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Full Length Research Paper

Comparative study of the chemical composition of the essential oils from organs of *Annona senegalensis* Pers. *oulotricha* le Thomas subspecies (Annonaceae)

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The chemical composition of the essential oils from leaves, stems bark, roots bark, epicarp and mesocarp of *Annona senegalensis* Pers., *oulotricha* Le Thomas subspecies (Annonaceae), growing in Brazzaville (Congo), were analyzed by CG and CG-MS. These oils essentially contain sesquiterpenic compounds (58.3 - 97.7%), dominated by oxygenated sesquiterpenes (21.8 - 88.3%), with elemol (13.2 - 35.0%), β and γ-eudesmols (3.7 - 58.3%) as characteristic components. The essential oils from roots and stems bark is distinguished by its high content in diterpenes (17.1 and 11.9% of the total), while the seeds presents a significant amounts of monoterpene hydrocarbons (25.8%) with α-pinene (6.2%) and β-phellandrene (11.5%) as major components accompanied by two oxygenated monoterpenes : bornyle acetate (4.5%) and smallest of 1,8-cineole. However, in the epicarp oil, the presence of about 5.8% of oxygenated monoterpenes as terpinen-4-ol and bornyle acetate in comparable rates (1.7%) was noted. The mesocarp oil is exclusively rich in aliphatic fatty acids (35.8%) which is absent in the other organs, but represented by lauric acid (18.0%), hexadecanoïc acid (8.6%), myristic acid (7.2%) and oleic acid (2.0%). Results were compared with same species collected in the democratic republic of Congo and in Cameroon essentially dominated by monoterpenes (84.2 and 87.6%).

Key words: *Annona senegalensis, Oulotricha* subspecies, organs, essential oil, chemical composition, sesquiterpenes.

INTRODUCTION

Annona senegalensis Pers. species belongs to the Annonaceae family. It is one of the natural and homogen vegetable families with essential oil cells (Le Thomas, 1969). It is found in the tropics and grow more often under low altitude (Lebrun and Stork, 1991). The Annonaceae's family contains about 120 genera. The *Annona* genus, originated from Central America and is constituted by 110 species. Several species of this family are used for the nutritive interest of their fruits (Eyog et al., 2006) or for their medicinal importance in traditional medicine. The *A. senegalensis* Pers., subspecies *oulotricha* Le Thomas, found in the African savanna, is limited in the dense and humid forest, particularly in Congo. It is a plant whose leaves, barks and roots are perfumed. Its ripe fruit which are orange yellow coloured, frozen, sugared and edible, also contains perfumed seeds (Dupont and Guignard, 2007). *A. senegalensis*

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Table 1. Preparation and color of the essential oil of *A. senegalensis* Pers., *oulotricha* Le Thomas subspecies.

Organs	Weight (g) of material	Essential oil weight (g)	Yield (%)	Color
Leaves	200	1.2	0.6	Pale yellow
Stems-bark	200	0.4 (*)	0.2	uncolor
Roots-bark	150	1.5 (*)	1.0	uncolor
Epicarp of fruit	100	0.1	0.1	Orange yellow
Seeds of fruit	300	0.6 (*)	0.2	uncolor
Mesocarp of fruit	100	0.1	0.1	Orange yellow

*Crystallized at ordinary temperature.

species is used in Central and West African folk medicine as anthelmintic (Nwude et al., 1980; Ibrahim et al., 1983; Alawa et al., 2003; Fall et al., 2003), andiarrheic, anticonvulsivant, antibacterial, antifungal, antitussif, anti-flammatory, antipyretic, as well as for cancer and leukemia treatments (Kanta, 1999; Noumi and Fozi, 2003; Abubakar et al., 2007; Magassouba et al., 2007). The association of the leaves of *A. senegalensis*, *Aframomum latifolium, Andropogon gayanus, Anthocleista* sp., *Desmodium adsendens, Gardenia ternifolia, Harungana madagascariensis, Imperata cylindrica, Achomanes difformis, Maytenus senegalensis, Pseudarthria hookeri, Merremia* sp., *Mostuea brunonis* and *Olax subcarpioidea,* are also used for malaria treatment (Haxaire, 1979). Traditionally, the plant is used in Congo against diarrhea, epileptics and other infections (Bouquet, 1969).

Several works have being carried out on the essential oils of species from DR of Congo and Cameroon. In the case of *Annona,* Xylopia, *Monodora*, *Cananga* species, *Annona* species from DR Congo showed the presence of majority of compounds such as sabinene (39.9%), α and β-pinene (5.1 and 9.2%), α-phellandrene (4.9%), limonene (5.8%) and germacrene D (9.0%) in the *Annona acuneta* barks; and α-phellandrene (25.0%), limonene (4.8%), α-pinene (8.3%), Z-sabinol (6.9%) and p-cymene (36.0%) in the *A. senegalensis* barks (Farid et al., 2002). Whereas, in the different organs of the species of *Xylopia aethiopica* (Dunal) A. Rich., the authors found common compounds such as terpinen-4-ol (23.4%), 1,8-cineole (16.3%), β-pinene and α-terpineol (11.1%) (Mohamed et al., 1979); sabinene (35.0%) (Masotti et al., 1997), β-pinene (36.2 - 40.2%); α-pinene (13.6 - 15.4%) and sabinene (7.2%) (Malo et al., 1994). The essential oils analysis obtained from five cameroonian species, showed that *Monodora myristica* (Gaertn.) and *Monodora brevipes* Benth. seeds, *A senegalensis var senegalensis and Cananga odorata* (Lam.) Hook., leaves and fruits are rich in monoterpenes of which the majority compounds are α-phellandrene, sabinene and myrcene. On the other hand, the essential oils of *M. brevipes* Benth., contained equal quantities of monoterpenes and sesquiterpenes of which the majority compounds are (Z)-β-ocimene and (E),(E) α-farnesene (Fekam et al., 1996). Several works have been carried out on *A. senegalensis* without indicating the studied subspecies (Eshiet, 1971; Kayode and Durodola, 1976; Fatope et al., 1996).

In an attempt to obtain the best knowledge and judicious use of *A. senegalensis* Pers. *oulotricha* Le Thomas species, the present work proposes to identify the chemical constituents of the essential oils from the different organs (leaves, barks, roots, epicarp, mesocarp and seeds) collected in Congo Brazzaville.

MATERIALS AND METHODS

Plant material

The different samples of leaves, roots barks, stem barks, seeds, mesocarp and epicarp of the fruits of *A. senegalensis* Pers., studied, originating from Brazzaville (Congo), were collected in January 2009. Voucher specimens were identified at the Laboratory of Botany of the Centre des Resources Végétales (CERVE) and deposited at the National Herbarium of Brazzaville (IEC, Nkounkou n° 1, 1bis, 1ter).

Essential oils

The air-dried plant material were hydrodistillated for five hours using a Clevenger-type apparatus (Ouamba et al., 1990). The oils were obtained after decantation and drying over anhydrous sodium sulphate. The weights of vegetal material used, the amounts, extraction yields and colour of essential oils obtained are reported in Table 1.

Analysis

The quantitative analysis was carried out using a Hewlett-Pacard HP 5890 chromatograph equipped with flam ionization detectors. Separation were performed on silica capillary column (30 m x 0.25 mm x 0.25 µm) coated with DB-5, using the following experimental conditions: oven temperature 50°C (5 min) – 300°C (5 min) at 5°C/min; injection temperature 280°C; detector temperature 280°C, carried gas helium at flow rate of 1.0 ml/min. The qualitative analysis was carried out using a GC/MS Hewlett-Packard apparatus (model 6890/5973) equipped with the same capillary column using same experimental conditions.

Component identification was carried out by comparison with authentic reference compounds, spectrometric electronic library (Wiley), published mass spectra and retention indices (Jennings and Shibamoto, 1980; McLafferty, 1989; Davies, 1990; Kondojoyan and Berdague, 1996; Adams, 2001).

Table 2. Comparative study of the percentage composition of the essential oil from different organs of *Annona senegalensis* Pers, *oulotricha* Le Thomas subspecies.

RI	Compounds (*)	Leaves	Stems bark	Roots bark	Epicarp (fruit)	Seeds (fruits)	Mesocarp (fruit)
939	α-pinene	-	-	t	-	6.2	-
953	camphene	-	-	t	-	3.3	-
975	sabinene	-	-	-	-	0.5	-
979	β-pinene	-	-	-	-	1.3	-
991	myrcene	0.6	-	0.1	-	2.3	-
1003	α-phellandrene	-	-	-	-	0.3	-
1011	δ-3-carene	-	-	-	-	0.3	-
1026	p-cymene	0.1	-	-	-	0.3	-
1029	limonene	0.8	-	-	-	-	-
1030	β-phellandrene	-	-	0.4	-	11.9	t
1031	1.8 cineole	0.5	-	-	1.8	0.3	0.5
1097	linalool	-	-	-	0.8	-	1.9
1177	terpinen-4-ol	-	-	-	-	-	1.7
1189	α-terpineol	-	-	-	-	-	1.5
1200	dodecane	-	-	-	-	0.8	-
1289	bornyl acetate	-	-	-	-	4.5	t
1351	α-cubebene	-	-	0.1	-	-	-
1371	cyclosativene	-	-	0.3	-	0.6	2.3
1377	α-copaene	0.5	0.5	2.3	-	4.4	0.4
1389	β-elemene	2.2	0.4	-	0.8	4.6	1.0
1399	cyperene	-	-	-	-	3.5	-
1400	tetradecane	-	-	-	-	1.9	-
1419	E-β-caryophyllene	4.9	1.4	7.6	1.9	14.9	2.4
1434	α-bergamotene	-	-	0.6	-	-	-
1436	β-copaene	-	-	-	-	0.8	-
1437	γ-elemene	-	-	0.1	-	-	-
1455	α-humulene	1.1	0.4	1.7	0.9	2.4	0.5
1457	E-β-farnesene	-	0.5	-	-	1.1	-
1461	rotundene	-	-	-	-	0.4	-
1477	γ-muurolene	-	-	0.3	-	0.4	-
1485	germacrene D	1.3	-	0.3	0.6	3.0	-
1486	β-selinene	0.6	0.4	0.8	-	0.6	0.5
1489	E-β-Ionone	-	-	-	-	-	1.0
1498	α-selinene	0.6	0.6	1.5	-	-	-
1500	α-muurolene	-	-	0.2	-	-	-
1500	pentacane	-	-	-	-	2.0	-
1506	β-bisabolene	-	-	-	-	2.0	-
1509	germacrene A	0.5	-	0.3	0.4	1.4	-
1514	γ-cadinene	-	0.3	0.9	-	0.4	-
1523	β-sesquiphellandrene	-	-	-	-	0.6	-
1523	δ-cadinene	0.3	0.8	0.1	0.4	-	-
1533	E-γ-bisabolene	-	-	-	0.4	-	-
1550	elemol	29.0	25.4	34.1	35.0	17.8	13.2
1561	germacrene B	-	-	1.3	-	0.2	-
1564	E-nerolidol	-	-	0.1	0.5	-	-

Table 2. Continued.

1568	Lauric acid	-	-	-	-	-	18.0
1583	oxyde de β-caryophyllene	6.2	1.0	4.5	9.3	-	5.9
1585	globulol	0.4	-	0.4	-	-	-
1601	guaiol	0.7	0.4	0.5	5.8	-	-
1606	1,2-epoxyde d'humulene	0.6	-	0.7	3.0	-	-
1608	5-epi-7-epi-α-eudesma	-	-	-	1.0	-	-
1620	hedycaryol	-	-	-	1.0	-	-
1624	10-epi-γ-eudesma	-	-	0.5	1.8	-	0.5
1630	γ-eudesmol	13.8	11.7	3.8	11.3	1.2	9.8
1631	eremoligenol	-	-	-	1.0	-	-
1641	α-epi-muurolol	-	-	0.7	-	-	-
1642	hinesol	-	1.0	0.4	1.3	-	-
1651	β-eudesmol	34.5	42.1	14.0	18.3	2.5	19.9
1672	bulnesol	-	0.8	0.7	0.9	0.2	-
1714	14-hydroxy-α-humulene	0.5	-	0.4	0.8	-	0.5
1762	Myristic acid	-	-	-	-	-	7.2
1963	hexadecanoic acid	-	-	-	-	-	8.6
1974	dolabradiene	-	-	0.3	-	-	-
2017	epi-13-manoyl oxide	-	-	0.3	-	-	-
2034	kaurene	-	-	2.4	-	t	-
2133	oleic acid	-	-	-	-	-	2.0
2225	kauren-16-ol	-	4.6	5.2	-	0.5	-
2299	4-epi-abietal	-	-	8.2	-	-	-
2310	Isopimarol	-	2.4	-	-	0.2	-
2321	β-hydroxy-16-kaurene	-	4.7	2.7	0.7	0.2	0.5
2360	manool	-	0.2	-	-	-	-
2368	kaur-16-en-19-ol	-	-	0.6	-	-	-
2500	pentacosane	-	0.2	-	-	-	-
Total identified compounds		99,7	99.8	99.4	99.7	99.8	99.8

*Classed in elution order on DB5; RI = retention index; t = traces.

RESULTS AND DISCUSSION

The hydrodistillation of *A. senegalensis* gave a pale yellow or orange yellow essential oil with an average yield of 1.0% (for the stems-bark), 0.5% (for the leaves), 0.2% (for the roots-bark and the seeds) and 0.1% (for epicarp and mesocarp of the fruit).

Table 2 showed the results of the chromatographic analyses, allowing the identification of 78 products, representing more of 99% of the total oil. These analyses showed that *A. senegalensis* essential oils contained mostly sesquiterpenes (58.3 - 97.7%), mainly oxygenated sesquiterpenes (31.1 - 88.3%), dominated by two compounds : elemol (29.0% in the leaves ; 25.4 and 34.1% in the stems-bark and roots-bark; 35.0, 17.8 and 13.2%, respectively, in the epicarp, seeds and mesocarp of the fruits), β and γ-eudesmols (34.5 and 13.8% in the leaves; 42.1 and 11.7% in the stems-bark; 14.0 and 3.8% in the roots-bark; 18.3 and 11.3% in the epicarp; 19.9 and 9.8% in the mesocarp); the percentage of eudesmols is smaller of 4.0% in the seeds oil. All organs contained small amounts of sesquiterpene hydrocarbons (5.3 - 19.0%), with α-copaene (0.4 - 2.3%), E-β-caryophyllene (1.4 - 7.6%), α-humulene (0.5 - 1.7%) and α-selinene (0.4 - 0.8%) as characterized components. Some particularities were observed between these oils. Diterpenes were in large amounts exclusively in the roots and stem bark oil (17.1 and 11.9%) with kauren-16-ol and β-hydroxy-16-kaurène, respectively (5.2 and 4.6% or 2.7 and 4.7%). In the seeds, the presence of eight important monoterpene hydrocarbons (25.8%) characterized by β-pinene (6.2%), camphene (3.3%), sabinene (0.5%), myrcene (2.3%), α-phellandrene, δ-3-carene and p-cymene (0.3%) were found. In the epicarp, a small proportion of oxygenated monoterpenes (5.8%) as 1,8-cineole, linalool and terpineol and in the seeds, the presence of bornyle acetate (4.5%) accompanied by the trace of 1,8-cineole was noted. The mesocarp oil is exclusively rich in aliphatic fatty acids (35.8%) represented by lauric acid (18.0%), myristic acid (7.2%), hexadecanoïc acid (8.6%) and oleic acid (2.0%). The Congolese species is distinguished by its important content of sesquiterpenic compounds from the same species collected in Africa. The essential oil of *A. senegalensis* gathered in the democratic republic of

Congo and in Cameroon, obtained in 0.2 and 0.1% yield, are essentially dominated by monoterpenes (84.2 and 87.6%) with p-cymène as major compounds (36.0%) (Ekundayo and Oguntimein, 1986; Farid et al., 2002). The smallest proportion of the sesquiterpenes (8.2%), represented by β-caryophyllene and α-humulene in these two samples, clearly differentiate the congolese essential oils.

Several compounds (monoterpenes in particular) identified to different percentages in the essential oils of Annonacea' family species (*A. senegalensis, X. aethiopica, A. acuneta, M. myristica, M. brevipes* and *C. odorata*) such as α and β-pinenes, p-cymene, myrcene, limonene, linalool, germacrene D, α-phellandrene, terpinen-4-ol, 1,8-cineole, α-terpineol and sabinene have been identified too well as the loud rates, in the *A. senegalensis* per., *oulotricha* Le Thomas subspecies (Mohamed et al, 1979; Ekundayo and Oguntimein, 1986; Nianga et al., 1994; Masotti et al., 1997; Farid and al., 2002).

The presence of elemol, β and γ-eudesmols, with important rates in the congolese samples, showed that this compounds are characteristic of the *Oulotricha* sub-species. It was noted that elemol and eudesmols could be absent or present at louder proportions in the Annonaceae oils (Masotti et al., 1997; Farid et al., 2002). The presence of alcohol function compounds justified the antibacterial, antifungal and antioxidant activities of *A. senegalensis* Pers. *oulotricha* Le Thomas species (Baratta et al., 1998; Dorman and Deans, 2000).

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