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Chemical composition and *in vitro* antibacterial activity of essential oil from *Eupatorium africanum* Oliv. & Hiern

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Abstract
A hydrodistilled oil from the aerial part of *Eupatorium africanum* Oliv. & Hiern collected from D.R. Congo was analyzed by GC and GC/MS. Thirty three compounds representing 97.34% of the oil were identified. The major components were *β*-eudesmol (49.1%), 10-*epi*-*γ*-eudesmol (11.30%), *α*-humulene (8.9%), hedycaryol (8.9%), and elemol (5.2%). The antibacterial activity of the essential oil and its dominant constituent was evaluated against four bacteria strains (*Staphyloccocus aureus* ATCC 25952, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Proteus vulgaris* ATCC 4635) using the micro-dilution method. The results indicate that the two samples are active against all tested microorganisms. The oil has a moderate activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with a minimum inhibitory concentration (MIC) value of 125 µg/mL. The isolated major constituent (β-eudesmol) exhibits the highest activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with a MIC value of 62.5 µg/mL. Both samples exhibited a MIC value of 250 µg/ml against *Escherichia coli*. and *Proteus vulgaris.*

Keywords: *Eupatorium africanum* Oliv. & Hiern, Asteraceae, essential oil, chemical composition, biological activity.

1. Introduction
Eupatorium africanum Oliv. & Hiern. [syn. *Stomatanthes africanus* (Oliv. & Hiern) R.M. King & H. Rob] belongs to the genus *Stomatanthes*, Asteraceae family, Eupatorieae tribe. This genus comprises 17 species distributed in South America (13 species) and in Africa (4 species). But according to Grossi, the genus *Stomatanthes* would be formed by only three of the four African species (*S. africanus*, *S. meyeri* and *S. helenae*). *Stomatanthes zambiensis*, the fourth African species, is excluded from *Stomatanthes* genus. It would be related with *Eupatorium sensu stricto* [1].

This plant is a perennial herb (sub-shrub 20–120 cm high) of hilly grassland throughout the region from Guinea to West Cameroon and widespread in tropical Africa [2]. It is used as herbal medicine against headache, diarrhea, dysentery and naso-pharyngeal affections [2, 3]. In Congo, a leaf decoction is made as a mouthwash for aphthera, and a tisane of the boiled root is used as a cough medicine and an anti-diarrhetic [4].

Phytochemical investigations and chemical composition of *Eupatorium* species essential oils (including many species now reclassified into other closely related genera) have been carried out by several researchers. These studies revealed the presence of a variety of monoterpernes, sesquiterpenes, sesquiterpene lactones, flavonoids, phenolic and acetylenic compounds, triterpenes, and alkaloids with cytotoxic, antitumoral, antimicrobial, antioxidant and anti-inflammatory activities has been reported [5-6]. A review of antimicrobial activity of the genus *Eupatorium* L. (Asteraceae) has been recently published and *Eupatorium africanum* is not mentioned in this paper [7]. The following terpenoids were reported as major constituents of *Eupatorium* species essential oils: α-pinene (50.98 %), β-pinene (10.5%) sabinene (46.7%), limonene (9.63-23.3%), camphene (8.9%), (+)-camphor (15.46%), 2,5-dimethoxy-*p*-cymene or thymohydroquinone dimethyl ether (20.8-60.7%), thymol, *p*-cymene (23.7%), thymol methyl ether (36.3%), aristolone+laevigatin (23.6%), globulol (16.2-25.1%), caryophyllene oxide (17.4–30.1%), germacrene D (8.6–21.6%), spathulenol (14.2%), (-)-spathulenol

(0.48-25.16 %), α-zingiberene (57.5%), α-gurjunene (19.5%), (*E*)-β-bisabolene (9.7%), α-selinene (9.0%), β-caryophyllene (1.11-41.7%), ocimene (4.78 %), δ-elemene (10.57%), α-humulene (0.25-14.6%), patchoulene (9.24%), viridiflorol (9.16%), γ-elemene (5.92%), (+)-δ-cadinene (5.83%), (-)-δ-cadinol (2.67%), α-santalene (2.53%), β-cubebene (1.64%), (+)-nerolidol (1.63%), selina-4(15),7(11)-dien-8-one (36.6%), γ-muurolene (10.4-19%), myrcene (15.7%), valencene (10.5%), cyperone (16.9%), and pregeijerene (14.3%), phellandrene (3.85%), α-bisabolol (9.53%), bornyl acetate (8.98-15.6%), β-bisabolene (6.16%), amorph-4-en-7-ol (9.6%), 3-acetoxyamorpha-4,7(11)- dien-8-one (7.8%) and amorph-4,7(11)-dien-8-one (5.7%), neryl isobutyrate (17.6%). The major non-terpenoid compounds reported were methyl chavicol (42.2%) and 1-naphthalenol (17.50%).

Amorph-4-en-7-ol (9.6%), 3-acetoxyamorpha - 4,7(11) - dien-8-one (7.8%) and amorph-4,7(11)-dien-8-one (5.7%) were identified in *Eupatorium adenophorum* oil. Amorphene derivatives (19.8–41.4%) may be considered as characteristic constituents of *E. adenophorum* [5, 8-23].

The present investigation reports the results of GC and GC-MS analyses of the essential oil from the leaves of *Eupatorium africanum* Oliv. & Hiern growing wild in D.R. Congo, and its antibacterial activity evaluation. To the best of our knowledge, this is the first report on the chemical composition and the biological activity of the *Eupatorium africanum* essential oil.

2. Materials and methods

2.1 Plant material

The *Eupatorium africanum* leaves were collected in the *Bombo-Lumene* Game reserve, Kinshasa Province, D.R. Congo, and authenticated by a voucher specimen (H. BREYNE 2785) kept at the INERA herbarium, Department of Biology, University of Kinshasa, D.R. Congo.

The collected fresh leaves were dried at room temperature in a well-ventilated room and preserved in tightly closed bumper bags at room temperature in the absence of light.

2.2 Isolation of essential oil

Dried plant material (300 g) was subjected to hydro-distillation for 4 hours using a Clevenger type apparatus to produce an essential oil at a yield of 0.4%. The oil was dried over anhydrous sodium sulfate and stored in sealed vials at low temperature (4 °C).

2.3 Gas Chromatographic (GC) Analysis

GC analysis was performed using a Hewlett-Packard 5880A gas chromatograph equipped with a flame ionization detector (FID) and data-handling system. The GC was fitted with a DB-5 fused silica capillary column (30m × 0.25 mm, film thickness 0.25 µm). The oven temperature was programmed as follows: 40 °C for 5 min, from 40 to 200 °C at a rate of 4 °C/ min, and 10 °C/min. ramp to 300 °C. Helium was used as carrier gas at the flow rate of 1 mL/min. Injector and detector temperatures were maintained at 280 and 295 °C, respectively. The volume of sample injected was 0.2 µL and the split ratio was 1:30.

2.4 GC/MS analysis

GC/MS analysis was performed with Varian Saturn 2100T GC/MS fitted with a DB-5 fused silica capillary column (30m × 0.25 mm, film thickness 0.25 µm). The GC conditions were as described for GC analysis. The mass spectrometer was operated at an ionization energy of 70 eV with a mass scan range of 40-400 amu.

2.5 Identification of oil constituents

The identification of constituents was based on a comparison of their retention indices and mass spectra with spectral data from several sources, by searching in the GC-MS library, and by matching their fragmentation patterns in mass spectra with those of published [24-28]. The percentage of the individual constituent was obtained from FID area percentages without the use of correction factors. Retention indices were calculated relative to C_8-C_{24} *n*-alkanes, and compared with values reported in the literature [24-26].

2.6 Antibacterial Activity

2.6.1 Microorganisms

Four strains of microorganisms from the American Type Culture Collection (ATCC, Rockville MD, USA) were used in the present study: *Staphylococcus aureus* (*S. aureus* ATCC 25952), *Escherichia coli* (*E. coli* ATCC 27195), *Pseudomonas aeruginosa* (*P. aeruginosa* ATCC 9027), and *Proteus vulgaris* (*P. vulgaris* ATCC 4635) strains.

2.6.2 Determination of Minimum inhibitory concentration (MIC)

The antibacterial activity of *Eupatorium africanum* essential oil and its major constituent was assessed against selected bacteria strains by the micro-well dilution method and the minimum inhibitory concentration (MIC) values, which represent the lowest sample concentrations that completely inhibit the growth of microorganisms were obtained using this method [29].

The 10 mg samples (essential oil and isolated major constituent) were each dissolved in DMSO (250 µL) and diluted with Mueller–Hinton Broth (MHB) in order to reach concentrations of 2000 µg/mL and a 5 ml solution (final volume, and 5% DMSO final concentration). These solution were used as stock solutions.

The inocula of microorganisms were prepared from 24h old MHB cultures. The microbial suspensions were prepared by adding five colonies of each of the test bacteria to 2 mL of with sterile physiological solution (0.9% NaCl) and adjusted with this sterile physiological solution to match that of a 0.5 McFarland standard solution (10^8 cells/ml). They were then diluted (1/100) to achieve 10^6 CFU/mL.

The assay was carried out using sterile clear polystyrene 96-well microtiter plates (round bottom). The wells in the columns 2 to 8 and those in columns 11 and 12 were filled with 100 µL MHB (Mueller Hinton Broth). Briefly 200 µL of stock solution of each *Eupatorium* sample were added to the wells in column 1 (A1 to H1), and two-fold serial dilutions were made from column 1 to column 8. Then 5 µL of the inoculum were dispensed to all the wells except those in column 12. The wells in columns 11 and 12 we used as positive and negative controls. The negative control wells (growth control) contained MHB and bacteria suspension without test sample (column 11) and the positive control wells contained only MHB (control of MHB sterility: column 12).

The microplates (96 wells) were incubated at 37°C for 24 hours. After the incubation, 5 µL de colorant resazurin 1% (7-Hydroxy-3H-phenoxazin-3-one 10-oxide) were added to each well and the microplates were then incubated for 5 hours. The minimum inhibitory concentration (MIC) was determined as the lowest essential oil concentration at which no growth were observed after 24 and 48 hours. All

experiments were performed in triplicate.

3. Results & Discussion

The oil isolated by hydrodistillation of the *Eupatorium africanum* leaves were found to be yellow and yields 0.40% (w/w), based on dry weights. Its composition is summarized in Table I. Constituents are listed in order of their elution from a DB-5 capillary column and were identified by GC/MS analysis in combination with retention indices. The main constituent was isolated and identified as β-eudesmol.

This study reports the presence of 42 components, 33 of which were identified and quantified, representing 97.34% of the total *Eupatorium africanum* oil composition. This oil was constituted mainly by oxygenated sesquiterpenes (82.64%). Sesquiterpene hydrocarbons represented 12.77%) and monoterpenes accounted only for 1.93% of the oil. β-Eudesmol made up the largest component of the oil (49.1%) and the second largest component was 10-*epi*-*γ*-eudesmol (11.30%). Other major constituents (> 5.0 %) of the oil were hedycaryol (8.9%), α-humulene (8.9%) and elemol (5.2%). The minor constituents (< 5.0 and > 0.03 %) of the oil were eremoligenol (1.90%), allo-spathulenol (1.90%), humulene oxide II (1.70%), (*E*)-β-caryophyllene (1.47%), limonene (1.10%), δ-cadinene (0.86%), 4-*epi*-cubebol (0.63%), caryohyllene oxide (0.63%), myrcene (0.47%), β-cubenene (0.47), cubebol (0.44%), germacrene D (0.37%), valencene (0.33%), (*E*)-β-ocimene (0.32%), α-eudesmol (0.31%) and *trans*-cadina-1(6),4-diene (0.27%). One minor (0.31%) and eight trace components (<0.03%) were not identified. The mass spectra (reported in Table 1) of the unidentified components(UI) UI 2, UI 3, UI 5 to UI 9 suggest that these compounds are polyoxygenated sesquitepenes. UI2 is a sesquiterpenediol (expected MW=238). The peaks at m/z=223 (M^+-CH_3), 220 (M^+-H_2O), 207 (M^+-CH_2OH), 205 (M^+-CH_3,-H_2O), 189 (M^+-H_2O,-CH_2OH), 177 (M^+-CH_2OH, -$2CH_3$) account for the presence of two -OH groups in the molecule, one of which is part of a -CH_2OH group. Unidentified constituents UI3, UI5 to UI9 are monoacetylated sesquiterpenetriols (expected MW=296). They all show a peak at m/z=281 corresponding to M^+-CH_3. The peak at m/z=59 in UI3 (base peak), UI6 (90%) and UI7 (base peak) suggests the presence of a hydroxyisopropyl group in these compounds. UI8 and UI9 mass spectra exhibit the similar fragmentation patterns with different relative intensity for corresponding peaks. These two components might be isomers. Although we did not identify most of the unknown constituents of the *Eupatorium africanum* oil, it is worth nothing that this is the first report indicating the presence of polyoxygenated sesquiterpenes in *Eupatorium species* essential oil.

Previously reported biologically active sesquiterpene lactones, cadinenes, chromenes and thymol derivatives were not detected in present investigation. The result of this investigation suggests that the essential oil of *Eupatorium africanum* can serve as a source of β-eudesmol that constitutes 49.1% of the oil.

Table 1: The chemical composition of *Eupatorium africanum* essential oil

No	Components[a]	RI[b]	(%)	Identification
1	Tricyclene	925	0.04	A, B
2	*α*-Fenchene	951	tr[c]	A, B
3	Sabinene	972	tr	A, B
4	*β*-Pinene	981	tr	A, B
5	Myrcene	993	0.47	A, B
6	Limonene	1032	1.10	A, B
7	(*E*)-*β*-Ocimene	1041	0.32	A, B
8	Linalool	1091	tr	A, B
9	*δ*-Elemene	1330	0.06	A, B
10	*α*-Ylangene	1365	0.04	A, B
	β-Cubebene	1382	0.47	A, B
12	UI1	1386	0.03	
13	7-*epi*-sesquithujene	1392	tr	A, B
14	(*E*)-*β*-Caryophyllene	1412	1.47	A, B
15	*trans*-Muurola-3,5-diene	1453	tr	A, B
16	*α-Humulene*	*1459*	*8.90*[d]	A, B
17	*trans*-Cadina-1(6),4 diene	1471	0.27	A, B
18	Germacrene-D	1474	0.37	A, B
19	4-*epi*-Cubebol	1487	0.63	A, B
20	γ-Amorphene	1492	0.33	A, B
21	Cubebol	1510	0.44	A, B
22	*δ*-Cadinene	1524	0.86	A, B
23	Zonarene	1526	tr	A, B
24	*Hedycaryol*	*1542*	*8.90*	A, B
25	*Elemol*	*1551*	*5.83*	A, B
26	Caryophyllene oxide	1581	0.63	A, B
27	Humulene oxide II	1611	1.70	A, B
28	Allo-spathulenol	1621	1.90	A, B
29	10-*epi*-*γ*-Eudesmol	*1624*	*11.30*	A, B
30	Eremoligenol	1631	1.90	A, B
31	*β-Eudesmol*	*1650*	*49.1*	A, B
32	*α*-Eudesmol	1654	0.31	A, B
33	UI2 (Sesquiterpenediol)	1738	tr	
34	*α*-Costol	1773	tr	A, B

35	Eudesma-11-en-4α,6α-diol	1799	tr	A, B
36	UI3 (Monoacetylated sesquiterpenetriol)	1882	tr	
37	UI4 (Sesquiterpene alcohol: (acetylated?))	2031	tr	
38	UI5 (Monoacetylated sesquiterpenetriol)	2046	tr	
39	UI6 (Monoacetylated sesquiterpenetriol)	2105	tr	
40	UI7 (Monoacetylated sesquiterpenetriol)	2183	tr	
41	UI8 (Monoacetylated sesquiterpenetriol)	2395	0.31	
42	UI9 (Monoacetylated sesquiterpenetriol)	2472	tr	

Total identified 97.34%

Group Components

- Monoterpene hydrocarbons : 1.93%
- Sesquiterpene hydrocarbons : 12.77%
- Oxygenated sesquiterpenes : 82.64%

a. Compounds listed in order of elution from DB-5 column
b. RI: retention index relative to *n*-alkanes (C8–C20) on the DB-5 column.
c. Traces: (<0.03%)
d. Italic/Boldface designates the major constituents

A**:** RI

B**:** MS

Mass spectra of unidentified constituents: m/z (relative intensity %)

- UI1: 174 (100, M^+), 161 (1), 142 (1), 137(1), 121 (3), 105 (1), 93 (5), 91(3), 83 (3), 81(1), 55 (3), 51 (3), 49 (7), 41(3)
- UI2 (Sesquiterpenediol: 223 (30, M^+-CH_3), 220 (13, M^+-H_2O), 207 (21, M^+-CH_2OH), 205 (15, M^+-CH_3,-H_2O), 189 (8,M^+-H_2O,-CH_2OH), 177 (10, M^+-CH_2OH, -2CH_3), 161 (18), 159 (16), 149 (8), 141 (22), 137 (15), 124 (20), 123 (25), 121 (18), 107 (25), 95 (19), 93 (18), 90 (20), 80 (22), 79 (26), 77 (18), 69 (39). 59 (17), 55 (54), 43 (100), 41(89)
- UI3 (Monoacetylated sesquiterpenetriol): 281(7, M^+-CH_3), 222 (8, M^+-CH_3,-59), 205 (4, M^+-CH_3,-59,-17), 191 (14), 189 (10), 180 (14), 175 (9), 162 (21), 147 (18), 135 (18), 125 (26), 121 (17), 109 (26), 107 (48), 105 (24), 96 (22), 95 (32), 93 (39), 91(25), 81 (40), 78 (28), 67 (44), 59 (100), 56 (33), 49 (39), 43 (74), 41(52).
- UI4 (Sesquiterpene alcohol: acetylated?): 207 (20, M^+-CH_3?), 164 (17, M^+-CH_3,-43 ?), 161(17), 159 (7), 149 (18), 147 (20), 124 (26), 119 (45), 109 (21), 107 (22), 105 (21), 95 (37), 93 (34), 91 (26), 81(49), 78 (42), 67 (22), 59 (49), 55 (41), 43 (96), 41(100).
- UI5 (Monoacetylated sesquiterpenetriol): 281 (39, M^+-CH_3), 207 (43, M^+-CH_3,-74), 162 (14), 148 (14), 142 (10), 130 (14), 128 (15), 121 (13), 108 (15), 107 (15), 103 (10), 93 (18), 90 (17), 83 (32), 80 (26), 79 (49), 67(18), 65 (22), 59 (55), 49 (66), 43(100).
- UI6 (Monoacetylated sesquiterpenetriol): 281(37, M^+-CH_3), 220 (24, M^+-17,-59), 207 (73, M^+-2CH_3,-59), 202 (24, 220-H_2O), 191 (27), 187 (58), 162 (25), 159 (36), 147 (44), 135 (22), 133 (20), 131 (25), 119 (38), 118 (23), 109 (19), 105(100), 95 (44), 90 (43), 80 (27), 78 (53), 76 (41), 72 (17), 69 (32), 67 (33), 62 (38), 59 (90), 43(69).
- UI7 (Monoacetylated sesquiterpenetriol): 281 (37, M^+-CH_3), 221(14, M^+-CH_3,-AcOH), 220 (10), 207 (47), 205 (8), 165 (10), 162 (24), 159 (7), 134 (21), 123 (10), 119 (27), 109 (10), 107 (20), 105 (19), 95 (27), 93 (22), 91(14), 84 (20), 80 (25), 78 (18), 76 (4), 59 (100), 55 (13), 53 (25), 49 (36), 43 (65).
- UI8 (Monoacetylated sesquiterpenetriol): 281(17, M^+-CH_3), 267(3), 265(3, M^+-CH_2OH), 207 (32, M^+-2CH_3,-AcO^-), 191(8), 177(2), 161(1), 141(2), 131(5), 107(2), 93 (5), 90(11), 80 (28), 78 (30), 76 (20), 73(8), 67 (22), 55 (34), 43 (100).
- UI9 (Monoacetylated sesquiterpenetriol): 281 (44, M^+-CH_3), 267 (15), 265 (11, M^+-CH_2OH), 207 (96, M^+-2CH_3,-AcO^-), 190 (21), 161 (25), 149 (4), 131(15), 121 (13), 119 (27), 105 (27), 93 (14), 90 (28), 86 (31), 84 (37), 80 (27), 78 (14), 76(15), 72 (22), 67 (14), 55 (16), 50 (29), 49 (66), 47 (45), 43 (100)

The results of the evaluation of antibacterial activity of the essential oil and its main constituent are reported in Table 2. The oil exhibited moderate antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with MIC values of 125 µg/mL and a weak antibacterial activity against *Escherichia coli* and *Proteus vulgaris* with MIC values of 250 µg/mL while the main constituent of the oil showed a strong antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with MIC values of 62.5 µg/mL, and a weak antibacterial activity against *Escherichia coli* and *Proteus vulgaris* with MIC values of 250 µg/mL.

Several studies report that β-eudesmol exhibit multiple pharmacological activities, including anti-angiogenic anti-mutagenic, anti-tumor, antipeptic, antisalmonella, hepato-protective, sedative, antiepileptic, anticonvulsant activities, and its neuromuscular blocking effect of succinylcholine [30-35].

Table 2: Antimicrobial activity of the *Eupatoriun africanum* essential oil and its major constituent.

Microorganism (Gram)	Samples concentrations (µg/mL)			
	Essential Oil		β-eudesmol	
	250	125	250	62.5
Escherichia coli (-)	MIC		MIC	
Staphylococcus aureus (+)		MIC		MIC
Pseudomonas aeruginosa (-)		MIC		MIC
Proteus vulgaris (-)	MIC		MIC	

4. Conclusions

The chemical analyses of the *Eupatorium africanum* essential oil by GC/MS allowed the identification of 33 constituents, representing 97.34% of the total oil. The main constituent was β-eudesmol (49.10%) and the yield of essential oils was 0.4%. Oxygenated sesquiterpenes were the major components of the oil (82.64%). Sesquiterpenes (12.77%) and monoterpenes (1.93%) represented the minor constituents. Polyoxygenated sesquiterpenes, although in very low concentrations, have been detected in this oil. Aromatic compounds were not present in this oil. The oil exhibited moderate antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and a weak antibacterial activity against *Escherichia coli* and *Proteus vulgaris.* The major constituent of the oil was isolated and identified. It showed a strong antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and a weak antibacterial activity

against *Escherichia coli* and *Proteus vulgaris.*
The present study represents the first comprehensive analyses of the oil obtained from the leaves of *Eupatorium africanum.*

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