Chemical Composition and *in Vitro* Antimicrobial Activities of the Essential Oils from Endemic *Psiadia* Species Growing in Mauritius

Joyce GOVINDEN-SOULANGE,^{*,a} Naresh MAGAN,^b Ameenah GURIB-FAKIM,^c Anne GAUVIN,^d Jaqueline SMADJA,^d and Hippolyte KODJA^e

^a Department of Agriculture and Food Science, Faculty of Agriculture, University of Mauritius; Reduit, Mauritius;

^b Cranfield Biotechnology Centre, Cranfield University; Cranfield Bedford, MK43 0AL, United Kingdom; ^c Department of Chemistry, Faculty of Science, University of Mauritius; Reduit Mauritius; ^d Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments, Université de La Réunion, Faculté des Sciences; 15 Avenue René Cassin, BP 7151, St Denis Messag Cedex 9, La Réunion, France; and ^e Laboratoire de Biologie et Physiologie Végétale, Université de La Réunion, Faculté des Sciences; 97715 St. Denis Messag Cedex 9, La Réunion, France.

Received May 14, 2004; accepted July 22, 2004

The composition of the leaf oils, obtained by hydrodistillation, of five endemic *Psiadia* species of the Asteraceae family were studied by GC/MS on both polar and non-polar columns. The analysis showed that the volatile components of the oils were made up essentially of monoterpenes, sesquiterpenes, aliphatics and other shikimic acid derivatives. With respect to the non-volatile components, great variations were observed: *P. lithospermifolia* contained (*E*)-isoasarone (51.5%); *P. penninervia*: eugenol (5.1%); *P. terebinthina*: eugenyl-acetate (4.0%); *P. viscosa*: pentyl-4-(1-methylethyl benzoate) (25.8%); *P. arguta*: isoeugenol (56.5%). *In vitro* antimicrobial assays using the agar-well diffusion method, revealed that most of the oils were not very active against the tested microorganisms except for that of *P. lithospermifolia*, which significantly inhibited the growth of *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomonas aureofaciens*, *Aspergillus ochraceus*, *Candida pseudotropicalis*, *Kluyveromyces lactis* and *Fusarium moniliforme*. This activity has been attributed to the presence of δ -elemene, (*E*)-farnesene, α -curcumene, selina-4,7(11)-diene, (*E,Z*)- α -farnesene, β -bisabolene some of which have established antimicrobial profiles. Likewise, the fungi toxic action of the oil of *P. arguta* against *Aspergillus ochraceus*, *Candida pseudotropicalis*, and *Fusarium moniliforme*, may be attributed to the presence of isoeugenol, eugenol being known to be mycotoxic especially against *Aspergillus* species.

Key words antimicrobial activity; essential oil; chemical composition; *Psiadia* species; Mauritius

Essential oils are among the secondary metabolites of higher plants. These fragrant compounds comprise volatile terpenes, sesquiterpenes as well as their derivatives. The antimicrobial attributes of essential oils have been recognized long ago but only recently it has been established scientifically.^{1,2)}

Among the essential-oil bearing families are the Asteraceae (Compositae). This family is one of the largest plant family with over 1000 genera and 25000 species and growing in several different habitats. Many of the plants from this family are reputed to have medicinal properties and enter the local pharmacopeias of countries and for example, several *Tagetes* species have been found to possess anti-microbial activity.^{3,4)} The anti-microbial activity of *Tagetes minuta* is well established against organisms such as *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *S. epidermidis* and *Pseudomonas aeruginosa*. This property has been ascribed to the presence of flavonoids in the leaf extract.⁵⁾

Several species of the genus *Psiadia* also belonging to the Asteraceae family are used in the traditional pharmacopoeia of Africa to treat several ailments such as abdominal pains and colds in the head,⁶⁾ as expectorant for bronchitis and asthma,⁷⁾ a poultice for rheumatoid arthritis,⁸⁾ and as a plaster cast for broken bones by the Bedouin.⁹⁾

In Mauritius, *Psiadia* species have been reported to be used medicinally. The species are: *P. viscosa*, *P. terebinthina*, *P. arguta*, *P. lithospermifolia* and *P. penninervia*. They are all known under one common vernacular name, which is "Baume de l'Ile Plate". In the local pharmacopoeia, leaves of *Psiadia* plants are usually employed as poultice or in herbal teas to treat pulmonary infections and minor wounds and

burns.¹⁰⁾

In the literature, there exists limited information on the essential oil composition of *Psiadia* species. *P. arabica* from Saudi Arabia has been reported to contain β -pinene as most abundant constituent (37%).¹¹⁾ Other components are present albeit as trace amounts. *P. salviifolia* has been reported to contain β -pinene, limonene, γ -terpinene, *p*-cymene, α -copaene, linalool, β -bourbonene, α -himachalene, γ -cadinene, δ -cadinene, γ -elemene and a hydroxy derivative of calarene while *P. altissima* contains sesquiterpenoids such as T-muurolol and β -eudesmol (2.2%) and δ -cadinol (1.2%).^{12,13)}

The *Psiadia* species which grow on Mauritius have also been studied and the composition of the leaf oil of three of them has already been reported in the literature.^{10,14,15)} *P. lithospermifolia* has been reported to contain (*E*)-isoasarone as the major component (51.55%). *P. viscosa* has been reported to contain pentyl-4-(1-methylethyl benzoate) (25.86%), and (*Z*)-isoasarone (13.35%) as major components. The leaf oil of *P. arguta*, the most endangered species locally, is characterized by the presence of isoeugenol (56.5%) as the major component.^{14,15)}

It can already be observed that the composition of the *Psiadia* species varies extensively. This may be attributed to several factors and one of them could be geographic origin.

The work reported here focuses on the remaining 2 endemic *Psiadia* species namely *Psiadia penninervia* and *P. terebinthina*. A correlation will also be established between the *in vitro*, biological activity of the leaf oil of the five endemic *Psiadia* species with their chemical composition.

* To whom correspondence should be addressed. e-mail: joyces@uom.ac.mu

MATERIALS AND METHODS

Plant Material The leaves from the five *Psiadia* species were collected from the Nature Reserve of Mondrain (Mauritius). The identity of the plants was confirmed by Mr J. Guého, the then Curator of the National Herbarium (M.S.I.R.I., Reduit, Mauritius). A voucher specimen was deposited at the Herbarium of the Faculty of Science, Chemistry Department, University of Mauritius. The voucher specimen number of the plants as at the National Herbarium were give accession numbers as follows: *P. arguta* 23490; *P. lithospermifolia* 23493; *P. penninervia* 23491; *P. terebinthina* 23489; *P. viscosa* 23492.

Extraction of Essential Oils The leaves (2.0 kg) of all five *Psiadia* species, were hydrodistilled in a Clevenger-type apparatus.¹⁶ After 3 h of distillation, the oil was recovered as a film floating on the surface of the water. The water-soluble components were further extracted into hexane and the combined fractions were dried over anhydrous Magnesium sulphate, filtered and concentrated *in vacuo*, at a temperature not exceeding 40 °C. The samples were sealed and kept in dark glass vials in the refrigerator for further analysis.

Gas Chromatography Analyses The oil was analyzed by GC and GC/MS. The GC (Intersmat DN 200FID instrument) was used with detector and injector temperature at 250 °C. It was fitted with capillary glass column (50 m × 0.32 mm wall coated open tubular fused silica, Permabond SE-30-DF, film thickness 0.25 µm). The column was temperature programmed as follows: 60 °C (5 min), 65–220 °C at 5 °C/min and 220 °C (30 min) using 250 °C, N₂ as carrier gas.

The GC/MS (HP 5971 instrument) was fitted with a 50 m × 0.22 mm, HP 101 (film thickness 0.32 µm) fused silica column operating under the following conditions: 70–110 °C at 1.5 °C/min then 110–250 °C at 3.5 °C/min with helium as the carrier gas. Injector temperature was 250 °C.

Characterization and Dosage The constituents were identified by matching their mass spectra with those recorded in the MS library of the Université de La Reunion, and confirmed by comparison of mass spectra and relative retention times with authentic compounds and literature data.^{17–20}

Bioassay Growth inhibitory activity of the essential oils was tested against 3 bacteria. (e.g., *Bacillus cereus* NCD 0577, *Pseudomonas aureofaciens* NCD 02178, *Staphylococcus aureus* NCIMB 3251), 4 fungi (*Aspergillus flavus* Udl. 3.37, *Aspergillus ochraceus* NRRL 3174, *Aspergillus niger* Udl. 3.37, *Fusarium moniliforme* C25) and 2 yeasts (*Candida pseudotropicalis* NCYC 143, *Kluyveromyces lactis* NCYC 416). Yeast and bacterial strains were obtained from the UK National Culture Collections, the NRRL strains from American Type Culture Collection (ATCC) and other fungi from the Microbiology laboratory of the University of Lleida, Food Technology Department, Culthou Collection, Spain. Anti-microbial screening of crude extracts was carried out at Cranfield Biotechnology Centre (U.K.)

The fungal and yeast strains were stored at 4 °C in 25 ml of full strength malt extract agar (Oxoid). Bacterial strains were incubated on 50% nutrient agar (Oxoid) at 4 °C. Spore suspensions were prepared from colonies of the above microorganisms cultured on the same media for 7–8 d at 30 °C. Two loops full (3 mm diameter) of spores of each species were

Table 1. Concentration and Spore Counts of Microorganisms Spore Suspensions

Microorganism	Concentration
<i>Aspergillus flavus</i>	19.0 × 10 ⁵ /ml
<i>Aspergillus niger</i>	11.5 × 10 ⁵ /ml
<i>Aspergillus ochraceus</i>	35.0 × 10 ⁵ /ml
<i>Fusarium moniliforme</i>	42.5 × 10 ⁵ /ml
<i>Candida pseudotropicalis</i>	230.0 × 10 ⁵ /ml
<i>Kluyveromyces lactis</i>	150.0 × 10 ⁵ /ml
<i>Bacillus cereus</i>	1.6 c.f.u./ml
<i>Pseudomonas aureofaciens</i>	3.1 c.f.u./ml
<i>Staphylococcus aureus</i>	3.5 c.f.u./ml

transferred aseptically to small vials containing 0.5 ml of 0.1% tap water agar and mixed. The spore suspensions were freshly prepared for each series of *in vitro* tests. Optical density was determined at 700 nm. Spore counts were effected using an haemocytometer (Table 1).

Detection of Anti-microbial Activity 0.1 ml of 1 g/l agar media containing 30 µl of overnight culture of each microorganism was overlaid on the plates containing 15 ml full strength malt extract agar (anti-fungal tests) and 15 ml, 50% nutrient agar (antibacterial tests). The agar well diffusion method previously described in literature,²¹ was used to detect anti-microbial activity. Nystatin at concentration of 1.5 mg/l or Chloramphenicol at 0.25 mg/l was used as positive controls. Plates injected with yeasts and fungi were incubated at 25 ± 0.1 °C for 24 h and those injected with bacteria were incubated at 37 ± 0.1 °C for 24 h.^{22,23} After this period, inhibition zones were measured and recorded in mm.

RESULTS AND DISCUSSION

Chemical Composition We isolated leaf oils from five *Psiadia* species and analyzed the composition of the essential oils such as monoterpenes, sesquiterpenes, shikimic acid derivatives, and aliphatics (Table 2). *P. terebinthina* has been found to be characterized by the presence of α- and β-curcumene (2.3%), germacrene-D (1.8%), β-elemene (1.5%), caryophyllene oxide (1.3%), eugenyl acetate (4.0%), eugenol (3.5%), tetradecane (2.4%) and β-asarone (2.1%). *P. penninervia*, on the other hand, is characterized by the presence of the following phytochemicals: linalool (1.5%), myrcene (1%), germacrene-D (2.8%), δ-selinene (1.0%), β-elemene (2.0%), γ-eudesmol (5.6%), eugenol (5.1%), tetradecane (1.3%) and undecane (1.0%).

Antimicrobial Activity The essential oil of *P. arguta*, at 0.03% v/v of oil showed moderate activity against the tested bacteria. Besides minor fungitoxic action of *P. arguta* leaf oil was noted against *Aspergillus ochraceus*, *Candida pseudotropicalis*, and *Fusarium moniliforme*.

A similar concentration of the essential oil of *P. lithospermifolia*, on the other hand, showed significant activity against most microorganisms assayed except for *A. flavus* and *A. niger*. However, *P. lithospermifolia* showed at the same time showing better antibacterial profile than other *Psiadias* against the tested organisms. When compared to the reference antibiotic, (Chloramphenicol, at recommended doses), *P. lithospermifolia* oil showed better anti-microbial activity against *B. cereus* and *S. aureus* although *P. aureofaciens*

Table 2. Essential Oil Composition of *Psiadia* Species
Monoterpenes (%)

<i>Psiadia</i>	<i>terebinthina</i>	<i>penninervia</i>	<i>viscosa</i>	<i>arguta</i>	<i>lithospermifolia</i>
Linalool	1.1	1.5	0.14	0.24	—
Linalool oxide	Trace	0.5	—	0.028	—
Terpinen-4-ol	0.6	0.7	1.1	0.114	—
α -Terpineol	0.3	0.6	0.3	0.13	—
β -Pinene	—	0.1	—	—	—
p -Cymen-8-ol	0.1	0.3	—	0.043	—
β -Phellandrene + 1,8-cineole	0.2	0.4	0.12	—	—
Ocimene	—	0.2	—	0.01	—
Myrcene	0.1	1.0	—	0.01	—
Limonene	—	0.1	—	Trace	—
Geraniol	—	0.2	—	0.05	—
Cryptene	—	0.1	—	—	—
α -Thujene	—	—	0.1	—	—
α -Terpinene	—	—	0.17	—	—
β -Cyclocitral	—	—	—	0.014	—
Nerol	—	—	—	0.024	—
Total	2.4	5.7	1.93	0.663	

Sesquiterpenes (%)

<i>Psiadia</i>	<i>terebinthina</i>	<i>penninervia</i>	<i>viscosa</i>	<i>arguta</i>	<i>lithospermifolia</i>
$\alpha + \beta$ -Curcumene	2.3	—	—	—	—
α -Curcumene	—	—	—	0.2	1.71
Germacrene-D	1.8	2.8	—	—	—
δ -Guaiene	—	0.7	—	—	—
β -Maaliene	0.8	—	—	—	—
δ -Selinene	—	1.0	—	0.11	3.69
Isoledene	0.7	—	—	—	—
β -Caryophyllene	0.2	0.5	—	—	—
α -Humulene	—	0.4	—	—	0.87
δ -Cadinene	0.3	0.2	—	—	—
β -Elemene	1.5	2.0	—	—	—
δ -Elemene	—	—	—	—	5.16
γ -Elemene	—	—	—	—	0.94
α -Genjumene	0.1	—	—	—	—
γ -Eudesmol	3.7	5.6	—	—	—
β -Eudesmol	—	—	—	0.2	—
Caryophyllene-oxide	1.3	0.8	—	—	—
Selina-4,7(11)-diene	—	—	1.68	—	4.80
β -Patchoulene	—	—	4.2	—	—
β -Bisabolene	—	—	—	—	1.59
β -Cedrene	—	—	3.00	—	—
α -Cedrene	—	—	—	—	1.84
α -Himachalene	—	—	1.15	—	—
γ -Cadinene	—	—	5.3	—	1.85
α -Patchoulene	—	—	7.5	—	—
Cadina-1,4-diene	—	—	2.5	—	—
Calarene	—	—	7.45	—	—
Agarospinol	—	—	1.94	—	—
Guiaol	—	—	1.94	—	—
Aristolone	—	—	1.05	—	—
Elemol	—	—	—	0.17	—
(<i>E</i>)-Farnesene	—	—	—	—	8.12
(<i>E,Z</i>)- α -Farnesene	—	—	—	—	2.2
Total	12.7	14.0	37.71	0.68	32.77

Shikimic acid derivatives (%)

<i>Psiadia</i>	<i>terebinthina</i>	<i>penninervia</i>	<i>viscosa</i>	<i>arguta</i>	<i>lithospermifolia</i>
Eugenol	3.5	5.1	—	—	—
Methyl eugenol	0.2	—	2.4	0.15	—
Eugenyl acetate	4.0	—	—	—	—
Phenethyl acetate	—	0.2	—	—	—

Table 2. (Continued)
Shikimic acid derivatives (%)

<i>Psiadia</i>	<i>terebinthina</i>	<i>penninervia</i>	<i>viscosa</i>	<i>arguta</i>	<i>lithospermifolia</i>
β -Asarone	2.1	—	—	—	—
Methyl salicylate	0.2	0.4	—	0.2	—
Methoxy-vinyl phenol	—	0.4	—	—	—
Theaspirane	—	0.2	—	—	—
Pentyl 4-(1-methylethyl benzoate)	—	—	25.86	—	—
(Z)-Isoasarone	—	—	13.35	—	—
(E)-Isoasarone	—	—	—	—	51.55
Acetophenone	—	—	—	Trace	—
Ethyl benzoate	—	—	—	0.09	—
Benzyl acetate	—	—	—	0.053	—
Benzyl alcohol	—	—	—	0.012	—
Isoeugenol	—	—	—	56.5	—
Isoeugenyl acetate	—	—	—	5.62	—
Vanillin	0.8	0.1	—	4.74	—
Vanillin acetate	—	—	—	0.14	—
Acetovanillone	—	—	—	0.042	—
Total	10.8	6.4	41.61	67.54	51.55

Aliphatics (%)

<i>Psiadia</i>	<i>terebinthina</i>	<i>penninervia</i>	<i>viscosa</i>	<i>arguta</i>	<i>lithospermifolia</i>
Octen-3-ol	0.2	0.4	—	—	—
Hexenyl acetate	—	0.8	—	—	—
Nonane	0.3	0.7	—	—	—
Undecane	—	1.0	—	—	—
Dodecane	1.0	0.9	—	—	—
Tridecane	—	0.1	—	—	—
Tetradecane	2.4	1.3	—	—	—
Pentadecane	—	0.4	—	—	—
Hexadecane	—	0.5	—	—	—
Cyclohexanol	—	—	—	0.75	—
2-Ethylhexan-1-ol	—	—	—	0.024	—
2-Ethylhexanoic acid	—	—	—	0.013	—
(E,E)-2,4-Decandienal	—	—	—	0.02	—
Total	3.9	6.1	0	0.807	0

Table 3. Antimicrobial Activities of the Essential Oils of *Psiadia* Species from Mauritius along with Those of Positive Controls

Microorganism	Inhibition zone (mm)					Chloramphenicol (0.25 mg/l)	Nystatin (1.5 mg/l)
	<i>P. arg.</i>	<i>P. lithos.</i>	<i>P. penn.</i>	<i>P. terebin.</i>	<i>P. visc.</i>		
<i>Bacillus cereus</i>	14.5±0.3	27.2±0.2	R	11.4±0.3	8.0±0	17.0±0.2	
<i>Staphylococcus aureus</i>	10.5±0.3	20.8±0.2	R	9.5±0.2	8.4±0	17.1±0.2	
<i>Ps. aureofaciens</i>	17.5±0.3	28.1±0.1	R	10.3±0.3	8.6±0.1	R	
<i>Aspergillus flavus</i>	R	R	R	R	R		16.0±0.2
<i>Aspergillus niger</i>	R	8.3±0.3	R	R	R		27.8±0.5
<i>Aspergillus ochraceus</i>	21.0±0.4	24.8±0.2	R	14.1±0.4	19.9±0.2		23.4±0.2
<i>Candida pseudotropicalis</i>	19.2±0.6	22.0±0.3	R	13.8±0.1	13.0±0.3		36.0±0.2
<i>Kluyveromyces lactis</i>	14.4±0.4	22.9±0.3	R	16.3±0.4	12.6±0.3		34.6±0.3
<i>Fusarium moniliforme</i>	20.2±0.6	24.6±0.1	R	15.9±0.2	R		12.9±0.2

R: Microorganism is resistant.

seems to resist the doses of antibiotic (Chloramphenicol) assayed.

The essential oil of *P. penninervia* did not seem to exhibit any anti-microbial activity against the microorganisms assayed.

The essential oil of *P. terebinthina*, on the other hand, was found to be moderately active only against some fungi namely *Aspergillus ochraceus*, *Candida pseudotropicalis*, *Kluyveromyces lactis* and *Fusarium moniliforme* at 0.03% (v/v) dilution.

Tests performed on *P. viscosa* leaf oil have shown it to possess none or very poor antimicrobial effects at equivalent 0.03% v/v except in the case of *Aspergillus ochraceus*, *Candida pseudotropicalis*, and *Kluyveromyces lactis* whereby moderate inhibition of growth was observed.

It is noteworthy that these results are in agreement with the unique report on the pharmacological activity of the volatile components of species of the genus *Psiadia*. In fact previous work on the pharmacological activities of *P. altissima* (from Madagascar) report considerable bacteriostatic action against *S. aureus*, *Sarcina lutea*, *Bacillus subtilis*, *Branhamella catarrhalis* (with more potent activity against the latter as indicated by lowest MIC 0.05 mg/l as opposed to other strains 0.166—0.333 mg/l).²⁴⁾

In addition these observations concur with the reviews on the antibacterial characteristics of several aromatic plants such as *Pimenta dioica*, *Ocimum basilicum*, *Laurus nobilis*, *Capsicum* spp, *Piper betel*, *Aloysia triphylla*, *Rosmarinus officinalis*, *Tanacetum vulgare*, *Curcuma longa*, due to the presence of essential oils.²⁵⁾

All *Psiadia* species screened in this study have been shown to contain terpenes. However the fact that *P. lithospermifolia* shows better antibacterial activity than the positive control represents interesting potential in further applications. The mechanism of action of terpenes is still not fully elucidated but is speculated to involve membrane disruption by lipophilic compounds.²⁵⁾ Another feature that is worth pointing out, as far as essential oil is concerned is that there may be synergistic effects operating at the level of the oil and which may give a different antimicrobial profile should individual molecules be tested.

Furthermore, it must be highlighted that the oil of *P. arguta* showed moderate pharmacological activity against the tested bacteria. This may be explained partly by the absence of or near insignificant content of sesquiterpenes with established antimicrobial profile such as camphene, limonene, pinene or curcumenone.

Alternatively, the fungi toxic action of the oil of *P. arguta* against *Aspergillus ochraceus*, *Candida pseudotropicalis*, and *Fusarium moniliforme*, is probably due to the presence of isoeugenol, eugenol, being known to be mycotoxic especially against *Aspergillus* species.^{26—28)}

These results on the essential oil composition and antimicrobial properties of *Psiadia* species constitute a step towards the validation of their use in the Mauritian traditional medicine.

REFERENCES

- 1) Deans S. G., Ritchie G. A., *Int. J. Food Microbiol.*, **5**, 165—180 (1987).
- 2) Janssen A. M., Scheffer J. J. C., Baerheim Svendean A., *Planta Medica*, **53**, 395—398 (1987).
- 3) Ross S. A., El-Keltawi N. E., Megalla S. E., *Fitoterapia*, **52**, 201—205 (1981).
- 4) Penna C. A., Radice M., Gutkind G. O., Van-Baren C., Brousalis A., Muchietti L., Martino V., Ferraro G., *Fitoterapia*, **65**, 172—174 (1994).
- 5) Tereschuk M. L., Riera M. V. Q., Castro G. D., Abdala L. R., *J. Ethnopharm.*, **56**, 227—232 (1997).
- 6) Kokwaro J. O., "Medicinal Plants of East Africa," East African Publishing Bureau, Nairobi, 1976, p. 69.
- 7) Al-Yahya M. A., *Fitoterapia*, **57**, 179—182 (1986).
- 8) Daruty C., "Plantes Médicinales de l'Ile Maurice et des Pays Intertropicaux," 2nd ed., Mauritius Stationary and Printing, Mauritius, 1911.
- 9) Aboud-Zaid M. N., El-Karemy Z., El-Negouny S. I., Altosaar I., Saleh N. A. M., *Bull. Chem. Soc. Ethiop.*, **5**, 37—40 (1991).
- 10) Gurib-Fakim A., Gueho J., Bissoondoyal M. D., "Plantes Médicinales de Maurice," Tome 1, Editions de L'Océan Indien, Maurice, 1995.
- 11) Mekki A. G., Mossa J. S., Hifnawy M. S., Karawya M. S., *Pharmazie*, **39**, 419—420 (1984).
- 12) Dennis R., *Phytochemistry*, **12**, 2705—2708 (1973).
- 13) Ramanoelina P. A. R., Rasoarahona J. R. E., *J. Essent. Oil Res.*, **6**, 565—570 (1994).
- 14) Gurib-Fakim A., Smadja A., Gauvin A., Govinden-Soulange J., Kodja H., *J. Essent. Oil Res.*, **12**, 533—535 (1995).
- 15) Gurib-Fakim A., Smadja A., Gauvin A., Govinden-Soulange J., Kodja H., *J. Essent. Oil Res.*, **12**, 556—558 (2000).
- 16) Denny E. F. K., *Perfumer Flavorist*, **14**, 57—63 (1989).
- 17) Kondjoyan N., Berdague J. L., "A Compilation of Relative Retention Indices for the Analysis of Aromatic Compounds," 1st ed., Editions du Laboratoire Flaveur, France, 1996.
- 18) Davies N. W., *J. Chromatogr.*, **503**, 1—24 (1990).
- 19) "The Sadtler Standard Gas Chromatography Retention Index Library," Sadtler Research Laboratories, Philadelphia, U.S.A., 1982.
- 20) Mc Lafferty F. W., Stauffer D. B., "The Wiley/NBS Registry of Mass Spectra," J. Wiley and Sons, New York, 1989.
- 21) Bennett J. V., Brodie J. L., Benner J. L., Kirby W. M. M., *Appl. Microbiol.*, **14**, 2170—2177 (1966).
- 22) Collins C. H., Lyne P. M., Grange J. M., "Microbiological Methods," Butterworths & Co. Ltd., London, 1989, p. 410.
- 23) Bradshaw L. J., "Laboratory Microbiology," 4th ed., Saunders College Publishing, U.S.A., 1992, p. 436.
- 24) Ramanoelina P. A. R., Terrom G. P., Bianchini J. P., Coulanges P., *Arch. Inst. Pasteur, Madagascar*, **53**, 217—226 (1987).
- 25) Cowan M. M., *Cli. Microbiol. Rev.*, **12**, 1—30 (1999).
- 26) Hitokoto M., Morozumi S., Wauke T., Sakai S., Kurata H., *Appl. Environ. Microbiol.*, **39**, 812—822 (1980).
- 27) Reiss J., *Getreide Mehl Brot.*, **36**, 50—53 (1982).
- 28) Bullerman L. B., Lieu F. Y., Seier S. A., *J. Food Sci.*, **42**, 1107—1109 (1977).