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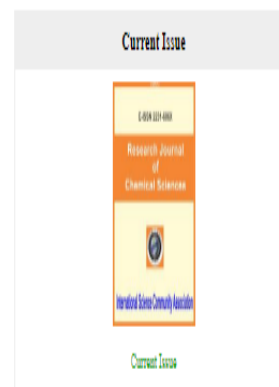
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Volatile oil composition and antiproliferative activity of *Hyptis spicigera* Lam against human breast adenocarcinoma cells MCF-7

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Abstract

This study reported the chemical composition and the evaluation of the *in vitro* antiproliferative activity of volatile extracts of *Hyptis spicigera* (HS) on breast cancer cells MCF-7. Essential oils (EO) HS1 and HS2 extracted from leafy stems of *H. spicigera* by hydrodistillation after different harvesting periods were characterized by gas chromatography/flame ionization detector (GC/FID) and gas chromatography/mass spectrometry (GC/MS). The cytotoxicity of these volatile extracts was tested in order to assess their antiproliferative activity on cancer cells MCF-7 by the resazurin test. Chromatographic analysis revealed several major compounds depending on the harvest period of vegetable. Sample HS1 was characterized by α -pinene (16.9%), sabinene (13.8%), β -pinene (9.6%) and 1,8-cineole (9.6%), while HS2 was potentially rich in β -caryophyllene (53.2%), caryophyllene oxide (5.4%) and α -humulene (3.8%). Biological investigations performed have shown that HS1 and HS2 were cytotoxic against MCF-7 cells. Inhibitory concentrations values (IC_{50}) determined for this purpose were 170 and 84 μ g/mL respectively for HS1 and HS2. Major compounds of the EO should be responsible for this inhibitory activity.

Keywords: *Hyptis spicigera*, volatile extract, MCF-7, β -caryophyllene, α -pinene, Benin.

Introduction

Hyptis spicigera (Lamiaceae) is an aromatic plant annual, hairy, smartweed, woody at the base. Leaves are opposite and lanceolate. Inflorescences are terminal, dense, cylindrical spike consisting to very small flowers with white corolla and mauve mark on the lips¹. *H. spicigera* is a ruderal herbaceous. It grows in savannas periodically flooded and fields. Although endemic to Brazil, it is widespread in tropical Africa and in Asia (figure-1).

The decoction is used as bath water or tea, as eupneic, or expectorant to treat bronchial secretions. The powder obtained from the aerial organs is used as an antimigraine drug. Fresh inflorescences of this plant are used to treat headaches and coryza. In most Southern-Africa countries, this species is exploited by farmers to control grain infestations in storage. *H. spicigera* is also used by farmers against insect pests and to fight mosquitoes²⁻⁵. Phytochemical investigations lead to the isolation of seven (7) new labdane diterpenes⁶. Due to its aromatic character, essential oil produced from this plant has been subjected to numerous studies. Indeed, a high proportion of β -caryophyllene (57-66%) was revealed in the volatile extract

of *H. spicigera* from Burkina Faso⁷. On the other hand, the proportion of β -caryophyllene (23-28%) of *H. spicigera* reported from the sample of Mali was down compared to those of this sesquiterpene compound noted from variety harvested in Nigeria (68%)^{8,9}. Otherwise, the presence of β -caryophyllene (33.8%), α -bergamotene (11.3%) and α -caryophyllene (7.4%) had been revealed in majority in the sample from Togo¹⁰. The sample of *H. spicigera* from Cameroon contained a large proportion of terpenes with a relatively low level of β -caryophyllene (19.1%)¹¹. Further investigations on the insecticidal, anticonvulsant, sedative and antimicrobial properties of the plant have been highlighted in the literature¹²⁻¹⁵. Spicigerolide (6-tetraacetyl oxyheptenyl-5,6-dihydro- α -pyrone), isolated from *H. spicigera* of Mexico was found to be active on cancer cells line KB¹⁶. Nevertheless, the antiproliferative character of the essential oil of *H. spicigera* has not been studied in Benin. The aims of the present work is to determine the chemical composition by GC/FID and GC/MS of volatile oils extracted from leafy stems of *H. spicigera* harvested in Benin and to evaluate their cytotoxic activity on cancer cells line MCF-7.



Figure-1
Hyptis spicigera Lam

Material and Methods

Plant Material: The aerial parts of *H. spicigera* were harvested at Tele (Benin) in January (HS1) and October (HS2) 2010. In the laboratory, plant material was stored at 20°C and protected from sunlight during the extractions. The identification and authentication were made at the National Herbarium of Abomey-Calavi University (Benin). Voucher number was assigned to the specimen.

Extraction of essential oils: Essential oils were obtained by hydrodistillation using a Clevenger-type apparatus for a period of three hours. The volatile oils collected after decantation was dried over anhydrous sodium sulfate and then stored at 4°C in amber glass vials until analysis.

Gas chromatography-mass spectrometry analysis of the volatile oils: The essential oils were analysed on a AGILENT gas chromatograph Model 7890, coupled to a AGILENT mass spectrometer model 5975 equipped with a DB5 MS column (20m X 0.20 mm. 0.20 μ m) programming from 50°C (5 min) to 300°C at 8°C/min with a 5 min hold. Helium was used as the carrier gas (1.0 mL/min); injection in split mode (1: 250); injector and detector temperature 250 and 280°C respectively. The mass spectrometer worked in electron impact mode at 70 eV; electron-multiplier: 1500 eV; ion source temperature: 230°C; mass spectra data were acquired in the scan mode in *m/z* range 33-450. The essential oil was diluted in hexane: 1/30.

Cancer cells: The cell line under investigation was human breast adenocarcinoma (MCF-7). MCF-7 stands for Michigan Cancer Foundation-7, in reference to the Detroit Institute where the line was established^{17,18}.

Cytotoxicity assays: Biological investigations were performed on cancer cells of human breast adenocarcinoma MCF-7. 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 hours, the cells were treated with volatile oils initially dissolved in dimethylsulfoxide (DMSO) and sonicated.

In parallel, for each tested essential oil, a control of dimethylsulfoxide DMSO (solvent of dilution of the EO) is produced. Plates were returned into the incubator for 72 hours 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 resofurin, 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.

The antiproliferative activity was evaluated using the following concentration ranges: 0.04% to 0.01% for HS1 and 0.04% to 0.002% for HS2. 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

Yield and chemical composition of essential oils: The yield of essential oil extracted from leafy stems of *H. spicigera* varied from 0.23% (HS1) to 0.2% (HS2) depending on harvest periods. These values were different from those obtained respectively from varieties of *H. spicigera* from Mali (0.1-0.3%), Cameroon (0.12%), Nigeria (0.1%) and Togo (1.2%)⁸⁻¹¹. These differences could be attributed to the geographical characteristics of the ecological zone and the vegetative state of the plant species. The chemical composition of the essential oils is given in table-1.

According to table-1, 58 to 60 compounds were identified representing 98.9 to 92.8% of the total identified in investigated essential oils. Depending on the period of collection of vegetable, chemical compositions of the essential oils, analyzed by GC/MS, varied remarkably in hydrogenated and oxygenated terpenes. Indeed, a large proportion of hydrogenated monoterpenes (62.5%) and oxygenated monoterpenes (21.5%) were noted in HS1. Contrary, sample HS2 was dominated by hydrogenated sesquiterpenes (68.1%) and oxygenated sesquiterpenes (8.5%). The results of the chromatographic analysis showed that essential oils HS1 and HS2 were characterized by α -thujene (6.5%), α -pinene (2.5-16.9%), sabinene (13.8%), β -pinene (9.6%), 1,8-cineole (9.6%), γ -terpinene (6.0%); terpinolene (5.3%), β -caryophyllene (7.0-53.2%), α -humulene (3.8%), caryophyllene oxide (2.1-5.4%) and cembrene A (2.6%). These main components, apart from their concentrations in each of the two samples, had already been revealed in earlier works⁸⁻¹¹. On the other hand, the rate of α -thujene (6.5%) in HS1 is higher than those obtained in HS2 and essential oils extracted from *H. spicigera* collected in Mali, Togo, Cameroon and Nigeria⁸⁻¹¹.

Table-1
Chemical composition of essential oils of HS1 and HS2

N°	Compounds	KI	HS1	HS2
1	α-thujene	923	6.5	0.6
2	α-pinene	931	16.9	2.5
3	sabinene	970	13.8	1.1
4	β-pinene	974	9.6	1.4
5	octen-3-ol	978	0.1	0.2
6	myrcene	987	1.0	0.1
7	octan-3-ol	995	-	0.1
8	α -phellandrene	1003	1.7	0.4
9	α -terpinene	1014	1.1	0.1
10	p-cymene	1023	3.6	1.4
11	limonene	1027	2	0.5
12	β -phallandrene	1029	-	0.3
13	1,8 cineole	1033	9.6	-
14	(E)- β -ocimene	1046	0.2	0.1
15	γ-terpinene	1058	6.0	1.2
16	sabinene cis hydrate	1071	0.4	-
17	terpinolene	1085	5.3	1.6
18	p-cymenene	1089	0.2	0.1
19	linalool	1099	-	0.1
20	sabinene trans hydrate	1101	0.5	-
21	2-methyl-1-butyle 2-methylbutanoate	1103	0.3	-
22	2-methyl-1-buthyle isovalerate	1109	0.7	-
23	(E)-thujone	1117	0.1	-
24	cis-p-menth-2-en-1-ol	1125	0.1	-
25	campholenal	1127	0.1	-
26	nopinone	1138	0.1	-
27	(E)-pinocarveol	1141	0.4	-
28	(E)-p-menth-2-en-1-ol	1143	0.5	-
29	(E)-verbenol	1147	0.5	-
30	sabinacetone	1158	0.1	-
31	pinocarvone	1162	0.1	-
32	δ -terpineol	1172	0.2	-
33	borneol	1174	0.2	-
34	terpinen-4-ol	1181	1.9	-
35	p-cymen-8-ol	1187	0.5	0.1
36	myrtenal	1196	0.6	0.1
37	myrtenol	1197	0.6	-
38	verbenone	1207	0.1	-
39	cuminic aldehyde	1243	0.1	-
40	carvacrol	1303	0.1	-
41	δ -elemene	1336	-	0.1
42	eugenol	1351	-	0.1
43	α -ylangene	1370	0.1	0.3
44	α -copaene	1376	-	0.2
45	β -bourbonnene	1384	0.1	0.3
46	β -elemene	1389	0.1	0.3
47	isocaryophyllene	1405	0.1	0.4
48	α -cedrene	1410	-	0.1
49	β-caryophyllene	1426	7.0	53.2
50	γ -elemene	1430	-	0.7
51	α -guaiene	1436	-	0.3

52	guaia-6,9-diene	1443	0.1	0.5
53	guaia-1(5),6-diene	1448	0.2	-
54	selina-4(15),6-diene	1451	-	0.9
55	α-humulene	1459	0.4	3.8
56	γ -himachalene	1478	-	0.2
57	(E)- β -ionone	1479	-	0.1
58	germacrene-D	1484	0.2	1.3
59	δ -amorphene	1505	-	0.3
60	γ -cadinene	1516	0.3	1.3
61	δ -cadinene	1520	-	0.3
62	α -cadinene	1540	-	0.1
63	germacrene-B	1562	0.1	0.7
64	caryophyllene oxide	1586	2.1	5.4
65	γ -gurjunene	1592	-	0.3
66	γ -muurolene	1596	-	0.1
67	curzerenone	1601	-	0.2
68	humulene-epoxyde II	1613	0.1	0.3
69	1,10-di-epi-cubenol	1618	0.1	0.4
70	isospathulenol	1630	-	0.2
71	caryophylla-4(12),8(13)-dien-5- β -ol	1641	0.2	0.9
72	α -cadinol	1658	0.2	0.8
73	6,10,14-trimethyl pentadecan-2-one.	1842	-	0.2
74	thumbergene	1926	-	0.2
75	cembrene A	1957	0.1	2.6
76	isophyllocladene	1966	-	0.1
77	palmitic acid	1964	0.5	-
78	bicyclogermacrene	2022	-	1.0
79	valenrena-4,7(11)-diene	2045	-	0.9
80	phytol	2106	-	0.3
81	oleic acid	2144	0.4	0.5
82	octadecanoic acid	2167	1.0	-
Hydrogenated monoterpenes			62.5	9.9
Oxygenated monoterpenes			21.5	1.9
Hydrogenated sesquiterpenes			7.9	68.1
Oxygenated sesquiterpenes			2.6	8.5
Others			4.4	3.8
Total identified			98.9	92.2

Anti-proliferative activities against MCF-7 cells: The cytotoxicity activities of test samples (HS1 and HS2) were performed against MCF-7. Analysis of the results revealed a difference of behavior of MCF-7 cells in the presence of various volatile extracts of *H. spicigera*. IC₅₀ values of volatile extracts HS1 and HS2 were respectively 170 and 84 μ g/mL. Based on these values, it appeared that the two essential oils had inhibited the growth of MCF-7 cells. However, HS2 had a higher efficiency than HS1. This activity was justified by the presence of α -pinene, sabinene, β -pinene, 1,8-cineole, β -caryophyllene, caryophyllene oxide, α -humulene which were identified as having antiproliferative properties on cancer MCF-7¹⁹⁻²². Indeed, essential oil of *Eugenia zuchowskiae* from Costa Rica, rich in α -pinene (23.3%), β -caryophyllene (13.2%), α -humulene (13.1%) was cytotoxic to MCF-7 cells¹⁹. Also, 1,8-cineole, the main component of essential oils extracted from of *Laurus nobilis* and *Salvia tribola* had demonstrated significant

inhibitory activity on cells²⁰. The antiproliferative ability of essential oils of *Heteropyxis dehniae* and *Pentadesma butyraceae*, respectively rich in caryophyllene oxide and β -caryophyllene, was also demonstrated on the mammary adenocarcinoma cells MCF-7^{21,22}. Volatile extracts of current work were less effective than that of *Origanum vulgare* (IC50 = 30.1 μ g/mL) and the methanolic extract of *Vitex trifolia*, but HS2 possessed higher antiproliferative activity on MCF-7 cells than that of essential oil of *Laurus nobilis* (IC50 = 101.7 μ g/mL)^{20,23}.

Conclusion

This work was devoted to the determination of the chemical composition by GC/FID and GC/MS and has evaluated the antiproliferative activity of two volatile extracts (HS1 and HS2) of the leafy stems of *H. spicigera* harvested in Benin. HS1 is potentially rich in monoterpene compounds (α -pinene: 16.9% sabinene: 13.8% β -pinene: 9.6%, 1,8-cineole: 9.6% α -thujene: 6.5%, γ -terpinene: 6.0% and terpinolene: 5.3%) while that of HS2 is mainly composed by sesquiterpene compounds (β -caryophyllene: 53.2%, caryophyllene oxide: 5.4%, α -humulene: 3.8%). The significant antiproliferative activity of HS2 essential oil could be due to its high content in sesquiterpene compounds.

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