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Comparative study of the chemical composition of the essential oils of the aerial part of *Acanthospermum hispidium* D.C. 1836 collected in three towns in Ivory Coast

Adéyolé TIMOTOU[1,2], Logopho Hyacinthe OUATTARA[1]*, Didjour Albert KAMBIRE[1], Moussa SISSOUMA[2], Doumadé ZON[1], Ahmont Landry Claude KABLAN[1], Jean Brice BOTI[2]

[1] *UPR Organic Chemistry, Department of Mathematics-Physics-Chemistry, UFR Biological Sciences, University Peleforo GON COULIBALY, BP 1328 Korhogo, Côte d'Ivoire*

[2] *Matter Constitution and Reaction Laboratory, UFR Sciences of the Structures of Matter and Technology, University Félix HOUPHOUËT- BOIGNY, 22 BP 582 Abidjan 22, Côte d'Ivoire*

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*Address for Correspondence:

OUATTARA Logopho Hyacinthe, UPR Organic Chemistry, Department of Mathematics-Physics-Chemistry, UFR Biological Sciences, University Peleforo GON COULIBALY, BP 1328 Korhogo, Côte d'Ivoire

Abstract

This research work concerns the comparative study of the chemical composition of essential oils from the aerial part of the *Acanthospermum hispidium* plant collected in three towns in Côte d'Ivoire. The aim of this study is to enhance the value of aromatic and medicinal plants through the chemical analysis of essential oils (EO). The essential oils extracted using a Clevenger-type apparatus were analysed using several analytical methods, including Ir-GPC, MS-GPC and ^{13}C-NMR. The results indicate that the essential oils of *Acanthospermum hispidium* are largely dominated by hydrocarbon sesquiterpenes (69.2 to 72.9%). The majority compounds identified are (E)-β-caryophyllene (33.3 to 34.9%), α-bisabolol (6.5 to 9.9%), bicyclogermacrene (6, 3 to 9.5%), germacrene D (7.3 to 8.4%), α-pinene (4.6 to 7.9%), β-lemene (3.7 to 4.3%) and α-copaene (2.5 to 3.2%). The co-presence of these compounds with multiple pharmacological properties could justify the use of *A. hispidium* in traditional medicine in the treatment of various pathologies.

Keywords: Acanthospermum hispidium, chemical composition, essential oil, Ivory Coast.

INTRODUCTION

The use of medicinal and edible plants has always been an integral part of human culture. Indeed, mankind has always taken advantage of its plant environment to satisfy its daily needs, whether for treatment or food [1].

Today, despite advances in modern medicine, the therapeutic use of medicinal plants is still widespread in many countries around the world, especially in the developing world [2]. In Africa, these plant species are the main means by which people are treated [3]. In Côte d'Ivoire, the use of phytotherapy has become widespread due to the ever-increasing number of dangerous pathologies, as well as the inaccessibility of health centres, the high cost of pharmaceutical products [4,5] and the resistance of certain pathogenic strains [6, 7].

Among the plant species used in traditional medicine are aromatic and medicinal plants (MAP), which are a source of high added-value natural products (essential oils, extracts, resins, etc.) that are easily accessible and have a range of biological properties [4, 8].

Aromatic plants are also used extensively in various sectors, including pharmaceuticals, perfumery, cosmetics, organic farming and the agri-food industry (pastries, confectionery, culinary by-products, etc.), giving them significant economic value [9]. In this context, scientific research programmes have been initiated with a view to enhancing the value of aromatic and medicinal plants from Côte d'Ivoire's flora through the chemical characterisation of their essential oils [10, 11, 12]. With this in mind, this study looked at *Acanthospermum hispidum,* an aromatic annual plant [13] in the Asteraceae family, still known in Côte d'Ivoire as "Saraka-weini" in Malinké and "Gnéakeyébêko" in Bété [14]. This plant is used in traditional medicine in a number of countries to treat a variety of illnesses. It is used to treat malaria, headaches, abdominal pain, convulsions, stomach disorders, snake bites, epilepsy, blennorrhoea, hepatobiliary disorders, microbial infections [15, 16, 17], haemorrhoidal attacks [18] and benign prostatic hypertrophy [13].

The aim of this study is therefore to enhance the value of *Acanthospermum hispidum* by gaining a better chemical understanding of its essential oil. Specifically, the chemical composition of the essential oils of the aerial parts of *A. hispidum* from three towns in Côte d'Ivoire will be determined using gas chromatography combined with retention indices GPC(Ir), then coupled with mass spectroscopy (GPC-MS) and nuclear magnetic resonance spectroscopy of carbon 13 (^{13}C NMR).

I- MATERIAL AND METHODS

I.1- Material

I.1.1- Plant material

The plant material consisted of fresh aerial parts (leaves and stems) of *Acanthospermum hispidium,* collected in three different towns in Côte d'Ivoire, namely:

- The locality of Adiopodoumé (5°20'12" North and 4°7'57" West) located in the city of Abidjan in the south of Côte d'Ivoire;

- The town of Bouaké (7°69" North and 5°03" West) in central Côte d'Ivoire ;

- The town of Lakota (5° 50′ 59″ North and 5° 41′ 01″ West) in the south-west of Côte d'Ivoire.

The various specimens of the plant were collected during the rainy season between June and July 2021. The various specimens collected in the three towns concerned were identified at the National Floristics Centre (CNF) of the University Félix HOUPHOUËT-BOIGNY (UFHB).

I.1.2- Technical material

The technical equipment consists of laboratory utensils, standard laboratory glassware and apparatus such as a Clevenger-type extractor mounted on a pressure cooker, a Perkin-Elmer Clarus 500 gas chromatograph (GC), a Perkin Elmer autosystem XL chromatograph and a Bruker 400 AVANCE NMR spectrometer.

II.2- Methods

II.2.1- Extraction of essential oils from Clevenger

The essential oils of the plant species studied were extracted by steam distillation using a Clevenger-type apparatus mounted on a pressure cooker. The fresh plant material was weighed and placed on a grid in the matrix (pressure cooker), which acted as a boiler and contained water in such a way that it was not in contact with the water. As soon as the first drop of essential oil has condensed, three hours are counted before the extraction is stopped by turning off the heat. The essential oil separated from the water by decantation is taken into a tube using a Pasteur pipette and dried with anhydrous sodium sulphate (Na_2SO_4) to remove any traces of water. The essential oils are then weighed and collected in glass pillboxes, sealed and stored in the refrigerator. Finally, the essential oils are analysed to determine their chemical composition.

II.2.2- Analysis of essential oils

In order to determine their chemical composition, the essential oil samples were analysed by GPC(Ir), GPC-MS and ^{13}C NMR, at the Environmental Sciences Laboratory, University of Corsica (Ajaccio, France). These analytical techniques were used to identify and quantify the constituents of the various oils in the aerial parts of *Acanthospermum hispidium.*

II.2.2.1- Analysis by GPC(Ir)

The gas chromatographic analyses were carried out using a Perkin-Elmer Clarus 500, equipped with a splitter injector, two columns (50 m x 0.22 mm; film thickness: 0.25 µm), apolar (BP-1, polymethylsiloxane) and polar (BP20, polyethylene glycol) and two flame ionisation detectors.

The operating conditions were as follows: carrier gas, hydrogen (0.8 mL/min); column head pressure: 20 psi; injector and detector temperature: 250 °C; temperature programming: from 60 °C to 220 °C (80 min) at 2 °C/min, with a 20-minute pause at 220 °C; injection: divider mode with a 1/60 ratio. The quantity of sample injected is 0.5 µL from a solution containing 50 µL of essential oil mixture in 350 µL of Chloroform. This technique is used to quantify the molecules present in essential oils and to identify them on the basis of their retention indices (Ir). Retention indices are calculated using Perkin-Elmer's "Target Compounds" software, by linear interpolation of the retention times of a series of n-alkanes (C8-C29).

II.2.2.2- Analysis by MS-GPC

Analyses were carried out using a Perkin Elmer Clarus 580 Autosystem XL chromatograph, equipped with an automatic injector and a BP-1 (polydimethylsiloxane) capillary column (50 m × 0.22 mm; film thickness: 0.25 µm), coupled to a Clarus SQ8S PerkinElmer TurboMass (quadrupole) mass detector. The operating conditions were as follows: carrier gas, helium (1 mL/min); injector and ion source temperature: 250°C; temperature programming: from 60°C to 220°C (80 min) at 2°C/min, with a 20 min plateau at 220°C; injection: divider mode with a 1/80 ratio; injected volume: 0.5 µL; ionisation energy: 70 eV; acquisition of mass spectra between 35 and 350 Da. GPC coupled with electron impact mass spectrometry (MS) can be used to identify the constituents of a mixture using structural information obtained from the fragmentations observed.

II.2.2.3- Analysis by ^{13}C-NMR

The NMR spectra were recorded on a Brücker 400 AVANCE spectrometer, 9.4 Tesla, operating at 100.623 MHz for carbon-13. The spectra were recorded with a 5 mm probe. The solvent was deuterated chloroform ($CDCl_3$) with the addition of tetramethylsilane (TMS). Chemical shifts are given in parts per million (ppm, δ scale) relative to TMS taken as the internal reference.

^{13}C NMR was used to confirm the presence of constituents previously identified by mass spectroscopy (MS) in the essential oils. The principle of this method is to observe the resonance lines belonging to a given compound in the spectrum of the mixture and to identify that compound [19].

II- RESULTS AND DISCUSSION

II.1- Results

II.1.1- Appearance and yield of *Acanthospermum hispidum* essential oils

The various samples of *Acanthospermum hispidum* essential oils studied have the same appearance (oleaginous liquid), with a light yellow colour.

The yields of the essential oils were calculated according to the following formulae and recorded in Table I.

$$\text{Yield (\%)} = \frac{\text{Mass of essential oil obtained (g)} \times 100}{\text{Mass of plant matter (g)}} = \frac{M_{HE} \times 100}{M_{MV}}$$

Yield error calculations: $\Delta\text{Yield} = \text{Yield} \times \left(\frac{\Delta M_{HE}}{M_{HE}} + \frac{\Delta M_{MV}}{M_{MV}}\right)$

ΔYield : yield error ;

ΔM_{HE} : error in the mass of essential oil = ± 0,0001g ;

M_{HE} : mass of essential oil ;

ΔM_{MV} : error in the mass of plant matter = ± 0,0001g ;

M_{MV} : mass of plant matter.

Table I: Distillation yields of essential oils

Plant species	Yields (%)
***Acanthospermum hispidum* (Adiopodoumé)**	0.1737 ± 0.0007
***Acanthospermum hispidum* (Bouaké)**	0.1418 ± 0.0006
***Acanthospermum hispidum* (Lakota)**	0.0890 ± 0.0005

II.1.2- Chemical composition of *Acanthospermum hispidum* essential oils

The chemical composition of the essential oils of the aerial part of *Acanthospermum hispidium* was determined using a combination of chromatographic and spectroscopic techniques (GC(Ir), GC-MS and ^{13}C NMR). The concentrations of the compounds were taken from the apolar column of the GC, except in the case of compounds showing co-elutions on the apolar column, for which the concentrations were taken from the polar column.

After analysing the three samples of *A. hispidium* essential oil, 63 compounds were identified, of which 56 were in the Adiopodoumé essential oil, 54 in the Bouaké essential oil and 57 in the Lakota essential oil. All the compounds identified are listed in Table II and most of the major compounds are shown in Figure 1.

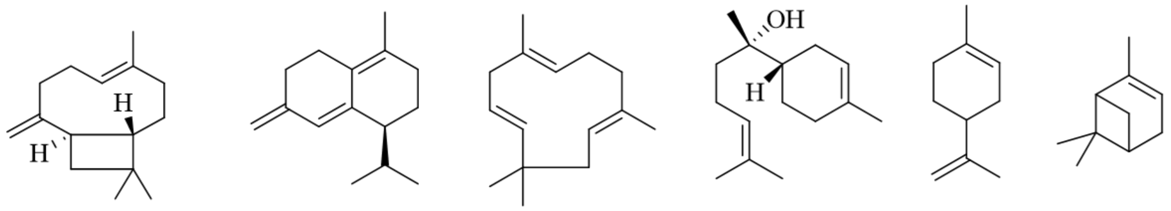

Figure 1: Some compounds identified in the essential oils of *A. hispidum*

Table II: Chemical composition of essential oils from the aerial parts of *A. hispidum*

N°	Compounds	Ir Apol	Ir pol	Ame	Bke	Lta	Identification
1	pentanal	743	1084	-	0.1	0.1	Ir, MS
2	**α-pinene**	932	1 020	**4.6**	**7.3**	**7.9**	Ir, MS, ^{13}C NMR
3	sabinene	966	1 127	tr	tr	0.1	Ir, MS
4	β-pinene	972	1 116	0.6	0.8	1.0	Ir, MS, ^{13}C NMR
5	myrcene	982	1 166	0.2	0.2	0.4	Ir, MS, ^{13}C NMR
6	α-phellandrene	998	1 171	-	0.1	tr	Ir, MS
7	*p*-cymene	1 013	1 277	tr	tr	0.1	Ir, MS
8	limonene	1 022	1 206	1.3	1.7	2.3	Ir, MS, ^{13}C NMR
9	terpinolene	1 079	1 288	0.2	0.2	0.3	Ir, MS
10	nonanal	1 083	1 397	0.8	0.4	1.3	Ir, MS, ^{13}C NMR
11	linalol	1 085	1 549	0.1	0.1	0.1	Ir, MS
12	4,8-dimethyl-1,3,7-nonatriene	1 105	1 311	tr	tr	0.1	Ir, MS
13	oxyde de methyle et de thymyle	1 215	1 593	0.9	1.5	1.5	Ir, MS, ^{13}C NMR
14	decan-1-ol	1 256	1 762	0.1	tr	0.1	Ir, MS
15	thymol	1 268	2 197	tr	0.1	tr	Ir, MS, ^{13}C NMR
16	δ-elemene	1 334	1 483	0.2	0.3	0.3	Ir, MS
17	(*7αH*)-silphiperfol-5-ene	1 345	1 453	0.2	0.2	0.2	Ir, MS
18	α-cubebene	1 348	1 460	0.2	0.1	0.1	Ir, MS
19	silphiperfol-6-ene	1 360	1 497	0.1	0.1	0.1	Ir, MS
20	**α-copaene**	1 375	1 494	**3.2**	**2.6**	**2.5**	Ir, MS, ^{13}C NMR
21	pethybrene	1 379	1 510	0.5	0.6	0.5	Ir, MS, ^{13}C NMR
22	β-bourbonene	1 383	1 520	0.3	0.2	0.3	Ir, MS

23	**β-elemene**	1 387	1 593	**4.3**	**4.2**	**3.7**	Ir, MS, ^{13}C NMR
24	sativene	1 390	1 528	0.1	tr	tr	Ir, MS
25	cyperene	1 399	1 529	0.1	tr	tr	Ir, MS
26	α-gurjunene	1 409	1 520	0.1	0.1	0.1	Ir, MS
27	**(*E*)-β-caryophyllene**	1 419	1 601	**34.9**	**33.3**	**34.6**	Ir, MS, ^{13}C NMR
28	β-copaene	1 426	1 593	0.2	0.1	0.2	Ir, MS
29	(*E*)-β-farnesene	1 447	1 670	0.4	0.3	0.3	Ir, MS
30	**α-humulene**	1 450	1 670	**6.5**	**6.7**	**7.0**	Ir, MS, ^{13}C NMR
31	*allo*-aromadendrene	1 457	1 645	0.3	0.2	0.2	Ir, MS
32	4-methoxy-safrole (sarisan)	1 463	2 174	0.9	1.5	0.6	Ir, MS, ^{13}C NMR
33	γ-muurolene	1 470	1 689	0.5	0.3	0.3	Ir, MS, ^{13}C NMR
34	**germacrene D**	1 476	1 710	**8.4**	**7.3**	**7.7**	Ir, MS, ^{13}C NMR
35	*trans*-β-bergamotene	1 478	1 685	0.2	0.1	0.1	Ir, MS
36	β-selinene	1 481	1 714	0.1	0.1	0.1	Ir, MS
37	α-zingiberene	1 485	1 724	0.2	0.2	0.2	Ir, MS
38	cubebol	1 487	1 885	0.3	0.2	0.3	Ir, MS
39	**bicyclogermacrene***	1 491	1 734	**6.3**	**9.5**	**8.0**	Ir, MS, ^{13}C NMR
40	α-muurolene*	1 491	1 725	0.8	0.3	0.5	Ir, MS, ^{13}C NMR
41	β-bisabolene	1 500	1 728	0.9	0.6	0.6	Ir, MS, ^{13}C NMR
42	γ-cadinene	1 506	1 758	0.4	0.2	0.4	Ir, MS, ^{13}C NMR
43	δ-cadinene*	1 514	1 758	1.7	1.2	-	Ir, MS, ^{13}C NMR
44	β-sesquiphellandrene*	1 514	1 770	1.7	1.1	1.1	Ir, MS, ^{13}C NMR
45	α-calacorene	1 527	1 916	0.1	0.1	0.1	Ir, MS
46	β-elemol	1 536	2 080	0.9	2.4	0.5	Ir, MS, ^{13}C NMR
47	γ-asarone	1 539	2 278	0.2	0.2	0.2	Ir, MS
48	(*E*)-nerolidol	1 547	2 041	0.1	0.1	0.2	Ir, MS
49	spathulenol	1 563	2 120	0.2	0.3	0.2	Ir, MS
50	oxyde de caryophyllene	1 569	1 978	1.3	1.3	1.4	Ir, MS, ^{13}C NMR
51	globulol	1 573	2 055	0.1	0.1	0.1	Ir, MS
52	humulene oxyde II	1 593	2 034	0.1	0.2	0.1	Ir, MS
53	alismol	1 610	2 262	0.1	tr	tr	Ir, MS
54	*epi*-cubenol	1 614	2 047	0.2	0.1	0.1	Ir, MS
55	γ-eudeMSol	1 620	2 178	0.2	0.2	0.1	Ir, MS
56	τ-cadinol*	1 625	2 167	0.1	tr	0.1	Ir, MS
57	τ-muurolol*	1 625	2 183	0.2	0.1	0.1	Ir, MS
58	β-eudeMSol	1 634	2 224	0.2	0.1	0.1	Ir, MS
59	α-cadinol	1 637	2 228	0.9	1.1	0.3	Ir, MS, ^{13}C NMR
60	α-bisabolol	1 666	2 214	**9.9**	**6.5**	**7.7**	Ir, MS, ^{13}C NMR
61	tetradecanol	1 674	2 169	0.1	0.1	0.2	Ir, MS
62	pentadecanal	1 694	2 023	-	tr	0.5	Ir, MS, ^{13}C NMR
63	(*E*)-phytol	2 097	2 609	0.1	0.2	0.1	Ir, MS

Elution order and percentages given on apolar column (BP-1), except for compounds whose names are followed by an asterisk (*) for which the percentages are given on polar column (BP-20) ; Ir : retention indices on apolar (Ir apol) and polar (Ir pol) columns ; tr = traces ; MS: compound identified by GC-MS ; ^{13}C NMR: compound identified by ^{13}C NMR ; Amé: Adiopodoumé ; Bké: Bouaké; Lta: Lakota; in bold: majority compounds.

The various compounds identified represent 97.8, 97.2 and 97.4% of the total chemical composition of the essential oils of Adiopodoumé, Bouaké and Lakota respectively. These compounds were grouped into five categories of families and listed in Table III according to their percentage presence in the different essential oils.

Table III: Composition (percentage) of the chemical families of the essential oils of *A. hispidum*

Families of compounds	**Adiopodoumé**	**Bouaké**	**Lakota**
Hydrocarbon monoterpenes	6,9	10,3	12,1
Oxygenated monoterpenes	1,0	1,7	1,6
Hydrocarbon sesquiterpenes	72,9	70,0	69,2
Oxygenated sesquiterpenes	14,8	12,7	11,3
Other compounds	2,2	2,5	3,2
Total identified	**97,8**	**97,2**	**97,4**

II.2- Discussion

This study was conducted to determine the chemical composition of the essential oils of the aerial part of *Acanthospermum hispidium* harvested in three towns in Côte d'Ivoire. The essential oils of the aerial organs (leaves and stems) of *A. hispidium* harvested in the period from June to July 2021 in the localities of Adiopodoumé, Bouaké and Lakota were extracted using the steam stripping technique with a Clevenger-type apparatus. Asteraceae, the botanical family to which *A. hispidium* belongs, are known to contain essential oils [20]. The yields of essential oils obtained from the aerial parts of the plant studied varied between 0.0890 ± 0.0005 and 0.1737 ± 0.0007%. This difference in yields could be explained by the environmental differences between the harvesting sites [20, 21, 22].

Chromatographic analysis of the three essential oils from the aerial parts of *Acanthospermum hispidium* identified a total of 63 compounds, with identification rates of 97.8%, 97.2% and 97.4% respectively for the Adiopodoumé, Bouaké and Lakota sites. These rates are virtually identical for the three essential oils studied. The compounds identified can be divided into five classes: hydrocarbon monoterpenes (6.9 to 12.1%), oxygenated monoterpenes (1.0 to 1.7%), hydrocarbon sesquiterpenes (69.2 to 72.9%), oxygenated sesquiterpenes (11.3 to 14.8%) and other compounds (2.2 to 3.2%). Hydrocarbon sesquiterpenes are the predominant class in these three essential oils, with a rate of 72.9% for the Adiopodoumé plant, 70.0% for the Bouaké plant and 69.2% for the Lakota plant.

(E)-β-caryophyllene is the main compound in the three essential oils tested with percentages of 34.9, 34.6 and 33.3% respectively for the Adiopodoumé, Lakota and Bouaké samples. It is followed in all three samples by α-bisabolol (6.5 to 9.9%), bicyclogermacrene (6.3 to 9.5%), germacrene D (7.3 to 8.4%), α-pinene (4.6 to 7.9%), β-lemene (3.7 to 4.3%) and α-copaene (2.5 to 3.2%). From this point of view, the chemical composition of the essential oils of the aerial parts of *Acanthospermum hispidium* collected at the Adiopodoumé, Bouaké and Lakota sites is fairly homogeneous.

Several studies have been carried out on essential oils from the aerial part of *A. hispidum*, but in other countries.

In Nigeria a study showed that the essential oil of the aerial part of *A. hispidum* is composed mostly of β-caryophyllene (28.0%), bicyclogermacrene (11.0%), α-bisabolol (8.9%), germacrene-D (6.9%) and α-humulene (6.0%) [23]. The chemical composition of Côte d'Ivoire essential oil is close to that of Nigeria reported in the literature. This can be explained by similar climatic conditions, vegetation and relief in these two countries.

In Congo, a study on the composition of the essential oil of the aerial part of *Acanthospermum hispidum* revealed that it is rich in β-caryophyllene (34.0 to 42.7%), α-humulene (8.9 to 12.7%), α-bisabolol (3.7-11.2%). It also indicates the presence of carvacrol and methyl carvacrol [24]. The chemical composition of essential oil from Congo differs from that of Côte d'Ivoire. Among the main compounds, the absence of bicyclogermacrene and germacrene D was noted, together with the presence of methyl carvacrol in appreciable quantities, a compound absent in the essential oil of Côte d'Ivoire.

In Argentina, the essential oil from a harvest of the aerial part of the same plant showed the presence of β-caryophyllene (35.2%), α-bisabolol (11.4%) and germacrene D (11.1%) as major constituents [25]. These compounds have practically the same percentages as those in Côte d'Ivoire essential oil.

The co-presence of all these identified compounds is thought to be responsible for *Acanthospermum hispidium*'s diverse biological properties. In Côte d'Ivoire, it is thought to have a number of therapeutic properties, including antimalarial, antihypertensive, antispasmodic, vermifuge, abortive and antimicrobial properties [14, 26]. In Burkina-Faso, a study showed that the methanolic extract of flowering stems had significant cytotoxic activity against certain cell lines [27]. In Mali, studies showed that a decoction of the aerial parts of *A. hispidum*, at a dose of 230 mg/kg, had a significantly higher peripheral analgesic activity than paracetamol at a dose of 100 mg/kg in mice. This same decoction at doses of 115 mg/kg and 230 mg/kg also showed anti-inflammatory activity in the carrageenan oedema test in mice [13]. Studies in Nigeria have shown that hydro-methanolic extracts of *A. hispidum* leaves and stems have a pharmacological action against diarrhoea. These studies also showed that the extract at low doses induced a smooth muscle relaxation effect in the rabbit jejunum [28].

Indeed, (E)-β-caryophyllene has antioxidant [29], anti-inflammatory [30], anticancer [31], antimicrobial [32], neuroprotective, nephroprotective and cardioprotective properties [32, 33]. As for α-bisabolol it is antibacterial [34], antifungal [35], antioxidant [36] (Fırat et al., 2018), antiseptic, anti-inflammatory and has skin soothing and moisturising properties [37, 38, 39]. Germacrene D, α-pinene and β-elemene are all antibacterial [40, 41, 42]. As for α-copaene, it has cytotoxic, genotoxic and antioxidant properties [43].

CONCLUSION

The study of essential oils from the aerial part of *Acanthospermum hispidium* is part of a contribution to the development of medicinal aromatic plants in Côte d'Ivoire. This plant, which belongs to the Asteraceae family, is used by local people for therapeutic and dietary purposes. The aim of this study was to determine the chemical composition of the essential oils of the aerial part of *A. hispidium* harvested in three localities in Côte d'Ivoire, namely Adiopodoumé, Bouaké and Lakota, with a view to its development.

The aerial part of the plant was first used to extract the essential oils using a Clevenger-type apparatus. These essential oils were then analysed by GPC(Ir), MS-GPC and ^{13}C-NMR, with a view to identifying and quantifying the various constituents.

Analysis of *Acanthospermum hispidium* essential oil samples shows that they are mainly dominated by hydrocarbon sesquiterpenes (69.2 to 72.9%). These essential oils are mainly made up of (E)-β-caryophyllene (33.3 to 34.9%), α-bisabolol (6.5 to 9.9%), bicyclogermacrene (6.3 to 9.5%), germacrene D (7.3 to 8.4%), α-pinene (4.6 to 7.9%), β-lemene (3.7 to 4.3%) and α-copaene (2.5 to 3.2%). The co-presence of these chemical compounds with multiple pharmacological properties in these essential oils could justify the use of *A. hispidium* for the traditional treatment of conditions such as malaria, headaches, abdominal pain, convulsions, stomach disorders, snake bites, epilepsy, blennorrhoea, hepatobiliary disorders, microbial and viral infections, haemorrhoidal attacks and benign prostatic hypertrophy.

The first step is to study the chemical variability of these essential oils by harvesting them at several sites in the country and at different seasons. Next, a wide range of biological tests will be carried out (antibacterial, antifungal, antioxidant activities and toxicity).

CONFLICT OF INTEREST

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