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Seasonal variations of volatile constituents of *Hemizygia bracteosa* (Benth.) Briq. aerial parts from Benin

Variations saisonnières de constituants volatils de la partie aérienne de Hemizygia bracteosa *(Benth.) Briq. du Bénin*

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ABSTRACT

Essential oils from fresh aerial parts of *Hemizygia bracteosa* (Benth.) Briq. were extracted by steam distillation. The oil yield from plants collected during the hot season (February) and during the cold season (August) were 0.12 ± 0.01% and 0.25 ± 0.02%, respectively. GC/FID and GC/MS analyses allowed us to identify a total of 65 compounds, representing 97% of the hydrodistillate. The main components of the oil from the hot period were (*E*)-β-farnesene (64 ± 0.04%), β-elemene (7.4 ± 0.05%), *trans*-nerolidol (6.2 ± 0.04%), and α-muurolene (2.7 ± 0.03%). The essential oil from the cold season was characterized by the presence, as major compounds, of (*E*)-β-farnesene (67 ± 0.04%) along with β-caryophyllene (3.6 ± 0.06%), β-elemene (3.3 ± 0.05%), 7-epi-α-selinene (3.1 ± 0.01%) and *p*-cymene (2.5 ± 0.04%). This is the first report of these components in the essential oil of *Hemizygia bracteosa* (Benth.) Briq.

RÉSUMÉ

Les huiles essentielles de parties aériennes fraîches de *Hemizygia bracteosa* (Benth.) Briq. ont été extraites par distillation à la vapeur. Les rendements en huile de la plante récoltée pendant la saison chaude (février) et la saison froide (août) étaient respectivement de 0,12 ± 0,01% et de 0,25 ± 0,02%. Les analyses GC/FID et GC/MS ont permis d'identifier 65 composés, représentant 97% de l'hydrodistillat. Les principaux composants de l'huile obtenue dans la période chaude étaient : (*E*)-β-farnésène (64 ± 0,04%), β-élémène (7,4 ± 0,05%), *trans*-nérolidol (6,2 ± 0,04%) et α-muurolène (2,7 ± 0,03%). L'huile essentielle de la saison froide est caractérisée par la présence, comme composés majoritaires, de

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(*E*)-β-farnésène (67 ± 0,04%), β-caryophyllène (3,6 ± 0,06%), β-élémène (3,3 ± 0,05%), 7-épi-α-selinène (3,1 ± 0,01%) et *p*-cymène (2,5 ± 0,04%). C'est la première observation de ces composants dans l'huile essentielle de *Hemizygia bracteosa* (Benth.) Briq.

1. Introduction

Hemizigia bracteosa (Benth.) Brip. (ex *Orthosiphon bracteatus* (Benth.) Baker) (Lamiaceae) is an erected annual and sometimes perennial herb of about 90 cm tall, widespread in tropical and South Africa, in marshy grasslands in Senegal, Benin (ex Dahomey), North and South Nigeria and West Cameroon [1]. The flowers are white and fairly inconspicuous but the colorful purple of its bracts makes the plant a striking feature [2].

In Southern Africa the plants are burnt and the smoke or vapors inhaled to treat mental illnesses, for narcotic or divination purposes [3]. The leaves are smoked or chewed by the San in Botswana to give energy for dancing and as a stimulant [4]. The Shonas of Zimbabwe are reported to use powdered leaves orally to treat fits [5]. In Zimbabwe, the plant is used in association with other plants for treating or preventing HIV infection in general and maybe to reduce the viral load of patients infected with HIV and/or exhibiting symptoms of acquired human immunodeficiency syndrome (AIDS) [6]. The plant is very useful in West Africa [7]. It is used to treat malaria, to foment the body of patients suffering from fever and its leaves are used as a mosquito repellent [8]. The fresh aerial parts of the plant are also traditionally used by fumigation in Benin and the decoction of the leaves in addition with *Dialium guineense*, *Pavetta corymbosa*, *Rytigynia canthiodes* and *Uvaria chamae* is used orally to treat malaria [9,10]. Leaves are also used in drink preparation and were shown to possess some antimicrobial activities [11].

To the best of our knowledge there are no reports concerning the volatile components of the aerial parts of *Hemizygia bracteosa* (Benth.) Briq. The aim of this study is to describe the chemical composition of essential oils extracted from fresh aerial parts of this plant from Benin and the impact of the harvesting period on this chemical composition and on the essential oil extraction yield.

2. Experimental

2.1. Plant material

Aerial parts of *Hemizygia bracteosa* (Benth.) Briq. were collected in the morning, in the Botanical Garden of the Abomey-Calavi University (Republic of Benin). The fresh aerial parts 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°AA6391/HNB) of these aerial parts has been deposited at the University of Abomey-Calavi Herbarium.

2.2. Essential oil isolation

Five hundred grams (500 g) of fresh aerial parts were steam distilled for 3 h in an improved Clevenger-type apparatus [12]. The extraction of each aerial part (I and II) was carried out in triplicate. The essential oil yields were based on the fresh material.

2.3. Chemical analysis

Analysis of the oils was performed by GC/FID and GC/MS [13,14].

2.3.1. GC/FID analysis

The GC/FID analysis was carried out on a FOCUS GC (ThermoFinigan; Milan, Italy) using the following operating conditions: A DB5 column (25 m × 0.25 mm, df: 0.25 μm) (J&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 was programmed as following: 50 °C – 250 °C at 6 °C/min and maintained at 250 °C for 5 min; carrier gas was helium with a constant flow of 1.2 mL/min; FID detector temperature was: 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 normalization process) and the identification of compounds by comparison of the retention indices with references.

2.3.2. GC/MS analysis

GC-MS analysis was 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. A HP5 column (30 m × 0.25 mm, df: 0.25 μm) was used under the same operating conditions 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 using the Xcalibur 1.1 software (Thermo-Quest). The mass spectra of the peaks were analyzed and compared with references and NIST/EPA/NIH database [15].

2.4. 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 [15,16] and home-made library mass spectra made from pure substances and components of known oils [14]. Mass spectrometry literature data were also used for the identification, which was confirmed by comparison of the GC retention indices (RI) on a non-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 [16]. A quantitative analysis of each oil component (expressed as percentages) was carried out by normalization measurements of peak area obtained by FID.

2.5. Chemicals

α-Pinene, β-pinene, camphene, *p*-cymene, myrcene, α-terpinene, γ-terpinene,1,8-cineol, terpinolene, borneol, citronellyl acetate, terpinene-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 FlukaChemie (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 the chemicals were of analytical standard grade. *tert*-Butyl methyl ether and anhydrous Na_2SO_4 analytical grade were purchased from FlukaChemie and from UCB (Bruxelles, Belgium) respectively.

2.6. 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.

3. Results and discussion

3.1. Variation of essential oil yields

The oils extracted from samples I and II were obtained in small quantities with different yields (0.12 ± 0.01% and 0.25 ± 0.02%, respectively). The cold period would be favorable for quantity production of essential oil by *Hemizygia bracteosa* (Benth.) Briq. from Benin.

3.2. Variation of oil composition

A total of 65 compounds, representing 97% of hydrodistillate, were identified (Table 1).

Table 1
Volatile compounds identified in the aerial part essential oils of *Hemizygia bracteosa* (Benth.) Briq. (Lamiaceae) from Benin.

N°	Compounds	[a]KI	KI	I	II
				% ± [b]SD	% ± [b]SD
1	4-Hydroxy-4-methyl-pentan-2-one***°	835	835	–	0.1 ± 0.04
2	α-Thujene*[h]	925	931	–	0.2 ± 0.05
3	α-Pinene*[h]	932	939	–	0.1 ± 0.08
4	Sabinene*[h]	972	975	tr	0.1 ± 0.01
5	β-Pinene*[h]	976	977	0.1 ± 0.02	0.1 ± 0.02
6	Myrcene*[h]	989	991	0.4 ± 0.13	0.3 ± 0.03
7	α-Terpinene*[h]	1017	1017	tr	0.1 ± 0.01
8	*p*-Cymene*[h]	1025	1026	tr	2.5 ± 0.04
9	Limonene*[h]	1029	1031	tr	0.3 ± 0.04
10	1,8-Cineole*°	1033	1033	0.1 ± 0.01	0.2 ± 0.01
11	(*Z*)-β-Ocimene*[h]	1038	1036	–	0.2 ± 0.05
12	(*E*)-β-Ocimène*[h]	1047	1050	tr	0.1 ± 0.03
13	γ-Terpinène*[h]	1059	1062	0.4 ± 0.01	1.6 ± 0.04
14	*cis* Sabinene hydrate*°	1067	1067	tr	–
15	*p*-Cymenene*[h]	1091	1091	0.1 ± 0.03	0.1 ± 0.03
16	Linalool*°	1097	1096	tr	–
17	Lavandulol*°	1165	1165	1.1 ± 0.01	0.1 ± 0.01
18	Terpinene-4-ol*°	1182	1178	0.6 ± 0.05	tr
19	Terpinen-4-ol acetate*°	1300	1300	0.1 ± 0.02	0.1 ± 0.02
20	α-Cubebene**[h]	1348	1347	0.3 ± 0.04	0.2 ± 0.04
21	α-Copaene**[h]	1378	1379	2.3 ± 0.02	1.5 ± 0.05
22	β-Panasinsene**[h]	1383	1383	0.1 ± 0.05	0.2 ± 0.05
23	β-Bourbonene**[h]	1386	1388	0.1 ± 0.01	0.1 ± 0.01
24	β-Elemene**[h]	1391	1391	7.4 ± 0.05	3.3 ± 0.05
25	β-Caryophyllene**[h]	1422	1418	tr	3.6 ± 0.06
26	(*Z*)-β-Farnesene**[h]	1426	1426	0.1 ± 0.03	0.1 ± 0.01
27	β-Copaene**[h]	1430	1430	0.1 ± 0.01	tr
28	Guaia-6,9-diene**[h]	1443	1443	0.4 ± 0.02	tr
29	(*E*)-β-Farnesene**[h]	1456	1454	64 ± 0.04	67 ± 0.04
30	α-Humulene**[h]	1458	1457	1.7 ± 0.03	0.6 ± 0.03
31	(2*E*) Dodecenal***°	1466	1466	–	0.1 ± 0.03
32	Massoïlactone***°	1474	1474	0.2 ± 0.04	0.4 ± 0.04
33	γ-Himachalene**[h]	1477	1477	1.8 ± 0.03	tr
34	Germacrene-D**[h]	1484	1480	0.3 ± 0.04	1.5 ± 0.04
35	β-Selinene**[h]	1486	1485	0.3 ± 0.06	0.8 ± 0.02
36	α-Selinene**[h]	1492	1491	0.2 ± 0.01	0.5 ± 0.01
37	Valencene**[h]	1495	1494	0.2 ± 0.01	0.8 ± 0.01
38	α-Muurolene**[h]	1498	1496	2.7 ± 0.03	0.3 ± 0.03
39	β-Himachalene**[h]	1503	1501	0.1 ± 0.01	0.1 ± 0.01
40	Germacrene-A**[h]	1510	1508	0.1 ± 0.01	0.3 ± 0.01
41	γ-Cadinene**[h]	1514	1513	0.1 ± 0.01	0.1 ± 0.01
42	δ-Cadinene**[h]	1519	1519	tr	1.9 ± 0.03
43	7-*epi*-α-Selinene**[h]	1522	1522	0.1 ± 0.01	3.1 ± 0.01
44	Cadina-1,4-diene**[h]	1534	1533	tr	0.1 ± 0.03
45	*cis*-Cadineneether**[h]	1554	1554	0.1 ± 0.01	0.1 ± 0.01
46	Elemicin***°	1557	1557	0.2 ± 0.02	0.2 ± 0.02
47	Germacrene-B**[h]	1560	1559	0.2 ± 0.01	0.5 ± 0.01
48	*cis*-Caryophyllene oxyde**°	1566	1564	0.3 ± 0.01	0.1 ± 0.01
49	Dendrolasin**[h]	1568	1569	0.1 ± 0.02	0.2 ± 0.04
50	Spathulenol**°	1580	1581	0.3 ± 0.01	0.3 ± 0.03
51	*trans*-Caryophyllene oxyde**°	1584	1584	0.1 ± 0.05	0.9 ± 0.05
52	Humulene-1,2-epoxyde**°	1612	1608	0.7 ± 0.05	0.2 ± 0.05
53	1,10-*diepi*-Cubenol**°	1616	1616	0.1 ± 0.03	0.1 ± 0.08
54	*trans*-Nerolidol**°	1625	1625	6.2 ± 0.04	0.1 ± 0.01
55	*epi*-Cubenol**°	1629	1629	0.2 ± 0.01	0.1 ± 0.02
56	Ledol**°	1632	1632	tr	0.1 ± 0.01
57	*epi*-α-Cadinol**°	1643	1643	0.5 ± 0.04	0.4 ± 0.01
58	Himachalol**°	1654	1653	0.1 ± 0.01	tr
59	α-Cadinol**°	1656	1655	1 ± 0.04	0.2 ± 0.04
60	*neo*-Intermedeol**°	1660	1660	0.1 ± 0.01	0.1 ± 0.01
61	14-Hydroxy-9-epi-(*E*)-caryophyllene**°	1670	1670	1.6 ± 0.05	0.1 ± 0.05
62	(*Z*)-α-*trans*-Bergamotol**°	1708	1705	0.2 ± 0.03	0.1 ± 0.03
63	Khusimol**°	1713	1713	0.1 ± 0.03	tr
64	Crysolide***°	1723	1720	0.1 ± 0.01	0.1 ± 0.01
65	*p*-Cresoloctanoate***°	1777	1775	tr	0.2 ± 0.03
	Total			**97.7 ± 1.12**	**96.9 ± 1.59**

IK = Kovats indices; * = monoterpenes; ** = sesquiterpenes; *** = non terpenes; [h] = hydrocarbons; ° = oxygenated; [a] = calculated; [b] $n = 3$; I = Sample of *Hemizygia bracteosa* (Benth.) Briq. harvested in February 2009; II = sample of *Hemizygia bracteosa* (Benth.) Briq. harvested in August 2009; tr = traces (inferior or equal to 0.05%); (–) = absence or not identified.

The oils were characterized by four major chemical groups: hydrocarbon and oxygenated monoterpenes; hydrocarbon and oxygenated sesquiterpenes with high amounts of hydrocarbon sesquiterpenes in all studied seasons (86.9 ± 0.61% in the cold season and 82.8 ± 0.56% in the hot season).

We observed the presence of a higher percentage of monoterpenes (and particularly hydrocarbons) in the sample collected in August (5.7 ± 0.37%) as compared to the sample collected during the hot season (1 ± 0.09%). The opposite is observed concerning oxygenated sesquiterpenes (2.8 ± 0.4% and 11.5 ± 0.41%, respectively) (Table 2). Non terpenic compounds represented 0.5 ± 0.07% of the essential oil collected during the hot season and comprised massoïlactone (0.2 ± 0.04%) and crysolide (0.1 ± 0.01%), while we found massoïlactone (0.4 ± 0.04%), *p*-cresol octanoate (0.2 ± 0.03%), 4-hydroxy-4-methyl-pentan-2-one (0.1 ± 0.04), (2*E*) dodecenal (0.1 ± 0.03%) and crysolide (0.1 ± 0.01%) representing 1.1 ± 0.17% of the extract of the cold season sample (Table 2).

Extract I (59 compounds) obtained from the aerial parts harvested during the hot season was characterized by the presence as main constituents of (*E*)-β-farnesene (64 ± 0.04%), β-elemene (7.4 ± 0.05%), *trans*-nerolidol (6.2 ± 0.04%), α-muurolene (2.7 ± 0.03%), α-copaene (2.3 ± 0.02%) together with γ-himachalene (1.8 ± 0.03%), α-humulene (1.7 ± 0.03%), and 14-hydroxy-9-epi-(*E*)-caryophyllene (1.6 ± 0.05%).

Extract II obtained during the cold season (63 constituents) was characterized by a high concentration of (*E*)-β-farnesene (67 ± 0.04%) along with β-caryophyllene (3.6 ± 0.06%), β-elemene (3.3 ± 0.05%), 7-epi-α-selinene (3.1 ± 0.01%), *p*-cymene (2.5 ± 0.04%), δ-cadinene, (1.9 ± 0.03%), γ-terpinene (1.6 ± 0.04%), α-copaene (1.5 ± 0.05%) and germacrene-D (1.5 ± 0.04%).

The concentration of all other constituents was less than 1.2%. Each extract was thus characterized by known compounds but the main components may differ quantitatively; for I, (*E*)-β-farnesene, β-elemene, and *trans*-nerolidol, and for II (with different levels), (*E*)-β-farnesene and β-caryophyllene were the major constituents. This is the first report of these components in the essential oil of *Hemizygia bracteosa* (Benth.) Briq.

If we compare the essential oils of the two samples, we can see that the differences between samples were noticed especially on the level of three sesquiterpenes: β-elemene, β-caryophyllene and *trans*-nerolidol. (*E*)-β-farnesene (Fig. 1) was the predominant compound in both samples with a level higher than 60% (67% in sample II and 64% in sample I). This acyclic sesquiterpene olefin, was previously identified in high levels (>70%) in the essential oil of *Hemizygia petiolata* Ashby (Lamiaceae) from South Africa [17] and was found in small amounts in essential oils of hundreds of species of both gymnosperms [18] and angiosperms [19–21].

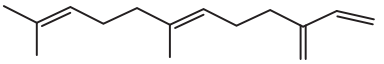
(*E*)-β-Farnesene is also released by aphids as an alarm pheromone upon death to warn away other aphids. Several plants, including potato species, have been shown to synthesize this pheromone as a natural insect repellent [22]. Recently, this compound has been reported to be involved in pea aphid (*Acyrthosiphon pisum*) wing induction [23] while its insecticidal activity at high doses has also been demonstrated [24].

β-Elemene (7.4%, Fig. 1) is an anticancer drug extracted from the traditional Chinese medicinal herb *Rhizoma zedoariae*. It has been used efficiently in China to treat certain types of tumors [25] and was evaluated in clinical trials in the United States. Studies indicated that β-elemene had a broad-spectrum anticancer activity, with effectiveness in treating leukemia, brain tumor, breast cancer and liver cancer [25]. Furthermore, it had low toxicity, was easy to administer, and was well tolerated and accepted by cancerous patients [25]. It also showed therapeutic potential for rheumatoid arthritis [26].

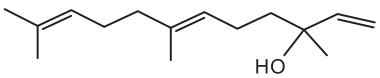
Table 2
Seasonal variation of the composition of the essential oils of *Hemizygia bracteosa* (Benth.) Briq.

Chemical groups	I	II
	% ± [a]SD	% ± [a]SD
Hydrocarbon monoterpenes	1 ± 0.09	5.7 ± 0.37
Oxygenated monoterpenes	1.9 ± 0.09	0.4 ± 0.04
Monoterpenes	**2.9 ± 0.18**	**6.1 ± 0.41**
Hydrocarbon sesquiterpenes	82.8 ± 0.56	86.9 ± 0.61
Oxygenated sesquiterpenes	11.5 ± 0.41	2.8 ± 0.4
Sesquiterpenes	**94.3 ± 0.97**	**89.7 ± 1.01**
Others	**0.5 ± 0.07**	**1.1 ± 0.17**

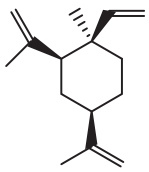
I = Sample of *Hemizygia bracteosa* (Benth.) Briq. harvested in February 2009; II = Sample of *Hemizygia bracteosa* (Benth.) Briq. harvested in August 2009; [a]$n = 3$.



(*E*)-β-farnesene (67%, II)



trans-nerolidol (6.2%, I)



β-elemene (7.4%, I)

Figure 1. Major essential oil constituents of *Hemizygia bracteosa* (Benth.) Briq. samples harvested in February 2009 (I) and in August 2009 (II).

The third major constituent of our oils, nerolidol (6.1%, Fig. 1), was approved by the U.S. Food and Drug Administration as a food flavoring agent. It exhibited antineoplastic, antinociceptive and anti-inflammatory activities [27,28], and it was tested as a skin penetration enhancer for the transdermal delivery of therapeutic drugs [29]. Rodrigues-Goulart et al. [30] reported its activity against the malaria parasite. Arrudan et al. [31] described the leishmanicidal activity of nerolidol and its inhibitory effect on the biosynthesis of isoprenoids.

4. Conclusion

GC/FID and GC/MS analyses allowed us to identify in the essential oils of *Hemizygia bracteosa* (Benth.) Briq., 65 compounds. The main constituents were (*E*)-β-farnesene (67 ± 0.04%), β-elemene (7.4 ± 0.05%) and *trans*-nerolidol (6.2 ± 0.04%). Biological studies of these essential oils could help to clarify their biological properties.

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