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Research Article

Chemical composition, enantiomeric analysis, and bactericidal activities of sesquiterpene-rich essential oil of *Acanthospermum hispidum* DC. from northwestern Nigeria

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Abstract

Acanthospermum hispidum (Asteraceae), a medicinal plant indigenous to Nigeria and known locally as "Dagunro" in Yoruba and "Kasihinyawo" in Hausa, has been a traditional remedy in local healthcare practices. This study explores the chemical composition, enantiomeric analysis, and bactericidal activities of the sesquiterpene-rich essential oil of *A. hispidum* from Nigeria. The essential oil of *A. hispidum* was obtained through hydrodistillation and chemical constituents and enantiomeric distributions were identified using gas chromatography-mass spectrometry (GC-MS) analysis. The antibacterial activity was assayed using the micro-dilution method against selected pathogenic bacterial strains. The essential oil was dominated by sesquiterpenes. The major constituents identified in the essential oil include (*E*)-β-caryophyllene (21.8%), α-bisabolol (20.7%), bicyclogermacrene (7.9%), caryophyllene oxide (6.6%), α-humulene (5.9%), and germacrene D (6.1%). Chiral GC-MS analysis further elucidated the enantiomeric distribution of chiral terpenoid components, which includes (+)-α-pinene 92.8% : (–)-α-pinene 7.2%;(+)-sabinene 14.7%: (–)-sabinene 85.3%; (+)-β-pinene 52.4% : (–)-β-pinene 47.6%; (+)-limonene 17.3% : (–)-limonene 82.7%; and (+)-linalool 8.6% : (–)-linalool 91.4%. The antibacterial activity of *A. hispidum* essential oil revealed notable inhibitory activity with Minimum Inhibitory Concentrations (MIC) ranging from 125 µg/mL to 1250 µg/mL. The essential oil showed marked activity against *Staphylococcus aureus* (MIC = 125µg/mL), moderate activity against *Salmonella typhi* and *Proteus vulgaris* (MIC = 625µg/mL). The presence of bioactive compounds such as α-bisabolol and β-caryophyllene may have implications for the potential therapeutic application of *A. hispidum* essential oil.

1. Introduction

The genus *Acanthospermum* belongs to the family Asteraceae (Compositae), which is known to be the largest family of flowering plants comprising over 1600 genera and 25,000 species widely distributed across the world [1]. *Acanthospermum* originates from the two Greek words *akanthos* (spiny) and *sperma* referring to seed. The specific epithet *hispidum* is derived from the Latin word *hispidus* meaning rough, hairy, or bristly [2]. *A. hispidum* is popularly known as bristly starbur or goat head. it is an annual herb characterized by dichotomous (Y-shaped) branching with hairy stems. The plant has elliptic and obovate leaves that range from 1.5 to 7.0 cm long, though some may be as long as 11.5 cm. It produces yellow flowers. The fruits of the plant are spiny and flattened, and are shaped like triangles that range from 5 cm to 10 cm in

length [3]. It is native to tropical America, but has been introduced to Europe, Africa, India, and Asia [4] and is locally called *kashin-yaawoo* (Hausa), *dagunro* (Yoruba) and *yaawoo* ("Ron" tribe) in Nigeria. In Kandlemullu it is commonly referred to as *kannada* [4]. *A. hispidum* has been traditionally used in ethnomedicine to treat various ailments, including headaches, abdominal pains, convulsions, coughs, eruptive fevers, snake bites, scabies, asthma, bronchitis, dysentery, and fevers, and as an expectorant [5, 6].

Sesquiterpene lactones, including melampolides, germacranolides, and guaianolides have been isolated and characterized from *A. hispidum* [7, 8]. *A. hispidum* has been reported to exhibit various pharmacological activities such as molluscicidal [5], antibacterial [5], anthelmintic [9], antitumor [10], and antitrypanosomal activity [4]. Ethnopharmacological activities of two major sesquiterpene lactones isolated from *A. hispidum*, have been reported to show effective antiparasitic activities against *Trypanosoma brucei* [11] and serve as a promising anticancer, antimicrobial and antioxidative agent [12]. This study is therefore aimed at investigating the chemical composition, enantiomeric distribution, and bactericidal activities of the essential oil of *A. hispidum*from Nigeria.

2. Materials and methods

2.1. Plant material and identification

The fresh plant of *A. hispidum*was collected in June 2023 from a local farm in Hayin Liman, Sabon Gari village (11°06′60.00″ N, 7°43′59.99″ E), located in Kaduna South Local government area of Kaduna State, Nigeria. The plant was authenticated by Mr. Namadi Sunusi of the Botany Department, Ahmadu Bello University, Zaria, with voucher number ABU07053. The fresh leaves of the plant were air-dried in the shade for 7 days and then pulverized using an electric blender before extraction.

2.2. Isolation of the essential oils

The air-dried leaves (500g) were introduced into a 5-L flask and distilled water was added until it covered the sample. Hydrodistillation was carried out for four hours in an all-glass Clevenger apparatus according to the British pharmacopeia. The distillate was extracted with *n*-hexane, transferred to a pre-weighed amber sample bottle and dried using anhydrous sodium sulfate to eliminate traces of water. The oils were kept under refrigeration (4 °C) until ready for analysis.

2.3. Gas chromatographic–mass spectral analysis

The essential oil was analyzed by Gas Chromatography – Mass Spectrometry (GC-MS) using Shimadzu GCMS-QP2010 Ultra operated in the electron impact (EI) mode (electron energy = 70 eV), scan range = 40-100 atomic mass units, scan rate = 3.0 scan/s. The GC column was a ZB-5 fused silica capillary column (30 m length × 0.25 mm inner diameter with a 5% phenyl polydimethylsiloxane stationary phase and a film thickness of 0.25 µm. The carrier gas was helium with a column head pressure of 553 kPa and a flow rate of 1.37 mL/min. The injector temperature was 250 °C and the ion source temperature was 200 °C. The GC oven temperature was programmed for 50 °C initial temperature, then increased at the rate of 2 °C/min to 260 °C. A 5% w/v solution of the sample in CH_2Cl_2 was prepared and 0.1 µL was injected with a splitting mode (30:1). Identification of the volatile oil constituents was achieved based on their retention indices and by comparison of their mass spectral fragmentation pattern with those reported in databases [13–16]. The quantification of the constituents of the essential oil was done using an external standard method using calibration curves generated by running a GC analysis of representative standard compounds for each class [17].

2.4. Chiral gas chromatographic–mass spectral analysis

Chiral GC-MS of the essential oil of *A. hispidum* was carried out using a Shimadzu GC-MS QP2010S (Shimadzu Scientific Instruments, Columbia, MD, USA) operated in the EI mode (electron energy = 70 eV) with a scan range of 40–400 amu and scan rate of 3.0 scans/s. The GC was equipped with a Restek B-Dex 325 capillary column (Restek Corp, Bellefonte, PA, USA) (30 m × 0.25 mm ID × 0.25 µm film). The oven temperature was programmed as follows: Start at 50 °C, temperature increased to 120 °C at a rate of 1.5 °C/min, then increased to 200 °C at 2 °C/min, and kept at 200 °C for 5 min. Helium was the carrier gas with a flow rate of 1.8 mL/min. The sample was diluted to 3% w/v with CH_2Cl_2, and a 0.1 µL sample was injected in a split mode at a split ratio of 1:45. The terpenoid enantiomers were identified by comparison of retention indices with authentic samples obtained

from Sigma-Aldrich (Milwaukee, WI, USA). Relative enantiomer percentages were determined based on peak areas.

2.5. Antibacterial activity

The essential oils were screened for antimicrobial activity against the bacteria *Staphylococcus aureus* (ATCC No. 25923), *Bacillus subtilis* (ATCC No.6633), *Streptococcus faecalis* (ATCC No.9790), *Salmonella typhi* (ATCC No. 6539), *Proteus vulgaris* (ATCC No. 6380), *Escherichia coli* (ATCC No.25922), and *Pseudomonas aeruginosa* (ATCC No. 27853) using the micro-broth dilution technique as previously described [18, 19]. All bacteria were cultured using tryptic soy agar (Sigma-Aldrich, St. Louis, MO). A 1% stock solution of the essential oil in DMSO (50 µL) and 50 µL of cation–adjusted Mueller Hinton broth (CAMHB) (Sigma-Aldrich, St. Louis, MO) was added to the top wells of a 96-well microdilution plate. The essential oil solution was diluted serially in CAMHB (1:1) to obtain concentrations of 2500, 1250, 625, 312.5, 156.3, 78.1, 39.1, and 19.5 µg/mL. All microbes were harvested from a fresh culture, and then added to each well at a concentration of approximately 1.5×10^8 CFU/mL The 96-well microdilution plates were incubated at 37°C. The minimum inhibitory concentration (MIC) was determined as the lowest concentration with no turbidity. The positive antibiotic control used was streptomycin obtained from (Sigma-Aldrich, St. Louis, MO) while DMSO was used as the negative control (50 µL DMSO diluted in 50 µL broth medium, and then serially diluted as above). (*E*)-β-Caryophyllene, caryophyllene oxide, and α-bisabolol (Sigma-Aldrich, St. Louis, MO) were individually screened for activity.

3. Results and discussion

3.1. Essential oil composition

Hydrodistillation of *A. hispidum* air-dried leaves yielded a pale-yellow essential oil, 0.46% (v/w) yield. The chemical constituents of the essential oil were identified using gas chromatography-mass spectrometry. The GC-MS analysis revealed a total of 70 chemical components presenting 99.2% of the total oil composition (Table 1). The essential oil was dominated by monoterpenes (9.5%) and sesquiterpenes (87.6%). The predominant sesquiterpenes were (*E*)-β-caryophyllene (21.8%), α-bisabolol (20.7%), bicyclogermacrene (7.9%), caryophyllene oxide (6.6%), germacrene D (6.1%), α-humulene (5.9%), *trans*-β-elemene (3.6%), α-copaene (2.1%) and δ-cadinene (1.8%). The major monoterpenes wereα-pinene (3.0%), 1,8-cineole (1.8%), thymylmethyether (1.4%), sabinene (1.1%), and limonene (0.6%).

Previous reports on the essential oil of *A. hispidum* obtained from Nigeria [20], Congo [21], and Argentina [5] revealed similar major constituents in varying quantities with some different constituents as shown in Table 2. Carvacryl methyl ether was identified in the previous study [21] but was absent in this study. Differences in the essential oil composition observed in the current and previous research studies may be attributed to certain factors, which include climatic, altitude, geographical, and environmental factors (temperature, day length, light, fertilizers) [22, 23]. The essential oil of *A. hispidum* obtained from Nigeria in this present study also showed similarity in the essential oil constituents obtained from Congo [21] and Argentina [5], which were dominated by sesquiterpenes and represent the uniqueness of the class of chemical constituents present in this essential oil.

The enantiomeric distribution of terpenoid components of *A. hispidium* is summarized in Table 3. The chiral GC-MS analysis of *A. hispidum* essential oil showed the presence of five pairs of enantiomeric monoterpenoids and six enantiomerically pure chiral sesquiterpenoids (α-copaene, *trans*-β-elemene, (*E*)-β-caryophyllene, germacrene D, β-bisabolene and δ-cadinene). The ratio of the enantiomer presence in the essential oil gives information which could be related to the biosynthesis of the plant essential oil components and the biological activity of the essential oil [24, 25]. The enantiomeric excess (ee, %) was determined for α-pinene (85.7%), sabinene (70.6%), β-pinene (4.8%), limonene (65.4%) and linalool (82.7%). β-Pinene showed almost a racemic mixture with 52.38% (+) and 47.08% (–).

3.2. Antibacterial activity

The essential oil of *A. hispidum*was screened for antibacterial activity using the micro-dilution method against selected pathogenic bacteria, namely *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Streptococcus faecalis* ATCC 9790, *Salmonella typhi* ATCC 6539, *Proteus vulgaris* ATCC 6380, *Escherichiacoli* ATCC 25922, and *Pseudomonas*

Table 1. Chemical composition of the essential oil of *Acanthospermum hispidum* from north-central Nigeria

RT (min)	RI_{calc}	RI_{db}	Compounds	Composition (%)
12.1	925	925	α-Thujene	0.1
12.5	933	933	α-Pinene	3.0
13.4	949	950	Camphene	tr
14.2	963	964	Benzaldehyde	tr
14.7	972	972	Sabinene	1.1
15.0	978	978	β-Pinene	0.4
15.4	985	984	3-Octanone	tr
15.6	989	989	Myrcene	0.2
15.6	990	991	2-Pentylfuran	0.1
16.7	1007	1007	α-Phellandrene	tr
17.3	1017	1017	α-Terpinene	0.1
17.9	1025	1025	*p*-Cymene	0.1
18.2	1029	1030	Limonene	0.6
18.3	1031	1031	β-Phellandrene	0.1
18.4	1033	1032	1,8-Cineole	1.8
18.5	1035	1034	(*Z*)-β-Ocimene	0.1
19.2	1045	1045	(*E*)-β-Ocimene	tr
20.0	1058	1058	γ-Terpinene	0.1
21.8	1085	1086	Terpinolene	0.1
22.2	1090	1091	1-Undecene	tr
22.2	1091	1093	*p*-Cymenene	0.1
22.8	1101	1101	Linalool	0.2
23.2	1106	1107	Nonanal	0.1
28.5	1182	1180	Terpinen-4-ol	0.1
29.6	1198	1198	α-Terpineol	0.1
31.1	1220	1211	β-Cyclocitral	tr
31.8	1230	1229	Thymyl methyl ether	1.4
38.6	1331	1335	δ-Elemene	0.5
39.4	1343	1349	7-*epi*-Silphiperfol-5-ene	0.1
39.6	1346	1348	α-Cubebene	0.2
40.0	1353	1357	Eugenol	0.2
41.0	1368	1367	Cyclosativene	0.1
41.5	1376	1375	α-Copaene	2.1
41.9	1382	1383	*cis*-β-Elemene	0.1
42.3	1388	1387	β-Cubebene	0.9
42.5	1390	1390	*trans*-β-Elemene	3.6
42.6	1392	1394	Sativene	tr
43.4	1404	1405	(*Z*)-β-Caryophyllene	0.9
44.6	1420	1417	(*E*)-β-Caryophyllene	21.8
45.0	1430	1430	β-Copaene	0.2
45.1	1432	1432	*trans*-α-Bergamotene	0.1
45.5	1438	1438	Aromadendrene	0.1
46.4	1453	1452	(*E*)-β-Farnesene	0.3
46.7	1457	1454	α-Humulene	5.9
46.9	1460	1458	*allo*-Aromadendrene	0.1
47.3	1467	1466	Dehydrosesquicineole	0.1
47.6	1471	1471	β-Acoradiene	0.1
47.7	1473	1476	γ-Gurjunene	tr
47.8	1476	1475	γ-Muurolene	0.5
48.3	1482	1480	Germacrene D	6.1
48.4	1485	1483	*trans*-β-Bergamotene	0.2

Table 1 *(Continued)*

RT (min)	RI_{calc}	RI_{db}	Compounds	Composition (%)
48.7	1490	1489	β-Selinene	0.2
48.9	1492	1490	γ-Amorphene	0.2
49.2	1497	1497	Bicyclogermacrene	7.9
49.8	1508	1508	β-Bisabolene	0.9
50.1	1513	1512	γ-Cadinene	0.2
50.5	1519	1518	δ-Cadinene	1.8
50.8	1525	1524	β-Sesquiphellandrene	1.5
52.4	1552	1551	(*Z*)-Caryphyllene oxide	0.5
54.3	1581	1578	Spathulenol	1.4
54.4	1586	1587	(*E*)-Caryophyllene oxide	6.6
54.7	1590	1590	Globulol	0.3
55.9	1611	1611	Humulene epoxide II	0.7
56.4	1619	1624	*cis*-Calamenene	0.2
58.5	1656	1656	α-Bisabolol oxide B	0.2
58.8	1663	1661	*neo*-Intermedeol	0.3
60.6	1688	1688	α-Bisabolol	20.7
61.8	1716	1715	Pentadecanal	0.4
68.4	1841	1841	Phytone	0.5
74.5	1961	1958	Palmitic acid	0.9
Compound Classes				
Monoterpene hydrocarbons				5.9
Oxygenated monoterpenoids				3.6
Sesquiterpene hydrocarbons				56.6
Oxygenated sesquiterpenoids				31.0
Benzenoid aromatics				0.2
Others				1.9
Total identified				***99.2***

RT = Retention time in minutes. RI_{calc} = Retention index determined using a homologous series of *n*-alkanes on a ZB-5ms column. RI_{db} = Reference retention index from the databases. tr = trace (< 0.05%).

Table 2. Major components (%) of *Acanthospermum hispidum* leaf essential oils from different geographical locations.

Compounds	Nigeria This work	Nigeria [20]	Congo [21]	Argentina [5]
α-Pinene	3.0	15.9	nd	nd
Carvacryl methyl ether	nd	4.1	0.2-2.4	nd
α-Copaene	2.1	nd	2.3-3.5	3.5
trans-β-Elemene	3.6	1.0	2.0-3.1	10.0
(*E*)-β-Caryophyllene	21.8	28.0	34.0-42.7	35.2
α-Humulene	5.9	6.0	1.4-12.7	9.7
Germacrene D	6.1	6.9	6.4-10.1	11.1
Bicyclogermacrene	7.9	11.0	5.3-10.6	9.7
(*E*)-Caryophyllene oxide	6.6	1.5	2.8-4.7	0.8
α-Bisabolol	20.7	8.9	3.7-11.2	11.4

nd = not detected.

aeruginosa ATCC 27853 (Table 4). The antimicrobial assay revealed a wide spectrum of activity with minimum inhibitory concentrations (MICs) ranging from 125 to1250 µg/mL. Antimicrobial activities of plants have been defined to exhibit good anti-microbial activity with MIC < 100 µg/mL, moderate activity (MIC 100-500 µg/mL), weak activity (MIC 500-1000 µg/mL), and considered inactive(MIC > 1000 µg/mL) [26]. Notably, the essential oil of *A. hispidum* showed good antimicrobial activity against all selected pathogenic bacteria with *Salmonella typhi* and *Proteus vulgaris* moderately susceptible (MIC = 625

Table 3. The enantiomeric distributions of chiral terpenoid constituents of *Acanthospermum hispidum* essential oil from north-central Nigeria.

Compounds	RT (min)	RI_{calc}	RI_{db}	ED (%)	ee (%)
(–)-α-Pinene	15.4	979	976	7.2	
(+)-α-Pinene	15.6	981	982	92.8	85.7
(+)-Sabinene	19.2	1021	1021	14.7	
(–)-Sabinene	20.0	1028	1030	85.3	70.6
(+)-β-Pinene	19.8	1026	1027	52.4	4.8
(–)-β-Pinene	20.2	1031	1031	47.6	
(–)-Limonene	24.6	1075	1073	82.7	65.4
(+)-Limonene	25.5	1082	1081	17.3	
(–)-Linalool	44.9	1228	1228	91.4	82.7
(+)-Linalool	45.5	1231	1231	8.6	
(–)-α-Copaene	62.3	1390	1381	100.0	100.0
(–)-*trans*-β-Elemene	65.7	1429	1420	100.0	100.0
(–)-(*E*)-β-Caryophyllene	68.7	1465	1461	100.0	100.0
(+)-Germacrene D	nd	nd	1519	0.0	
(–)-Germacrene D	73.4	1523	1522	100.0	100.0
(+)-β-Bisabolene	74.9	1543	1546	100.0	100.0
(–)-β-Bisabolene	nd	nd	1549	0.0	
(–)-δ-Cadinene	nd	nd	1563	0.0	
(+)-δ-Cadinene	76.8	1568	1576	100.0	100.0

RT = Retention time in minutes. RI_{calc} = Retention index determined using a homologous series of *n*-alkanes on a Restek B-Dex 325 capillary column. ED = Enantiomeric distribution. ee = enantiomeric excess. RI_{db} = Retention index from our in-house database. nd = compound not detected.

Table 4. Antibacterial activities (MIC, µg/mL) of *Achanthospermum hispidum* leaf essential oil from north-central Nigeria.

Organism	*Acanthospermum hispidum* leaf EO	β-Caryophyllene	Caryophyllene oxide	α-Bisabolol	Streptomycin
Staphylococcus aureus	125	312.5	78.1	nt	<19.5
Bacillus subtilis	2500	312.5	312.5	312.5	<19.5
Streptococcus faecalis	2500	312.5	312.5	312.5	<19.5
Salmonella typhi	625	312.5	312.5	312.5	<19.5
Proteus vulgaris	625	312.5	312.5	312.5	< 19.5
Escherichia coli	1250	312.5	625	312.5	< 19.5
Pseudomonasaeruginosa	1250	312.5	312.5	312.5	< 19.5

EO = essential oil. nt = not tested.

µg/mL) and strong susceptibility against *Staphylococcus aureus* (MIC = 125 µg/mL). The antimicrobial activity displayed by the essential oil of *A. hispidum* may be attributed to the presence of identified (*E*)-β-caryophyllene and α-bisabolol constituents. (–)-α-Bisabolol, a monocyclic sesquiterpene alcohol has been reported to have promising activities as anti-inflammatory, anti-irritant, and antibacterial [27, 28]. Synergistic effects likely play a role in the activities [29, 30].

4. Conclusions

In conclusion, chiral GC-MS is an efficient method of analysis of reporting enantiomeric distribution of chiral terpenoids present in the essential oil. The results revealed the importance of enantiomers of sesquiterpenoid compounds as antibacterial agents. The major compounds observed in the present study, (*E*)-β-caryophyllene (21.8%) and α-bisabolol (20.7%), complement the previous investigations of *A. hispidum* essential oil. Future studies should be considered for the prospect of *A. hispidum* as an antimicrobial agent. That is, the essential oil may be considered for formulation as an antibacterial in the pharmaceutical or cosmetics industries.

Authors' contributions

Conceptualization, M.S.O; Methodology, D.S.R.O., M.S.O., P.S., and W.N.S.; Software, P.S.; Validation, L.A.O., W.N.S., Formal Analysis, A.P., and W.N.S.; Investigation, D.S.R.O., M.S.O., P.S., A.P., and W.N.S.; Resources, D.S.R.O., M.S.O., P.S. and W.N.S.; Data Curation, W.N.S.; Writing – Original Draft Preparation, D.S.R.O. and M.S.O; Writing – Review & Editing, M.S.O. and W.N.S.; Project Administration, D.S.R.O. and M.S.O.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest.

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