Characterization of volatile compounds from the gum-resin of Mangiferaindica L. trunk bark using HS-SPME-GC/MS.

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Abstract:
Mangifera indica L. is a tree that exudes a gum-resin which is highly valued in traditional medicine of several central African countries. Gum-resin samples of M. indica L. were collected in Brazzaville (Congo), in the same area and their volatile compounds were extracted by headspace solid phase microextraction (HS-SPME). The chemical composition of the extracts obtained by gas chromatography-mass spectrometry (GC/MS) showed a predominance of sesquiterpenes. The major constituents were: β-élemene (6.7- 65.7%), β-caryophyllene (8.7-14.6%), α-humulene (3.1-5.3%), β-chamigrene (0.7-4.6%), β-selinene (7.7-31.4%) and α-selinene (9.0-39.4%) with quantitative differences from sample to another. This variability was discussed. Chemical composition of the volatile components of gum-resin of M. indica L. was compared with those of leaves and fruits which have been reported in the literature. The results showed significant differences.

Key words: gum-resin; *Mangiferaindica*; exudate; headspace solid phase microextraction; gas chromatography/mass spectrometry

Caractérisation des composés volatils de la gomme-résine d'écorce de tronc de *Mangiferaindica* L. par HS-SPME/GC/MS

Résumé:
Mangifera indica L. est un arbre qui exsude une gomme-résine, très utilisée dans la médecine traditionnelle de plusieurs pays d'Afrique centrale. Des échantillons de gomme-résine de *M. indica* L. ont été collectés à Brazzaville (Congo), dans une même zone géographique et leurs composés volatiles ont été extraits par microextraction en phase solide de l'espace de tête (HS-SPME). La composition chimique des extraits obtenue par chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC/MS) a montré une prédominance de sesquiterpènes avec des différences quantitatives d'un échantillon à l'autre. Les constituants majoritaires de ces échantillons sont le β-élémène (6,7- 65,7%), le β-caryophyllène (8,7-14,6%), l'α-humulène (3,1-5,3%), le β-chamigrène (0,7-4,6%), le β-sélinène (7,7-31,4%) et l'α-sélinène (9,0-39,4%). La composition chimique des composants volatils de la gomme-résine de *M. indica* L. a été comparée à celles des feuilles et des fruits, qui ont été rapportés dans la littérature. Ces résultats ont montré des différences significatives.

Mots clés: gomme-résine; *Mangiferaindica*; exsudat; microextraction en phase solide de l'espace de tête; sesquiterpènes; chromatographie en phase gazeuse/spectrométrie de masse

Introduction

Mangifera indica L. is well known as a source of the most important tropical fruits in the world, with the status of "king of fruits" as a result of its unique flavour, fragrance and appearance. The *M. indica*L. species, belonging to the *Mangifera genus*, Anacardiaceae family and Sapindales order, is a large evergreen fruit tree from 10 to 45 meters of height. Native to Southeast Asia, this species is widely found in many tropical and subtropical countries, including the Republic of Congo. The trunk bark of this tree gives a milky liquid that oozes and freezes slowly, giving a reddish gum-resin. Various parts of *M. indica* L. are used in traditional medicine to treat several diseases (Sulaiman et al., 2012). In some of the Caribbean Islands, the leaf decoction is taken as a cure for diarrhea, hemorrhages and bleeding hemorrhoids, fever, chest complaints, diabetes, hypertension and other illnesses (Núñez et al., 2002; Abu Dakar et al., 2009). In Nigeria the plant is used to treat ailments such as asthma and malaria which is one of the most important tropical diseases (Ene et al., 2010). In Congo, the Bark decoction is believed to cure cancerous sores, gingivitis, diarrhea and dysentery (Boullard, 2001). The gum-resin of *M.indica* L. has been used traditionally not only to treat scabies (Abu Dakar et al., 2009) but also as sudorific and against syphilis (Raponda, 1961). Moreover, it was demonstrated recently that this exudates can be used as a drug release retardant in the formulation of sustained release dosage forms (Singala et al., 2010). Several investigations on the phytochemical composition of differentorgansof

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M. indica L. were carried out, given their medicinal applications (Scartezzini and Speroni 2000; Masibo et al., 2009; Dzamic et al., 2010; Garrido et al., 2010). Essential oils from leaves and fruits were also extensively investigated (Dzamic et al., 2010; Wang et al., 2010; Gebara et al., 2011; Pino et al., 2006; 2012). However, to the best of our knowledge, the chemical composition of volatile compounds from the exudate of *M. indica* L. tree has not yet been investigated.From a health care point of view, the evaluation of the exudate composition could provide information on the potential health benefits of this gum-resin. This work aims therefore to establish the volatile profile of *M. indica* L. exudate and to compare its composition to the volatile composition of other organs of the tree. In this work the volatile fractions of *M. Indica* L. exudate samples were analyzed by means of headspace Solid-phase microextraction (HS-SPME) coupled with gas chromatography/mass spectrometry (GC/MS). HS-SPME has been widely used successfully in the characterization of a wide range of resins and gum-resins (Hamm et al., 2003). This method is efficient and easy to implement, allowing for the simultaneous extraction of compounds within a wide range of volatility and polarity.

Materials and methods

Plant materials: The exudate samples of *M. indica* L. were collected from the trees after natural exudation. Gum-resins samples were collected on six trees located in the Faculty of Sciences of Bacongo, Brazzaville (Congo), in May 2010. The plants were authenticated by Jean Marie Moutsambote, Assistant Professor in the Department of Forestry Techniques (University MarienNgouabi, Congo). A voucher specimen of the plant (collection n° 286) was deposited at the Herbarium of this Center.

Headspace solid-phase microextraction: The extraction procedure was inspired by a previous work (Hamm et al., 2003). The volatile compounds were extracted in headspace mode by a DVB/CAR/PDMS (50/30 µm) fiber supplied by Supelco (Bellefonte, PA, USA). Before each extraction, the fiber was conditioned at 250 °C during 30 min. Extractions were performed on 3.2 mg of exudate samples from *M. indica* L. placed in 2 ml glass vials. The vial was placed in an oil bath at 80 °C for 30 min.

Gas Chromatography/Mass Spectrometry analysis: A Hewlett-Packard 6890 gas chromatograph system, coupled with a HP MD 5973 quadrupole mass spectrometer was used. The chromatograph was equipped with a column RTX-5ms (5% phenyl, 95 % polydimethyl siloxane, 30 m length x 0.25 mm i.d., 0.25 µm film thickness). The carrier gas was helium (constant pressure, 70 kPa; initial flow, 1.2 ml/min). Volatiles obtained by HS-SPME technique were injected at 250 °C using splitless mode. The oven temperature program was from 60 °C to 240 °C, increasing at 3 °C/min. The mass spectrometer source was at 150 ºC. Mass spectra were acquired in the full scan mode from m/z 29 to m/z 300 at 2 scans per second. Ionization energy was 70 eV.**Identification of Components**: The compounds were identified by comparison of their mass spectra with those of standards available in the commercial NIST'05 database. The identification of most of the molecules was confirmed by comparison of their experimental retention indices (RI) with those provided by Adams' book (Adams, 2001). The retention times of n-alkanes to calculate retention indices were obtained with a commercial mixture of C8-C20 in n-hexane. Relative amounts of individual components were calculated on the basis of their GC peak areas.

Results and discussion

Volatile compounds identified in exudate samples of *M. indica* L.: The Kovats retention indices and the main EIMS fragments of volatile compounds of gum-resin samples from trunk bark of *M. indica* L. are presented in table 1. According samples, 16 to 23 main compounds were detected in GC-MS. All compounds detected in the volatile fraction of the gum-resin samples of *M. indica* L. are terpenes and most of them are non-oxygenated sesquiterpenes. Actually, use of DVB/CAR/PDMS fiber allows the simultaneous extraction of compounds within a wide range of volatility and polarity if they are present in the sample. The volatiles extracted from the six gum-resins samples headspaces showed a similar qualitative composition. Comparison of their chromatographic profiles showed that gum-resins samples from *M. indica* L. were characterized by the dominance of hydrocarbon sesquiterpenes with predominance of β-elemene, β-caryophyllene, α-humulene, β-chamigrene, α-selinene and β-selinene. This is due to the fact that these samples were collected from trees growing in the same geographical area.

Table 1: Compounds identified in the volatile fraction of the of gum-resin exudate from trunk bark of *M. Indica* L.

N°	Compound	EIMS Fragmentation	RI Calc
1	α-pinene	53; 67; 77; 91; **93** ; 105; 121; **136**	934
2	β-pinene	53; 69; 79; **93**; 107; 121; **136**	980
3	α-phellandrene	65;77;**93**;119;**136**	1007
4	3-carene	53 ; 68 ; 77 ; **93** ; 107 ; 121 ; **136**	1015
5	p-cymene	51 ; 65 ; 77 ; 91 ; 103 ; **119** ; **134**	1025
6	1,8-cineole	55 ; 71 ; 81 ; 93 ; 108 ; 125 ; 139 ; **154**	1031
7	β-elemeneisomer	53 ; 67 ; 81 ; **93** ; 107; 121; 133; 147; 161; 189; **204**	1387
8	β-elemene	53 ; 67 ; 81 ; **93** ; 107; 121; 133; 147; 161; 189; **204**	1396
9	α-gurjunene	55 ; 69 ; 77 ; 91 ; 105 ; 119 ; 147 ; 161 ; 189 ; **204**	1413
10	β-caryophyllene	69 ; 79 ; **93** ; 105 ; 121 ; 133 ; 147 ; 161 ; 189 ; **204**	1423
11	γ-elemene	67 ; 79 ; **93** ; 107 ; **121** ; 133 ; 147 ; 161 ; 189 ; **204**	1429
12	allo aromadendrene	55 ; 67 ; 79 ; 91 ; 105 ; 119 ; 133 ; 147 ; **161**; 189 ; **204**	1454
13	α-humulene	55 ; 67 ; 79 ; **93** ; 107 ; 122 ; 161; **204**	1457
14	drima-7,9(11)-diene	55 ; 67 ; 79 ; 93 ; **105** ; 121 ; 133 ; 161; **204**	1472
15	β-chamigrene	55 ; 67 ; 79 ; 93 ; 105 ; 119 ; 133 ; 161; **189** ; **204**	1479
16	β-selinene	55 ; 67 ; 79 ; 91 ; **105** ; 119 ; 133 ; 147 ; 161; 189 ; **204**	1489
17	α-selinene	55 ; 67 ; 79 ; 91 ; 107 ; 119 ; 133 ; 147 ; 161; 175 ; **189** ; **204**	1498
18	germacrene A	53 ; **69** ; 81 ; 93; 107 ; 119 ; 133 ; 147 ; 189 ; **204**	1510
19	sesquiterpene NI	53 ; 67 ; 79 ; **93** ; 107; 121; 133; 147; 161; 175; 189; **204**	1515
20	7-epi-α-selinene	55 ; 67 ; 81 ; 93 ; 107 ; 122 ; **161**; 189 ; **204**	1520
21	sesquiterpene NI	55 ; 67 ; **81** ; 93 ; 105; 121; 133; 147; 161; 175 ; 189; **204**	1532
22	caryophylleneoxide	69 ; 79 ; 93 ;109 ; 121 ;135 ;149 ;161 ; 177 ; (**220**)	1584
23	selin-11-en-4-alpha-ol	55 ; 71 ; **81** ; 93 ; 109 ; 135 ; 161 ; 189 ; 204 ; (**222**)	1662

RIcalc: Kovats retention indices determined by GC–MS analysis on non-polar RTX-5 MS column.
- Base peak in bold. - Molecular mass in bold and underlined. - Molecular absent on the spectrum between parenthesis, in bold and underlined.

However, no volatile compounds (esters, aldehydes, acids, ketones, lactones, and phenols) reported by some authors (Pino et al., 2006; 2012; Torres et al., 2007) in different extract of *M.indica* L. were found in the gum-resin. Besides, compositions of saps (latex) flowing from seven varieties of Indian mangoes after detachment of the pedicel from the mature fruit reported by Saby John (1999) were consistent with those described for fruits from *M. indica* L. although they were very different from the volatile composition obtained for gum-resin exudate from the trunk of the *Mangiferaindica* L. tree.

Proportion of volatiles in gum-resin samples of *M. indica* L.: Each gum-resin sample from M. Indica L. was extracted and analyzed in triplicate, at least. By way of illustration, the table 2 shows relative composition (%) in volatile compounds for three extractions from exudate sample n°1 as well as the average value and its relative standard deviation (RSD). Sixteen volatile components were identified inthe headspace from this sample n°1. The main constituents were α-selinene (39.1 %) and β-selinene (31.3 %), then β-caryophyllene (9.8 %), β-elemene (6.7 %), β-chamigrene (4.5 %) and α-humulene (3.4 %). Relative standard deviations for these values were up to 0.07 %, except for the main compound where it reaches 0.33 %. Relative standard deviations obtained showed a good repeatability for analysis of this sample.

We focused on these six major compounds and we reported the values of their relative proportion in samples from n°1 to n°6 in Table 3. The results show a good repeatability on measurement of each compound in each sample but there is great variability in the composition of the different samples and, depending on the sample, the major compound is not the same. For samples n°1 to 3, α-selinene is the main constituent with a percentage between 34 % and 40%. For samples n°4 to 6, β-elemene is the main constituent with a percentage between 34 % and 65 %. Samples were collected from n°1 to 6 on *M. indica* L. trees of the same cultivar in the same geographical zone. We observe only a decrease in β-elemene content but not a global decrease in respect of the most volatile compounds in samples n°6 to n°1.

Table 2 : Gum-resin exudate sample n°1 from *MangiferaIndica* L.: volatile relative content determined by HS-SPME GC/MS.

N°	compound	Relative content (%)			Average %	RSD
		1st extraction	2nd extraction	3rd extraction		
1	α-pinene	0.46	0.40	0.41	0.42	0.03
2	β-pinene	0.35	0.31	0.31	0.32	0.02
3	α-phellandrene	0.25	0.22	0.25	0.24	0.02
5	p-cymene	0.85	0.78	0.75	0.79	0.05
6	1.8-cineol	0.17	0.15	0.17	0.16	0.01
7	β-elemeneisomer	1.11	1.00	1.07	1.06	0.06
8	β-elemene	6.75	6.74	6.67	6.72	0.04
9	α-gurjunene	0.55	0.55	0.54	0.55	0.01
10	β-caryophyllene	9.89	9.77	9.78	9.81	0.07
13	α-humulene	3.45	3.45	3.42	3.44	0.02
14	drima-7.9(11)-diene	0.85	0.86	0.84	0.85	0.01
15	β-chamigrene	4.52	4.54	4.49	4.52	0.03
16	β-selinene	31.38	31.30	31.27	31.32	0.06
17	α-selinene	38.68	39.19	39.30	39.06	0.33
20	7-epi-α-selinene	0.74	0.74	0.73	0.74	0.01
22	caryophylleneoxide	0.46	0.40	0.41	0.42	0.03

Table 3: Percentage of predominant volatiles for the six samples of gum-resins from *MangiferaIndica* L. (average value ± RSD).

		Sample n°					
N°	**Compound**	**1**	**2**	**3**	**4**	**5**	**6**
8	β-elemene	6.72 ±0.04	10.89 ±0.58	11.90 ±0.21	34.53 ±0.43	44.49 ±0.97	64.82 ±0.88
10	β-caryophyllene	9.81 ±0.07	11.15 ±0.50	11.57 ±0.36	14.52 ±0.15	9.26 ±0.52	9.18 ±0.26
13	α-humulene	3.44 ±0.02	4.00 ±0.08	4.14 ±0.06	5.25 ±0.03	3.71 ±0.01	3.24 ±0.09
15	β-chamigrene	4.52 ±0.03	4.30 ±0.01	4.04 ±0.01	1.75 ±0.04	1.51 ±0.04	0.75 ±0.01
16	β-selinene	31.32 ±0.06	30.63 ±0.45	29.05 ±0.19	17.36 ±0.01	17.39 ±0.58	7.76 ±0.08
17	α-selinene	39.06 ±0.33	37.69 ±0.74	36.65 ±0.42	19.89 ±0.03	18.98 ±0.80	9.05 ±0.08

The decrease of β-elemene cannot be due only to partial evaporation and loss of samples. Analyses of samples two years later corroborate a good preservation of samples. Moreover, increase of β-elemene in samples n°1 to n°6 comes with a proportional decrease in α-selinene, β-elemene and β-chamigrene relative content. Discussions with international researchers led us to conclude that the variability in chemical composition of the exudate samples may be due to a chemical transformation of some gum-resin exudates components in specific thermodynamically conditions.

The results demonstrate that even using an accurate method, there is a significant variability in volatile chemical composition of exudates from *M. indica* L. The main components of the exudate samples were β-elemene, α-selinene, β-selinene, β-caryophyllene, α-humulene and β-chamigrene. But, the predominent constituent of the extracts depends of the freshness of the exudate sample. From a health care point of view, the presence of up to 64 % β-elemene in samples of *M. indica* L. could provide health benefits. Indeed, experiments performed in vitro show that β-elemene has anti-proliferative effects against some cancer cell types.

Comparison of volatiles composition of M. indica L. samples: The study of volatile compounds of gum-resin exudate from *M. indica* L. extracted using headspace solid-phase microextraction allowed us to determine an average chemical composition of these exudates (Table 4). Like Andrade (2000), Pino (2005, 2006), Torres (2007) and Liu (2013) we found that terpene hydrocarbons are the major volatiles of *Mangifera indica* organs.

Table 4. Comparative composition in volatile compounds of gum-resins exudates, leaves and fruits from *Mangiferaindica* L. t = traces of compound not quantifiable. Data for leaves and fruits originate from Gebara S.S. et al (2011)

Substances	Relative composition (%)								
	Exudate fromtrunk	Leaf				Fruit			
		Immature		Mature		Immature		Mature	
	SPME	SPME	HD	SPME	HD	SPME	HD	SPME	HD
α-pinene	**t - 0.5**	–	–	–	–	–	–	–	–
β-pinene	**t - 0.4**	0.2	0.2	0.1	–	–	–	–	–
α-phellandrene	**t - 0.2**	0.8	t	0.3	0.2	0.3	–	2.0	–
3-carene	**t - 0.2**	–	2.0	–	–	2.3	5.5	1.2	–
p-cymene	**t - 1.3**	–	–	–	–	–	–	–	–
1,8-cineole	**t - 1.2**	–	–	–	–	–	–	–	–
β-elemeneisomer	**0.3 - 2.1**	–	–	–	–	–	–	–	–
β-elemene	**6.7 - 65.7**	0.6	0.8	0.9	0.8	0.2	–	0.1	0.3
α-gurjunene	**0.1 - 0.6**	1.0	1.0	–	24.0	6.1	10.2	11.3	11.5
β-caryophyllene	**8.7 - 14.6**	14.34	16.4	0.1	25.5	8.5	8.9	16.0	26.6
γ-elemene	**t - 0.1**	–	–	0.1	–	–	–	–	–
allo aromadendrene	**t - 0.2**	–	–	–	–	–	–	–	–
α-humulene	**3.1 - 5.3**	5.4	10.4	2.4	15.6	4.2	6.2	–	19.6
drima-7,9(11)-diene	**0.3 - 0.9**	–	–	–	–	–	–	–	–
β-chamigrene	**0.7 - 4.6**	–	–	–	–	–	–	–	–
β-selinene	**7.7 - 31.4**	T	0.3	–	–	–	–	–	–
α-selinene	**9.0 - 39.4**	–	–	–	1.24	–	–	–	0.6
germacrene A	**t - 2.4**	–	–	–	–	–	–	–	–
sesquiterpene NI	**t - 4.6**	–	–	–	–	–	–	–	–
7-epi-α-selinene	**0.3 - 0.9**	–	–	–	–	–	–	–	–
sesquiterpene NI	**t - 0.2**	–	–	–	–	–	–	–	–
caryophylleneoxide	**t - 0.3**	–	4.5	–	6.0	–	7.9	–	3.3
selin-11-en-4-alpha-ol	**t - 0.4**	–	–	–	–	–	–	–	–
Monoterpenes	**< 1.3**	28.2	7.2	10.9	1.3	78.2	17.6	65.2	–
Oxygenatedmonoterpenes	**< 1.2**	0.1	3.6	–	1.1	0.2	23.9	–	–
Sesquiterpenes	**> 96.8**	60.3	76.8	74.2	80.2	20.2	28.5	29.2	63.2
Oxygenatedsesquiterpenes	**< 0.7**	0.1	8.5	0.2	11.7	–	11.1	–	5.4

After analysis of volatile flavor compounds from mangoes, theseauthors reported that the dominant terpenes of mango were α-terpinolene, limonene, myrcene, α-phellandrene and δ-3-carene depending on different cultivars. Among these monoterpenes, only α-phellandrene and 3-carene were found to be minors in our exudate samples. α-pinene, p-cymene, α-gurjunene, β-caryophyllene, 7-epi-α-selinene and α-selinene were also identified in both pulp fruit and exudate samples from *Mangifera indica* L. No β-elemene was found in fruits and α-selinene was found only in Jin Hwang mango as reported by Liu (2013). Gebara (2011) studied volatiles from mature and immature fruits and leaves of *M. indica* var. coquinho. The major compounds found in fruit of *M. indica* var. coquinho were terpinolene using HS-SPME and p-cymen-8-ol, α-gurjunene, α-humulene, β-caryophyllene and hexadecanol analyzing essential oil. The predominant compound reported was cyperene in the immature leaf and in the mature leaf using HS-SPME. From a general point of view, this study showed a predominance of monoterpenes in fruits when volatiles were extracted using HS-SPME, while there was a predominance of sesquiterpenes in leaves regardless of the mode of extraction (Table 4). Comparing our results with those obtained by Gebara (2011), eleven volatile compounds were found in common : β-pinene, α-phellandrene, 3-carene, β-elemene, α-gurjunene, β-caryophyllene, α-humulene, α-selinene, β-selinene and caryophyllene oxide. Among these compounds β-elemene and α/β-selinene were found only in small amounts while β-caryophyllene, α-humulene and α-gurjunene were found in significant percentages, particularly using hydrodistillation.

Nevertheless, we are surprised by the low values obtained by Gebara (2011) for β-caryophyllene in mature leaf and α-humulene in mature fruit using SPME. From one party to the other of the shaft, the volatile chemical composition varies. Different volatiles may make different contribution to *M. indica* L. organs aroma; Pino& Mesa (2006) reported that 3-carene has a sweet odor and β-caryophyllene makes a minor contribution to mango. Moreover, we can note the absence of monoterpene responsible for the aroma of different varieties of mango, such as cis-ocimene, myrcene and limonene. This could justify the weak smell of the exudate samples from *M. indica* L. where elemene and selinene may contribute to the floral and herbal aromas, respectively. Several studies have reported the biological activities of some terpenes. For example, β-caryophyllene has been reported to possess antibacterial activity (Oztürk et al., 2009), while β-elemene has been shown to have antiproliferative effects, effectively suppressing tumor cells survival (Li et al., 2013). These compounds are present in significant amounts in our gum-resin samples of *M. indica* L. It may be interesting to evaluate the antibacterial activity of different extracts of these gum-resins.

Conclusion

Our characterization showed that the extracts from the gum-resin samples consist essentially of hydrocarbon sesquiterpenes. This result is consistent with chemical compositions of *M. indica* L. leaf extracts although it is very different from chemical compositions of *M. indica* L. fruits and sap of fruit extracts reported in literature. The main components of the exudate samples were β-elemene, α-selinene, β-selinene, β-caryophyllene, α-humulene and β-chamigrene but depending to the freshness of the exudate sample β-elemene or α-selinene was the prominent constituent of the extracts. The results obtained demonstrate that even using an accurate method, there is a significant variability in volatile chemical composition of gum-resin exudate from *M. indica* L. Moreover, there is a significant variability in qualitative and quantitative compositions in volatile compounds from the different organs of *M. indica* L.

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