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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OILS OF *AGERATUM CONYZOIDES* (ASTERACEAE) HARVESTED IN THE REGION OF THE MOUNTAIN DISTRICT IN CÔTE D'IVOIRE

Konan N'dri Séraphin[1], Kouamé Bosson Antoine[2], Traoré Lanciné[1]*, Gooré Kouakou Serge[1], MamyrBékova-Békro Janat Akhanovna[2] and Békro Yves-Alain[2]

[1]UFR Sciences et Technologies, Université de Man, BP 20 Man, Côte d'Ivoire.
[2]Laboratoire de Chimie bio-Organique et de Substances Naturelles (LCBOSN), UFR SFA, Université Nangui Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire.

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***Corresponding Author**
Traoré Lanciné
UFR Sciences et Technologies, Université de Man, BP 20 Man, Côte d'Ivoire.

ABSTRACT

Essential oils (EO) from the organs of *Ageratum conyzoides* were extracted by hydrodistillation with a yield of (0.17±0.02) % for fresh flowers and (0.27±0.03)% for stems and leaves. The chemical compositions of the organs were highlighted by GC/MS. Eighteen (18) compounds corresponding to 99.98% of the total constituents were identified in the stems and leaves. It consists of hydrocarbon (1.49%) and oxygenated (0.24%) monoterpenes, hydrocarbon (3.4%) and oxygenated (0.33%) sesquiterpenes and other compounds (0.76%). The majority phytocompound is precocene I (93.76%). That of the flowers is made up of 21 compounds also representing 99.98%) of the total chemical composition. It has the same phytochemical composition as the EO of stems and leaves but the proportions are different. The major phytoconstituent is also precocene I (90.38%). The in vitro evaluation of the antioxidant activity with the radical DPPH at 0.3 mM, showed a weak antioxidant power of the essential oil extracts. The IC50 values determined are 9.78 µg/mL for the EO extract of leaves and stems and 12.5 µg/mL for that of the flowers of the species.

KEYWORDS: *Ageratum conyzoides*, essential oils, Precocene I, antioxidant activity.

INTRODUCTION

Human beings have always used plants for food but also for healing. Considerable economic advantages in the development of this medicine and in the use of medicinal plants for the treatment of various diseases have been observed.[1] Plants and essential oils (EO) are endowed with powers to cure certain diseases. Articles concerning well-being and health through plants and essential oils are flourishing.[2] Essential oils are also used as anti-inflammatory, antioxidant, healing, etc.[3] Indeed, this rush towards herbal medicine can be explained by the fact that plants are accessible and abundant, thus making medicine through the use of plants affordable, especially in developing countries.[4] It is in this order of idea that we were interested in *Ageratum conyzoides* (Asteraceae) a medicinal plant and essential oil widely used in traditional medicine by populations for the treatment of many diseases. *Ageratum conyzoides* is an annual, erect, softly pubescent herb that can reach 1m in length.[5] Witchweed is a tropical plant with multiple uses in folk medicine. The leaves are used as an anti-bacterial to treat ulcers, wounds, dental pain, dermatoses and burns. The roots are used in a decoction to cure diarrhea.[5-7] Many important works have been carried out on the organs of *A. conyzoides*. The 70% ethanolic extract of leafy twigs exhibits mildly toxic effects on proliferating human HFF cells.[8] Ethanolic extracts from the whole plant, methanol from the stems and aqueous from the leaves are used to treat ulcers.[9] The aqueous extracts of the leaves have an antifungal activity against *A. niger* and *A. ustus* with an average inhibition of 20 mm each[10] and an anti-diarrheal activity.[11] At a dose of 5 g/kg bw of EAqAc, it seems to normalize the AST and ALT levels of treated diabetic rats.[12]

Recent work on the chemical composition and antimicrobial and antioxidant activities of the essential oil of the aerial parts of witch weed has been carried out. In Côte d'Ivoire, EOs, extracted by hydrodistillation, from organs (flowers and stems), harvested in Gagnoa in December, with a pale yellow color, have respective yields of 0.22% for flowers and 0.19% for the stems. The majority compounds are precocene I with the proportions of 58.78% for the flowers and 74.46% for the stem bark followed by β-caryophyllene with 15.2% for the flowers and 8.06% for the stem bark. The antioxidant activity was evaluated with the radical DPPH. The IC_{50} values determined are 1970 µg/ml for the flowers and 3020 µg/ml for the stems. It has moderate antibacterial activity against GRAM + bacteria.[13,14] The main compounds of that of the leaves harvested in Abidjan on the Cocody campus are pecocene I (51.81%), 7-bromo-2,2-dimethylhept-3-yne (15.52%) and 1-methylcyclohex- 1-ene (11.5%). EO has a higher antibacterial activity than ampicillin, which is the reference antibiotic.[15]

Precocene I (80%) and β-caryophyllene (6%) are also the major constituents of that of the species collected in Abidjan.[16] In Burkina Faso, the EO from fresh leaves, harvested in January, is extracted by hydrodistillation. It has a pale yellow color and a yield of 0.75%. The results of the analyzes made it possible to identify 32 compounds, representing more than 99% of the total chemical composition of the essential oil of the species. Precocene I (86.44%) and β-caryophyllene (8%) are the main compounds of the essential oil.[17] The major constituents of that of leafy twigs, harvested in June 2010, are precocene I (82.09%) and β-caryophyllene (8.49%).[18] The yield of EO from fresh flowers, extracted by hydrodistillation from the Nigerian species, is 0.25%. The majority compounds are precocene I (57.2%) and β-caryophyllene (18.5%).[19] Work has been carried out by Vietnamese researchers. The main compounds of the EO of the leaves of the species are precocene II (45.75%), precocene I (14.09%) and β-caryophyllene (12.13%). It is endowed with antibacterial and anti-radical properties. The IC_{50} value is 8 mg/mL.[20] Work on the geographical variation of the chemical composition of EO in wizard grass has been done in Brazil. The essential oils from the leaves harvested in three regions were extracted by hydrodistillation. The main compounds in Campinas are precocene I (81.25%) and β-caryophyllene (13.36 %) In Rebeirao Pires, these are precocene I (79.11%), precocene II (10.39%) and β-caryophylene (8.39%). In Ibiuna, these are precocene II (54.99%), precocene I (29.13%) and β-caryophyllene (11.45%). These essential oils have antifungal properties.[21] This is why, in this work for the purpose of contributing to the development of medicinal and aromatic plants of the Ivorian flora, we propose to determine the chemical composition and to evaluate the antioxidant activity by spectrophotometry of the essential oil of *Ageratum conyzoids* collected at Man.

MATERIALS AND METHODS

Plant material

The plant material consists of the organs (flowers, stems and leaves) of *Ageratum conyzoides* (Asteraceae) of region of the Mountain District in Côte d'Ivoire. The plant was identified and authenticated by a technician at the Center National de Floristique (CNF) in Abidjan (Côte d'Ivoire) using the existing herbarium under number H UCJ003411.

Method of extraction of essential oils

The extraction was carried out by hydrodistillation using a Clevenger-type device. To extract the essential oils from the organs of witch weed, we used a 6 liter round bottom flask

containing approximately 3 L of distilled water, plant material and a condenser. The stems and leaves of *Ageratum conyzoides* were cut into small pieces when the flowers were carefully cut. A cooler is mounted on the flask containing the plant material. The whole is brought to the boil with a heating cap. The water vapor carries the volatile products towards the condensation column. Condensed vapor is a binary azeotropic mixture composed of floral water and essential oil. This is separated from the water by decantation. It is dried over anhydrous sodium sulphate. The different EO samples are put in vials covered with aluminum foil and stored in a refrigerator at -9°C.[22,23]

Determination of phytochemical composition

The analysis of EO diluted in dichloromethane (1:100) was carried out on a GC chromatograph (7890A, Agilent Technologies) coupled to a mass spectrometer (5975C, Agilent Technologies). A sample of HE (1µL) was injected into an HP-5MS capillary column at 250°C. The oven temperature was programmed at 40°C for 5 min, then at 2°C/min for 15 min up to 250°C, with a flow rate of 10°C/min up to 300°C. Helium was used as the carrier gas with a flow rate of 1 mL/min. The MS detector had a temperature of 280°C and a voltage of 1.4 kV. Only ions whose mass/charge ratio is between 40 and 500 can be detected. The identification of the compounds was carried out by comparing the retention indices, calculated from the retention times and the mass spectra obtained with those of the National Institute of Standards and Technology (NIST) database and literature.[13,24]

$$RI = 100\left[n + \frac{t_R(C_i) - t_r(C_n)}{t_r(C_{n+1}) - t_R(C_n)}\right]$$ *RI* : Retention indice

C_i: Unknown compound of EO

C_n: linear alkane (comprising n C atoms) whose retention time is just before that of the unknown EO compound;

C_{n+1}: Linear alkane (comprising n C atoms) whose retention time is just after that of the unknown compound;

n: carbon number of the linear alkane.

$t_R(C_n)$: retention time of the linear alkane with n carbon atoms.

Evaluation of antioxidant activity. The antioxidant potential of the extracts was assessed using the Blois method

The DPPH is dissolved in absolute ethanol to obtain a solution with a molar concentration of 0.3 mM. The solutions to be tested: are diluted in absolute ethanol in order to have the following concentrations in µg/mL: 2.5; 5; 10; 25; 50; 125; 250 and 500.

2.5 mL of solution to be tested are introduced into dry and sterile hemolysis tubes and 1 mL of ethanolic solution of DPPH is added. After shaking, the tubes are placed in the dark for 30 min away from light.

For each solution to be tested, a blank consisting of 2.5 mL of pure absolute ethanol to which 1 mL of ethanolic DPPH solution is added is prepared.

For the negative control, a DPPH solution is prepared by diluting 1 mL of the ethanolic DPPH solution in 2.5 mL of ethanol. For the positive control, a solution of vitamin C (ascorbic acid) is used, the absorbance of which is measured under the same conditions. The residual absorbance is measured at 517 nm. It is translated into percentage inhibition by the following formula[25]:

$$\%I = (1 - \frac{\text{Abs test}}{\text{Abs DPPH}}) \text{ x100}$$

%I: Inhibition percentage. Abstest: Absorbance of the ethanolic solution of EO and DPPH; AbsDPPH: absorbance of the blank (ethanolic solution of DPPH).

RESULTS AND DISCUSSION

Result of the extraction

Essential oils obtained by hydrodistillation have a pale yellow color with an aromatic odor. The yields of essential oils extracted from the organs of A. conyzoides are (0.17±0.02) % for the flowers and (0.27±0.03)% for the stems and leaves. By comparing our results with those reported in the literature, we find that: for the flowers and stems + leaves of *A. conyzoides* (0.19% and 0.27%). Our results are of the same order of magnitude as the studies reported by Daouda, and by Wandji.[26] These authors have shown that the essential oil content of the species varies from 0.11 to 0.58% for the stems and leaves, from 0.2% for the fresh flowers. Similar yields were also obtained by Albersger et al.,[27] and Usman and[19] on flowers of the same plant acclimatized in Fiji Islands and Nigeria respectively. This similarity is due to the fact that Nigeria and Ivory Coast are located in the same tropical zone. According to some authors, the harvest period and place of harvest and the distillation technique have an influence on the yield of essential oil.[28,29]

Phytochemical composition of essential oils extracted by GC-MS from organs

Table I: Chemical composition of the essential oils of the organs of Ageratum conyzoides.

N°	IR	Composés	M/Z	%(Fl)	%(T+Fe)
1	924	α-pinene	136	0.13	0.07
2	938	Camphene	136	1.39	0.48
3	966	β-Pinene	136	0.11	-
4	988	Myrcene	136	0.04	-
5	994	4-carene	136	0.87	0.42
6	1022	D-limonene	136	0.43	0.12
7	1054	γ-terpinene	136	0.06	0.20
8	1220	bornyl isoformate	182	0.92	0.17
9	1279	Bornyl acetate	196	1.97	0.41
10	1292	Thymol	150	0.09	0.24
11	1363	3,7-dimethyl-2,6-octadienyl-3-phenylpropanoate	286	0.06	-
12	1282	β-cubebene	204	0.09	0.06
13	1408	β-Caryophyllene	204	1.71	2.28
14	1444	α-Caryophyleène	204	0.19	0.13
15	**1457**	**precoceneI**	**190**	**9.,38**	**93.76**
16	1471	Germacrène D	204	0.75	0.45
17	1478	2,2'-ethylidene bis (5-methylfurane)	190	-	0.18
18	1480	1-ethyl-3-vinyladamantane	190	0.14	-
19	1489	bicyclogermacrene	204	0.34	0.26
20	1516	copaene	204	0.11	0.22
21	1570	(+)- eremophilene	204	0.15	-
22	1575	caryophyllene oxide	220	-	0.33
23	1587	Humulan-1,6-dien-3-ol	222	0.05	-
		Hydrocarbons monoterpene		**3.03**	**1.49**
		Oxygeneted monoterpene		**0.09**	**0.24**
		Hydrocarbons sesquiterpenes		**3.34**	**3.4**
		Oxygeneted sesquiterpènes		**0.05**	**0.33**
		others		**3.09**	**0.76**
		PrecoceneI		**9.38**	**93.76**
		Total		**99.98**	**99.98**

%(Fl): Percentage of flower EO

%(T +Fe): Percentage of EO of leaves and stems

M/Z: Relative mass

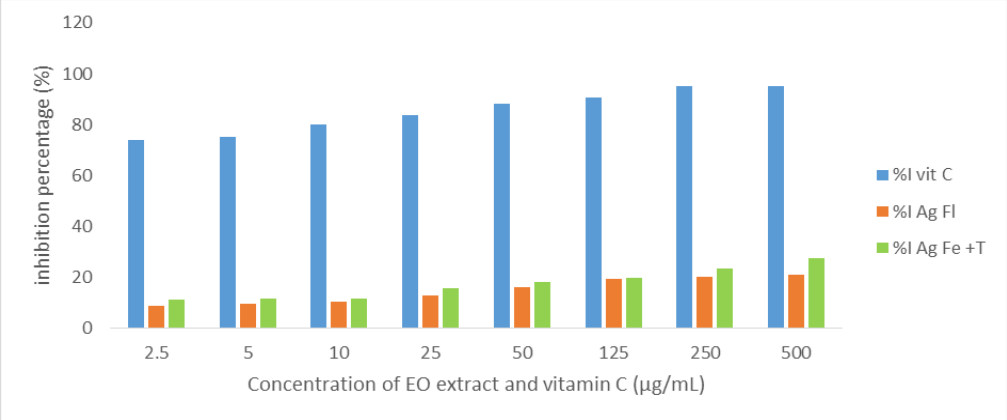
Analysis of the chromatogram and mass spectra of the essential oil from the stems and leaves of *A. conyzoides* identified 18 compounds. This corresponds to 99.98% of the total constituents of the essential oil. It consists of hydrocarbon (1.49%) and oxygenated (0.24%) monoterpenes, hydrocarbon (3.4%) and oxygenated (0.33%) sesquiterpenes and other compounds (0.76%). The majority phytocompound is precocene I (93.76%). That of the

flowers is made up of 21 compounds also representing 99.98%) of the total chemical composition. It has the same phytochemical composition as the EO of the stems and leaves but the proportions are different (Table I). The major phytoconstituent is also precocene I (90.38%). It can be seen that the chemical composition of the EO of the flowers and the stems+leaves of *A. conyzoides* is the same. The chemotype is of the Precocene I type. The results of this study are similar to those obtained in Nigeria and the Fiji Islands from the essential oils of the flowers of the species.

Indeed, the species native to Nigeria contains 57.2% and that of the Fiji Islands contains 16-60% of precocene I. Our work is in agreement with that of certain authors.[13,15-19,27] They are characterized by precocene I as the major phytocompound. This similarity is explained by the fact that these countries are located in the same tropical zone. However, various works on the chemical composition of the essential oils of the leaves of *A. Conyzoides* acclimatized in Brazil and Vietnam have shown that these essential oils mainly contain ageratochromene (precocene II) and/or derivatives.[20,21]

Antioxidant activity of essential oils by spectrophotometry

The percentage inhibition of DPPH expressed as a function of the concentration is measured at 517 nm. The extracts show less activity than that of vitamin C taken as a reference antioxidant. We expressed the antioxidant activity in IC50 (inhibitory concentration 50%). The IC_{50} is the concentration of the substrate which causes the loss of 50% of the activity of DPPH **(figure1).**



% vit C: percentage inhibition of vitamin C

% Ag Fl: percentage of inhibition of the EO of the flowers of A. conyzoides

% Ag Fe+T: percentage of inhibition of EO in the leaves and stems of A. conyzoides

Figure 1: Antioxidant power of EO extracts and vitamin C.

In view of these results, we can say that the essential oils of the organs of the species (flowers, stems and leaves) have a capacity to reduce free radicals. This result is confirmed by the IC50 values (the concentration necessary for neutralization and stability of 50% of the concentration of DPPH). Its value is 9.78 µg/mL for the EO extract of leaves and stems and 12.5 µg/mL for that of the flowers of the species. This attests that the capacity for reduction of the DPPH radical by the EO extract of the stems and leaves is higher than that of the flowers. The IC50 of vitamin C, the reference antioxidant, is 0.7 µg/mL. The IC50 of the EO of the flowers is 17 times higher than that of vitamin C and that of the stems and leaves is 14 times higher than that of vitamin C. The lower the IC50, the more effective the extract. So vitamin C has a DPPH reduction power 17 times higher than that of flowers and 14 times higher than that of stems and leaves. This low capacity for radical reduction by EO extracts of A. conyzoides could be justified by the presence of phenolic compounds and terpenes.[30] We compared our results with those of some authors. They are 1970µg/mL for the flowers and 3020µg/mL for the stems.[13] This value is 8000 µg/mL for the leaves.[20] These values are vastly different from ours. This difference could be explained by the phytochemical composition and the harvest period.

CONCLUSION

In this study, we were interested in the valuation of an Asteraceae of the Ivorian flora used in traditional medicine.

Yields of HE extracted from Witch weed organs are low.

The EO of flowers is made up of 21 compounds. That of the stems and leaves contains 18. The study showed that the essential oils of the organs are essentially made up of precocene I. This study also showed that the essential oils have a low capacity for reducing free radicals. This activity would be due to the presence of terpenes and precocene I. It would be desirable to carry out a toxicity study and to carry out other biological studies to justify its use in traditional medicine.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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