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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL FROM LEAFY TWIGS OF *ACANTHOSPERMUM HISPIDUM* FROM THE MOUNTAIN DISTRICT OF CÔTE D'IVOIRE

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ABSTRACT

Acanthospermum hispidum DC. (Asteraceae) is a medicinal plant used in traditional medicine. Although antioxidant activity has been reported for solvent extracts of this species, information on its essential oil remains limited. This study aimed to characterise the essential oil obtained by hydrodistillation from leafy twigs collected in Kassiapleu, Côte d'Ivoire, and to evaluate its in vitro radical-scavenging activity using the DPPH assay. The oil yield was 0.039% (dried-mass basis). GC–MS analysis led to the tentative identification of 95 compounds, representing 90.06% of the reported composition. Sesquiterpenes predominated (79.39%); the main reported constituents were β-caryophyllene (25.64%), α-bisabolol (12.75%), bicyclogermacrene (11.91%), and germacrene D (8.03%). In the DPPH assay, the reported EC_{50} was 8 µg/mL for the essential oil and 6 µg/mL for vitamin C. These values suggest that, under the assay conditions used, the oil showed radical-scavenging activity close to that of the reference. However, the contribution of individual constituents cannot be established from the present data, and the compound assignments and assay results require confirmation through appropriate validation and reporting of replicate variability.

Keywords: *Acanthospermum hispidum*, Essential Oil, GC–MS, Antioxidant Activity, DPPH

1 INTRODUCTION

Since prehistoric times, plants have formed the basis of various therapies [1]. They are crucial for drug development as they contain numerous bioactive molecules [2]. According to the World Health Organization (WHO), nearly 80% of the populations of developing countries relies on traditional medicine for primary healthcare [3]. The use of plants in medicine has evolved over time. Nowadays, modern medicines are the result of lengthy and costly research processes

ranging from plant harvesting to the identification, isolation, and synthesis of active principles [4]. Numerous studies on the antioxidant activities of essential oils from a wide variety of aromatic plants demonstrate that these properties are linked to their chemical composition [5-6]. Khan et *al.* reported that antioxidant activity is linked to the presence of molecules such as sesquiterpenes [7]. Antioxidants play a significant role in the food industry as food preservatives and contribute to the prevention of chronic diseases combating oxidative stress [8-10]. In this regard, essential oils, with their antioxidant properties, offer a promising alternative in the search of new bioactive natural products. It is against this background that we turned our attention to *Acanthospermum hispidum*, a member of the Asteraceae family used in traditional medicine. The species is an annual (sometimes perennial) suffrutescent herb with a hairy stem, forming small bushes 50 cm or more in height. The interest of this plant lies in its widespread use by local populations to treat several diseases. Indeed, the plant is used in folk medicine to treat asthma, bronchitis, jaundice, malaria, vomiting, dysentery, fevers, intestinal pains and viral infections [11-14]. In Benin, leaf decoction and maceration are taken orally to treat abdominal pain; the maceration is also used orally to treat female infertility [15]. In Togo, leaf sap is prescribed as eye drops for eye conditions and applied topically for migraines and headaches; chewed leaves are used to treat animal bites and stings; and a stem decoction is taken orally to treat liver disorders [16]. Studies conducted on a sample from Burkina Faso revealed the presence of alkaloids in the powdered whole plant, as well as organic acids [17]. Other compounds such as flavonoids, tannins, and saponins have also been highlighted in solvent extracts of this plant [18-20]. Numerous studies have investigated the biological and pharmacological properties of the plant, including anti-inflammatory [21], antiviral [22, 23] Antimicrobial [24, 25], antiplasmodial [20, 26], antitrichomonal [27], and antibacterial [28]. The aim of this study was to contribute to the valorisation of the aromatic and medicinal plants of the Ivorian flora. In this context, we determined the chemical composition of the essential oil and evaluated its antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

2 MATERIAL

2.1 Plant material

Leafy twigs of *Acanthospermum hispidum* were collected in December 2022 at Kassiapleu, in the Man Department, Côte d'Ivoire, in a gallery forest near the University of Man. The plant was identified by a technician at the National Floristics Centre (CNF), Abidjan, using an existing herbarium specimen (voucher number H UJC 003383).

3 METHODS

3.1 Essential oil extraction

The essential oil (EO) was extracted from the leafy twigs by hydrodistillation. A condenser was fitted to a 6-litre round-bottomed flask containing 3 litres of water and 1271 g of three-day-dried plant material, cut into small pieces. The assembly was brought to the boil using a heating mantle for 2.5 h. The water vapour carried the volatile compounds towards the condenser. The condensed vapour, a heterogenous azeatropic mixture of water and essential oil, collected in a burette containing a small amount of distilled water. The EO was separated from the water by decantation, dried over anhydrous sodium sulphate (Na_2SO_4) to remove traces of water, and stored in a freezer until analysis. The extraction yield was calculated as the ratio of the mass of oil obtained to the mass of dried plant material (w/w).

3.2 Essential oil analysis

The essential oil was diluted in dichloromethane (1:100, v/v) and analysed using an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass spectrometer. A 1-µL aliquot was injected into an HP-5MS capillary column at 250 °C. The manuscript reports an initial oven temperature of 40 °C for 5 min, followed by a ramp of 2 °C/min for 15 min to 250 °C and a further ramp of 10 °C/min to 300 °C. Helium was used as the carrier gas at 1 mL/min; the MS source/detector temperature was 280 °C and the reported voltage was 1.4 kV. Ions with m/z 40–500 were recorded. Compounds were tentatively assigned by comparison of mass spectra and calculated retention indces with the NIST library and published data.

$$RI = 100\left[n + \frac{t_R(C_i) - t_R(C_n)}{t_R(C_{n+1}) - t_R(C_n)} \right]$$ [29] with :

- RI: Retention index of the unknown compound;
- n: carbon number of the linear alkane preceding the unknown compound;
- $t_R(C_i)$: retention time of the unknown compound;
- $t_R(C_n)$: retention time of the linear alkane preceding the unknown compound;
- $t_R(C_{n+1})$: retention time of the linear alkane following the unknown compound.

3.3 Evaluation of antioxidant activity of essential oil

The DPPH radical-scavenging assay was based on Brand-Williams et *al.* [30], with modifications. DPPH was dissolved in absolute ethanol to a final concentration of 0.3 mM. Essential-oil solutions were prepared at 2.5, 5, 10, 20, 50, 125, 250, and 500 µg/mL. For each measurement, 2.5 mL of test solution was mixed with 1.0 mL of DPPH solution and incubated in the dark for 30 min. A sample blank containing 2.5 mL of test solution and 1.0 mL of ethanol was prepared to correct for sample absorbance. The negative control contained 1.0 mL of DPPH solution and 2.5 mL of ethanol. Vitamin C was used as the positive reference. Absorbance was measured at 517 nm [31], and percentage inhibition was calculated using the equation provided in the manuscript [32].

$$\%I = \left[1 - \frac{(Abs_{test} - Abs_{blank})}{Abs_{control}}\right] \times 100$$

- Abs_{test}: Absorbance of the ethanolic solution of EO and DPPH.
- Abs_{blank}: Absorbance of the sample blank
- $Abs_{control}$: Absorbance of the ethanolic solution of DPPH.
- %I: Percentage of inhibition

The EC_{50} value was determined from the dose-response curve (%I versus concentration) obtained via Excel (from Microsoft Office LTSC 2021).

4 RESULTS AND DISCUSSION

4.1 Essential oil extraction

The essential oil extracted from the leafy twigs of *Acanthospermum hispidium* hydrodistillation was pale orange in colour, with an aromatic odour. The extraction yield, calculated on the dried material weight basis (w/w), was 0.039%. Yields reported for the same part of the plant by Timotou *et al.* [33] (0.0890 - 0.1737%) in Côte d'Ivoire and by Menut et *al.* [34] (0.1 - 0.2%) in Congo are higher than those obtained in the present study. The low yield, obtained in this study, may be explained by factors such as genotype, environmental conditions, harvest time and geographical origin [35].

4.2 Essential oil chemical composition

The chemical composition of the essential oil derived from leafy twigs of *A. hispidum* was analysed by gas chromatography coupled to mass spectrometry (GC/MS). Analysis of the chromatogram and mass spectra of the essential oil led to the tentative identification of 95 compounds, representing 90.06% of the total oil composition (Table 1). The chemical composition is dominated by sesquiterpene hydrocarbons (60.58%) followed by oxygenated sesquiterpenes (18.81%). Monoterpenes were present in small proportions: monoterpene hydrocarbons accounted for 3.20% and oxygenated monoterpenes for 2.33%. Other compounds accounted for 2.33% whereas non-determinated compounds represented 2.81% . β-caryophyllene (25.64%), α-bisabolol (12.75%), bicyclogermacrene (11.91%) and germacrene D (8.03%) were major compounds of the essential oil (Figure 1).

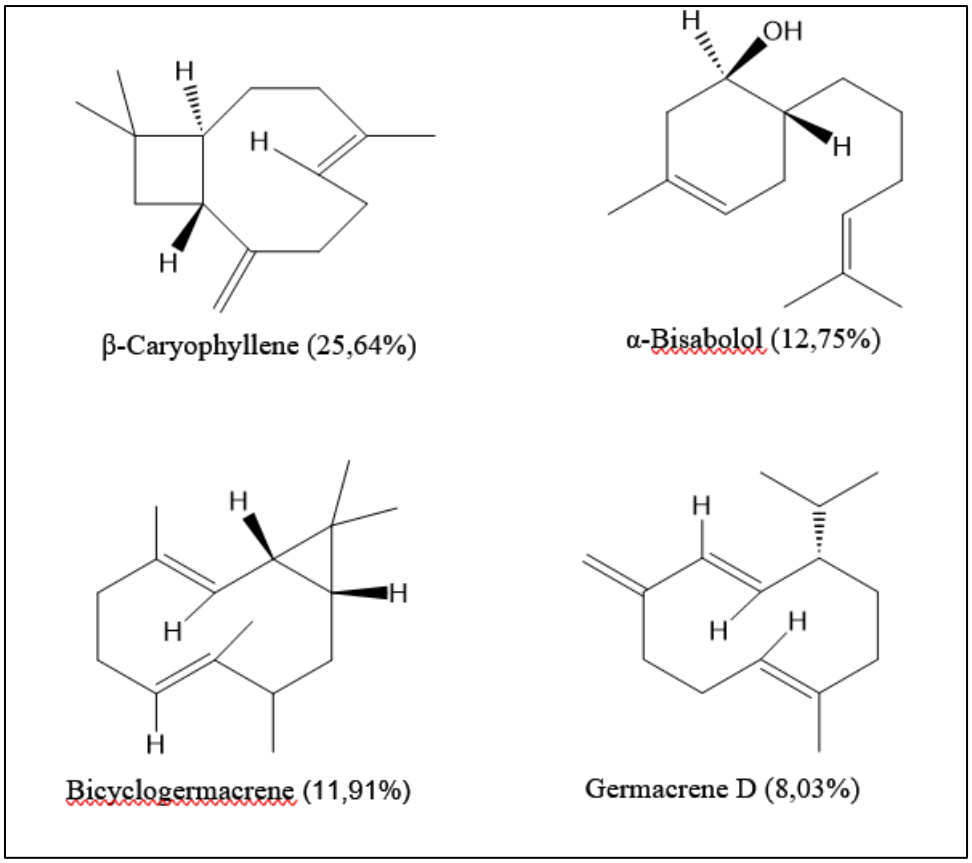

Figure 1: Main compounds of leafy twigs essential oil from of *A. hispidium*

Other sesquiterpenes were present at significant levels: β-cubebene (2.36%); δ-cadinene (2.13%); α-selinene (1.91%); α-copaene (1.85%) and caryophyllene oxide (1.10%). The monoterpene hydrocarbon fraction was led by α-pinene (1.65%). Among the oxygenated monoterpenes, the most abundant was thymol methyl ether (2.24%), while precocene I (1.28%) was the most abundant of the other compounds.

Table 1: Chemical composition of the essential oil of *A. hispidum* leafy twigs oil

N°	Components[a]	RT[b]	RI[c]	M/Z	%
1	α-Pinene	12.524	923	136	1.65
2	β-Pinene	15.256	966	136	0.31
3	Myrcene	16.638	987	136	0.10
4	α-Phellandrene	17.290	997	136	0.06
5	*p*-Cymene	18.757	1018	136	0.10
6	Limonene	19.005	1021	136	0.83
7	*(E)*-β-Ocimene	20.679	1045	136	0.02
8	Terpinolene	23.279	1081	136	0.13
9	Linalool	24.423	1097	154	0.03
10	Isothymol methyl ether	33.477	1226	164	0.02
11	Thymol methyl ether	33.790	1231	164	2.24
12	cis-3-Hexenyl valerate	33.991	1234	184	0.02
13	Bornyl acetate	37.020	1279	196	0.02
14	Carvacrol	38.074	1294	150	0.02

15	δ-Elemene	40.314	1329	204	0.12
16	Longifolene	40.552	1332	204	0.21
17	α-Cubebene	41.140	1342	204	0.15
18	Isoledene	41.553	1348	204	0.10
19	Cyclosativene	41.924	1354	204	0.01
20	α-Muurolene	42.061	1356	204	0.08
21	Isoledene	42.543	1358	204	0.02
22	α-Copaene	42.750	1367	204	1.85
23	α-Patchoulene	42.893	1369	204	0.35
24	β-Bourbonene	43.263	1375	204	0.15
25	daucene	43.427	1377	204	0.18
26	β-Cubebene	43.724	1382	204	0.89
27	β-Elemene	43.915	1385	204	2.36
28	α-Gurjunene	44.825	1399	204	0.10
29	β-Caryophyllene	45.593	1410	204	25.64
30	Epi-bicyclosesquiphellandrene	46.043	1419	204	0.21
31	β-Gurjunene	46.297	1427	204	0 05
32	Aromadendrene	46.589	1428	204	0.14
33	γ-Muurolene	46.954	1434	204	0.06
34	α-humulene	47.558	1443	204	0.58
35	*allo*-Aromadendrene	47.913	1450	204	0.15
36	Precocene I	48.257	1456	190	1.28
37	selina-4,11-diene	48.945	1467	204	0.24
38	Germacrene D	49.289	1473	204	8.03
39	β-Selinene	49.512	1476	204	0.16
40	Bicylosesquiphellandrene	49.591	1477	204	0.13
41	1-Epibicyclogermacrene	49.824	1481	204	0.25
42	Bicyclogermacrene	50.258	1488	204	11.91
43	α-Amorphene	50.518	1492	204	0.28
44	α-Selinene	50.671	1495	204	1.91
45	Himachala-2,4-diene	50.814	1498	204	0.11
46	γ-Cadinene	51.116	1502	204	0.33
47	Cadina-1(10),4-diene	51.370	1508	204	0.65
48	δ-Cadinene	51.884	1516	204	2.13
49	Calamenene	52.207	1522	202	0.24
50	α-Epizonarene	52.625	1529	204	0.04
51	α-Calacorene	52.917	1534	200	0.37
52	8,9-Epoxycedrane	53.192	1539	220	0.04
53	Germacrene B	53.367	1542	204	0.19
54	6,7-Dimethyltetralin	53.479	1544	160	0.05

55	Aristolene epoxyde	53.711	1548	220	0.04
56	β-maalième	54.193	1555	204	0.17
57	(*E*)-Nerolidol	54.442	1560	222	0.26
58	Diepicedrene-1-oxide	54.680	1565	220	0.05
59	Spathulenol	54.897	1568	220	0.73
60	Caryophyllene oxide	55.082	1572	220	1.10
61	Globulol	55.209	1574	222	0.51
62	Bulnesol	55.453	1578	222	0.11
63	Ledol	55.649	1581	222	0.32
64	1,3,3-Trimethyl-2-(2-methyl-cyclopropyl)-cyclohexene	55.813	1584	178	0.14
65	Ylangenol	56.268	1592	220	0.21
66	Valencene	56.442	1595	204	0.05
67	*trans*-Longipinocarveol	56.576	1597	220	0.24
68	Aromadendrene epoxide	56.745	1600	220	0.06
69	α-Copen-3-ol	56.904	1603	220	0.07
70	Humulan-1,6-dien-3-ol	57.052	1606	222	0.08
71	2-tert-1,4-benzoquinone	57.428	1613	164	0.08
72	3'-Amino-4-ethoxy-[(1,1'-biphenyl)]-4-ol	57.529	1615	229	0.06
73	β-Cadin-4-en-10-ol	57.767	1619	222	0.21
74	Thujopsene	57.995	1623	204	0.04
75	4,4-Dimethyltetracyclo[(6.3.2.0)(2,5).0(1,8)]tridecan-9-ol	58.196	1627	220	0.12
76	8-α-3-Copen-8-ol	58.344	1630	220	0.13
77	Agarospirol	58.561	1634	222	0.43
78	10-Epi-α-Muurolol	58.842	1639	222	0.14
79	τ-Muurolol	59.239	1647	222	0.81
80	dehydrocyclolongifolene oxide	59.562	1652	218	0.10
81	γ-Gurjunenepoxide	59.795	1656	220	0.05
82	Germacra-1(10),5-diene-4-ol	59.954	1659	222	0.13
83	2,3,4,5-tetramethyltricyclo[3.2.1.$0^{2,7}$]oct-3-ene	60.367	1667	202	0.14
84	α-Bisabolol	61.156	1682	222	12.75
85	2,2-dimethyl-3-(3-methyl-2-butenyl)-6-methylenecyclohexanecarbaldehyde	61.331	1684	220	0.18
86	1,2,3,4-tetrahydro-1,5,7-trimethylnaphthalene	62.189	1699	174	0.17
87	Nd	62.459	1705	250	1.00
88	Nd	62.755	1711	192	1.05
89	6-Isopropenyl-4,8a-dimethyl-1,2,3,4,5,6,7,8,8a-trihydronaphtalen-2,3-diol	63.370	1722	236	0.05
90	Nd	63.920	1733	218	0.12
91	Nd	67.256	1796	264	0.29
92	α-santalol	71.731	1887	220	0.07
93	Cinerolone	74.548	1945	166	0.14
94	Phytol	82.115	2109	296	0.07

95	Nd	106.807	2739	390	0.35
	Yield (%)				0.039
	Monoterpenes hydrocarbons				3.20
	Oxygenated monoterpenes				2.33
	Sesquiterpenes hydrocarbons				60.58
	Oxygenated sesquiterpenes				18.81
	Other compounds				2.33
	Nd				2.81
	Total				90.06

Nd: Non-determinated; [a] Order of elution and contents determined on the apolar column (HP-5MS); [b] Retention time [c] Retention indice measured on apolar capillary column (HP-5MS); M/Z : Molecular weight

A previous study of essential oils extracted from the aerial parts of *A. hispidum* collected at three different locations (Abidjan, Bouaké and Lakota) in Côte d'Ivoire showed that β-caryophyllene was the main constituent, at higher proportions (33.3-34.9%) than in our sample (25.64%) [33]. Other studies carried out elsewhere in Africa [34, 36, 37, 38] and in Argentina [39] have also identified β-caryophyllene as the predominant compound. With respect to its main components, the composition of our oil sample was consistent with those described in these studies. indeed, the other three main compounds in our essential oil, α-bisabolol, bicyclogermacrene and germacrene D were consistently found in significant amounts in oils from this species in all these studies. Therefore, β-caryophyllene, accompanied by α-bisabolol, bicyclogermacrene and germacrene D could be proposed as chemical markers for the essential oil of *A. hispidum*.

4.3 Antioxidant activity of essential oil

The percentage of DPPH inhibition, measured at 517 nm, is expressed as a function of concentration. The essential oil and vitamin C showed virtually the same inhibition percentages between 2.5 and 10 µg/mL, indicating that their antioxidant activities are comparable within this concentration range (Figure 2). Vitamin C was used as the reference antioxidant. Above 10 µg/mL, vitamin C showed higher inhibition (≈ 85-92%) than the essential oil (≈ 55-59%).

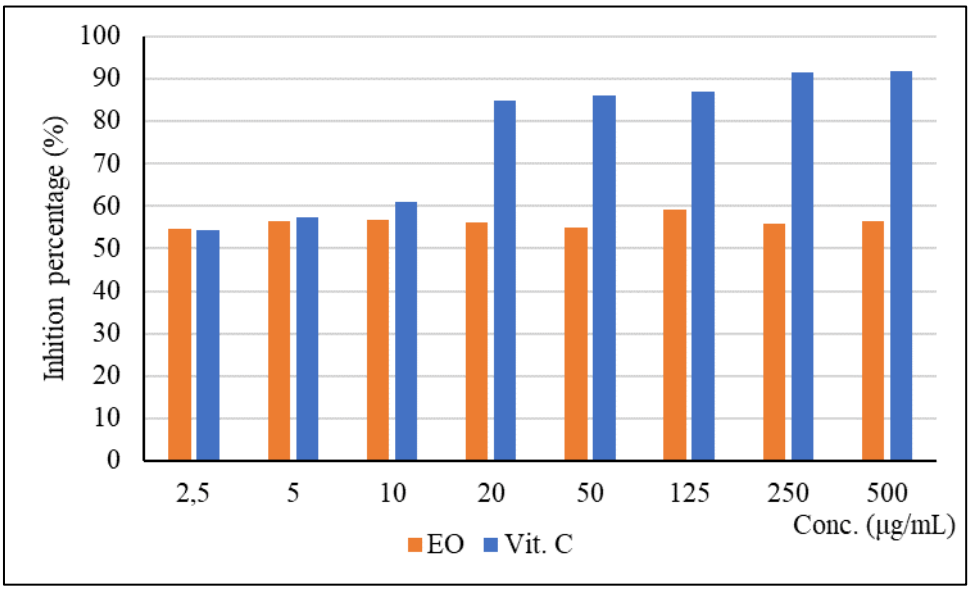

Figure 2: Antioxidant capacity of essential oil extract and vitamin C

These results indicate that the essential oil from the leafy twigs of this species exhibits free radical scavenging capacity. This is supported by the EC_{50} values (the concentration required to scavenge 50% of the DPPH radicals). The EC_{50} was 8 µg/mL for the essential oil versus 6 µg/mL for vitamin C (Figure 3).

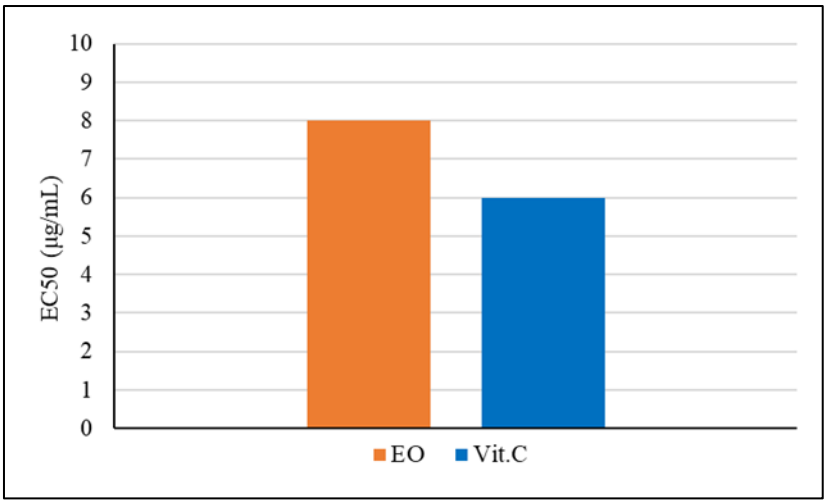

Figure 3: EC_{50} values for EO and vitamin C

The EC_{50} of the leafy twig essential oil is 1.33 times higher than that of the reference antioxidant. The lower the EC_{50}, the more effective the extract. Therefore, vitamin C has a DPPH scavenging capacity 1.33 times greater than that of essential oil. These EC_{50} results confirm that the EO extract has an anti-radical activity close to that of vitamin C. This radical scavenging capacity of the essential oil could be related to the high proportion of sesquiterpenoids, especially β-caryophyllene. Indeed this molecule has been reported to have a strong radical scavenging activity with very low IC50 values [40]. The potent antioxidant capacity of β-caryophyllene has been implicated in reducing oxidative stress induced by carbon tetrachloride (CCl_4) in rats [41]. Another study has shown that this sesquiterpene accelerates cutaneous wound healing in rats, partly due to its antioxidant action, which is mediated by an increase in glutathione peroxidase (GPx) activity [42]. These reports support the value of β-caryophyllene as a natural antioxidant.

5 CONCLUSION

The essential oil extracted from the leafy twigs of *Acanthospermum hispidum* by hydrodistillation was obtained in low yield (0.039%). Its chemical composition is dominated by sesquiterpene (79.39%), with β-caryophyllene (25.64%), α-bisabolol (12.75%), bicyclogermacrene (11.91%) and germacrene D (8.03%) as the major compounds. The essential oil thus belongs to a sesquiterpene-rich chemotype, with β-caryophyllene as a potential chemical marker. The DPPH assay showed that the essential oil of *A. hispidum* has promising antioxidant activity (EC_{50} = 8 µg/mL), close to that of vitamin C (6 µg/mL). Further studies (complementary antioxidant assays such as ABTS and FRAP, toxicological assessment and *in vivo* evaluation) are needed before this essential oil can be used in the manufacture of plant-based pharmaceutical products and food preservatives.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

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