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Chemical Constituents and Antibacterial Activity of Essential Oils from Flowers and Stems of *Ageratum conyzoides* from Ivory Coast

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Abstract: The essential oils (EOs) obtained by hydro-distillation of flowers and stems of *Ageratum conyzoides* L. (Asteraceae) growing in Ivory Coast were investigated. The oils were analyzed and characterized by GC and GC–MS. Analyses of the EOs led to the identification and quantification of 48 constituents in the flower oil and 44 from the stem oil, respectively. Characterization of the EOs revealed the predominance of 6-demethoxyageratochromene or precocene I (flower: 58.8%, stem: 76.5%) and the sesquiterpene *β*-caryophyllene (flower: 15.2%, stem: 8.1%). Six of the identified compounds *β*-copaene, hexanal, trans-cadina-1(6),4-diene, *α*-calacorene, caryophylla-4(12),8(13)-diene-5-β-ol and 1,10-di-epi-cubenol are reported for the first time as constituents of *A. conyzoides*. Comparative analysis with data from Nigeria, Pakistan, Fiji and Brazil is reported. The antibacterial activity of EOs from of *A. conyzoides* was tested against seven bacteria. The inhibition zones and minimum inhibitory concentration (MIC) for bacteria strains which were sensitive to *A. conyzoides* EOs were in the range of 6.7 to 12.7 mm and 64 to 256 µg/mL, respectively. The EOs showed moderate activity against *Staphylococcus aureus* and *Enterococcus faecalis*.

Keywords: *Ageratum conyzoides*; essential oils; flowers; stems; precocene I; antibacterial activity; Ivory Coast.

1. Introduction

Ageratum conyzoides L. belongs to the family Asteraceae, tribe Eupatoriae. The genus *Ageratum* consists of approximately 30 species but only a few species have been phytochemically investigated [1], *A. conyzoides* is a tropical plant that is very common in West Africa as well as in some parts of Asia and South America. It is an annual branching herb which grows to approximately 1 m in height [2]. This plant is known mainly for its therapeutic qualities and has traditionally been used in various parts of Africa, Asia and South America for curing a variety of diseases. In West Africa, in addition to its popular use for skin diseases and wound healing [3, 4], *A. conyzoides* is also employed as an antimalarial agent [5, 6]. Additionally, in Ivory Coast, *A. conyzoides* is used against gastrointestinal pain [7], in the treatment of epilepsy, measles, eye diseases, headache, as an anthelminthic, an antidiabetic, and to facilitate childbirth [8-13]. *A. conyzoides* found in India, China, Brazil and Colombia has been used in folk medicine for the treatment of several diseases such as boils, eczema, diarrhea, leprosy, metrorrhagia, dermatitis, fever and inflammation. It has also been used as anti-hemorrhagic, analgesic, diuretic, antipyretic, insecticide and to treat rheumatism [14-

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18]. In Central and Southern Africa (Cameroon, Congo and Kenya), *A. conyzoides* is used in traditional medicine to treat pneumonia [19], pain [20], for its anti-asthmatic, antispasmodic, haemostatic, emetic, analgesic and anesthetic properties, as well as for uterine issues [21-23].

Widespread traditional use of this plant in the cure of several diseases has led to the investigation of the biological activities of its extracts. Assays using whole plant hexane extracts in Colombia, or petroleum ether and acetone extracts of leaves in India, have demonstrated insecticidal activity against *Musca domestica* and *Culex quinquefasciatus* larvae [15, 24, 25]. Similar results were observed for *Anopheles stephensi* larvae in others assays, confirming the anti-juvenile hormone potential of *A. conyzoides* [26, 27]. In Cameroon, Nigeria, Sudan, Kenya and Ivory Coast, the methanol, ethanol, chloroform, petroleum ether and aqueous extracts of the plant have shown the following properties: cicatrizing [14, 28], antiurolithic [29], antioxidant [30], antidiabetic [8], antibacterial [19, 31], haemostatic [32], antispasmodic [33], antimalarial and antidiarrheal [5, 6, 34].

A large number of pharmacological activities have been attributed to the essential oil (EO) of *A. conyzoides* [17]. The plant produces a volatile oil with a strong odor for which chemical analysis in different countries has shown significant variation in chemical composition. In the west of India, the EO obtained from all part of *A. conyzoides* has shown a dominance of precocene I (52.2%) and caryophyllene (26.2%). This EO has demonstrated fungicidal activity by inhibiting the growth of *Aspergillus parasiticus* and the production of aflatoxin. The strongest antibacterial activity was observed against the bacteria *Staphylococcus aureus* and *Bacillus subtilis* [35]. The main components of the EOs of flowers and a combination of leaves plus stems of *A. conyzoides* from Pakistan were *β*-caryophyllene (14.4% and 17.0%), 6-demethoxy-ageratochromene (30.3% and 26.6%) and ageratochromene (34.9% and 36.9%), respectively [36]. Eos extracted from leaves of *A. conyzoides* collected in Ibiúna, Ribeirão Pires and Campinas in the São Paulo state, southeastern Brazil have shown some differences in chemical constitution [37]. There is also a significant difference between the amounts of the major compounds (precocene I and II) in the EOs of leaves and aerial parts of the plants growing in Ibiuna [38]. This indicates the chemical composition of the EO may vary depending on the part of the plant studied. A similar observation has been made for volatiles oils extracted from the different parts (inflorescence, leaf, stem, root) of the plant collected in Santarém Novo (State of Pará, northern Brazil) [39]. Chemical compositions of the EOs extracted from the leaves of *A. conyzoides* acclimatized in the western zone of Africa display a similar pattern. The leaves of Ghanaian plants contain 80.3% of precocene I [40], while those from Burkina-Faso, Benin, Lagos and Ibadan (Nigeria) respectively contain 86.0% [41], 85.6%, 63.1% [42] and 82.2% [43]. The EO exhibited remarkable insecticidal activity-against the cowpea weevil, *Callosobruchus maculatus* F. [44]. Work on EOs extracted from the leaves of *A. conyzoides* acclimatized in Ivory Coast showed a different chemical composition with only 46.5% of precocene I [41]. There is no comparative information on the EOs extracted from the flowers and stems of plants from Ivory Coast and elsewhere in Africa.

From the literature review it is evident that: (i) geographical variations have a major quantitative and/or qualitative impact on the chemical constituents of EOs; (ii) the chemical composition of the EO may vary depending on the part of the plant studied; (iii) there is little research on the antibacterial activity of *A. conyzoides* EOs. This work presents the chemical compositions and antibacterial activities of the EOs extracted from the stems and flowers of the same *A. conyzoides* plants originating from Ivory Coast.

2. Materials and Methods

2.1. Plant Material

Flowers and stems of *Ageratum conyzoides* were collected at 8 am in Bobia village in the west of Ivory Coast. Plant material was identified by Professor Ake Assi in the National Floristic Center of the University of Felix Houphouët Boigny, Cocody-Abidjan, Department of Botany, Ivory Coast where voucher specimens were deposited (Herbarium Voucher Number: CNF-14229).

2.2. Isolation of the Essential Oil

The EOs were isolated from fresh flowers and stems by hydrodistillation using a Clevenger-type apparatus. The obtained oils were dried over anhydrous sodium sulfate and, after filtration, stored in a sealed sample tube at 0°C until GC and GC-MS analysis.

2.3. Chemical Analyses of the Essential Oil

EO composition was investigated first by Gas Chromatography (GC) with a flame ionization detector (FID) and then by GC coupled with a Mass Spectrometer (GC-MS).

2.3.1. GC-FID Conditions

GC analysis was carried out using a Delsi DI 200 instrument equipped with a FID and a DB5 column (25 m x 0.25 mm, df: 0.25 µm) with a split flow rate of 60 mL/min. Nitrogen was used as carrier gas; temperature programming was 5 min at 50°C and 30°C/min up to 220°C, injector and detector temperatures were respectively set to 220°C and 250°C.

2.3.2. GC-MS Conditions

GC-MS analysis was performed using a Hewlett-Packard gas Chromatograph Model 6890 coupled to a Hewlett-Packard MS Model 6890 equipped with an HP5 column (30 m x 0.25 mm, df: 0.25 µm). Initial oven temperature was maintained at 50°C for 5 min and then programmed at 50 °C/min to 300 °C (held 50 min). The carrier gas was helium (1.0 mL/min); a split injection with a split ratio 1:10 was chosen. Injector and detector temperatures were respectively set to 250°C and 320°C. The electron multiplier was set at 2200 V with an applied electron ionization voltage of 70 eV, with the ion source temperature at 230°C. Mass spectral data were acquired in the scan mode in the *m/z* range of 33-450. Identification of compounds was carried out by calculating Retention Indices (RI) or Kováts Indices (KI) and comparing mass spectra with those in data banks, i.e. Adams [45] or Mc Lafferty and Stauffer [46]. For quantification purposes, relative area percentages obtained by field ionization detection (FID) were used.

2.4 Bacterial strains

Pure bacterial strains were obtained from the Institut Pasteur de Côte d'Ivoire (IPCI) and were either from the ATCC or clinical isolates. Bacteria strains were cultured overnight at 37°C in nutrient agar (NA, Oxoid). EOs were tested against the following microorganisms:

Gram-positive: *Enterococcus faecalis* USSURMI 469C/13, *Staphylococcus aureus* USSURMI 524C/13, *Staphylococcus aureus* ATCC 25923.

Gram-negative: *Klebsiella pneumonia* USSURMI 444C/13, *Shigella sp.* USSURMI 434C/13, *Escherichia coli* ATCC 25922, *Citrobacter koseri* USSURMI 745C/13, *Enterobacter aerogenes* USSURMI 746C/13.

2.5 In vitro Antimicrobial Evaluation

The *in vitro* antimicrobial activity of EOs was evaluated by the disc diffusion method using Mueller-Hinton Agar with determination of inhibition zones (IZ) according to the Committee of Clinical Laboratory Standards [47]. Freshly-grown microbial suspensions in Mueller-Hinton broth were standardized to a cell density of 1.5×10^8 (McFarland 0.5). In the disc sensitivity test, three different concentrations of the EOs were prepared by dissolving at 10% (v/v) in dimethyl sulfoxide (DMSO). Discs of 6 mm diameter (BioRad) were sterilized at 121°C for 15 min. An aliquot (20 µL) of EO solution was applied to a disc and allowed to dry. After 18 to 24 h of incubation at 37°C, the diameters of the growth inhibition zones were measured. The results are reported as mean ± standard deviation (SD) for three repeats. Gentamicin (30 µg/disc) and DMSO (10%) were used as positive and negative control, respectively. Gentamicin standard was purchased from Sigma-Aldrich.

2.6 Determination of Minimum Inhibitory Concentration

For minimum inhibitory concentration (MIC), a micro broth dilution method in broth media Mueller-Hinton (Difco) susceptibility assay was used. In these experiments, EO dissolved in Tween 80 (Merck,

Germany) was first diluted to the highest concentration at the concentration 10% (v/v) in order to enhance EO solubility. Geometric dilutions ranging from 0.5 to 1024 µg/mL of EOs were prepared in 96-well microtiter plates, the volume being 100 µL. Bismaleimidohexane (90 µL) was added. As a final step, 10 µL of 1×10^6 UFC/mL (according to 0.5 McFarland turbidity standards) of standardized microorganism suspensions were inoculated into each well. The test was performed in a volume of 200 µL with concentrations of 0.25 to 512 µg/mL. All tubes were incubated in air at 37°C for 24 h before being read. The same test was performed simultaneously for the grow control (Mueller-Hinton broth + test microorganism) and sterility control (Mueller-Hinton broth + test oil). The MIC is the lowest concentration of the sample that prevented visible growth. Microorganism growth was indicated by the turbidity and a pellet on the well bottom.

3. Results and Discussion

3.1. Composition of Essential Oils

The hydrodistillation of flowers and stems of *A. conyzoides* afforded EOs (yellow) with yields of 0.22% and 0.19% (v/w), respectively. The yield of EOs of the flowers compared favorably with the yields from flower of *A. conyzoides* from Fiji [48] and Nigeria [49]. GC and GC-MS analysis of *A. conyzoides* flower EOs led to the identification and quantification of a total of 51 major components accounting for 95.9% of the total components present. Forty-four constituents representing 98.2% were identified in EO from the stems (Table 1). Flower and stem oils of the plants from Ivory Coast were dominated by chromenes and sesquiterpene derivatives. The main components were 6-demethoxyageratochromene or precocene I (flower 58.8%, stem 76.5 %) and *β*-caryophyllene (flower 15.2%, stem 8.1%). The EOs of the flowers and stems of *A. conyzoides* from Ivory Coast both showed a precocene I and *β*-caryophyllene chemotype. The amount of precocene I was lower in the flowers and higher in the stems. Oil from the stems contained the lowest amount of *β*-caryophyllene. Among the identified compounds, seven have not been reported previously in *A. conyzoides*. These are *β*-copaene (flowers 0.03%, stems 0.02%), hexanal (flowers 0.03%, stems 0.03%), *trans*-cadina-1(6),4-diene (flowers 0.50%, stems 0.11%), *α*-calacorene (flowers 0.02%, stems 0.02%), caryophylla-4(12),8(13)-diene-5-β-ol (flowers 0.29%, stems 0.06%), 1,10-di-epi-cubenol (flowers 0.08%, stems 0.04%) and (*E*)-nerolidol (flowers 0.61%, stems 0.29%).

Although, studies on the comparative percentage composition of *A. conyzoides* EOs from different regions and different parts of the plant are well documented [36, 39, 48, 49], this is the first report for EOs of both flowers and stems of *A. conyzoides* L. from Ivory Coast. Results presented in Table 2 indicate that, whilst the flower oils of *A. conyzoides* from Ivory Coast, Nigeria, western Fiji and northern Brazil have the same chemotype dominated by precocene I and *β*-caryophyllene, this chemotype is different from that obtained for flowers oils from Pakistan and eastern Fiji, which have precocene I, *β*-caryophyllene and precocene II as the main components. In addition, stems oils of the plants of Ivory Coast and northern Brazil have a chemotype with precocene I and *β*-caryophyllene. However, although precocene I and *β*-caryophyllene are present in all samples, their amounts vary from plant to plant. Quantitatively, whilst only a trace of precocene II is found in the EOs of Ivory Coast plants (flower: 0.12%, stem: 0.06%) and western Fiji (flower: 0.4%), this component is completely absent in oils from Nigeria and northern Brazil. In contrast, the percentages of precocene II in the flower oils of Pakistan and eastern Fiji are substantial: 34.9% and 15.5%, respectively.

The difference in chemical composition of EOs can be attributed to climatic variations specific to each location, which shows the importance of geo-ecological factors in the production of metabolites of the plant.

Table 1. Chemical constituents of essential oil extracted from flowers or stems of *Ageratum conyzoides*

			Flower	**Stem**
N°	**KI**	**Compound**	**Content (%)**	
1	839	hexanal	0.03	0.03
2	934	*α*-pinene	0.07	0.21
3	951	camphene	0.36	1.60
4	979	*β*-pinene	0.04	0.17
5	990	myrcene	0.05	0.05
6	999	*δ*-2-carene	0.30	0.91
7	1007	*α*-phellandrene	nd	0.03
8	1030	limonene	0.13	0.47
9	1086	terpinolene	nd	0.05
10	1100	linalool	0.04	nd
11	1113	(*E*)-4,8-dimethyl-nona-1,3,7-triene	0.37	0.38
12	1175	endo borneol	0.03	0.08
13	1231	bornyl formate	0.20	0.49
14	1286	bornyl acetate	0.68	1.29
15	1290	thymol	0.06	0.24
16	1310	2-methoxy-4-vinylphenol	0.10	nd
17	1334	*δ*-elemene	0.03	nd
18	1349	*α*-cubebene	0.07	nd
19	1353	*α*-longipinene	0.25	0.03
20	1358	eugenol	0.04	0.04
21	1379	*α*-copaene	0.13	0.03
22	1387	*β*-bourbonene	0.07	nd
23	1390	*β*-cubebene	1.06	0.22
24	**1425**	***β*-caryophyllene**	**15.20**	**8.06**
25	1430	*β*-copaene	0.03	0.02
26	1435	trans-α-bergamotene	0.22	0.08
27	1442	(*Z*)-β-farnesene	0.19	0.15
28	1454	α-humulene	1.68	1.43
29	1460	(*E*)-β-farnesene	1.58	0.54
30	**1469**	**6-demethoxyageratochromene (precocene I)**	**58.78**	**76.46**
31	1474	trans-cadina-1(6),4-diene	0.50	0.11
32	1479	*γ*-cadinene	0.14	0.07
33	1486	germacrene-D	2.84	0.93
34	1496	trans-muurola-4(14),5-diene	0.89	0.21
35	1499	bicyclogermacrene	1.65	0.87
36	1504	*α*-muurolene	0.08	nd
37	1509	*β*-bisabolene	0.11	0.06
38	1520	*γ*-cadinene	0.48	0.07
39	1526	*β*-sesquiphellandrene	1.82	0.88
40	1536	trans-cadina-1,4-diene	0.04	nd
41	1545	*α*-calacorene	0.02	0.02
42	1561	(*E*)-nerolidol	0.61	0.29
43	1581	spathulenol	0.22	0.07
44	1587	caryophyllene oxide	0.73	0.35
45	1599	6-acethyl-2,2-dimethylchroman	0.08	0.04
46	1609	humulene-1,2-epoxide	0.04	nd
47	1615	1,10-di-epi-cubenol	0.08	0.04
48	1632	1-épi-cubenol	0.09	nd
49	1642	caryophylla-4(12),8(13)-diene-5-β-ol	0.29	0.06
50	1653	desmethoxyencecalin	1.61	0.22
51	1660	Ageratochromene (precocene II)	0.12	0.06
52	1676	androencecalinol	2.34	0.87
53	2107	phytol	0.09	0.05
		TOTAL	**95.85**	**98.15**

nd: not detected

Table 2. Comparative percentage composition of flowers and stem oils of *Ageratum conyzoides* from Ivory Coast, Nigeria Pakistan, Fiji and Brazil.

	Ivory Coast		Nigeria [49]	Pakistan [36]	Eastern Fiji [48]	Western Fiji [48]	Northern Brazil [39]	
Part of the plant	**Flower**	**Stem**	**Flower**	**Flower**	**Flower**	**Flower**	**Flower**	**Stem**
Main components (%)								
6-demethoxyageratochromene (precocene I)	58.78	76.46	57.20	30.30	38.30	53.80	55.50	71.60
β-Caryophyllene	15.20	8.06	18.50	14.35	20.50	18.70	19.40	12.80
Ageratochromene (precocene II)	0.12	0.06	-	34.90	15.50	0.40	-	-

3.2 Antibacterial Activity

The antibacterial activities of the EOs extracted from the flowers and stems of *A. conyzoides* and evaluated on 3 gram-positive and 5 gram-negative bacteria are presented in Table 3. The EOs exhibited a strong broad-spectrum antimicrobial activity against all these organisms when tested with the disk diffusion bioassay.

Table 3. Antibacterial activity of essential oils of *A. conyzoides* on some selected microorganisms

Microorganisms	**Sources**	**Zone of inhibition (mm)**			**MIC (µg/mL)**	
		Essential oils (20µg/disc)		**Gentamicin**	**Essential oil**	
		Flower	**Stem**	**(30µg/disc)**	**Flower**	**Stem**
Staphylococcus aureus	ATCC 25923	12.7±0.0	12.0±0.7	35.0±1.00	64	64
Enterococcus faecalis	USSURMI 469C/13	8.3±1.4	8.0±1.4	-	256	256
Staphylococcus aureus	USSURMI 524C/13	9.7±0.0	9.0±0.0	63.7±0.6	256	256
Klebsiella pneumoniae	USSURMI 444C/13	7.0±0.0	6.7±0.0	26.3±1.2	> 512	> 512
Shigella sp	USSURMI 434C/13	7.7±0.7	7.7±0.0	41.3±0.6	512	512
Escherichia coli	ATCC 25922	7.7±0.7	7.3±0.7	22.3±1.2	256	256
Citrobacter koseri	USSURMI 745C/13	7.3±0.7	7.3±0.0	27.3±0.6	256	256
Enterobacter aerogenes	USSURMI 746C/13	6.7±0.0	7.3±0.7	22.3±1.2	512	512

USSURMI: Clinical isolate from Institut Pasteur de Côte d'Ivoire.

The inhibition zone (IZ) values for bacterial strains which were sensitive to EOs, were in range of 6.7 to 12.7 mm. *A. conyzoides* EOs showed moderate antibacterial activity against strains of gram-positive bacteria *Staphylococcus aureus* and *Enterococcus faecalis* with zones of inhibition (IZ) between 8.3 and 12.7 mm. The strongest antibacterial activity was observed against *Staphylococcus aureus* ATCC 25923. The bacterial strain *Enterococcus faecalis* 469C/13 obtained from clinical isolates was not sensitive to the antibiotic Gentamicin, while the EOs of the flowers and stems of *A. conyzoides* showed antibacterial activity against this bacterial strain (flower IZ = 8.3 ± 1.4 mm; Stem IZ = 8.0 ± 1.4 mm). In contrast, only low or negligible activity was observed against the gram negative bacteria *Klebsiella pneumoniae, Shigella sp*, *Escherichia coli*, *Citrobacter koseri* and *Enterobacter aerogenes* with IZs of 6.7 to 7.7 mm. The bacteriostatic efficacy of *A. conyzoides* EOs estimated by minimum values of inhibitory concentration (MIC) was between 64 and 256 µg/mL. For *Klebsiella pneumoniae*, *Shigella sp* and *Enterobacter aerogenes* strains, the MIC values are greater than or equal to 512 µg/mL. The antibacterial activity of the EOs extracted from the flowers and stems of *A. conyzoides* observed in this study confirms the antimicrobial potential reported by Patil *et al*. [35] against other bacteria.

4. Conclusions

The essential oils of the flowers and stems of *A. conyzoides* collected in Ivory Coast both show a chemotype dominated by precocene I and *β*-caryophyllene. Amongst the 53 compounds identified, six are reported for the first time as constituents of *A. conyzoides*. Tests of antibacterial activity show that the EO from *A. conyzoides* has moderate antimicrobial activity against gram-positive bacteria but is less effective

against gram-negative bacteria. The level of observed activity validates the use of the essential oils of this plant in traditional medicine for the treatment of infectious diseases. This study on the antibacterial activity of essential oils contributes to the search for new classes of antimicrobial agents needed due to the rapid increase in multidrug-resistant organisms [50].

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