/diethyl ether for elution. In the case of the preparation of the ester mixtures, a simple filtration on silica gel using such a type of eluent was sufficient. The pure or mixed esters were finally obtained as colorless oils.

*Ester Mixture **1***. The use of a mixture of acetyl, n-butyryl, n-valeryl and n-hexanoyl chlorides as acylating agents (respective massic percentages: 20, 25, 27, and 27%) furnished 49 mg of a mixture of isochavicol acetate, n-butyrate, n-valerate and n-hexanoate from 33 mg of isochavicol (Figure S1 of the Supporting Information).

*Ester Mixture **2***. The use of a mixture of propionyl, isobutyryl, and 2-methylbutyryl chlorides as acylating agents (respective massic percentages of 25, 47, and 28%) furnished 24 mg of a mixture of isochavicol propionate (**3**), isobutyrate (**2**), and 2-methylbutyrate (**4**) from 25 mg of isochavicol (Figure S2 of the Supporting Information).

4-(Prop-(1E)-enyl)phenyl Isobutyrate (***2***) (*64%*). ^{1}H NMR (Figure S3 of the Supporting Information) δ 1.31 [d, 6H, J = 7.0 Hz; $(CH_3)_2CH$], 1.87 [dd, 3H, J = 1.3, 6.3 Hz; H7], 2.79 [hept, 1H, J = 7.0 Hz; $(CH_3)_2CH$], 6.18 [dq, 1H, J = 6.3, 15.7 Hz; H6], 6.39 [br d, 1H, J = 15.7 Hz; H5], 6.99 [d, 2H, J = 8.6 Hz; H2], 7.32 [d, 2H, J = 8.6 Hz; H3]; ^{13}C NMR δ 175.54, 149.54, 135.49, 130.04, 126.56, 125.72, 121.38, 34.08, 18.84, 18.20; EI-MS *m*/*z* (%) 41 (5), 43 (27), 71 (5), 77 (8), 105 (5), 107 (5), 133 (38), 134 (100), 135 (10), 204 (5).

4-(Prop-(1E)-enyl)phenyl Propionate (***3***) (*67%*). ^{1}H NMR (Figure S4 of the Supporting Information) δ 1.26 [t, 3H, J = 7.5 Hz; CH_3CH_2], 1.87 [dd, 3H, J = 1.3, 6.3 Hz; CH_3CH_2], 2.58 [q, 2H, J = 7.5 Hz; CH_2CO], 6.18 [dq, 1H, J = 6.3, 15.7 Hz; H6], 6.38 [br d, 1H, J = 15.7 Hz; H5], 7.00 [d, 2H, J = 8.6 Hz; H2], 7.32 [d, 2H, J = 8.6 Hz; H3]; ^{13}C NMR (Figure S5 of the Supporting Information) δ 173.00, 149.40, 135.59, 130.00, 126.63, 125.88, 121.48, 27.73, 18.49, 9.08; EI-MS *m*/*z* (%) 57 (10), 77 (8), 105 (6), 107 (7), 115 (4), 117 (5), 133 (51), 134 (100), 135 (10), 190 (6) (Figure S7 of the Supporting Information).

Bacterial Strains and Culture Conditions. References strains of *Escherichia coli* (ATCC 9739), *Staphylococcus aureus* subsp. *aureus* (ATCC 6538), *Nocardia asteroides* (ATCC 19247), *Klebsiella pneumoniae* (IBMC Strasbourg), *Staphylococcus hemolyticus* (IBMC Strasbourg), *Salmonella enterica* subsp. *enterica*, Serovar Enteritidis (Institut de Biologie Moléculaire et Cellulaire, Strasbourg), *Mycobacterium bovis* BCG (Pasteur), and *E. coli*, PQ37 (F- thr leu his-4 pyrD thi galE galK lacΔU169 srl300::Tn10 rpoB rpsL uvrA rfa trp::Muc$^+$ sfiA::Mud (Ap, lac) cts), were obtained from the Collection of the Institute Pasteur, Paris, France. The essential oil was screened as toxic against *Plasmodium falciparum* FcB1, which was from the Muséum National d'Histoire Naturelle. Bacteria species were cultivated for 24 h in Mueller Hinton's medium (MH) at 37 °C except *N. asteroides* ATCC 19247, which was cultivated in heart−brain infusion (HBI) at 30 °C for 48 h, at 30 °C in malt extract agar for *Aspergillus niger*, and in Sabouraud dextrose medium (Sanofi Diagnostic Pasteur) for *Candida albicans*.

Disk Diffusion Assay. The antibacterial activity of the essential oil was evaluated using the agar diffusion test according to the Kirby−Bauer method (*24*) with small adaptations. Briefly, culture suspension of the tested microorganisms (approximately 10^6 CFU/mL) was spread on the solid medium plates (40 mL). Five microliters of serial dilutions in dimethyl sulfoxide (DMSO) (1/2, 1/5, and 1/10 v/v) of the essential oil were placed onto the solid media plates. The diameter of inhibition was measured after 24 or 48 h of incubation at 30 or 37 °C. Ampicillin, itraconazole, and DMSO were used as positive and negative controls, respectively.

Minimum Inhibitory Concentration (MIC) Determinations for Mycobacteria. MICs were determined using the new microdilution resazurin assay (MRA) (*25*). Resazurin salt powder (Sigma) was prepared at 0.01% (w/v) in distilled water, sterilized by filtration through a 0.22 μm membrane, and stored at 4 °C for a week. Drug stock solutions were prepared in DMSO at a concentration of 20 mg/mL and frozen until used. The inocula were prepared from *M. bovis* BCG strains grown in 7H9 medium supplemented with 10% albumin, dextrose, catalase (ADC) enrichment. Two microliters of 2-fold serial dilutions of each drug was prepared in 200 μL of 7H9 medium directly in 96-well plates at concentrations from 100 to 0.1 μg/mL. Growth controls containing DMSO and isoniazid (from 1 μg/mL to 1 ng/mL) were also included. The plates were covered, sealed, and incubated at 37 °C. After 8 days, 30 μL of resazurin solution was added to each well and plates were allowed to incubate at 37 °C for an additional 24 h. A change from blue to pink indicates reduction of resazurin and therefore bacterial growth. The MIC was defined as the lowest drug concentration that prevented this color change.

Cytotoxicity Evaluation. VERO cell lines were maintained in Dulbecco modified Eagle medium (DMEM) supplemented with 5% fetal calf serum (FCS), at 37 °C in air with 5% CO_2. Proliferating cells were seeded in 96-well microtitration plates at a density of 10^5 cells/mL, which were further incubated for 24 h at 37 °C under 5% CO_2 in air before each assay. Various concentrations of solutions of compounds in 1% DMSO were added and then incubated for 48 h as described above. At the end of this, 20 μL of dimethylthiazolyldiphenyl tetrazolium bromide solution (MTT, Sigma) (5 mg/mL) was added to each well and further incubated for 4 h at 37 °C to allow the formation of formazan. The crystals of formazan were then dissolved with 100 μL of a freshly prepared solution of sodium dodecyl sulfate (SDS) 10% (15 mL) and 1 N HCl (150 μL). The optical density of each well was measured at 595 nm using a multiwell plate

reader. The 50% inhibition concentration was then determined by curve fitting.

MIC Determination for Bacteria. Precultures of the tested microorganisms were made by inoculating 10 mL of Luria−Bertani (LB) and incubating for 24 h. A culture suspension were made by 1/1000 dilution from preculture and seeded in 96-well microtitration plates. One microliter of 2-fold serial dilutions of each drug was prepared in 100 μL of LB. The plates were incubated at 37 °C. After 24 h, the optical density of each well was measured at 595 nm using a multiplate reader. The 90% inhibition concentrations for the most active compounds were determined by curve fitting.

Antifungal Activity. Activities of the more active compounds were evaluated on the growth of *C. candida* and *A. niger* using the standardized filter paper disk (6 mm non-impregnated disk; Antibiotica assay disks, grade 2668 Schleider and Schuell) diffusion method according to the Kirby−Bauer method (*24*). Briefly, a suspension of *C. albicans* was spread on solid Sabouraud medium plates (20 mL). Filter paper disks were impregnated with 10 μL of serial dilutions in DMSO of the compounds and placed onto the solid medium plates. The diameter of inhibition was measured after 24 h of incubation at 30 °C. Itraconazole was used as positive control.

Genotoxicity Assay: SOS-Chromotest. The capacity of the drugs to induce DNA damage is monitored with the SOS-chromotest (*26*). This test is based on a genetically engineered *E. coli* strain, PQ37, licensed from the Institute Pasteur, which measures the primary response of a cell to genotoxic damage. This strain harbors the lacZ gene under the control of sfiA, a gene involved in the SOS response. DNA damage results in the activation of the SOS system, which in turn induces the transcription and synthesis of β-galactosidase. This enzyme is detected by a chromogenic reaction with the substrate X-gal.

Antiplasmodial Activity. The antiplasmodial activity was evaluated against the chloroquine-resistant FcB1/Colombia strain of *Plasmodium falciparum*. The test was performed using the method of Desjardins et al. (*27*). Extracts and pure compounds were diluted in DMSO at 20 μg/mL with culture medium to the expected concentrations in 96-well microplates. Asynchronous parasite cultures were then added (1% parasitemia and 1% final hematocrite), and plates were maintained for 24 h at 37 °C in a candle jar. [^{3}H]Hypoxanthine (0.5 μCi) was subsequently added to each well, and parasites were maintained for an additional 24 h. After freezing and thawing, the cells were harvested from each well onto glass fiber filters, and the dried filters were counted in a scintillation spectrometer. The growth inhibition for each well was determined by comparison of the radioactivity incorporated into the treated culture with that in the control culture maintained on the same plate. The concentration causing 50% inhibition (IC_{50}) was obtained from the drug concentration−response curve. The DMSO concentration never exceeded 0.1% and did not inhibit the parasite growth.

Determination of DPPH Radical Scavenging Activity. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was measured according to the procedure described by Lopes-Lutz et al. (*28*). Briefly, 0.2 mM DPPH (Sigma, St. Louis, MO) in methanol solution was prepared. Ten microliters of 2-fold serial dilutions of each drug in methanol was added to 190 μL of DPPH solution in 96-well microplates. After incubation for 30 min in the dark at room temperature, the absorbance was measured at 510 nm. Ascorbic acid was used as positive control, and DPPH plus methanol was used as negative control. The concentration causing 50% inhibition (IC_{50}) was obtained from the drug concentration−response curve.

RESULTS AND DISCUSSION

GC-MS analyses were performed on the essential oil of *D. crinitus*, as well as on the fractions obtained after flash chromatography of the whole oil, to identify further minor constituents and to separate coeluting compounds.

Our preliminary investigation of the GC-MS profile did not permit us to identify the main (39.0%) constituent at RI = 1546. The second most abundant component was octyl acetate (12.3%), followed by α-pinene (9.9%), and common constituents of essential oils, mainly mono- and sesquiterpenes present at lower amounts (< 6%). Another unidentified substance (RI = 1643) was detected at a relatively high percentage, and the examination of it mass spectrum suggested that it was structurally related with the main constituent: both had a base peak at *m*/*z* 134, and some of their other fragments were separated by 14 amu, as is typically the case between one-carbon homologues. To identify these two components, the oil was fractionated by flash chromatography, and a fraction containing only a mixture of these two compounds was obtained and studied by NMR. The combination of the MS and NMR data was consistent with the structures **2** and **4** (**Figure 1**), but to ensure their characterization, we first reduced this mixture with LAH and identified unambiguously isochavicol (**1**) as the main product of the reaction. Then, for definitive proof, we planned to esterify **1** back to **2** and **4**, but to allow a deeper investigation of the isochavicol esters potentially contained in the oil, we synthetised a series of mixed homologous esters by reacting **1** with mixtures of various acyl chlorides. Hence, isochavicol acetate, *n*-butyrate, *n*-valerate, and *n*-hexanoate constituted the first mixture, and isochavicol propionate (**3**), isobutyrate (**2**), and 2-methylbutyrate (**4**) the second one. Both of these mixtures were cleanly separated in the GC-MS conditions, and the attribution of each peak to its parent component was done easily from the MS pattern and the elution order on apolar column. These mixtures could then be used as standard in coelution experiments with the whole oil, which confirmed that the main component was isochavicol isobutyrate (**2**) and the compound at RI = 1643 was the homologous 2-methylbutyrate (**4**). However, we could not detect either the acetate, *n*-butyrate, *n*-valerate, or *n*-hexanoate in the essential oil, but we noted that the propionate (**3**) was an actual constituent, present at low amount (0.05%) (Figure S6 of the Supporting Information). The relative concentrations of the volatile components identified are presented in **Table 1**.

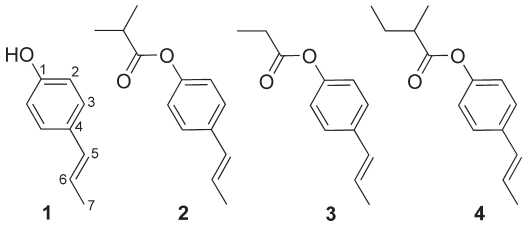

Figure 1. Structures of isochavicol (**1**) and of constituents **2**−**4** of *D. crinitus* essential oil.

Despite their relative structural simplicity, a bibliographical investigation of the natural occurrence of such isochavicol esters showed that they are rather rare: **2** and **4** were identified in a solvent extract of *Moonia heterophylla* Arn. (*29*), as well as in the essential oil of some Apiaceae (*30*), especially in several *Pimpinella* species (*23*). In the root oils of *Pimpinella corymbosa*, *Pimpinella olivieroides*, and *Pimpinella kotschyana*, the percentage of **4** reached approximatively the same level as in our sample, whereas in these species **2** was present in lower amounts (< 0.8%) but reached 1.8% in the fruit oil of *Pimpinella peucadanifolia* (*23*). To the best of our knowledge, isochavicol propionate (**3**) was never described in the literature.

In view of the recent evidence on the biological activity of related isochavicol derivatives (*22*, *23*), and as it is well-known that phenylpropanoids often show interesting pharmacological properties, we were interested to explore the activity of the essential oil and the pure esters. Therefore, for full spectral and biological characterizations, pure samples of **2** and **3** were prepared by esterification of **1** with isobutyryl and propionyl chloride, respectively. We scanned then the antibacterial, antifungal, antimalarial, and antiradical potential of the whole oil

Table 1. Composition of *D. crinitus* Essential Oil

compound	RI[a]	RI[b]	%[c]	identification[d]
α-thujene[e]	924		0.2	MS, RI
benzaldehyde	929		0.2	MS, RI, Std
α-pinene	932	1003	9.9	MS, RI, Std
oct-1-en-3-ol	963		0.1	MS, RI, Std
β-pinene	970	1090	0.2	MS, RI, Std
myrcene	983	1153	3.4	MS, RI, Std
(*Z*)-β-ocimene[e]	1021	1206	0.2	MS, RI
limonene	1028	1170	0.1	MS, RI, Std
octan-1-ol	1055		0.3	MS, RI, Std
linalool	1083	1515	0.3	MS, RI, Std
decanal	1180		0.2	MS, RI, Std
octyl acetate[e]	1188	1447	12.3	MS, RI
myrtenyl acetate[e]	1302		0.2	MS, RI
octyl 2-methylpropanoate[e]	1333		0.3	MS, RI
α-cubebene[e]	1347	1438	0.1	MS, RI
α-copaene[e]	1374		0.2	MS, RI
β-bourbonene[e]	1382		0.4	MS, RI
β-elemene[e]	1389		0.3	MS, RI
β-caryophyllene	1420	1548	5.4	MS, RI, Std
α-humulene[e]	1449		0.8	MS, RI
β-farnesene[e]	1451	1648	3.4	MS, RI
γ-muurolene[e]	1469		0.6	MS, RI
germacrene D[e]	1469	1652	2.3	MS, RI
α-selinene[e]	1486		0.4	MS, RI
α-muurolene[e]	1489		0.1	MS, RI
isochavicol propionate (**3**)	1495	2104	0.05	MS, RI, Std
n-pentadecane	1500	1500	2.8	MS, RI, Std
β-sesquiphellandrene[e]	1511	1741	2.6	MS, RI
isochavicol isobutyrate (**2**)	1546	2097	39.0	MS, RI, Std
caryophyllene oxide	1568	1932	0.5	MS, RI, Std
isochavicol 2-methylbutyrate (**4**)	1643	2189	6.5	MS, RI, Std
n-heptadecane	1700	1700	1.2	MS, RI, Std
total identified			94.6	

[a] Retention indices on HP-1 column relative to C7−C22 n-alkanes. [b] Retention indices on HP-20 column relative to C7−C22 n-alkanes. [c] Area FID (HP-1 column). [d] Method 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 co-injection of an authentic sample. [e] Compound tentatively identified according to its mass spectrum and by comparison of its retention indices with those of the literature.

and its major component (**2**). In this study, we included also the new compound **3**, which can be considered as a close analogue of **2** with probably a different reactivity toward enzymatic cleavage, as well as isochavicol itself (**1**), for comparison purposes, even if it is not a constituent of the oil.

The essential oil of *D. crinitus* was first assayed for its mutagenic potential using an SOS-chromotest. No mutagenic effect of the essential oil was observed at the different concentrations tested (crude extract, 1/2, 1/5, and 1/10 dilutions), thus underscoring the interest to pursue the investigations of the biological activities.

The antimicrobial activities of the essential oil of *D. crinitus* and of compounds **1**−**3** were then evaluated against a range of Gram-negative (*E. coli*, *K. pneumoniae*, *S. enteriditis*) and Gram-positive (*E. faecalis*, *S. aureus*, *S. hemolyticus*, *N. asteroides*) bacteria and two strains of fungi (*C. albicans* and *A. niger*). These activities were first determined by measuring the zone of growth inhibition of serial dilutions of the essential oil. Ampicillin and itraconazole were employed as positive controls.

The essential oil of *D. crinitus* turned out to be devoid of significant antimicrobial activity against the growth of the Gram-negative bacteria tested. As shown in **Table 2**, the 1/2 dilution of the essential oil displays a weak activity (inhibition zone = 15 mm) against *E. coli* compared to ampicillin control (26 mm).

Table 2. Antimicrobial Activities of *D. crinitus* Essential Oil and of Compounds **1**− **3**

	inhibition zone (mm)							
strain	EO[a] (1/2)	EO (1/5)	EO (1/10)	**1**[b]	**2**[b]	**3**[b]	amp[c]	itra[d]
E. coli	15	14	13	21	4	−[e]	26	NT[f]
S. aureus	22	17	14	7	−	−	44	NT
S. hemolyticus	15	12	9	7	−	−	32	NT
E. faecalis	20	14	10	NT	NT	NT	30	NT
N. asteroides	25	15	10	11	7	6	21	NT
K. pneumoniae	−	−	−	−	−	−	10	NT
S. enteriditis	06	06	06	NT	NT	NT	25	NT
A. niger	17	15	10	NT	NT	NT	NT	25
C. albicans	25	22	12	NT	NT	NT	NT	26

[a] Essential oil; 5 μL of diluted essential oil (v/v) in DMSO. [b] 5 μL of diluted compounds in DMSO (100 μg). [c] Ampicillin (15 μg). [d] Itraconazole (15 μg). [e] No activity. [f] Not tested.

Table 3. MIC Determinations for the Most Sensitive Strains and Antimycobacterial Activity of *D. crinitus* Essential Oil and of Compounds **1**−**3**

	MIC_{95} (μg/mL)					
strain	EO[a]	**1**	**2**	**3**	ampicillin	itraconazole
S. aureus	2.5×10^3	>200	>200	>200	0.05	NT[b]
E. faecalis	5×10^3	>200	>200	>200	0.4	NT
N. asteroides	310	15.8	60.5	42.5	2	NT
M. bovis	$>5 \times 10^3$	200	25	50	NT	0.4

[a] Essential oil. [b] Not tested.

Strains of *K. pneumoniae* and *S. enteriditis* were also particularly insensitive to the essential oil even at the highest amount used in this assay. This was also true for compounds **1**−**3** except for isochavicol **1**, which showed a good activity against *E. coli* (diameter of inhibition = 21 mm) at the concentration tested (100 μg). Moreover, as shown in **Table 2**, a more potent result was seen with *S. aureus* because diameters of inhibition of the essential oil dilutions were, respectively, 22, 17, and 14 mm, as well as with *S. hemolyticus* (diameters of, respectively, 15, 12, and 9 mm). The most strongly affected Gram-positive strain was *N. asteroides*, because the diameter of inhibition for the 1/2 dilution of the essential oil (25 mm) is fairly close to the effect of ampicillin (30 μg). Compounds **1**−**3** were also assayed for their inhibition of Gram-positive strain growth. Surprisingly, the major constituent of this essential oil, isochavicol isobutyrate (**2**), is devoid of significant activity against these strains. This does suggest that, although it is the main component, this compound is not the source of the antibacterial properties of the entire essential oil, but the presence of high quantities of terpenes such as α-pinene (9.9%), myrcene (3.4%), and β-caryophyllene (5.4%) could explain the growth inhibition observed.

The same lack of effect was observed for **3**, and only isochavicol **1** possesses a weak activity against *N. asteroides*.

At the same dilution range, the inhibition effect seen on fungus growth was of interest. Indeed, a diameter of inhibition of about 25 mm was seen against *C. albicans* at the highest concentration used, which is to be compared to itraconazole (26 mm).

The MIC of the compound against the most sensitive bacterial strains were then determined (**Table 3**). The results obtained were disappointing compared to the antibiogram. Indeed, no significant MIC was seen for the essential oil or for the derivatives **1**−**3** on the bacterial strains tested (MIC > 200 μg/mL), except on *N. asteroides*. Indeed, a MIC_{95} of 310 μg/mL was measured for the essential oil, which is fairly weak when compared to the control ampicillin (MIC_{95} = 2 μg/mL). On the other hand, the isochavicol derivatives **1**−**3** have very good activities, with MICs

Table 4. Antiplasmodial, Cytotoxic, and Antiradical Activities of *D. crinitus* Essential Oil and of Compounds **1**−**3**

	IC_{50} (μg/mL)						
strain	EO[a]	**1**	**2**	**3**	chloroquine	camptothecin	ascorbic acid
P. falciparum	26.7	1.9	14.0	13.3	0.2	NT[b]	NT
VERO cells	82	26.8	26.5	14.6	NT	0.4	NT
antiradical activity	85×10^3	1×10^3	$>1 \times 10^3$	$>1 \times 10^3$	NT	NT	12

[a] Essential oil. [b] Not tested.

ranging between 16 and 61 μg/mL. The essential oil and **1**−**3** were also evaluated for their antimycobacterial activity against *M. bovis* BCG. As for *N. asteroides*, a good activity was obtained, in particular for the isochavicol isobutyrate, with a MIC_{95} of 25 μg/mL. The inhibitory effect observed both on the actinomycete *N. asteroides* and on *M. bovis*, which share similar cell wall constituents (*31*), suggests an effect on the biosynthesis of these constituents, namely, the mycolic acids. Further studies will be necessary to confirm this hypothesis.

Compounds isolated from *Pimpinella* species are structurally related with chavicol derivatives and present good activity against *P. falciparum* (*22*, *23*). Accordingly, we investigated the antimalarial effect of **1**−**3**. The results of the parasite growth screening showed that all of these compounds present a good activity against *P. falciparum*, especially isochavicol **1**, with an IC_{50} of around 2 μg/mL (**Table 4**). The cytotoxicity against VERO cells was also investigated, and no effect was seen for the essential oil either for the isochavicol derivatives at 10 μg/mL (IC_{50} from 15 to 82 μg/mL). This is particularly interesting for the most active compound isochavicol **1** against *P. falciparum* as its cytotoxicity is around 27 μg/mL. This provides a relatively promising selectivity index (SI > 12).

In the course of this extensive screening of biological activities of the essential oil of *D. crinitus* and of some of its constituents and derivatives, we also investigated their antiradical activity using a DPPH test (**Table 4**). Unfortunately, no activity was seen, ($IC_{50} \geq 1 \times 10^3$ μg/mL) in comparison with the ascorbic acid used as control (IC_{50} = 12 μg/mL).

In conclusion, the essential oil of *D. crinitus* presents a modest antimicrobial activity against bacteria and fungi. Nevertheless, its main constituent, isochavicol isobutyrate **2**, as well as the related propionate (**3**), and isochavicol (**1**) have interesting activities against both *M. bovis* and *N. asteroides*, suggesting a possible specificity against mycolates biosynthesis. Further work on this hypothesis will be reported in due time.

On the other hand, isochavicol **1** displayed an effect on *P. falciparum* growth as well as a low cytotoxicity. The relatively good selectivity index (SI > 12) is of interest, and the chemical synthesis of analogues may lead to new series of antiparasitic compounds. Despite their rather simple structure, isochavicol (**1**) and its esters are very uncommon as essential oil constituents, compared, for example, with ubiquitous phenylpropanoids such as eugenol and anethole. Examination of their biological properties can permit the discovery of new drugs, which can be produced in large scale as their synthesis is rather straightforward. From the analytical point of view, we showed that the synthesis of mixtures of potential constituents followed by their co-injection is a potent methodology for the identification of new natural products in natural extracts, especially if these constituents are trace compounds, for which the traditional approach of fractionation/structural analysis is not well suited.

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Supporting Information Available: Figures S1−S8. This material is available free of charge via the Internet at http://pubs.acs.org.

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