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Full Length Research Paper

Chemical composition and seasonal variation of essential oil of *Sclerocarya birrea* (A. Rich.) Hochst subsp birrea leaves from Benin

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Essential oils from fresh leaves of *Sclerocarya birrea* (A. Rich.) Hochst. were extracted by steam distillation. The oil yield from plant collected during the hot season (February) was 0.10±0.02 and 0.24±0.01% from plant collected during the cold season (August). GC/FID and GC/MS analysis allowed us to identify a total of 49 compounds, representing 98% of the hydrodistillate. The oils contained about 96% sesquiterpenes among which 38±0.034% of 7-epi-α-selinene during the hot season and 51.7±0.12% of 7-epi-α- selinene during the cold. The main components of the oil from the hot period were 7-*epi*-α-selinene (38±0.03%), α-muurolene (25±0.03%), valencene (17±0.06%), β-selinene (4.3±0.01), β-caryophyllene (3.2±0.02) *allo*-aromadendrene-epoxide (1.5±0.03) and 14-hydrox-α-humulene (1.5±0.03). The essential oil from the cold season was characterized by 7-*epi*-α-selinene (51.7±0.12%), β-selinene (15.1±0.2%), valencene (12.9±0.05%), α-selinene (8.1±0.03) and β-caryophyllene (1.8±0.02%). This is the first report of these components in the essential oil of *Sclerocarya birrea*.

Key words: *Sclerocarya birrea* (A. Rich.), essential oils, seasonal variation, 7-*epi*-α-selinene, α-muurolene, valencene, β-selinene.

INTRODUCTION

Sclerocarya birrea (A. Rich.) Hochst. (Anacardiaceae) is a medium-sized to large deciduous tree with an erect trunk and rounded crown. It is one of the plants that played a role in feeding people in ancient times. It is widespread in Africa from Ethiopia in the north to KwaZulu-Natal in the south (Van Wyk et al., 1997). It is more dominant in the Baphalaborwa area in Limpopo in South Africa and in the woody vegetation of the Park W in Benin (Gouwakinnou et al., 2009). It occurs naturally in various types of woodland, on sandy soil or occasionally sandy loam. This tree grows easily from seed sown in washed river sand in spring. It can also grow from a truncheon planted in the early spring. It is fast-growing, with a growth rate of up to 1.5 m per year (Coates, 1983). In Southern African the plant fruit is edible, eaten either fresh or made into a delicious jelly. It also makes alcoholic beer known as Mukumbi by the Vhavenda people. This liqueur is available commercially (Venter and Venter, 1996). The white nut is highly nutritious and is eaten as it is or mixed with vegetables. Fruit-farming communities prefer planting a couple of these trees to attract pollinators to their farm in early spring. It is a dioecious fast-growing tree species in Benin. Flowering takes place in

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the dry season when the trees are leafless. The major pollinators (or flower visitors) of tree are honey bees (Chirwa and Akinnifesi, 2008). The tree bears plum-sized stone fruits with a thick yellow peel and translucent white flesh. They are eaten fresh and can be processed into things such as beverages, jams and jellies. The juice contains as much as four times the vitamin C of orange juice (National Research Council, 2008). In Benin, the species has a multitude of uses; all organs are used for more than 20 different purposes.

The kernels are eaten or the oil extracted; the leaves are browsed by livestock and have medicinal uses, as does the bark. The wood is carved into spoons, plates and decorative animal figures (Gouwakinnou, 2008; Gouwakinnou et al., 2011). The powdered bark is used to treat pregnant women to determine the gender of an unborn baby. If a pregnant woman wishes to have a girl, she will take a preparation from the female plant and for a boy she will use the male plant. Traditional healers use the hard nut in their divining dice (Mutshinyalo et al., 2003). A decoction of the bark treats dysentery, diarrhea and rheumatism and has a prophylactic effect against malaria. The bark is an excellent remedy for hemorrhoids. Roots and bark are also used as laxatives. A drink made from the plant leaves is used for the treatment of gonorrhea. Sometimes one finds a tree with a wound, probably caused by a traditional healer or someone who collected material for medicinal use (Gouwakinnou, 2008; Gouwakinnou et al., 2011). Previously, a quantitative study of the phenolic constituents of wild and cultivated leaves of *Sclerocarya birrea* (Anacardiaceae) was carried out by HPLC-UV/PDA and LC-MS. Phytochemical analysis of the methanol extract of wild plants led to the isolation of one flavonol glycoside, quercetin 3-O-α-L-(5”-galloyl)-arabinofuranoside, and eight known phenolic compounds; two epicatechin derivatives were also isolated from the same extract of the cultivated species.

The antioxidant activity of all isolated compounds was determined by measuring free radical scavenging effects using the Trolox equivalent antioxidant capacity assay and the coupled oxidation of β-carotene and linoleic acid (autoxidation assay) (Braca et al., 2003). The partial nutrient content of the edible part of the plant seed, a snack food eaten by children in rural Niger was also reported. The part contained relatively large amounts of copper (24.8 mg/g dry wt), magnesium (4210 mg/g dry wt), and zinc (62.4 mg/g dry wt). The protein content of the pit was high (36.4% of dry wt); however, the protein fraction contained relatively low proportions of leucine, phenylalanine, lysine, and threonine. Fatty acids accounted for 47 mg/g dry weight of the part, two-thirds of which was due to oleic acid. The essential fatty acid linoleic acid was present (24.5 mg/g dry wt), but the other essential fatty acid, α-linolenic acid, was absent (Glew et al., 2004). The tree inner bark extracts tended to be the most potent in antimicrobial activities; followed by outer bark and leaf extracts, but the differences were not statistically significant (Eloff, 2001). Stem bark ethanol extracts exhibited strong activity against *Candida albicans* (Adoum et al., 1997) and *Candida krusei* (Hamza et al., 2006; Samie et al., 2010). To our knowledge there are no literature reports to date concerning the volatile components of the leaves of *S. birrea* essential oils. Our main aim here was thus to study the chemical composition of essential oils extracted from fresh leaves of *S. birrea* of Benin, the variation of this chemical composition and extraction yields according to the season when the leaves were harvested.

MATERIALS AND METHODS

Plant material

Leaves of *S. birrea* were collected from the same place, in the morning, in the Botanical Garden of the Abomey-Calavi University. The fresh leaves were harvested in February 2009 (sample I), a period of very hot weather (35°C), and in August 2009 (sample II) (21°C), a colder period with occasional light rain. A voucher specimen (n°AA6384/HNB) of these leaves was conserved at the University of Abomey-Calavi Herbarium.

Essential oil isolation

Five hundred grams (500 g) of the fresh leaves were steam distilled for 3 h in an improved Clevenger-type apparatus (Clevenger, 1928; Bruneton, 1993). The extraction of each leaves (I and II) was carried out in triplicate. Each essential oil sample was dried over anhydrous sodium sulphate and preserved in sealed sample tubes and stored at 0°C until GC/FID and GC/MS analyses. The essential oil yields were calculated taking into account the fresh vegetable.

Essential oil analysis

The analysis of the essential oils was performed by GC/FID and GC/MS (AFNOR, 2000). *GC/FID*: The analysis was carried out on a FOCUS GC (Thermo Finigan; Milan, Italy) using the following operating conditions: capillary column, CP Wax 52 CB (15 m × 0.25 mm; film thickness: 0.25 µm) (J and W Scientific Column of Agilent Technologies, N° US167072Ã, USA); injection mode, splitless; injection volume, 1 µL (TBME solution); flow of split, 10 ml/min; splitless time, 0.80 min; injector temperature, 260°C; oven temperature programmed, 50 to 250°C at 6°C/min and held at 250°C for 5 min; carrier gas, helium with a constant flow of 1.2 ml/min; FID detector temperature, 260°C. The data were recorded and treated with the ChromCard software.

The quantification was completed by the calculation of the areas under curve of the peaks (GC/FID, by the process of normalization) and the identification of compounds by comparison of the retention indices with the references. *GC/MS*: with an aim of confirming the identifications obtained by the GC/FID method, GC-EIMS analysis were carried out on a TRACE GC 2000 series (Thermo-Quest, Rodano, Italy), equipped with an autosampler AS2000 Thermo-Quest. The GC system was interfaced to a Trace MS mass spectrometer (ThermoQuest) operating in the electronic impact mode. The same capillary column (CP Wax 52 CB) was used with the same conditions of injection, flow of helium and programming of the temperature of the oven as above. The coupling temperature

of the GC was 260°C. The energy of the electrons was 70 eV and the source of the electrons at 260°C. The data were recorded and analyzed with the Xcalibur 1.1 software (ThermoQuest). The mass spectra of the peaks were analyzed and compared with references and NIST/EPA/NIH database (1998, version 1.6).

Identification of oil constituents

Individual components of the volatile oils were identified by comparison of their relative retention times with those of authentic standard references, computer matching against commercial library (Sadler, 1986; Sandra, 1987; NIST, 1998; Adam, 2007) and home-made library mass spectra made from pure substances and components of known oils. Mass spectrometry literature data (Masada, 1976; Heneberg, 1995; Mclafferty, 1991) were also used for the identification, which was confirmed by comparison of the GC retention indices (RI) on a polar column (determined from the retention times of a series of *n*-alkanes "C8-C24" mixture). The Kovats indices (KI) calculated were in agreement with those reported by Adams (2007). A quantitative analysis of each oil component (expressed as percentages) was carried out by normalization measurement of peak area obtained by FID.

Chemicals

α-Pinene, β-pinene, camphene, p-cymene, myrcene, α-terpinene, γ-terpinene,1,8-cineol, terpinolene, borneol, citronellyl acetate, terpine-4-ol, α-terpineol, geraniol, verbenone, carvacrol, thymol, bornyl acetate, α-copaene, β-caryophyllene, fenchone, thujone, *trans*-pinocarveol, *trans*-verbenol, lavandulol, myrtenal, *trans*-carveol, carvone, aromadendrene, *allo*-aromadendrene, γ-gurjunene, *cis*-ocimene, camphor and *n*-alkanes "C8-C26" were obtained from Sigma-Aldrich chemie (Germany), Acros Organics (New jersey, USA), and Fluka Chemie (Switzerland); α-thujene, sabinene, δ-3-carene, limonene, linalool, α-humulene, *cis*-pinane, α-phellandrene, *p*-cymenene, myrtenyl acetate and valencene were purchased from Extrasynthese (Genay, France). All compounds were of analytical standard grade. Ter-Butyl methyl ether was an analytical grade solvent purchased from Fluka Chemie, and anhydrous Na_2SO_4 was of analytical reagent grade from UCB (Bruxelles, Belgium).

Statistical analysis

All data were expressed as mean±standard deviation of triplicate measurements. The confidence limit was set at $P<0.05$. Standard deviations did not exceed 5% for the majority of values obtained.

RESULTS AND DISCUSSION

The oils extracted from samples I and II were obtained in small quantities with different yields (0.10±0.02% and 0.24±0.01%, respectively). The cold period would be favourable for quantity production of essential oil by *S. birrea* from Benin. A total of 49 compounds, representing 98% of hydrodistillate, were identified by GC/FID and GC/MS analysis (Table 1).

The oils were characterized by four major chemical groups: hydrocarbon and oxygenated monoterpenes; hydrocarbon and oxygenated sesquiterpenes with high amount of hydrocarbon sesquiterpenes in all studied seasons (95.44±1.19% in cold season and 90.9±1.09% in hot season). We observed the presence of a higher percentage of monoterpenes (and particularly hydrocarbons) in the sample collected in February (1.6±0.51%) as compared to the sample collected during the cold season (1%). The same is observed concerning oxygenated sesquiterpenes (4.2±0.56% and 1.2±0.17%, respectively) and the contrary is observed concerning hydrocarbon sesquiterpenes (90.9±1.09% and 95.44±1.19%, respectively) (Table 2). Phytol is the only one diterpene identified in the two studied seasons with 0.3±0.01%. Non terpenic compounds represented 0.4±0.09% of the essential oil collected during the hot season and comprised 4-hydroxy-4-methyl-pentan-2-one (0.2±0.06%), phthalates (0.1±0.02%) and hexadecanoic acide (0.1±0.01%), while we found phthalates (0.1±0.02%) and hexadecanoic acide (0.1±0%) representing 0.2% of the extract of the cold season sample (Table 2).

The essential oil of *S. birrea* leaves contained more than 90% hydrocarbon compounds. The higher amount is found in the sample collected in August (96.53±1.27%) as compared to the sample collected during the hot season (92.5±1.6%). The contrary is observed concerning oxygenated compounds (1.99±0.27 and 5.3±0.72%, respectively) (Table 2). Extract I (45 compounds) obtained from the leaves harvested during the hot season was characterised by the presence as main constituents of 7-*epi*-α-selinene (38±0.03%), α-muurolene (25±0.03%) and valencene (17±0.06%) together with β-selinene (4.3±0.01%), β-caryophyllene (3.2±0.02%), *allo*-aromadendrene-epoxide (1.5±0.03%), 14-hydroxy-α-humulene (1.5±0.03%) and α-copaene (1.2±0.04%). Extract II obtained during the cold season (49 constituents) was characterised by a high concentration of 7-*epi*-α-selinene (51.7±0.12%) along with β-selinene (15.1±0.2%), valencene (12.9±0.05%), α-selinene (8.1±0.03%) and β-caryophyllene (1.8±0.02%). The concentrations of all the other constituents were less than 1%. Each extract was thus characterised by known but different main compounds; for I, 7-*epi*-α-selinene, α-muurolene and valencene and for II (with different levels), of 7-*epi*-α-selinene, α-selinene, β-selinene and valencene. This is the first report of these components in the essential oil of *S. birrea* (Anacardiaceae). If we compare the essential oils of the two samples, we see that the differences between samples are noted especially on the level of five sesquiterpenes: β-selenene, α-selinene, valencene, α-muurolene and 7-*epi*-α-selinene. 7-*epi*-α-selinene (Figure 1) was the predominant compound in the both essential oils of the studied seasons with a level of 51.7±0.12% in sample II and 38±0.03% in sample I. This sesquiterpene was previously identified in the essential oil of *Eugenia platysema* (10.4%) (Apel et al., 2002), *Stachys laxa* collected from north of Iran (8.3%) (Morteza-Semnani et al., 2006) and in low levels in the oil of other plants such

Table 1. Volatile compounds identified in the leaves essential oils of *Sclerocarya birrea* from Benin.

No	Compound	[a]KI	KI	(I)			(II)		
				%	±	[b]SD	%	±	[b]SD
1	4-hydroxy-4-methyl-pentan-2-one[&o]	835	835	0.2	±	0.06	tr		
2	α-thujene*[h]	925	931	0.1	±	0.05	0.07	±	0.01
3	α-pinene*[h]	932	939	0.1	±	0.05	0.07	±	0.01
4	Sabinene*[h]	972	976	0.2	±	0.08	tr		
5	β-pinene*[h]	977	980	0.2	±	0.1	tr		
6	Myrcene*[h]	989	991	0.1	±	0.02	0.08	±	0
7	*p*-cymene*[h]	1024	1026	0.5	±	0.13	0.48	±	0.04
8	Limonene*[h]	1029	1031	0.1	±	0.01	0.08	±	0
9	1,8-cineole*[o]	1033	1033	-			tr		
10	(*E*)-β-ocimene*[h]	1047	1050	0.2	±	0.04	0.2	±	0.01
11	γ-terpinene*[h]	1059	1062	tr			tr		
12	Linalol*[o]	1100	1096	0.4	±	0.05	0.19	±	0.03
13	(E)-4,8-dimethyl-1, 3,7-nonatriene*[h]	1113	1113	0.1	±	0.03	0.11	±	0.01
14	α-terpineol*[o]	1197	1196	tr			0.1	±	0.01
15	Thymol*[o]	1294	1298	0	±	0.01	tr		
16	Cyclosativene**[h]	1375	1378	0.3	±	0.03	0.3	±	0.01
17	α-copaene**[h]	1381	1379	1.2	±	0.04	0.74	±	0.43
18	β-bourbonene**[h]	1390	1388	0.2	±	0.01	tr		
19	β-elemene**[h]	1394	1391	-			0.6	±	0.1
20	β-caryophyllene**[h]	1424	1418	3.2	±	0.02	1.8	±	0.02
21	β-copaene**[h]	1433	1430	0.1	±	0.04	0.3	±	0
22	Selina-5,11-diene**[h]	1448	1444	0.1	±	0.05	0.1	±	0.01
23	Aromadendrene**[h]	1450	1441	0.1	±	0.05	0.1	±	0
24	α-humulene**[h]	1457	1454	0.1	±	0.01	0.8	±	0.02
25	4,5-*di*-*epi*-aristochene**[h]	1470	1470	0.2	±	0.05	0.2	±	0.02
26	Selina-4,11-diene**[h]	1473	1475	0.4	±	0.06	0.4	±	0.02
27	Germacrene-D**[h]	1481	1480	tr			0.4	±	0.04
28	β-selinene**[h]	1484	1485	4.3	±	0.01	15.1	±	0.2
29	α-selinene**[h]	1489	1494	0.4	±	0.2	8.1	±	0.03
30	Valencene**[h]	1492	1494	17	±	0.06	12.9	±	0.05
31	α-muurolene**[h]	1495	1496	25	±	0.03	1.7	±	0.09
32	7-*epi*-α-selinene**[h]	1522	1522	38	±	0.03	51.7	±	0.12
33	selina-3,7(11)-diene**[h]	1556	1557	0.3	±	0.4	0.1	±	0
34	(*E,E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene**[h]	1567	1565	-			0.1	±	0.03
35	Caryophyllene oxide**[o]	1580	1581	0.1	±	0.04	0.1	±	0.01
36	Humulene-1,2-epoxide**[o]	1607	1608	-			0.1	±	0.01
37	*epi*-cubenol**[o]	1624	1627	0.1	±	0.1	0.1	±	0.03
38	γ-eudesmol**[o]	1632	1632	0.1	±	0.1	tr		
39	*allo*-aromadendrene epoxide **[o]	1636	1641	1.5	±	0.03	0.1	±	0.03
40	*epi*-α-muurolol**[o]	1640	1641	0.1	±	0.01	0.1	±	0
41	α-muurolol**[o]	1643	1646	0.1	±	0.1	0.1	±	0.03
42	α-cadinol[**o]	1652	1654	0.2	±	0.1	0.2	±	0.03
43	Selin-11-en-4-α-ol**[o]	1655	1660	0.2	±	0.03	0.2	±	0.01
44	Intermedeol[**o]	1662	1667	0.2	±	0.01	0.2	±	0.02
45	14-hydroxy-α-humulene**[o]	1713	1714	1.5	±	0.03	tr		
46	Nootkatone**[o]	1797	1800	0.1	±	0.01	tr		
47	Phtalates[&o]	1851	1852	0.1	±	0.02	0.1	±	0.02

Table 1. Contd.

48	Hexadecanoïc acide[&o]	1950	1951	0.1	±	0.01	0.1	±	0
49	Phytol***[o]	2096	2097	0.3	±	0.01	0.3	±	0
	Total			97	±	0.06	98.1	±	0.03

KI = Kovats indices; a = calculated; bn=3; I = Sample of *Sclerocarya birrea* harvested in February 2009 ; II = Sample of *Sclerocarya birrea* harvested in August 2009; tr = traces (inferior or equal to 0.05%); (-) = absence or not identified; * = monoterpenes ; ** = sesquiterpenes; *** = diterpene; & = non terpenes; h = hydrocarbons ; o = oxygenetad.

Table 2. Seasonal variation of the composition of the essential oils of *Sclerocarya birrea*.

Chemical groups	(I)	II
	(%)±SD[b]	(%)±SD[b]
Hydrocarbon compounds	92.5±1.6	96.53±1.27
Oxygenated compounds	5.3±0.72	1.99±0.23
hydrocarbon monoterpenes	1.6±0.51	1.09±0.08
oxygenated monoterpenes	0.4±0.06	0.29±0.04
Monoterpenes	2±0.57	1.38±0.12
hydrocarbon sesquiterpenes	90.9±1.09	95.44±1.19
oxygenetad sesquiterpenes	4.2±0.56	1.2±0.17
Sesquiterpenes	95.1±1.65	96.64±1.36
Diterpenes	0.3±0.01	0.3±0
Non terpenes	0.4±0.09	0.2±0.02

I = Sample of *Sclerocarya birrea* harvested in February 2009, II = Sample of *Sclerocarya birrea* harvested in August 2009; [b]n=3.

as *Callicarpa americana* (1.3%) (Tellez et al., 2000), *Anthemis altissima* (0.2%) (Javidnia et al., 2004), *Zea mays* L. (1.13%) (El-Ghorab et al., 2007), fruit of *Mangifera indica* L. (0.2-0.5%) (Pino et al., 2005) and *Gomidesia tijucensis* (1.5%) (Limberger et al., 2003). 7-*epi*-α-selinene, α-selinene and β-selenene are isomers. α-selinene (8.1%, II; Figure 1) was previously found in high levels in the essential oil of *G. tijucensis* (27.1%) (Limberger et al., 2003), *Eugenia brasiliensis* (13.3 to 14.8%) (Fischer et al., 2005), *Eugenia uniflora* (15.1%) (Henriques et al., 1993), *Psidium guajava* (10.0%) (Ramos et al., 2006) and β-selenene (15.1%, II; Figure 1) in the essential oil of *E. uniflora* (25.9%) (Henriques et al., 1993), *G. tijucensis* (22.9%) (Limberger et al., 2003), *E. brasiliensis* (12.6 to 17.3%) (Fischer et al., 2005), *Eugenia platysema* (17.9%) (Apel et al., 2002), *Eugenia schuechiana* (10.5%) (Henriques et al., 1993) and *Psidium cattleyanum* (10.1%) (Marin et al., 2008). These essential oils showed antibacterial, antifungal, antioxidant, antinociceptive, cytotoxic, antilarvae, hypothermic and anthelmintic activities (Bhalke et al., 2008; Santos et al., 1998; Marin et al., 2008; Ogunwande et al., 2005; Adebajo et al., 1989; Lima et al., 1993; Adebayo et al., 1999; Amorim et al., 2009; Magina et al., 2009; Apel et al., 2006). α-muurolene (25%, I; Figure 1), second major constituent of *S. birrea* essential oils, was the first component of the essential oil of *Eryngium billardieri* F. Delaroche (42.0%) (Sefidkon et al., 2004). Valencene (17%, I, Figure 1) is an aroma component of citrus fruit and citrus-derived odorants. It is cheaply obtained from valencia oranges (Mai et al., 2005) and is used as a flavor and fragrance ingredient.

The majority of the applications are found in flavors for the beverage industry (especially in citrus flavors). Although minor, valencene also can be found in Fragrances applications (Elston et al., 2005). Some biological activities of these major compounds could explain a part of traditional uses of *S. birrea.* The lack of literature for most essential oils makes comparison in composition difficult.

Conclusion

This is the first report of sesquiterpenes: 7-*epi*-α-selinene, α-muurolene, valencene, β-selenene and α-selinene as major components of the essential oil of *S. birrea* leaves from Benin. Our work also showed that the season of harvest influenced the extraction yields and the chemical composition of the essential oil. The study of the biological activities of *S. birrea* essential oils could help to clarify a part of its traditional uses.

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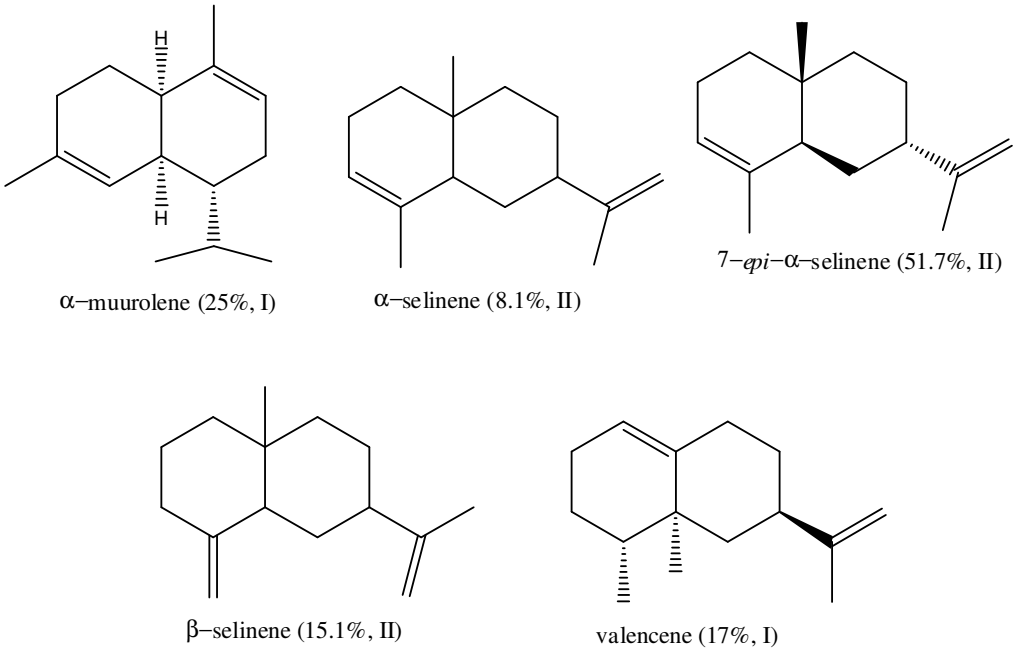

Figure 1. Major essential oil constituents of *Sclerocarya birrea* samples harvested in (I) February 2009 and (II) August 2009.

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