Chemical Composition and Antimicrobial Activity of the Essential Oil from the Edible Aromatic Plant *Aristolochia delavayi*

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The essential oil obtained by hydrodistillation from the aerial parts of *Aristolochia delavayi* FRANCH. (Aristolochiaceae), a unique edible aromatic plant consumed by the Nakhi (Naxi) people in Yunnan, China, was investigated using GC/MS analysis. In total, 95 components, representing more than 95% of the oil composition, were identified, and the main constituents found were (*E*)-dec-2-enal (52.0%), (*E*)-dodec-2-enal (6.8%), dodecanal (3.35%), heptanal (2.88%), and decanal (2.63%). The essential oil showed strong inhibitory activity (96% reduction) of the production of bacterial volatile sulfide compounds (VSC) by *Klebsiella pneumoniae*, an effect that was comparable with that of the reference compound citral (91% reduction). Moreover, the antimicrobial activity of the essential oil and the isolated major compound against eight bacterial and six fungal strains were evaluated. The essential oil showed significant antibacterial activity against *Providencia stuartii* and *Escherichia coli*, with minimal inhibitory concentrations (*MIC*) ranging from 3.9 to 62.5 μg/ml. The oil also showed strong inhibitory activity against the fungal strains *Trichophyton ajelloi*, *Trichophyton terrestre*, *Candida glabrata*, *Candida guilliermondii*, and *Cryptococcus neoformans*, with *MIC* values ranging from 3.9 to 31.25 μg/ml, while (*E*)-dec-2-enal presented a lower antifungal activity than the essential oil.

Introduction. – All plants are able to store and emit volatile organic compounds (VOCs), and all compounds contained in aromatic essential oils are, in principle, biological VOCs. Plant VOCs are released from leaves, flowers, and fruits into the atmosphere and from roots into the soil. The primary functions of plant VOCs are the defense against herbivores and pathogens, the attraction of pollinators, seed dispersers, and other beneficial animals and microorganisms, and to serve as signals in the plant–plant communication [1]. In some plants, released VOCs may also act as wound sealers [2]. Their role in these 'housekeeping' activities underlies agricultural applications that range from the search for sustainable methods for pest control to the production of flavors and fragrances. On the other hand, there is also growing evidence that plant VOCs are endowed with a range of biological activities in mammals, and that they represent a substantially underexploited and still largely untapped source of novel drugs and drug leads [3][4].

Within the framework of our ongoing research on volatile natural products and their biological activities of the native Chinese flora, *Aristolochia delavayi* FRANCH. was selected, because it is a unique and threatened wild, edible aromatic plant native to the dry and hot valley of Jinshajiang (Jinsha river) within the northwest Yunnan and the southwest Sichuan provinces of China. *A. delavayi* FRANCH. (local name: shan cao-guo) belongs to the plant family of the Aristolochiaceae, and the whole plant has a strong odor reminiscent of black cardamom (*Amomum tsao-ko*) or coriander leaves [5]. There is a long history on the use of *A. delavayi* by Nakhi people. It is cooked with beef, lame, fish, and pork to enhance the flavor of the foods and is also used as aromatic stomachic to increase appetite.

To the best of our knowledge, there are only two Chinese literature sources reporting the preliminary study of the chemical constituents of *A. delavayi* essential oil, with 18 and 37 components detected and (*E*)-undec-2-enal identified as the major compound [5][6]. No information on the biological activities of the essential oil is available. Therefore, in the present study, the essential oil isolated from the aerial parts of *A. delavayi* was investigated in more details; in particular, its chemical composition, its biological activities against several bacterial and fungal strains, as well as its inhibitory activity of the bacterial volatile sulfide compounds (VSCs) for oral care flavors were studied.

Numerous studies have shown that in addition to the major compounds, minor compounds may make a significant contribution to the activity of essential oils. Therefore, it is indispensible to detect and identify also the minor compounds, even if they are present in very low amounts [7]. Hence, a detailed study of the essential-oil constituents of *A. delavayi*, including the investigation of minor components in addition to the isolation and identification of its major compounds, which were different from previously reported data [5][6], was reported here.

Results and Discussion. – *Chemical Composition of the* A. delavayi *Essential Oil.* The average essential-oil yield obtained by hydrodistillation of the dry aerial parts of *A. delavayi* was 0.9% (*w*/*w*). Qualitative and quantitative analysis of the essential oil and its fractions by GC-FID and GC/MS allowed the identification of 95 components, representing 95.3% of the total oil composition. The oil was very rich in aliphatic saturated and unsaturated aldehydes, especially in (*E*)-dec-2-enal (52.00%), (*E*)-dodec-2-enal (6.80%), dodecanal (3.35%), heptanal (2.88%), and decanal (2.63%). Further details of the essential-oil composition and the relative amounts of the various components are shown in *Table 1*.

The composition pattern of the *A. delavayi* essential oil was quite unusual and very different from those of other *Aristolochia* species previously reported. *Francisco et al.* [9] reported the chemical constituents of the essential oils of ten *Aristolochia* species, and the most abundant compounds included camphene, *o*-cymene, limonene, cedrane-8,14-diol, viridiflorene, eudesm-7(11)-en-1-ol, (*E*)-nerolidol, α-phellandrene, limonene, and isobornyl acetate [9]. However, (*E*)-dec-2-enal, the main constituent of *A. delavayi* essential oil, was found neither in one of these ten oil samples, nor was it reported as essential-oil constituent of any of the following *Aristolochia* species: *A. elegans* [10], *A. gibertii* [11], *A. acutifolia* [12], *A. argentina* [13], and *A. contorta* [14].

Table 1. *Composition of the Essential Oil Isolated from the Aerial Parts of* Aristolochia delavayi

Compound	*RI*[a])	RI_{lit}[b])	Content [%][c])	Identification[d])
Hexanal	800	801	0.18	MS, *RI*, RC
Furfural	830	828	tr[e])	MS, *RI*
(*E*)-Hex-2-enal	850	846	0.02	MS, *RI*
(*E*)-Hex-2-en-1-ol	862	854	0.02	MS, *RI*
Hexan-1-ol	864	863	0.04	MS, *RI*, RC
Nonene	890	896	0.09	MS, *RI*
Nonane	900	900	tr	MS, *RI*
Heptanal	901	901	2.88	MS, *RI*, RC
α-Thujene	920	924	0.02	MS, *RI*, RC
α-Pinene	934	932	0.06	MS, *RI*, RC
Camphene	949	946	0.05	MS, *RI*, RC
Heptan-1-ol	967	959	0.04	MS, *RI*, RC
Sabinene	974	969	0.02	MS, *RI*, RC
Hexanoic acid	976	967	0.03	MS, *RI*
β-Pinene	978	974	0.13	MS, *RI*, RC
Myrcene	990	988	0.18	MS, *RI*, RC
Octanal	1003	998	1.71	MS, *RI*, RC
(*E*)-Hexenyl acetate	1015	1010	tr	MS, *RI*, RC
Limonene	1030	1024	0.06	MS, *RI*, RC
1,8-Cineole	1033	1026	0.02	MS, *RI*, RC
Phenyl acetaldehyde	1045	1048[f])	0.01	MS, *RI*, RC
Oct-2-enal	1057	1049	0.39	MS, *RI*, RC
(*Z*)-Oct-2-en-1-ol	1067	1069[f])	0.02	MS, *RI*, RC
Octan-1-ol	1069	1063	0.05	MS, *RI*, RC
(*Z*)-Linalool oxide	1073	1067	tr	MS, *RI*, RC
Heptanoic acid	1073	1074[f])	0.71	MS, *RI*, RC
Non-1-en-3-ol	1080	1081[f])	0.03	MS, *RI*, RC
(*E*)-Linalool oxide (furanoid)	1089	1084	0.21	MS, *RI*
Nonan-2-one	1092	1087	tr	MS, *RI*
2-Hexylfuran	1092	1094[f])	1.95	MS, *RI*, RC
Linalool	1100	1095	1.12	MS, *RI*, RC
Nonanal	1104	1100	1.27	MS, *RI*, RC
(*Z*)-*p*-Menth-2-en-1-ol	1123	1118	tr	MS, *RI*
(*E*)-*p*-Menth-2-en-1-ol	1142	1136	0.02	MS, *RI*
Camphor	1148	1141	tr	MS, *RI*, RC
Menthone	1157	1148	tr	MS, *RI*, RC
Non-2-enal	1160	1157	0.17	MS, *RI*, RC
Borneol	1170	1165	tr	MS, *RI*, RC
(*Z*)-Linalool oxide	1171	1170	tr	MS, *RI*
Octanoic acid	1175	1167	1.25	MS, *RI*, RC
(*E*)-Linalool oxide (pyranoid)	1177	1173	tr	MS, *RI*
Terpinen-4-ol	1180	1174	tr	MS, *RI*
p-Cymene-8-ol	1187	1179	0.04	MS, *RI*, RC
Cryptone	1190	1183	tr	MS, *RI*
α-Terpineol	1192	1186	tr	MS, *RI*
(*Z*)-Dec-4-enal	1194	1191[f])	0.51	MS, *RI*, RC
(*E*)-Dec-4-enal	1197	1193[f])	1.42	MS, *RI*, RC
Decanal	1206	1201	2.63	MS, *RI*, RC
(*E*)-Oct-2-enol acetate	1211	1208	0.05	MS, *RI*, RC
(*Z*)-Hex-2-enyl isovalerate	1238	1241	0.02	MS, *RI*, RC
(*Z*)-Dec-2-enal	1250	1229[f])	1.51	MS, *RI*
(*E*)-Dec-2-enal	1266	1260	52.60	MS, *RI*, NMR
(*E*)-Dec-2-enol	1271	1266	0.53	MS, *RI*, RC

Table 1 (cont.)

Compound	*RI*[a])	RI_{lit}[b])	Content [%][c])	Identification[d])
Decan-1-ol	1273	1266	0.60	MS, *RI*, RC
Bornyl acetate	1290	1287	0.15	MS, *RI*
2-Octylfuran	1296	1281	0.34	MS, *RI*
Undecanal	1307	1305	0.17	MS, *RI*, RC
(*E*,*E*)-Deca-2,4-dienal	1317	1315	0.10	MS, *RI*, RC
Hexyl tiglate	1331	1330	0.03	MS, *RI*, RC
Hex-2-enyl tiglate	1340	1338	0.37	MS, *RI*
Dec-9-enoic acid	1363	1359	2.53	MS, *RI*
Undec-2-enal	1365	1357	0.31	MS, *RI*, RC
Decanoic acid	1368	1363[f])	0.44	MS, *RI*, RC
3,4-Dehydro-γ-decalactone	1370	–	1.16	MS
Geranyl acetate	1383	1379	0.03	MS, *RI*, RC
Hexyl hexanoate	1387	1382	0.03	MS, *RI*
(*E*)-Hex-2-enyl hexanoate	1390	1383	0.03	MS, *RI*
Dodec-4-enal	1399	–	0.21	MS
Dodecanal	1409	1408	3.35	MS, *RI*, RC
Dec-2-enoic acid	1413	1413[f])	1.03	MS, *RI*, RC
Longifolene	1417	1407	tr	MS, *RI*
Oct-2-enyl 2-methylbutyrate	1434	1436[f])	0.01	MS, *RI* , RC
Dec-2-en-4-olide	1452	–	2.54	MS
(*E*)-Muurola-3,5-diene	1459	1451	tr	MS, *RI*
Undecanoic acid	1462	1462[f])	0.02	MS, *RI*, RC
(*E*)-Dodec-2-enal	1469	1464	6.81	MS, *RI*, RC
Alloaromadendrene	1471	1473[f])	0.15	MS, *RI*, RC
γ-Decalactone	1474	1465	tr	MS, *RI*
γ-Muurolene	1485	1478	tr	MS, *RI*
β-Ionone	1493	1487	tr	MS, *RI*
α-Muurolene	1509	1500	tr	MS, *RI*
Butyl hydroxytoluene	1518	1519[f])	0.02	MS, *RI*, RC
Zonarene	1533	1528	0.70	MS, *RI*
Oct-2-enyl tiglate	1534	1538	0.07	MS, *RI*, RC
α-Calacorene	1553	1544	0.38	MS, *RI*, RC
Dodecanoic acid	1559	1565	0.03	MS, *RI*, RC
Nerolidol	1567	1561	0.03	MS, *RI*, RC
β-Calacorene	1573	1564	0.08	MS, *RI*, RC
Calarene epoxide	1582	–	0.89	MS
Caryophyllene oxide	1596	1582	0.02	MS, *RI*, RC
(*E*)-Dodec-2-enoic acid	1599	–	0.40	MS
Dodecyl acetate	1613	1607	0.02	MS, *RI*, RC
β-Himachalene oxide	1622	1615	0.10	MS, *RI*, RC
Tetradecanol	1673	1671	0.08	MS, *RI*
Cadalene	1686	1675	0.05	MS, *RI*, RC
Total			95.30	

[a]) *RI:* Linear retention indices determined relative to a series of *n*-alkanes (C_8–C_{30}) on the *HP-5* capillary column. [b]) RI_{lit}: *RI* reported by *Adams* [8] determined on an apolar column, except those marked with [f]), which are reported in the *Givaudan* database. [c]) Relative content determined by integration of the peak-areas. [d]) Identification of the compounds: MS, comparison of mass spectra with those listed in commercial mass-spectral libraries; *RI*, comparison of *RI* with those of authentic samples or from the literature; RC, comparison of mass spectra and *RI* with those of reference compounds recorded in the author's laboratory; NMR, compound was isolated and characterized by NMR spectroscopy. [e]) tr: Traces ($\leq 0.01\%$). [f]) *RI* listed in the *Givaudan* database.

In the only two literature sources reporting the essential-oil constituents of *A. delavayi*, undec-2-enal was identified as the main constituent [5][6], which is not in agreement with the present findings. Hence, to confirm the chemical structure of the major compound, the *A. delavayi* essential oil was isolated and purified by different chromatographic techniques to afford the pure main compound. The structure of the compound was established as (2*E*)-dec-2-enal on the basis of the NMR and MS spectra, and confirmed by comparison of these spectroscopic data with those of the literature [15].

Bacterial Volatile Sulfide Compound (VSC)-*Inhibitory Activity of* A. delavayi *Essential Oil.* Oral malodor (or halitosis) is a non-life-threatening, but potentially psycho-socially distressing condition that is relatively common throughout the adult population. The great majority of halitosis results from bacterial degradation of endogenous or dietary organic compounds, to yield a mixture of malodorants. The major contributors to oral malodor are sulfides, nitrogenous compounds, and volatile fatty acids, of which sulfides account for *ca.* 60% of the organoleptically measured oral malodor [16]. Inhibition screening of oral bacterial VSC production *in vitro* can provide clues to find new oral care flavors to deliver breath-freshening benefits.

The essential oil of *A. delavayi* was tested in duplicate for its inhibitory potential of bacterial VSC production by *Klebsiella pneumoniae* ATCC 10031, and the results are showen in *Table 2*. Citral was used as positive control and untreated sample as negative control. The data showed that the essential oil strongly inhibited the production of bacterial VSC (96% reduction). The inhibitory activity of the oil was comparable with or even higher than that of citral (91% reduction), used as reference in the bioassay.

Table 2. *Bacterial Volatile Sulfide Compound* (VSC)-*Inhibitory Activity of the* Aristolochia delavayi *Essential Oil*

Sample	H_2S Production [ng/ml][a])		VSC Reduction [%][b])
	Replicate 1	Replicate 2	
Negative control	104	131	0
Citral (positive control)	12	10	91
A. delavayi oil	7	2	96

[a]) Quantity of H_2S present in the sample's headspace gas, estimated using GC analysis. [b]) The VSC-inhibition percentage was calculated by comparison with the untreated (negative) control.

Antibacterial and Antifungal Activities of A. delavayi *Essential Oil.* The *A. delavayi* essential oil and the major compound isolated, *i.e.*, (*E*)-dec-2-enal, were tested against several bacterial and fungal strains, and the results are summarized in *Tables 3* and *4*. The antimicrobial activities were evaluated by the broth microdilution method and expressed as minimal inhibitory concentration (*MIC*), minimal bactericidal concentration (*MBC*), or minimal fungicidal concentration (*MFC*). The data indicated that the essential oil and the pure compound exhibited varying levels of antimicrobial activity against the investigated pathogens.

There is no general agreement on the acceptable inhibition levels of plant material when compared with standards. According to the classification of *Ríos* and *Recio* [17]

Table 3. *Antibacterial Activity of the* Aristolochia delavayi *Essential Oil and Its Major Compound (*E*)-Dec-2-enal*

Microorganism	Essential oil		(*E*)-Dec-2-enal		Rifampicin[a])	
	MIC[b])	*MBC*[c])	*MIC*[b])	*MBC*[c])	*MIC*[b])	*MBC*[c])
Pseudomonas aeruginosa	250.00	500.00	125.00	250.00	25.00	50.00
Escherichia coli ATCC 8739	15.62	500.00	31.25	125.00	0.09	6.25
Escherichia coli ATCC 11775	62.50	250.00	62.50	500.00	0.39	1.56
Enterobacter aerogenes	250.00	500.00	15.62	125.00	0.19	0.19
Providencia stuartii	3.90	500.00	31.25	250.00	0.09	0.19
Salmonella typhi	125.00	500.00	15.62	500.00	0.09	6.25
Enterococcus faecalis	250.00	500.00	62.50	250.00	0.19	1.56
Staphylococcus aureus	250.00	500.00	15.62	125.00	0.19	0.78

[a]) Positive control. [b]) *MIC:* Minimal inhibitory concentration [μg/ml]. [c]) *MBC:* Minimal bactericidal concentration [μg/ml].

Table 4. *Antifungal Activity of the* Aristolochia delavayi *Essential Oil and Its Major Compound (*E*)-Dec-2-enal*

Microorganism	Essential oil		(*E*)-Dec-2-enal		Amphotericin B[a])	
	MIC[b])	*MFC*[c])	*MIC*[b])	*MFC*[c])	*MIC*[b])	*MFC*[c])
Trichophyton ajelloi	3.90	125.00	7.81	125.00	25.00	50.00
Trichophyton terrestre	31.25	62.50	250.00	250.00	6.25	50.00
Candida albicans	125.00	500.00	125.00	>500.00	25.00	>50.00
Candida glabrata	7.81	62.50	7.81	7.81	1.56	12.50
Candida guilliermondii	7.81	62.50	7.81	15.62	6.25	12.50
Cryptococcus neoformans	7.81	62.50	25.65	31.25	1.56	12.50

[a]) Positive control. [b]) *MIC:* Minimal inhibitory concentration [μg/ml]. [c]) *MBC:* Minimal bactericidal concentration [μg/ml].

and *Tekel et al.* [18], plant extracts and essential oils can be considered to possess significant, moderate, or weak activity against the corresponding pathogens, when the *MIC* values are <100 μg/ml, between 100 and 625 μg/ml, or >625 μg/ml, respectively. Meanwhile, the presence of activity is considered very interesting in the case of *MIC*s below 100 μg/ml for extracts and 10 μg/ml for isolated compounds. This classification is very useful to detect the potential of various plant materials with biological activity.

The *MIC* and *MBC* values obtained for the essential oil and (*E*)-dec-2-enal against eight bacterial strains are presented in *Table 3*. The essential oil showed strong activity against *Providencia stuartii* (*MIC* of 3.90 μg/ml), which is the most common *Providencia* species responsible of causing the purple urine bag syndrome [19]. The essential oil also showed strong activity against *Escherichia coli* ATCC 8739 and *E. coli* ATCC 11775, with *MIC* values of 15.62 and 62.50 μg/ml, respectively. The most susceptible bacteria to (*E*)-dec-2-enal were *Staphylococcus aureus*, *Enterobacter aerogenes*, and *Salmonella typhi* (responsible for typhoid fever), against which the compound exhibited the same activity (*MIC* of 15.62 μg/ml). The essential oil exhibited

significant to moderate activity against all tested microorganisms, with *MIC* values ranging from 3.9 to 250.0 μg/ml. The aldehyde (*E*)-dec-2-enal showed similar activity against the *Gram*-positive and *Gram*-negative microorganisms tested. However, the *A. delavayi* essential oil showed better activity against the *Gram*-negative than the *Gram*-positive bacteria. This is a rather unusual result, since most plant secondary metabolites show more potent activity against *Gram*-positive than *Gram*-negative bacteria [20].

The *MIC* and *MFC* values obtained for the essential oil and (*E*)-dec-2-enal against several yeasts and fungal strains are shown in *Table 4*. Except against *Candida albicans*, the essential oil showed strong activity against the other fungi tested. The *MIC* for the essential oil and (*E*)-dec-2-enal ranged from 3.9 to 125.0 μg/ml and 7.8 to 250.0 μg/ml, respectively. The strongest activity was observed against the dermatophyte *Trichophyton ajelloi*, one of the common pathogens of athlete's foot (*tinea pedis*), with *MIC* values of 3.9 and 7.8 μg/ml for the essential oil and (*E*)-dec-2-enal, respectively. The whole essential oil has greater antifungal activities than the isolated major compound against the tested fungi, which suggests that the minor components might be critical to the activity and might have a synergistic effect or potential. This hypothesis is supported by the research results of *Bisignano et al.* [21] on a combination of four olive aldehydes, including (*E*)-dec-2-enal, which was significantly more effective at inhibiting bacterial growth than any of the aldehydes singly [20]. This suggests that plant essential oils may be more effective antimicrobials than their purified components.

The *A. delavayi* essential oil was found to be very rich in α,β-unsaturated aldehydes, especially (*E*)-dec-2-enal (52%), its major constituent, being a potential industrial source of these interesting compounds. α,β-Unsaturated aldehydes may be good candidates of antimicrobial agents against bacteria responsible for human gastrointestinal and respiratory-tract infections, and they might be good alternatives to other highly toxic disinfectants, such as glutaraldehyde, for hospital equipment [21]. Some aldehydes showed broad applications as food preservatives [22]. According to *Kim et al.* [15], (*E*)-dec-2-enal, (*E*)-dec-2-en-1-ol, and decan-1-ol showed strong nematicidal activity against the pine wood nematode (*Bursaphelenchus xylophilus*) at 0.2 mg/ml, and among all the active compounds, dec-2-enal showed the highest nematicidal activity through all concentration ranges tested [15]. Unsaturated aldehydes are important minor constituents responsible for a variety of characteristic aroma of natural food, as they have a stronger fragrance odor than their corresponding saturated aldehydes. They are indispensable compounds in formula of fragrances and food flavors [5].

Conclusions. – The essential oil isolated from the aerial parts of *A. delavayi* from Yunnan, China is a new source of α,β-unsaturated aldehydes, compounds appreciated in the pharmaceutical as well as the flavor and fragrance industry. The essential oil exerted a broad antimicrobial spectrum with activity against all bacteria and fungi tested in this study. It might be a good candidate as antimicrobial agent against human pathogenic bacteria and fungi. In addition, the essential oil showed strong inhibitory activity of bacterial volatile sulfide compound production, which might imply its potential use as oral care flavor. Briefly, *A. delavayi* essential oil might be a potential industrial resource of new products.

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Experimental Part

General. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AM-400* NMR spectrometer, at 400 and 100 MHz, resp., in $CDCl_3$; δ in ppm rel. to Me_4Si, J in Hz.

Plant Material. The aerial parts of *Aristolochia delavayi* FRANCH. were collected in the Daju County of Lijian, Yunnan Province (China), in September 2008. The species was identified by Dr. *E. D. Liu*, Kunming Institute of Botany, and a voucher specimen (KUN1229504) has been deposited with the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction of the Essential Oil. The essential oil of the *A. delavayi* aerial parts (air-dried) was extracted using the standard apparatus described in the *Chinese Pharmacopeia* [23]. Each extraction was performed by hydrodistillation for 6 h of 100 g of ground sample placed inside a round-bottomed flask submerged in 600 ml of dist. H_2O. The essential oil was separated from the aq. phase produced during distillation and dried (anh. Na_2SO_4).

*Isolation of (*E*)-Dec-2-enal.* Flash column chromatography was used to obtain concentrated fractions for the subsequent analyses of the low-concentration components in the essential oil. An aliquot of 8 g of *A. delavayi* oil was applied to a silica gel *60* (SiO_2, 40–63 μm) flash-chromatography column (4 × 14 cm, *Biotage*) and eluted with a stepwise solvent gradient of AcOEt in hexane (0%, 1000 ml; 3%, 1000 ml; 6%, 500 ml; 10%, 1000 ml; 20%, 600 ml; 40%, 600 ml). Each eluted fraction was collected and concentrated on a rotary evaporator to afford seven fractions for the GC/MS analyses. *Fr. 2* was submitted to preparative TLC (SiO_2, Hexane/AcOEt 100:5) and further purified by column chromatography (*Sephadex LH-20*, acetone) to give (*E*)-dec-2-enal.

*(2*E*)-Dec-2-enal.* ^{1}H-NMR: 9.49 (*d*, J = 7.9, 1 H); 6.88 (*dt*, J = 13.7, 6.8, 1 H); 6.13 (*dd*, J = 15.6, 7.9, 1 H); 2.35 (*ddt*, J = 7.5, 7.0, 1.5, 2 H); 1.51 (*quint.*, J = 7.5, 2 H); 1.24–1.30 (*m*, 8 H); 0.89 (*t*, J = 7.0, 3 H). ^{13}C-NMR: 194.3 (CHO); 159.3 (CH); 132.9 (CH); 32.7 (CH_2); 31.7 (CH_2); 29.2 (CH_2); 29.0 (CH_2); 27.8 (CH_2); 22.6 (CH_2); 14.0 (Me). MS: 154 (1.6, M^+), 136 (6.2), 121 (12.8), 110 (26.0), 98 (33.2), 83 (78.9), 70 (100), 55 (93.3), 41 (90.4).

GC-FID and GC/MS Analyses. The GC-FID and GC/MS analyses of the essential oil and its subfractions were carried out with an *Agilent 7890A* gas chromatograph equipped with an *HP-5* (5% diphenyl polysiloxane) cap. column (50 m × 0.2 mm i.d., film thickness 0.33 μm). The column effluent was split equally between an *Agilent 5975* inert mass selective detector (MSD; ionization potential, 70 eV) and an FID detector *via* a capillary flow technology splitter plate. The oven temp. was programmed rising from 50 to 280° at 2°/min and then held isothermal at 280° for 20 min; injector temp., 225°; FID temp., 300°; MSD transfer-line temp., 280°; carrier gas, He (1.7 ml/min); initial head pressure, 46.3 psi. All injections were performed in split mode (50:1). Data was acquired and processed using MSD ChemStation software.

Compound Identification. The identification of the oil constituents was based on the comparison of their retention indices (*RI*s), determined relative to the retention times (t_R) of *n*-alkanes (C_8–C_{30}) according to *Van den Dool* and *Kratz* [24], and their fragmentation patterns in the mass spectra with those listed in both an in-house and commercial libraries including *NIST*, *Adams*, and *Wiley*, as well as with those published in the literature [8].

Bacterial Volatile Sulfide Compound (VSC)*-Inhibitory Activity.* The essential oil was tested for *in vitro* inhibition of bacterial VSC production by *Klebsiella pneumoniae* ATCC 10031 (*American Type Culture Collection*, Rockville, MD, USA). The essential oil and the positive control citral were tested at a final concentration of 500 ppm (0.05% *w*/*w*). A 250-ml overnight broth culture of *K. pneumoniae* was harvested by centrifugation at 4000 rpm for 20 min [16]. The cells were washed three times with sterile 0.3% tryptone soya broth (TSB) and resuspended in 10 ml of 0.3% TSB. The optical density of the cellular biomass was then adjusted to give an optical density equivalent to 39 at a wavelength of 610 nm.

Sterile cysteine was added to the cellular suspension to give a concentration of 0.1% (*w*/*v*) cysteine in the reaction vessel. The mixture was then incubated at 37° with shaking for 1 h, to induce the cysteine metabolism in the microorganisms. After incubation, the cells were washed twice with 0.3% TSB soln. to remove excess cysteine and resuspended in the same volume of 0.3% TSB soln.

The stock solns. of essential oil and citral were prepared in sterile TSB soln. at a concentration of 32600 ppm (3.26% (*w*/*w*)). Into sterile headspace vials, 11.55 ml (11.75 ml for control vials) of 0.3% TSB and 0.25 ml of 2% (*w*/*v*) cysteine soln. was added, giving a final cysteine concentration of 0.5% (*w*/*v*). To the test vials, 0.2 ml of the stock solns. to be tested was added, giving a final essential-oil or citral concentration of 500 ppm. The mixture was then thoroughly stirred and incubated at 37° for 1 h, to allow the mixture to equilibrate. Subsequently, 1 ml of the previously prepared bacterial suspension was added to the vials, and the vials were capped to produce an airtight seal and incubated at 37° with shaking for 1 h. The volume of H_2S produced in the headspace of the vials after incubation was then analyzed. An aliquot of 0.5 ml of the headspace gas was removed and the quantity of H_2S estimated by GC analysis. The VSC-inhibition percentage was calculated for each sample by comparison with an untreated control.

Microbial Strains. The microorganisms used in the bioassay study include six *Gram*-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 8739, *E. coli* ATCC 11775, *Enterobacter aerogenes* ATCC 13048, *Providencia stuartii* ATCC 29916, and *Salmonella typhi* ATCC 6539), two *Gram*-positive bacteria (*Enterococcus faecalis* ATCC 10541 and *Staphylococcus aureus* ATCC 25922), two dermatophytes (*Trichophyton ajelloi* and *T. terrestre* E 1501), and four yeasts (*Candida albicans* ATCC1663, *C. glabrata* IP 35, *C. guilliermondii*, and *Cryptococcus neoformans* IP 95026,). The reference strains (ATCC) were obtained from the *American Type Culture Collection* (Rockville, USA). The two IP fungal strains were obtained from the *Institute Pasteur* (Paris, France), and *T. terrestre* E 1501 was obtained from the Ecole Nationale Vétérinaire d'Aford, France. The clinical dermatophyte and fungal isolates were collected from the *Centre Pasteur* (Yaoundé, Cameroon).

Antimicrobial Assay. The broth microdilution method was used to determine the minimal inhibitory concentration (*MIC*) for bacteria and the minimal bactericidal concentration (*MBC*), according to the method described by *Michielin et al.* [25]. The *MIC* for fungi and the minimal fungicidal concentration (*MFC*) were determined in 96-well microtiter plates as recommended by the *Clinical and Laboratory Standards Institute* (CLSI, formerly *National Committee for Clinical and Laboratory Standards*), according to documents M27-A2 and M38-A for yeasts and filamentous fungi, resp., with slight modifications [26][27]. The tests were performed in *Mueller – Hinton* broth (MHB) for bacteria and in *Sabouraud* dextrose broth (SDB) for fungi. The inocula of the microorganisms were prepared from 24-h (bacteria), 48-h (yeasts), or 10-d (dermatophytes) old cultures, and suspensions were adjusted to 0.5 *McFarland* standard turbidity. The standardized inoculum was diluted to achieve a final inoculum concentration of *ca.* 10^7 CFU/ml for bacteria and 2×10^5 CFU/ml for yeasts and dermatophytes. The samples were dissolved in 10% DMSO to obtain the highest test concentration of 1 mg/ml. Serial dilutions of the samples were performed to reach final concentration ranges of 1000.0-7.8 and 500.0-3.9 μg/ml for the antibacterial and antifungal tests, resp. For each experiment, a sterility check (10% (*v*/*v*) aq. DMSO and medium), a negative control (10% aq. DMSO, medium, and inoculum), and a positive control (10% aq. DMSO, medium, inoculum, and rifampicin or amphotericin B for bacteria or fungi, resp., at concentrations ranging from 50.00-0.09 μg/ml) were included. All assays were performed in triplicate. The 96-well plates were covered with sterile lids and incubated at 37° for 24 h for bacteria, at 37° for 48 h for yeasts, and at 28° for 5 d for dermatophytes.

The growth of bacteria was monitored colorimetrically by using iodonitrotetrazolium chloride (INT; *Sigma*). Viable bacteria reduced the yellow dye to afford a pink color. For yeast and dermatophytes, *MIC*s were monitored by comparing the visual turbidity of the test wells with those of the positive and negative control wells. The lowest concentrations, at which no visible color or turbidity change was observed, indicating no growth of microorganism, were considered as the *MIC*s. The *MBC* or *MFC* were determined by adding 50-μl aliquots of the contents of the clear wells to 150 μl of freshly prepared broth medium and by incubation at the above-mentioned temps. and time periods. The *MBC* or *MFC* were regarded as the lowest concentration of test sample, which did not produce color or turbidity change. All tests were carried out in triplicates.

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