The Chemical Composition and Antibacterial Activities of the Essential Oils from Three *Aframomum* Species from Cameroon, and Their Potential as Sources of (*E*)-(*R*)-Nerolidol

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Essential oils obtained by hydrodistillation of seeds, pericarps, leaves and rhizomes of *Aframomum dalzielii*, *A. letestuianum* and *A. pruinosum* grown in Cameroon were analyzed by GC-FID and GC-MS. The seed oils of the three species were characterized by a high content of (*E*)-(*R*)-nerolidol (>88.0 %), which was fully characterized by NMR spectroscopy and chiral GC analysis. The main constituents of the pericarp and rhizome oils were monoterpene hydrocarbons, mainly β -pinene (0.8%-22.9%) and sabinene (29.0%-42.3%), along with 1,8-cineole (4.5%-23.7%); leaf oils were characterized by sesquiterpenes, namely (*E*)- β -caryophyllene (18.4%-82.4%) and caryophyllene oxide (4.5%-23.7%). The antibacterial activities of these essential oils and of nine pure compounds (sabinene, β -pinene, 1,8-cineole, linalool, racemic (*E*)-nerolidol, (*E*)-(*R*)-nerolidol, (*E*)- β -caryophyllene, α -humulene and caryophyllene oxide) were assessed against *Micrococcus luteus* and *Escherichia coli*. The strongest activities were observed against *E. coli*. The seed essential oils and their major component, (*E*)-(*R*)-nerolidol, exhibited the lowest MIC values (0.19-0.39 μ L/mL), justifying their traditional use and their potential application as natural food preservatives.

Keywords: *Aframomum dalzielii*, *Aframomum letestuianum*, *Aframomum pruinosum*, Zingiberaceae, Essential oils, (*E*)-(*R*)-Nerolidol, Antibacterial activity, Natural preservative.

The therapeutic potentials of aromatic and medicinal plants and of their extracts have been recognized since antiquity; their pharmaceutical properties are partially attributed to their volatile aroma components, composed mainly of terpenes, in addition to other non-terpene components that are currently extracted from the plant material by water or steam distillation. Numerous biological properties of essential oils have recently been reviewed [1-3]. Among these, the antimicrobial qualities of the essential oils from a wide variety of plants, as well as those of their constituents, have been assessed [4,5]. In spite of these data, the greatest use of essential oils is still in food (as flavorings), perfumes and pharmaceutical products for their functional properties. Up to now, only a few preservatives containing essential oils are commercially available [6]. In the meantime, there is a growing number of outbreaks of food-borne illness caused by less than 20 different bacterial species. Due to the resistance that some microorganisms have built up against antibiotics, there is a need for new biologically active components for the maintenance or extension of product shelf life [7]; spices that have been recognized for their antimicrobial properties [8] might represent interesting sources of additives for the reduction or elimination of food-borne pathogens.

The genus *Aframomum*, family Zingiberaceae, is mainly spread in the rainy forest areas of Africa. Over 20 species of this genus are found in Cameroon, where they are widely used for medicinal,

ethno-dietary, cultural and spiritual purposes [9a]. *Aframomum* species are well known in several countries for their odoriferous leaves and fruits with aromatic seeds which produce essential oils [9a, 10]. Among them, *A. melegueta* (Rosc.) K. Schum., called "maniguette" or "grains of paradise", is probably the most studied [11a]; its seeds contain the same pungent principles as ginger (6-paradol, gingerol and shogaol), which justifies their use as spices and their other designation as "Poivre de Guinée" [12].

As a contribution to our investigations on volatiles from *Aframomum* species growing in Central Africa [9a-9b, 11a-11b], we have focused on three species as potential sources of (*E*)-nerolidol in their seed essential oils: *A. dalzielii* Hutchinson (synonym: *A. leptoplepis*), *A. letestuianum* Gagnepain and *A. pruinosum* Gagnepain. In the eastern region of Cameroon, these species grow in the wild, while they are highly cultivated in the western highlands for the local trading of their fruits. These contain sweet and spicy seeds with pleasant scent, popularly consumed in culinary preparations. They are used as ingredients in "medicines" to cure several afflictions like sterility for women and schizophrenia, as reported by traditional healers; they might also have antisymphathetic properties and tranquilizing effects [13]; there is a general agreement on their properties to bring strength and peace in families with twins (data not published).

Regarding chemistry, Tane *et al.* [13] reported on eleven *Aframomum* species collected in Cameroon (among them *A. letestuianum* and *A. pruinatum*), which contained diterpenoids, flavonoids, sesquiterpenoids and arylalkanooids. To the best of our knowledge there were no previous chemical investigations on *A. dalzielii*. Finally, the only chemical investigations on essential oils from *Aframomum* species collected in Cameroon were performed on nineteen species from various geographical areas [9a]; all the essential oils contained mainly terpenic components obtained from the shikimic acid pathway, usually found in essential oils, but in minor amounts; among them, *A. letestuianum* and *A. pruinatum* seed oils were rich in (*E*)-nerolidol (67%; 91.7%) respectively, while α -pinene (31.4%) and (*E*)- β -caryophyllene (25.8%) were the main constituents of *A. pruinatum* leaf oil.

The cultural and medicinal interest attributed to the *Aframomum* species growing in Cameroon increases the commercial value of their fruits. To enhance the value of these species, we have systematically investigated the chemical constituents of the essential oils obtained from seeds, pericarps, leaves and rhizomes of *A. dalzielii*, *A. letestuianum* and *A. pruinatum*; the antibacterial activities of these essential oils, as well as those of their main components, were tested against two bacterial species: *Micrococcus luteus*, a Gram-positive human pathogen, and *Escherichia coli* (Gram-negative), a foodborne bacterium, also implicated in nosocomial infections.

Pericarps, seeds, leaves and rhizomes of the three species were submitted to hydrodistillation; the highest yields were obtained from pericarps (0.3-2.2%), followed by seeds (0.1-0.7%); the lowest contents of volatile components were observed in the rhizomes (0.06-0.1%), while the leaves of the three species afforded almost the same amounts of essential oils (0.1-0.3%). Globally, the best yields were obtained from *A. pruinatum* fruits (Table 1).

Table 1: Oil yields from different parts of three *Aframomum* species collected in Cameroon

Species	Date of harvest	Locality	Oil yields (w/w)			
			Seeds	Pericarps	Leaves	Rhizomes
<i>A. dalzielii</i>	August 26th 2008	Fontem	0.6	1.2	0.3	0.06
<i>A. pruinatum</i>	August 26th 2008	Fontem	0.7	2.2	0.1	0.1
<i>A. letestuianum</i>	March 15th 2008	Bamendjida	0.1	0.3	0.2	0.1

GC and GC-MS analyses of the essential oils allowed the identification of 73 components, amounting to 89.7-99.7% of the total chemical composition (Table 2). Important qualitative and quantitative variability was noticed according to the plant part and / or the botanical species; nevertheless, the seed oils were characterized by the same major component, (*E*)-nerolidol (88.0-95.1%), which could find its application as an agroalimentary product and in perfumery for its long lasting floral odor [14, 15].

This component was, therefore, isolated by liquid solid chromatography from the seed essential oil of *A. dalzielii* and fully characterized by ^1H and ^{13}C NMR spectroscopy. The data, which were in agreement with the literature, confirm the formal identification of (*E*)-nerolidol as the major component of the seed essential oil [16]; the specific optical rotation of this component was measured as $[\alpha]_D = -13.8$ (*c* 0.1, CHCl_3). Analysis by gas chromatography on a chiral column compared with that of the racemic sample (resolution factor 1.01) and (+)-(*E*)-nerolidol from Cabreuva oil (*Myrcarpus fastigiatus*) used as reference, indicated an enantiomeric excess of the levorotary (*E*)-nerolidol (96.4%); chiral analyses of the seed essential oils of *A. letestuianum* and *A. pruinatum*, performed under the same experimental conditions,

indicated an enantiomeric excess of (-)-(*E*)-nerolidol in both samples (respectively 99.5% and 98.3%). These results confirm those previously obtained with the seed oil of *A. pruinatum* collected in Cameroon [11b].

The accurate quantification of (*E*)-nerolidol in the seed oils was performed using a multilevel standardization method with tridecane as internal standard (see Experimental). The values obtained (g/100g of the seed oil) were 75.6 ± 0.3 for *A. letestuianum*; 84.6 ± 0.4 for *A. dalzielii*; and 88.3 ± 0.4 for *A. pruinatum*.

The known natural sources of this component are not so numerous: the same enantiomer was identified in the wood oil of *Dalbergia parviflora* (Leguminosae) [14]; on the other hand, the (+)-(*S*)-enantiomer is predominant in Cabreuva oil; finally, (*E*)-nerolidol is also the main headspace component of *Cymbidium goeringii* var. *goringii*, a species with cultural importance in Japan and Southern China, to which it provides a lily of the valley type contribution to the pleasant scent [17].

As previously published by Amvam Zollo *et al.* [9a], the leaf essential oil of *A. pruinatum* was dominated by sesquiterpenes, mainly β -caryophyllene, α -humulene and caryophyllene oxide (47.7%, 3.0% and 6.2%); these components remain the main volatile constituents of *A. letestuianum* (18.4%, 12.4% and 23.7%) and *A. dalzielii* leaves (81.4%, 4.3% and 3.3%), which are described here for the first time.

As far as the literature survey could ascertain, pericarp and rhizome essential oils from the three species have never been reported before. The essential oils from pericarps were rather dominated by monoterpenes, mainly sabinene and β -pinene (31.9%-56.5% all together) in variable relative proportions, along with 1,8-cineole (10.6%-14.0%); a relatively high content of linalool (10.1%) characterized the essential oil from *A. letestuianum* pericarps. Finally, the essential oils obtained from rhizomes, in all cases in very low yields, are very similar to those of the pericarp extracts; they are dominated by pinenes and sabinene, but with some qualitative and quantitative differences regarding the minor components.

The results of the antibacterial tests performed on all twelve essential oil samples are reported in Table 3; the MIC values varied from 0.19 to 50 $\mu\text{L/mL}$.

Globally, the strongest activities were obtained against the Gram-negative bacterium *E. coli*, with MICs varying from 0.19 to 1.56 $\mu\text{L/mL}$ according to the essential oil sample, while the antimicrobial activities against *M. luteus* were lower (MICs 0.39 to 50 $\mu\text{L/mL}$). These activities could be classified in three levels: Strong activity for MIC value ranging from 0.19 to 0.78 $\mu\text{L/mL}$; Moderate activity in the range 1.56-6.25 $\mu\text{L/mL}$; and Low activity between 12.5 and 50 $\mu\text{L/mL}$.

Considering the above, the essential oils tested showed either strong or moderate activity against *E. coli*, the most efficient samples being seed oils, along with the rhizome oils from *A. dalzielii* and *A. pruinatum*, and the volatile extract from *A. letestuianum* pericarps. The seed oils were the most efficient samples against *M. luteus*, with MICs ranging from 0.39 to 0.78 $\mu\text{L/mL}$; all the other samples showed weak inhibitory activity against *M. luteus*, except for the leaf oil of *A. letestuianum* (MIC = 0.78 $\mu\text{L/mL}$). A strong bactericidal activity against both microorganisms was observed with *A. letestuianum* seed oil (MBC= 0.39-0.78 $\mu\text{L/mL}$).

Table 2: Comparative percentage composition of essential oils from seeds, pericarps, leaves and rhizomes of *A. dalzielii*, *A. letestuanum* and *A. pruinosum*.

Identified compounds	LRI	<i>A. dalzielii</i>				<i>A. letestuanum</i>				<i>A. pruinosum</i>				Identification methods
		Se	Pe	Le	Rh	Se	Pe	Le	Rh	Se	Pe	Le	Rh	
Monoterpenes		1.6	91.1	5.0	79.7	2.8	82.9	17.3	75.2	2.0	82.9	34.7	79.4	/
Monoterpene hydrocarbons		1.0	70.7	4.6	71.9	0	50.7	15.9	58.0	1.6	49.5	33.5	57.6	/
α -Thuiene	928	/	0.3	t	0.4	/	0.9	t	0.6	/	0.7	/	1.9	MS, LRI
α -Pinene	938	/	7.8	0.5	7.8	/	6.4	2.2	5.9	/	5.9	3.9	5.2	GC, MS, LRI
Camphene	952	/	0.1	t	0.1	/	/	/	/	/	t	1.1	/	MS, LRI
Sabinene	977	/	14.2	t	22.9	/	0.8	/	11.6	/	2.9	0.3	9.8	GC, MS, LRI
β -Pinene	985	/	42.3	3.9	37.9	/	38.5	13.4	32.9	/	29.0	27.8	34.3	GC, MS, LRI
Myrcene	988	0.1	0.5	/	0.6	/	t	/	0.5	/	t	/	t	MS, LRI
α -Terpinene	1018	/	0.6	/	t	/	t	/	1.1	/	/	/	0.5	MS, LRI
<i>p</i> -Cymene	1022	/	0.5	t	0.3	/	1.2	/	0.5	/	4.0	/	2.7	MS, LRI
β -Phellandrene	1030	0.1	/	/	/	/	/	/	/	/	/	/	/	MS, LRI
Limonene	1030	/	2.7	0.2	1.6	/	2.5	0.3	2.2	/	7.0	0.4	1.5	GC, MS, LRI
(Z)- β -Ocimene	1045	0.4	/	/	/	/	/	/	0.1	/	/	/	/	MS, LRI
(E)- β -Ocimene	1050	0.4	/	/	/	/	/	/	/	1.6	/	/	/	GC, MS, LRI
γ -Terpinene	1057	/	1.3	/	0.3	/	0.4	/	2.0	/	/	/	1.2	MS, LRI
Terpinolene	1089	/	0.4	/	t	/	/	/	0.6	/	/	/	0.5	MS, LRI
Oxygenated-monoterpenes		0.6	20.4	0.4	7.8	2.8	32.2	1.4	17.2	0.4	33.4	1.2	21.8	/
1,8-Cineole	1032	0.1	10.6	0.1	3.2	t	11.2	/	4.3	0.3	14.0	0.2	2.5	GC, MS, LRI
<i>cis</i> -Sabinene hydrate	1067	/	0.1	/	0.5	/	0.7	/	0.6	0.1	/	/	/	MS, LRI
Linalool	1095	0.1	2.6	/	1.3	2.2	10.1	0.3	4.8	/	/	/	5.5	GC, MS, LRI
<i>trans</i> -Sabinene hydrate	1098	/	0.1	/	0.2	/	/	/	/	/	/	/	0.8	MS, LRI
α -Campholenal	1125	/	/	/	0.1	/	/	0.1	/	/	0.6	/	/	MS, LRI
<i>cis</i> - <i>p</i> -Menth-2-en-1-ol	1139	/	0.1	/	/	/	/	/	/	/	1.7	/	/	MS, LRI
<i>trans</i> -Pinocarveol	1143	/	/	/	0.2	/	1.9	/	/	/	0.7	/	1.7	MS, LRI
Pinocarvone	1155	/	0.1	/	0.1	/	0.8	0.2	0.3	/	0.9	/	0.8	GC, MS, LRI
<i>cis</i> -Pinocarveol	1167	/	/	/	0.1	/	/	/	/	/	t	/	/	MS, LRI
Terpinen-4-ol	1173	/	4.2	t	0.6	/	0.8	0.1	/	/	4.6	0.2	3.1	GC, MS, LRI
α -Terpineol	1177	0.3	0.1	0.3	0.3	/	0.8	0.4	3.5	/	4.4	0.2	0.7	GC, MS, LRI
Myrtenol	1189	/	2.0	/	0.6	/	2.7	/	2.0	/	1.7	0.4	3.7	MS, LRI
Verbenone	1219	/	/	/	/	/	/	/	0.3	/	/	/	/	MS, LRI
Cuminaldehyde	1231	/	/	/	0.1	/	/	/	0.3	/	0.5	/	/	MS, LRI
<i>cis</i> -Sabinyl acetate	1262	/	/	/	/	0.6	/	/	/	/	/	/	/	MS, LRI
α -Terpinen-7-yl	1265	/	/	/	0.1	/	0.2	/	/	/	/	/	/	MS, LRI
<i>trans</i> -Pinocaryyl acetate	1286	/	/	/	0.1	/	0.3	/	/	/	0.3	/	/	MS, LRI
<i>cis</i> -Pinocaryyl acetate	1290	/	/	/	/	/	0.1	/	/	/	0.5	/	/	MS, LRI
Myrtenyl acetate	1319	/	0.5	t	0.3	/	2.6	0.3	1.1	/	1.5	0.2	3.0	GC, MS, LRI
4-Terpinyol acetate	1325	/	/	/	/	/	/	/	/	/	1.3	/	/	GC, MS, LRI
α -Terpinyl acetate	1338	/	/	/	/	/	/	/	/	/	0.4	/	/	GC, MS, LRI
Dihydrocarveol acetate (neois)	1359	0.1	/	/	/	/	/	/	/	/	0.3	/	/	MS, LRI
Sesquiterpenes		92.9	8.5	92.8	17.7	92.3	15.0	74.6	21.0	97.5	14.0	61.8	10.3	/
Sesquiterpene hydrocarbons		0.7	5.5	87.5	9.4	0.9	0.4	32.8	12.3	1.6	1.0	52.0	5.2	/
α -Copaene	1371	/	/	t	t	/	/	0.1	/	/	0.4	/	/	MS, LRI
β -Cubebene	1387	/	/	/	/	0.5	/	/	/	/	/	/	/	MS, LRI
β -Elemene	1400	/	/	0.2	0.1	/	/	t	/	/	0.2	0.1	/	MS, LRI
Cyperene	1422	/	/	/	0.9	/	/	0.5	4.9	/	/	0.4	4.4	MS, LRI
α -Gurjunene	1424	/	/	0.4	/	/	/	/	/	/	/	0.4	/	MS, LRI
(E)- β -Caryophyllene	1439	/	4.8	81.4	6.4	0.2	0.4	18.4	2.9	/	/	47.7	0.8	GC, MS, LRI
(Z)- β -farnesene	1446	0.3	/	/	/	/	/	0.1	/	0.7	/	/	/	MS, LRI
α -Humulene	1471	/	0.3	4.3	0.4	/	/	12.4	2.1	/	0.4	3.0	/	GC, MS, LRI
γ -Murolene	1478	/	/	/	/	/	/	0.2	/	/	/	/	/	MS, LRI
Germacrene-D	1480	/	/	/	/	/	/	/	/	/	/	/	/	MS, LRI
α -Curcumene	1481	0.1	/	/	/	/	/	/	/	0.3	/	/	/	MS, LRI
Selina-4,11-diene	1489	/	0.1	0.3	0.4	/	/	0.1	0.9	/	/	0.3	/	MS, LRI
Eremophyllene	1513	/	0.1	0.2	0.3	/	/	0.5	0.4	/	/	/	/	MS, LRI
β -Selinene	1503	/	0.2	0.2	0.5	/	/	0.3	0.7	/	/	/	/	MS, LRI
(E,E)- α -Farnesene	1506	0.2	/	0.1	/	/	/	/	/	0.3	/	/	/	MS, LRI
β -Bisabolene	1506	0.1	/	0.1	/	/	/	/	/	0.3	/	/	/	MS, LRI
Viridiflorene	1513	/	/	0.3	0.4	/	/	/	0.4	/	/	/	/	MS, LRI
δ -Cadinene	1533	/	/	/	/	0.2	/	/	/	/	/	0.1	/	MS, LRI
α -Cadinene	1542	/	/	/	/	/	/	0.2	/	/	/	/	/	MS, LRI
Oxygenated-Sesquiterpenes		92.2	3.0	5.3	8.3	91.4	14.6	41.8	8.7	95.9	13.0	9.8	5.1	/
(E)-Nerolidol	1563	91.2	/	0.3	0.3	88.0	0.3	1.3	/	95.1	0.3	0.4	/	GC, MS, LRI
Caryophyllene oxide	1600	/	1.3	3.3	4.5	1.1	8.9	23.7	2.9	/	9.0	6.2	1.0	GC, MS, LRI
Humulene epoxide II	1623	/	0.1	0.3	0.3	1.3	0.4	10.0	1.7	/	0.3	0.2	1.3	MS, LRI
Caryophylla-4(12),8(13)-dien-5-ol	1643	/	/	0.1	0.1	/	1.5	0.5	/	/	0.3	0.3	1.1	MS, LRI
α -Bisabolol oxide	1658	0.4	/	/	/	1.0	/	0.4	/	/	0.8	/	/	MS, LRI
α -Selin-11-en-4-ol	1671	/	1.6	1.1	3.0	/	1.4	2.9	3.1	/	0.2	2.1	1.1	MS, LRI
α -Selin-3,11-dien-6-ol	1685	/	/	0.1	/	/	2.1	/	/	/	/	/	/	MS, LRI
α -Bisabolol	1691	0.3	/	0.1	/	/	1.4	0.6	0.5	1.2	0.6	/	/	GC, MS, LRI
Cyperenone	1709	/	/	/	0.1	/	/	0.7	0.4	/	/	/	0.6	MS, LRI
(2E,6E)-Farnesol*	1722	0.2	/	/	/	t	/	0.2	/	0.3	/	/	/	MS, LRI
(2E,6E)-Farnesyl acetate*	1822	0.1	/	/	/	t	/	0.7	/	/	0.9	/	/	MS, LRI
Aromatic compounds		1.0	0	0	0	0.3	0	0	0.0	0.0	0.4	0	0	/
<i>o</i> -guaiacol	1089	0.2	/	/	/	/	/	/	/	/	/	/	/	MS, LRI
2-Phenylethyl acetate	1259	0.5	/	/	/	0.3	/	/	/	/	0.4	/	/	MS, LRI
(E)-cinnamyl acetate	1452	0.3	/	/	/	/	/	/	/	/	/	/	/	GC, MS, LRI
Aliphatic compounds		3.7	0	0	0	1.5	0	0	0	0.2	0	0	0	/
2-heptanol	869	0.1	/	/	/	/	/	/	/	/	/	/	/	MS, LRI
2-Heptyl acetate	1042	2.8	/	/	/	0.7	/	/	/	/	/	/	/	MS, LRI
Octyl acetate	1196	0.1	/	/	/	0.8	/	/	/	0.2	/	/	/	MS, LRI
Decyl acetate	1408	0.7	/	/	/	/	/	/	/	/	/	/	/	MS, LRI
Total		99.2	99.6	97.8	97.4	96.9	97.9	91.9	96.2	99.7	97.3	96.5	89.7	/

t: traces (<0.1), Se: Seeds; Pe: Pericarps; Le: Leaves; Rh: Rhizomes; *correct isomer not identified; LRI: Linear retention index; Identification methods: GC, identification based on co-injection with authentic sample; MS, identification based on comparison of mass spectrum with literature data; LRI, identification based on comparison of retention index with those of published data.

Table 3: Correlation between chemical composition (relative percentages of the major constituents) and antibacterial activity (MIC and MBC in $\mu\text{L/mL}$) of the essential oils of *A. dalzielii*, *A. lesteuianum* and *A. pruinsum* and of their major components

	Antibacterial activity of pure compounds				Chemical composition (%)											
	<i>M. luteus</i>		<i>E. coli</i>		<i>A. dalzielii</i>				<i>A. lesteuianum</i>				<i>A. pruinsum</i>			
	MIC	MBC	MIC	MBC	Se	Pe	Le	Rh	Se	Pe	Le	Rh	Se	Pe	Le	Rh
Sabinene	50c	>50	1.56b	6.25	-	14.2	t	22.9	-	0.8	-	11.6	-	2.9	0.3	9.8
β -Pinene	3.12b	12.5	0.19a	0.78	-	42.3	3.9	37.9	-	38.5	13.4	32.9	-	29.0	27.8	34.3
1,8-Cineole	12.5c	>50	0.78a	3.12	0.1	10.6	0.1	3.2	t	11.2	-	4.3	0.3	14.0	0.2	2.5
Linalool	0.19a	0.78	0.19a	0.19	0.1	2.6	-	1.3	2.2	10.1	0.3	4.8	-	-	-	5.5
(<i>E</i>)- β -Caryophyllene	>50c	>50	50c	>50	-	4.8	-	6.4	0.2	0.4	18.4	2.9	-	-	47.7	0.8
α -Humulene	>50c	>50	6.25b	>50	-	0.3	4.3	0.4	-	-	12.4	2.1	-	0.4	3.0	-
(<i>E</i>)-Nerolidol (synthetic)	0.19a	0.19	0.19a	0.19	-	-	-	-	-	-	-	-	-	-	-	-
(<i>R</i>)-(<i>E</i>)-Nerolidol (natural)*	0.19a	0.19	0.19a	0.19	91.2	-	0.3	0.3	88.0	0.3	1.3	-	95.1	0.3	0.4	-
Caryophyllene oxide	12.5c	>50	25c	>50	-	1.3	3.3	4.5	1.1	8.9	23.7	2.9	-	9.0	6.2	1.0
Antibacterial activity of essential oils	<i>M. luteus</i>				0.39a	25c	25c	12.5c	0.39a	25c	0.78a	12.5c	0.78a	12.5c	25c	50c
	MBC				25	>50	50	50	0.78	50	6.25	25	12.5	12.5	25	50
	<i>E. coli</i>				0.19a	1.56b	1.56b	0.19a	0.39a	0.19a	0.78a	1.56b	0.19a	0.78a	1.56b	0.19a
	MBC				1.56	6.25	12.5	3.12	0.39	1.56	3.12	6.25	3.12	3.12	12.5	1.56

* ex *A. dalzielii*; Se: Seeds; Pe: Pericarps; Le: Leaves; Rh: Rhizomes; MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; Values followed by: a ("strong activity"), MIC ranging from 0.19-0.78 $\mu\text{L/mL}$; b ("moderate activity"), MIC ranging from 1.56-6.25 $\mu\text{L/mL}$; c ("low activity"), MIC ranging from 12.5-50 $\mu\text{L/mL}$.

The antibacterial activities should be expected to relay the chemical compositions of the essential oils, the structural configuration of the components of the oil, their functional groups and possible synergistic interactions between components [18, 19]. Single compounds, identified as major components of the tested essential oils, were then tested under the same experimental conditions against both microorganisms. Components with an alcohol functional group such as (*E*)-nerolidol and linalool were highly active against both microorganisms; the synthetic and purified nerolidol showing the same efficiency, contrary to the observations of Schmidt *et al.* [20] on *Staphylococcus aureus* with synthetic *cis*-nerolidol and natural *trans*-nerolidol. Furthermore, Brehm-Stecher & Johnson [21] showed that nerolidol induces a sensitization of *Staphylococcus aureus* and *Escherichia coli* to antibiotics. A strong bactericidal activity (MBC = 0.19 $\mu\text{L/mL}$) was observed with linalool and nerolidol for *E. coli* and with nerolidol in the case of *M. luteus*. On the other hand, (*E*)- β -caryophyllene and its epoxide showed the lowest activity (MIC = 12.5 to 50 $\mu\text{L/mL}$); among the other tested compounds, β -pinene was the most active against *E. coli* (MIC = 0.19 $\mu\text{L/mL}$), while sabinene and α -humulene were moderately active against the same microorganism (1.56 and 6.25 $\mu\text{L/mL}$); 1,8-cineole exhibited a good antibacterial activity on *E. coli* (MIC = 0.78 $\mu\text{L/mL}$).

These results are in accordance with previous results that indicated that oxygenated compounds exhibited the strongest antimicrobial activities, especially visible on whole cells, while hydrocarbon derivatives were less active because of their limited hydrogen bond capacity and water solubility [7]. Nevertheless, a high bactericidal activity was reported for terebenthine oil, which was attributed to pinenes [22, 23]. 1,8-Cineole was previously reported for its potential antimicrobial activity, either alone or in combination with other minor compounds in essential oils [24]. The strong activities observed against *E. coli* for the rhizome oils of *A. dalzielii* and *A. pruinsum*, as well as for the pericarps oil of *A. lesteuianum*, might be related to their high content of β -pinene, in combination with sabinene and 1,8-cineole or linalool.

In conclusion, regarding the relationship between the antibacterial activities of *Aframomum* essential oils and their main components, the strong activities of the seed oils against both microorganisms might be attributed to their high content of (*R*)-(*E*)-nerolidol; these essential oils could then be used as agro-alimentary products owing to their aromatizing and bactericidal properties and could be included in effective and inexpensive formulations to reduce the presence of pathogens in food in place of conventional chemical

antimicrobials. Finally, our study corroborates the use of *Aframomum* seeds in traditional culinary preparations.

Experimental

Plant material and extraction procedure: All plant materials (seeds, pericarps, leaves and rhizomes) were collected in the western area of Cameroon; both *A. dalzielii* and *A. pruinsum* were harvested in August 2008 at N'jenseh and Mbelah village of Fontem, respectively, while *A. lesteuianum* samples were collected in March 2008 at Bamendjida, a village of Mbouda. The botanical identifications were carried out at the National Herbarium of Cameroon where voucher specimens are kept: NKM 165639/HNC, 45194 HNC and 43138 HNC, respectively. A portion (200 g) of each plant material was submitted for 6 h to hydrodistillation, using a Clevenger-type apparatus. The obtained essential oils, separated from water by decantation, were dried over anhydrous sodium sulfate and, after filtration, stored in sealed flasks at 4°C until tested and analyzed. The yields (w/w) were calculated according to the weight of fresh plant material (Table 1).

Chemical analysis: The essential oils were analyzed by gas chromatography (GC) and gas chromatography combined with mass spectrometry (GC-MS).

Gas chromatography analysis: GC analyses were performed on a Varian gas chromatograph, model CP-3380, equipped with a flame ionization detector (FID) using an HP-5 J&W Agilent (5% phenyl-95% methylpolysiloxane) capillary column (30 m x 0.25 mm, film thickness 0.25 μm). Injector and detector temperatures were set at 220 and 250°C, respectively. Oven temperature was gradually raised from 50°C to 200°C at 5°C/min, then kept at 200°C for 10 min. N_2 was the carrier gas, at a flow rate of 0.8 mL/min; 0.1 μL of pure sample was injected manually and in a split mode (1:100).

GC-chiral analyses of racemic (*E*)-nerolidol, cabreuva oil containing mainly (+)-(*E*)-nerolidol (kindly afforded by "Laboratoire Rosier Davenne", Avignon, France) and (-)-(*E*)-nerolidol isolated from *A. dalzielii*, were carried out on a HP 5890 provided with a Cyclosil-B (J&W ref 112-6632) capillary column (30% 6-TBDMS-2,3-dimethyl β -cyclodextrine) having the following dimensions: 30 m x 0.25 mm i.d. x 0.25 μm film. The temperature program was: 80°C, held for 5 min, raising at 1°C/min to 130°C, then at 4°C/min to 175°C, finally kept at 175°C during 4 min; injection volume: 0.2 μL of a 10:100 CH_2Cl_2 solution (split ratio 1:100); carrier gas He at a constant pressure 10 psi; injector temperature 250°C, flame ionization detector temperature 250°C.

Gas chromatography-mass spectrometry: GC-MS analyses were performed using a Hewlett Packard 5890 II gas chromatograph, interfaced with a quadrupole detector (Model 5972) and equipped with a HP-5 MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm). Helium was the carrier gas, at a flow rate of 0.6 mL/min. Injector and MS transfer line temperatures were 220°C and 250°C, respectively. The oven programme temperature was the same as that used in the GC-FID analyses. Diluted samples (10:100 in CH₂Cl₂, v/v) of 1 µL were injected manually and in a split mode (1:100). The MS was operated in the EI mode at 70 eV, in the *m/z* range 35-300; electron multiplier 1460 eV; scan rate, 2.96 scan/s.

Qualitative analysis: The identification of the constituents was assigned on the basis of a comparison of their relative retention indices, calculated with reference to a series of *n*-alkanes (C₉-C₂₀), and their mass spectra with those of the standards (for main components), those found in the literature [25] and supplemented by the NBS75K database and Wiley 7th NIST 98 EPA/NIH Mass Spectral Library Upgrade (provided by Hewlett Packard with the GC/MS control and data processing software).

Quantification: The relative proportions of the essential oil constituents were obtained electronically from the GC-FID data by peak area normalization, taking response factors (RRF) as 1.0 for all compounds. Accurate quantification of the main component of the seed essential oils, (*E*)-nerolidol, was carried by the internal standard method. The response factor RRF of (*E*)-nerolidol (target) was calculated by using the following equation and adopting tridecane as internal standard (i.s.): $RRF = (C_{\text{target}} \times A_{\text{i.s.}}) / (A_{\text{target}} \times C_{\text{i.s.}})$, where C_{target} is the concentration of the referent solution, A_{target} its absolute peak area, $C_{\text{i.s.}}$ the concentration of the internal standard and $A_{\text{i.s.}}$ its absolute peak area [26]. A graphical representation expressing the area ratios $A_{\text{i.s.}}/A_{\text{target}}$ depending on the concentration ratio $C_{\text{i.s.}}/C_{\text{target}}$ ($C_{\text{i.s.}} = 2.5$ g/L, C_{target} ranging from 1 to 10 g/L in *n*-hexane) could be achieved; the calibration curve was linear over the concentrations tested, according to the regression equation $y = 1.1558x - 0.0099$ in which the slope corresponds to the response factor. The mean RRF value (3 replicates) calculated under our experimental conditions was: $RRF = 1.16 \pm 0.06$. Finally, contents of (*E*)-nerolidol in the 3 seed essential oils, expressed as g/100 g of essential oil, were calculated as: $\text{weight \%} = A_{\text{target}} \times m_{\text{i.s.}} \times RRF \times 100 / A_{\text{i.s.}} \times m_{\text{oil}}$ in which $m_{\text{i.s.}}$ and m_{oil} are expressed as g.

Isolation of (*E*)-(*R*)-nerolidol by liquid solid chromatography and characterization: Seed oil of *A. dalzielii* (1 g) was loaded onto a glass column packed with 60 g of silica gel 60 A (35-70 µm) as a slurry in *n*-hexane. Elution was performed with increasing polarity of the solvent mixture (*n*-hexane: diethyl ether 95:5; 90:10; 80:20; 70:30); solvents were dried and distilled before use. All fractions were screened by GC-FID and GC-MS analyses; those containing the single major constituent were gathered then concentrated to yield 0.82 g of pure (*-*)-(*E*)-nerolidol, a colorless liquid with a pleasant floral odor.

Optical rotation: The specific optical rotation was measured in CHCl₃ (*c* 0.1) at 25°C using a Perkin Elmer 241 polarimeter.

NMR data: ¹H and ¹³C NMR spectra of nerolidol were recorded with a Bruker DRX-400 spectrometer using CDCl₃ as solvent and TMS as internal standard (400 and 100 MHz, respectively). The assignment of ¹H and ¹³C signals was supported by one- and two-dimensional NMR experiments. 2D-NMR spectra were obtained from standard Bruker software.

Antimicrobial activities: Commercial chemical compounds: sabinene (Fluka, 84085, purity of 99%), β-pinene (Aldrich, 80609, purity of 98%), 1,8-cineole (Fluka, 29210, purity of 98%), linalool (Fluka, 74856, purity of 97%), (*E*)-β-caryophyllene (Fluka, 22075, purity of 98.5%), α-humulene (Extrasynthèse, 5424 S, purity >95%), synthetic (*E*)-nerolidol (Fluka 18143, purity >85%), caryophyllene oxide (Interchim, OC125765, purity of 95%).

The antibacterial activity of the essential oils and pure molecules were tested against *Micrococcus luteus* ATCC 4698 (Gram-positive) and *Escherichia coli* 363 ATCC 11775 363 (Gram-negative) using a broth dilution method [27]. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined for all samples. Stock solutions of essential oils, of 8 authentic commercial compounds (sabinene, β-pinene, 1,8-cineole, linalool, synthetic (*E*)-nerolidol (racemic mixture), (*E*)-β-caryophyllene, α-humulene, caryophyllene oxide), as well as of the isolated (*E*)-(*R*)-nerolidol, were prepared by geometric dilutions in sterile distilled water starting from a 1/2 to 1/512 dilutions, with vigorous shaking between each dilution. Then 100 µL of each solution was added to 900 µL of the bacterial culture previously prepared (the optical density of the inoculum was measured at $D_{600} = 0.1$ and then diluted to reach a final optical density of $D_{600} = 0.001$ in the assay), to reach a final concentration of the tested ingredient ranging from 0.19 to 50 µL/mL. The positive control was obtained with the bacterial culture without the antimicrobial agent, the negative control containing only the medium. After over-night incubation at 30°C with constant shaking (300 rpm), 100 µL of each dilution was transferred to microplates, then optical density was measured at 600 nm. The MIC, defined as the lowest concentration of the essential oil or pure compound at which the microorganism did not demonstrate visible growth, was evaluated by testing serial triplicate dilutions.

Cell suspensions (0.1 mL) from the tubes showing no growth were subcultured on Zobell and Poor broth agar plates to determine if the inhibition was reversible or permanent. MBC was determined as the highest dilution (lowest concentration) at which no growth occurred on the agar plates.

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