

Evaluation of the correlation between the chemical profile and the antalgic and anti-proliferative activities of essential oil of *Elionurus hensii* K. Schum

Aubin Nestor Loumouamou^{1,2*}, Kévin Bikindou^{1,2}, Hermeline Ntalani², Thomas Silou^{1,4}, Pierre Chalard^{3,5}, Ombeline Danton^{3,5}, Laetitia Delort^{6,7}, Caroline Decombat^{6,7}, Florence Caldefie-Chezet^{6,7}, Cathérine Rubat-Coudert⁸ and Gilles Figueredo⁹

¹Equipe Pluridisciplinaire de Recherche en Alimentation et Nutrition (EPRAN), Faculté des Sciences et Techniques, Université Marien Nguabi, BP 389, Brazzaville, Congo.

²Institut National de Recherche en Sciences Exactes et Naturelles (IRSEN), Cité scientifique de Brazzaville, BP 2400, Congo.

³Université Clermont Auvergne, SIGMA Clermont, Institut de Chimie de Clermont-Ferrand, BP 10448, F-63000 Clermont-Ferrand, France.

⁴Ecole Supérieure de Technologie (EST-Cataractes), Brazzaville, Congo.

⁵CNRS, UMR 6296, Institut de Chimie de Clermont-Ferrand, F-63178 Aubière, France.

⁶Université Clermont Auvergne, Université d'Auvergne, UFR Pharmacie, UMR 1019, Unité de Nutrition Humaine, Equipe ECREIN, CLARA, Clermont-Ferrand, France.

⁷INRA, UMR 1019, Unité de Nutrition Humaine, CRNH Auvergne, Clermont-Ferrand, France.

⁸Université Clermont Auvergne, Université d'Auvergne, NEURO-DOL, BP 10448, F-63000 Clermont-Ferrand, France.

⁹Laboratoire d'analyse des Extraits végétaux et des Arômes (LEXVA), Biopole, Clermont-Limagne, Saint-Beauzire, France.

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ABSTRACT

Elionurus hensii is usually used by peasant populations as a théiforme drink to relieve aches. The aim of this study was to evaluate the antalgic activity (AA) and antiproliferative activity (ATA) of essential oil of *Elionurus hensii* (VEH). Essential oils from the aerial parts and roots was extracted by hydrodistillation and analyzed by GC and GC/MS. AA was examined by using test cramps. This method consists in inducing cramps in the mouse by intraperitoneal injection of 0.6% acetic acid solution and to determine any inhibition of these cramps by the compounds contained in the VEH. Cytotoxicity of the essential oil was evaluated in order to assess their ATA on cancer cells MCF-7 using resazurin test. The study was carried out by considering 6 samples of VEH whose contents of major compounds vary. The major constituents are p-menthadienol isomers and limonene for samples from the aerial part, aristolone and limonene for samples from the roots. The most significant AA (inhibition percentage = 56.41%) was observed with a VEH containing p-menthadienol isomers (40.25%) and limonene (15.85%). The VEH containing limonene (20.21%) and aristolone (15.16%) also inhibit cramps with a percent inhibition of 48%. The pure aristolone extract of the essential oil inhibits to 36%. These first results confirm the traditional use of *Elionurus hensii* by peasant populations. The results of the resazurin test showed that the ATA is dose-dependent. VEH from roots, exhibited better anti-proliferative activity compared to the VEH from the aerial part. However, this activity is low.

Keywords: *Elionurus hensii*, essential oil, chemical compounds, GC/MS analysis, antalgic and anti-proliferative activities.

*Corresponding author. E-mail: aubinnestor@yahoo.fr.

INTRODUCTION

Elionurus hensii belongs to the genus *Elionurus* which contains about twenty species already identified. In Congo-Brazzaville, the geographical distribution of this species seems to be restricted to the "plateau des cataractes" (80 km south of Brazzaville) where it grows spontaneously. This is a transition zone between the southern and the northern part of Congo, limited to the north by the "plateaux Batéké", to the southeast of Congo river and DRC, to the west by the Ndouo (Niari) and to the northwest by the foothills of Chaillu massif (Bikindou, 2017). *E. hensii* is a perennial grass composed of culms 60 to 100 cm long, with strongly developed side branches forming blades 7 to 10 cm long, 2 to 3 cm wide that flower at maturity (Clayton et al., 2008). *E. hensii* is usually used by local people as a théiforme drink to relieve aches, which even justifies its vernacular name "tikoni". However, no scientific studies have been done to confirm these traditional practices.

This is the first study concerning the evaluation of the antalgic and antiproliferative activities of oils from *E. hensii*. The first explorations on the chemical composition showed that the essential oil from the aerial parts (stems and flowering tops) and roots of this species present two chemical profiles. The aerial part is rich in monoterpene alcohols which are the p-menthadienol isomers: cis- and trans-p-mentha-2,8-dien-1-ol, cis and trans p-mentha-1(7), 8-dien-2-ol. The roots are rather rich in sesquiterpene compounds which the main component is aristolone (Silou et al., 2006). This chemical composition has been recently confirmed through a study of the seasonal variation of the chemical profile (Loumouamou et al., 2016, 2017). The essential oils of the species rich in p-menthadienol isomers present many biological properties. It is the case of *Cymbopogon densiflorus* and *C. giganteus* whose essential oils exhibit antimicrobial properties. Indeed, these species are the subject of many uses: the crushed leaves of *C. densiflorus* are used as a treatment for rheumatism in Gabon, the flower head is smoked in a pipe as a cure brochial affections in Malawi, and the plant sap is used in the Congo-Brazzaville as a treatment for asthma and calm fits (Menut et al., 2000; Boti et al., 2006; Akhila, 2010). Aristolone is also an interesting constituent since it is likely to induce an antalgic activity (Wu et al., 2004). The probable influence of the p-menthadienol isomers on the biological properties of these species has inspired the recent study on the optimization of the extraction of this components contained in the essential oil from *E. hensii* (Loumouamou et al., 2017).

Furthermore, the antioxydant activities of essential oils and solvent extracts of *E. hensii* from Congo-Brazzaville was studied (Yang et al., 2013). The essential oil did not exhibit significant antioxydant activity in terms of DPPH and ABTS⁺ scavenging ability, and exhibited only slight Fe³⁺ reducing ability. However, the antioxydant activity of the solvent extracts was found important.

MATERIALS AND METHODS

Plant material

The plant material consists of the aerial parts (stems, leaves and flowering tops) and roots were collected on three sites: Loufoulakari (L) located at -4.4816 (latitude in decimal degrees), 14.9381 (longitude in decimal degrees), Campus rural (C) located at -4.4697 (latitude), 14.9450 (longitude) and Sese (S) located at -4.4125 (latitude), 14.6805 (longitude) and at different period of the year : February (f), May (m), June (j), November (n) and December (d). These harvest periods include periods of drastic reduction in rainfall (the dry season which covers the months of May and June) and periods of heavy rainfall (the rainy season which covers the months of November and December).

Extraction of the essential oil

Volatile extracts of *E. hensii* (VEH) has been obtained from dry plant by steam distillation using Clevenger type apparatus (Clevenger, 1928). Each time, 300 g of vegetable material, consisting either only of the roots or the aerial parts are placed in the plant tank above the balloon containing 500 ml of water and subjected to distillation. The organic phase from the distillation is separated from the aqueous phase by extraction with diethyl ether. The organic phase is dried over anhydrous sodium sulfate to remove traces of water and the essential oil is recovered after evaporation of the diethyl ether and then submitted to the chromatographic analysis.

GC analysis

The quantitative analysis of the essential oil was carried out using an Agilent gas chromatograph model 6890 equipped with a DB5 column (20 m x 0.18 mm; 0.18 µm). The oven temperature was programmed to 50°C for 3.2 min, then heated to 300°C at a rate of 10°C/min. The temperatures of the injector and the flame ionization detector (FID) were maintained at 280°C. The essential oils were diluted in acetone to 3.5% (v/v) and injected in split mode (1/60); hydrogen was used as the carrier gas (1 ml/min), and the injection volume was 1 µl. At the same time, a solution of n-alkanes (C8-C30) was analyzed under the same conditions to calculate retention indices (RI) using the Van den Dool and Kratz equation (Van Del Dool and Kratz, 1963). The relative concentrations of the compounds were calculated from the peak area obtained by gas chromatography without using correction factors.

GC/MS analysis

Qualitative analysis was performed using an Agilent gas chromatograph model 7890 coupled to a Agilent mass spectrometer model 5975 equipped with a DB5 MS column (20 m x 0.18 mm; 0.18 µm). The oven temperature was 50°C and remained constant for 3.2 min; then, it was increased to 300°C at a rate of 8°C/min. The injector temperature was 280°C. Ionization was obtained by electron impact at 70 eV, and the electron multiplier was maintained at 2200 eV. The temperature of the ion source was 230°C. Mass spectral data were acquired in the scan mode in the range m/z 33–450. The flow of carrier gas (helium) was set at 0.9 ml/min. The identification of the compounds was made by comparison of their mass spectra and RI with those of libraries such as Adams (2012), NIST (2008), König et al. (2001) and those of laboratory.

Antalgic activity (AA)

In order to verify the traditional practice of using *E. hensii* as theiforme drink to relieve pain, we have realized tests to evaluate the antalgic activity. The method used is that described by Collier et al. (1968) and Musa et al. (2008). This method, also called Writing test or test cramps, is still widely used (Gangwar et al., 2016). It consists of inducing cramps in the mouse by intraperitoneal injection of 0.6% acetic acid solution and to determine any inhibition of these cramps by the test compound. The pain is described as cramping and manifests itself in mice by a stretching movement of the hind legs and torsions of the dorsal-abdominal musculature.

The study was conducted at the Faculty of Pharmacy of the University of Auvergne (France) on male mice, in white color and swiss breed, weighing 30 g. The animals were coming from the livestock JANVIER and arrived to the pet store of laboratory one week before the test, with a weight between 16 and 18 g. The protocol was approved by the ethics committee for animal experimentation in Auvergne-France (No. record of this committee: C2E2A-O2).

The protocol implemented were composed of three batches of 8 mice distributed as follows: (i) the first batch had receive by gavage a solution of Tween 20 prepared in water for HPLC; (ii) the second batch had been treated with the essential oil at a dose of 100 mg/kg of body weight; (iii) the third batch had received the oral morphine at a dose of 1 mg/kg of body weight; 30 min after administration of the products, acetic acid prepared in the 0.6% of physiologic salt solution (0.3 ml/kg) is injected to mice by intraperitoneally. The number of cramps had been counted for 20 min. The antalgic activity was estimated as percent inhibition cramps by the following formula:

$$\% I = [(Average\ number\ of\ cramps\ in\ the\ control\ batch - average\ number\ of\ cramps\ in\ the\ test\ batch) / (average\ number\ of\ cramps\ in\ the\ control\ batch)] \times 100.$$

All the data of control and treated animals were analyzed using the student t-test, further the significance difference (P values <0.01) was calculated between mean values. The values expressed after the analysis were average \pm standard error of means (SEM).

Antiproliferative activity (ATA)

Cytotoxicity assays were conducted at the Laboratory of Plant Sciences and pharmaceutical Fungal EA 4233 team «Nutrition Carcinogenesis and therapy antitumorale» of the Faculty of Pharmacy of Auvergne University. The ATA was assessed using the resazurin reduction test as described by O'Brien et al. (2000). The tests were carried out on cancer cells of human breast adenocarcinoma MCF-7 (ATCC-HTB-22). According to the cells growth profile, MCF-7 cells were seeded into the wells of microplates at a concentration of 50,000 cells/ml. The microplates were kept in an incubator at 37°C, in an humidified atmosphere containing 5% carbon dioxide. After 24 h, the cells were treated with the VEH initially dissolved in dimethylsulfoxide (DMSO). In parallel, for each tested essential oil, a control of DMSO (solvent of dilution of the EO) is produced. Plates were returned into the incubator for 72 h under the same conditions. After this final period of incubation, the culture medium was replaced with a solution of resazurin (25 mg/ml) which in the presence of metabolically active cells is oxidized into resorufin, fluorescent at 590 nm. The intensity of fluorescence was proportional to the number of viable cancer cells. Fluorescence was then measured using a plate reader (Fluoroskan Ascent[®]FL, Thermo Electron Corporation France) at 590 nm.

ATA was evaluated using the following concentration ranges: 0.04% (372 μ g/ml), 0.02% (186 μ g/ml) and 0.01% (93 μ g/ml) for

essential oil and 10, 25, 50 and 100 μ g/ml for aristolone. The intensity of fluorescence obtained after reading with Fluoroskan Ascent (expressed in arbitrary units) was converted into percentage of inhibition of proliferation relative to cells proliferation in the control (DMSO). It was established that DMSO got no influence on cell proliferation, in comparison with a control performed without DMSO under the same conditions.

RESULTS AND DISCUSSION

Chemical composition of the volatile extracts studied

VEH involved in this study are from the aerial parts (Cd, Sm and Ln) and roots (Cf, Ln and Sn). The VEH of the aerial part are rich in p-menthadienol isomers, the overall content is of the order of 40%. The contents of cis- and trans-p-mentha-1(7), 8-dien-2-ol (23 to 39%) are twice as high as those of the cis- and trans-p-mentha-2,8-dien-1-ol (10 to 16%). These contents are also found in the same proportions in the essential oil of *C. giganteus* (Menut et al., 2000 ; Boti et al., 2006): cis- and trans-p-mentha-1(7),8-dien -2-ol (31.7 to 49.3%) cis- and trans-p-mentha-2,8-dien-1-ol (26.7 to 27.6%). However, in the essential oil of *C. densiflorus* the content of cis-p-mentha-1(7),8-dien-2-ol (11.1%) is low and that of trans-p-mentha-2,8-dien-1-ol (22.4%) is greater (Chisowa, 1997). The limonene is the other major constituent. In the Sm VEH the content of limonene is low (2%), but it is higher in the VEH of Cd and Ln (15.85 and 19.28%, respectively). Concerning the essential oil from the roots, the aristolone and the limonene are the two main major constituents. The Cf and Sn VEH are rather rich in aristolone (44.55 and 32.29%, respectively) whereas the Ln VEH is rich in the limonene (30.44%). It should be noted that the essential oil of the roots of *Elionurus muticus* is much richer in aristolone (72.1%) than that of *Elionurus hensii* (Chagonda et al., 2012). The complete chemical composition of the six VEH is presented in Table 1.

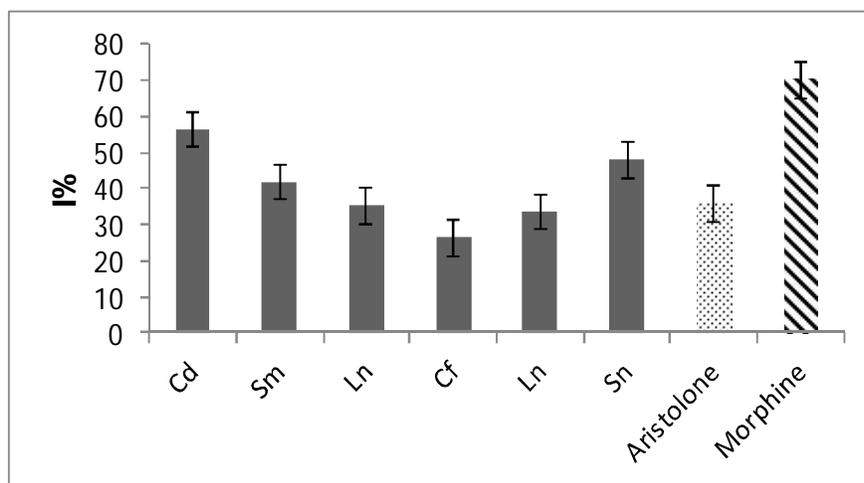
Antalgic activity

The AA of VEH from the aerial part and the roots of *Elionurus hensii* was evaluated by considering 6 samples whose chemical profile in major constituents are presented in Table 1. Analysis of the results shows that mice treated with VEH reducing significantly cramps caused by the injection of acetic acid, compared to the control group. The average number of cramps recorded for samples Cd, Sm, and Ln is respectively 20.13, 24.00 and 29.88, which corresponds to a percentage of inhibition respectively of 56.41, 41.83 and 35.29% (Figure 1). The t-test values of student ($p < 0.05$) are respectively 5.49, 2.33 and 2.30, the theoretical t-test is 2.16. One should note that morphine, which is the reference, exhibits a number of means cramps equal to 13.75 (70.22%). The antalgic effect is more important for sample Cd because it significantly inhibits cramps. The

Table 1. Chemical composition of the VHE.

Components	RI	Aerial part			Roots		
		Cd*	Sm	Ln	Cf	Ln	Sn
Tricyclene	922	0.37	0.41	0.35	0.80	0.35	0.23
α -thujene	925	-	-	-	0.22	0.22	-
α -pinene	933	0.27	-	0.25	1.45	0.78	0.4
camphene	950	1.16	6.07	1.25	2.57	1.24	0.88
Sabinene	973	-	-	0.05	0.27	0.21	-
myrcene	989	0.31	-	0.48	0.61	1.05	0.77
ortho-cymene	1025	-	1.82	0.95	0.35	1.11	0.70
limonene	1028	15.85	2.09	19.28	8.75	30.44	18.41
1.8-cineole	1032	-	0.21	-	2.61	4.51	2.48
Terpinolene	1085	-	-	0.06	-	0.27	0.17
trans-p-mentha-2,8-dien-1-ol	1125	10.65	6.16	16.22	0.19	2.15	0.89
cis-p-mentha-2,8-dien-1-ol	1137	5.84	4.56	6.39	0.13	1.63	0.62
Pinocarvone	1163	0.17	0.51	0.20	-	0.25	-
Terpinen-4-ol	1182	-	-	1.73	0.20	-	-
trans-p-mentha-1(7),8-dien-2-ol	1192	11.02	20.16	12.89	0.42	3.49	2.80
trans-carveol	1221	6.65	7.5	1.83	0.15	1.6	-
cis-p-mentha-1(7),8-dien-2-ol	1230	12.74	19.01	13.52	0.34	3.23	2.19
cis-carveol	1233	0.74	-	5.36	-	-	-
carvone	1245	3.34	4.49	3.45	0.15	1.28	0.90
piperitone	1255	1.47	0.61	0.41	0.27	1.02	0.64
Undecan-2-one	1292	2.98	9.03	3.52	0.80	6.20	-
α -gurjunene	1412	2.3	-	-	0.98	-	-
β -gurjunene	1435	-	-	0.14	9.98	4.71	4.43
Tridecan-2-one	1495	1.96	5.5	1.97	-	2.60	2.34
Nerolidol <E>	1561	2.3	-	0.28	-	-	0.60
maaliol	1575	-	-	-	2.51	1.83	-
intermedeol	1671	0.82	2.9	3.19	1.6	3.03	5.91
aristolone	1764	0.41	-	0.6	44,55	14.08	32.29
Total p-menthadienol isomers (%)		40.25	49.89	49.02	1.08	10.5	6.5

*Cd: campus-december; Sm: sese-may; Ln: loufoulakari-november.
Cf: campus-february; Sn: sese-november.

**Figure 1.** Inhibition percent of VEH, aristolone and morphine.

other two samples (Sm and Ln) also present a real inhibition, but moderate (the t-test values are low).

At the roots level, four samples of VEH was tested: three VEH (Cf, Sn and Ln) and one sample of the aristolone resulting from the splitting of VEH. The results holistically show a low average number of cramps for essential oil samples compared to the witness. Sn, Ln and Cf VEH inhibit cramps respectively with 48, 33.57 and 26.35%. This inhibition is significant with Sn sample because his t-test is equal to 3.29 ($p < 0.05$) and not significant for samples Ln and Cf with the t-test values equal to 2.20 and 1.05 (theoretical t-test is 2.16). The aristolone produced an average number of cramps equal to 40.33 with an inhibition percentage of 36%, the t-test value is 4.02. At a dose of 13 mg/kg (body weight), aristolone significantly inhibits abdominal cramps. The aristolone shows a real antalgic effect, but low compared to that of Sn VEH.

Comparing these results with those of the essential oil of *Ageratum conyzoides*, a species of the Congolese flora used in African traditional medicine for headaches and whose antalgic effect has been scientifically demonstrated (Abena et al., 1995, 1997), it turns out that the results obtained from the VEH are very promising.

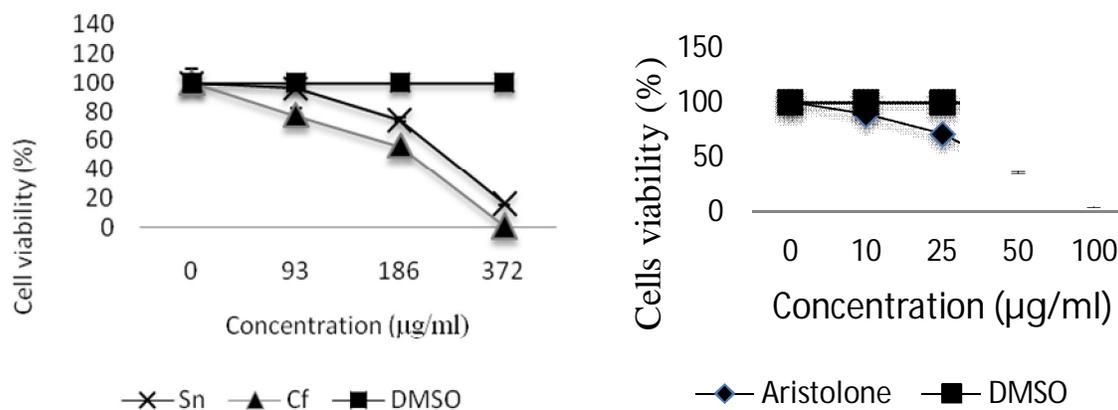


Figure 2. Dose-dependent anti-proliferative activity of essential oil and aristolone.

CONCLUSION

This study showed very interesting and promising results on the antalgic activity of the analyzed VEH. For the aerial part, the most significant antalgic effect (inhibition percentage = 56.41%) was observed with a VEH containing trans-p-mentha-2,8-dien-1-ol (10.65%), cis-p-mentha-2,8-dien-1-ol (5.84%), trans-p-mentha-1(7),8-dien-2-ol (11.02%), cis-p-mentha-1(7),8-dien-2-ol (12.74%), trans-carveol (6.65%) and limonene (15.85%). Concerning the roots, the VEH containing 20.21% of limonene and 15.16% of aristolone inhibit significantly cramps with an inhibition percentage of 48%. The

Anti-proliferative activity

The ATA from the aerial part and the roots of *Elionurus hensii* was evaluated by considering 6 VEH (the same samples considered for AA). The VEA of the roots is slightly found more active than that of the aerial part. Samples Cd, Sm and Ln showed little anti-proliferative activity against MCF-7 cells. The samples Sn ($IC_{50} = 244.45 \mu\text{g/ml}$), Cf ($IC_{50} = 244.3 \mu\text{g/ml}$) and aristolone ($IC_{50} = 37.77 \mu\text{g/ml}$), from roots, exhibited better anti-proliferative activity compared to VEH from the aerial part. The results show that the inhibition of MCF-7 cells is very important when the concentration is high: the ATA is dose-dependent (Figure 2). This activity can be justified by the fact that the samples from the roots are rich in sesquiterpene compounds such as aristolone (Wu et al., 2004; Shi et al., 2009) and intermedeol (Ercil et al., 2001).

Indeed, it seems that the high content in aristolone of essential oil from roots is largely responsible for the observed activity. Furthermore, the very low activity observed with essential oil can find the explanation in a potential antagonistic effect between the different constituents of the essential oil.

aristolone resulting from the splitting of VEH inhibits to 36%. These first results show a real antalgic effect of VEH, which makes it possible to validate the use of *Elionurus hensii* by local people for to relieve aches. It would be interesting to deepen the studies on VEH to better understand the correlation between the chemical composition and the antalgic activity and to identify the molecules responsible for this activity. The ATA from the aerial part and the roots of *Elionurus hensii* is low. The essential oil of the roots is slightly found more active than that of the aerial part. This difference activity observed may be largely due to the presence of sesquiterpenes which predominate in the essential oil of the roots.

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