International Journal of Biosciences (IJB)
ISSN: 2220-6655 (Print) 2222-5234 (Online)
Vol. 2, No. 8, p. 58-66, 2012
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Chemical composition and biological activities of essential oils of *Chenopodium ambrosioides* L. collected in two areas of Benin

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Received: 02 August 2012

Revised: 21 August 2012
Accepted: 22 August 2012

Key words: *Chenopodium ambrosioides*; essential oils; α-terpinene; ascaridole; biological activities.

Abstract

The chemical composition of the essential oils obtained by hydrodistillation from leaves of three samples of *Chenopodium ambrosioides* L. (Chenopodiaceae) growing in Benin were analyzed by GC and GC/MS. To date the biological functions of *Chenopodium ambrosioides* L. essential oils have not been demonstrated scientifically. We investigated the chemical composition of *Chenopodium ambrosioides* L. essential oils and their antiradical antimicrobial and anti-inflammatory activities. Twenty three compounds were identified and quantified in the oils. The essential oils are rich in hydrocarbons monoterpenic. The major compounds being α-terpinene (48.8%, 60.0%, 63.7%), ascaridole (19.7%, 13.8%, 11.7%), p-cymene (19.1%, 15.4%, 19.0%) and isoascaridole (2.5%, 1.6%, 0.7%). The antiradical and anti-inflammatory.

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Introduction

Chenopodium ambrosioides L. is an annual or long-lived plant, drawn up, reaching more than one meter height, more or less pubescent, odorous. It is fragrant when it was offend. The leaves are used for the treatment of abscesses, epilepsy, vomit (Adjanohoun *et al.*, 1986), cutaneous dermatoses, and neuralgias intercostales (Adjanohoun *et al.*, 1988; Adjanohoun *et al.*, 1989). It is used as vermifuge, anthelminthic (Adjanohoun *et al.*, 1995, 1989; Lavergne and Véra, 1989; Kerharo and Adam, 1974), against the scale, the jaundice (Lavergne and Véra, 1989). Extracts of *Chenopodium ambrosioides* exhibit molluscidal activity (Hmamouchi *et al.*, 2000). The oil has been reported to have insecticidal, nematocidal and antimicrobial properties (Morishita *et al.*, 1997). They are used to treat some tumours, rheumatisms articulars (Leung and Foster, 1996).

The objective of the present work is to characterize *Chenopodium ambrosioides* L. leaves oils growing in Benin by GC, GC/MS and to evaluate their antiradical, anti-inflammatory and antimicrobial properties.

Material and methods

Plants material and isolation of the essential oils

The plant material was collected in two areas of Benin at Cotonou (Samples 1 in December 2003) and at Sehoue (Sample 2 and 3 respectively in May 2004 and in February 2006). A voucher specimen was deposited in the Herbarium of the University of Abomey-Calavi. Batches of 200 g of fresh leaves were submitted to hydrodistillation for 2h using a Clevenger-type apparatus; after decantation, the oils were dried with anhydrous sodium sulfate and stored at 4 °C until used.

Chemical analyses of essential oils

Quantitative and qualitative analysis of essential oils were carried out by gas chromatography/flame ionization detection (GC/FID) and gas chromatography/mass spectrometry (GC/MS). GC/FID analyses were performed using a Varian CP-3380 GC equipped with a DB1 (100% dimethylpolysiloxane) fitted with a fused silica capillary column (30 m x 0.25 mm, film thickness 0.25 µm) and Supelcowax 10 (polyethylene glycol) fused capillary column (30 m x 0.25 mm, film thickness 0.25 µm); temperature program 50 °-200 °C at 5 °C/min, injector temperature 220 °C, detector temperature 250 °C, carrier gas N_2 at a flow rate of 0.5 mL.min^{-1}. Diluted samples (10/100, v/v, in methylene chloride) of 2.0 µL were injected manually in a split mode (1/100). The percentage compositions were obtained from electronic integration measurements without taking into account relative response factors. The linear retention indices of the components were determined relatively to the retention times of a series of n-alkanes (C_9-C_{20}).

GC/MS analyses were performed using a Hewlett Packard apparatus equipped with a HP1 fused silica column (30 m x 0.25 mm, film thickness 0.25 µm) and interfaced with a quadruple detector (Model 5970). Column temperature was programmed from 70° to 200 °C at 10 °C/min; injector temperature was 220 °C. Helium was used as carrier gas at a flow rat of 0.6 mL.min^{-1}, the mass spectrometer was operated at 70 eV. 2.0 µL of diluted samples (10/100, v/v, in methylene chloride) were injected manually in the split mode (1/100).

The identification of individual compounds was based on the comparison of their relative retention times with those of authentic samples on the DB1 column and by matching the linear retention indices and mass spectra of peaks with those obtained from authentic samples and/or the NBS75K.L and NIST98.L libraries and published data (Adams, 2007; Joulain and König, 1998).

Biological evaluation

Free radical-scavenging activity: DPPH test

Antiradical activity was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) following the Mellors and Tappel method (Mellors, Tappel, 1996) adapted to essential oil screening (Avlessi *et al.*, 2005).

1,1-diphenyl-picrylhydrazyl [1898-66-4] was purchased from Sigma-Aldrich and the solutions were prepared with analytical grade solvents purchased from standard commercial sources.

DPPH, was dissolved in ethanol to give a 100 µM solution. To 2.0 mL of the ethanolic solution of DPPH were added 100 µL of a methanolic solution of the antioxidant reference BHT (butylate hydroxy toluene) at different concentrations. The essentials oils and the fractions were tested with the same manner. The control, without antioxidant, is represented by the DPPH ethanolic solution containing 100 µL of methanol. The decrease in absorption was measured at 517 nm after 30 min, at 30 °C. All the spectrophotometric measures were performed in triplicate with a SAFAS UV mc2 spectrophotometer, equipped with a multicells/multikinetics measure system and with a thermostated cells-case.

The free radical-scavenging activity of each solution was calculated according to the following equation:

$$SC\% = \frac{A\,(blank) - A\,(sample)}{A\,(blank)} \times 100$$

Antiradical activity, defined as the concentration of test material required to cause a 50% decrease of the initial DPPH absorbance, was determined graphically and expressed as SC_{50} (mg. L^{-1}).

Anti-inflammatory activity: lipoxygenase test

Soybean lipoxygenase (EC.1.13.11.12) was purchased from Fluka whereas nordihydroguaiaretic acid (NDGA) [500.38.9] and linoleic acid sodium sulfate (822-17-3) were obtained from Sigma Chemical Co; potassium phosphate buffer 0.1 M, pH=9 was prepared with analytical grade reagent purchased from standard commercial sources. Deionized water was used for the preparation of all solutions.

Lipoxygenase is known to catalyse the oxidation of unsaturated fatty acids containing 1,4-diene structures. The conversion of linoleic acid to 13-hydroperoxy linoleic acid was followed spectrophotometrically by the appearance of a conjugate diene at 234 nm. Nordihydroguaiaretic acid (NDGA), a known inhibitor of soybean lipoxygenase, was used as a reference drug. The experimental conditions were adapted from those previously used and fully described (Alitonou *et al.*, 2010).

Antibacterial activity

Essential oil emulsion

2 mL of Mueller Hinton broth added with 0.02 g/L (w/v) of phenol red were added 40 µL of essential oil and 2 drops of Tween 80 and has been introduced in a hemolyse test tube and homogenized (Yehouenou *et al.*, 2010a).

Preparation of bacteria suspensions

This preparation was carried out from the two stocks of tested bacteria. A pure colony of each stock was suspended in 5 mL of Mueller Hinton broth. After incubation at 37 °C for 2 hours, we obtained 10^6 cfu/mL corresponding to the scale 2 of McFarland standard (Yehouenou *et al.*, 2010a).

Determination of Minimal Inhibitory Concentration (MIC)

The method used was reported by Yèhouenou et al. (2010b). 100 µL of bubble Mueller Hinton broth containing of phenol red to 0.02 g/L were distributed in all the 96 wells of microplate. 100 µL of essential oil emulsion (initial solution) were added to the well of the first column except that of the second line and successive dilutions of reason 2 were carried out well by well till the 12th one and the remaining aliquot (100 µL) were rejected. 100 µL of Mueller Hinton which not containing phenol red were introduced in the first well of the first columns and successive dilutions of reason 2 were carried out as before. All the wells of the second column received 100 µL of bacteria suspension except the first line which represents the negative control and the second line, the positive control. The microplate one was finally covered with parafilm paper and was incubated at 37 °C during approximately 18 hours.

Minimum bactericidal concentration (MBC)

MBC were appreciated by method proposed by Oussou *et al.* (2004) reported by Kpadonou *et al.* (2012). To determine the MBC, each microliter-plate well content 50 µL in which no color change occurred, the mixture of essential oil and the strain was isolated on sterile MHA poured in Petri dishes. These plates were incubated at 37°C for 24 hours. The MBC is the lowest concentration of essential oil which 99.9% of the microorganisms were killed. The tests were carried out in triplicate.

Statistical analysis

Data from three independent replicate trials were subjected to statistical analysis using Statistica version 6.0. Differences between means were tested using Z-test.

Results and discussion

Chemical composition of Chenopodium ambrosioides L. essential oils

The hydrodistillation of the leaves of *Chenopodium ambrosioides* collected in Cotonou and Sehoue in 2003, 2004, 2006 permitted to obtain the essential oils with yields included between 0.3 to 1.2% (m/m) what represents a relatively significant variability of the values (Table 1). These yields are comparable with those of the literature (Sagrero-Nieves and Bartley, 1995; Muhayimana *et al.*, 1998; Gupta *et al.*, 2002; Pino *et al.*, 2003; Kasali *et al.*, 2006).

Table 1. Yields (w/w percentage) of essential oils obtained from fresh leaves of *Chenopodium ambrosioides* L. from Benin.

Samples	Date and place of harvest	Yields (w/w percentage)
1	December 2003 (Cotonou)	0.3
2	May 2004 (Sehoue)	1.2
3	February 2006 (Sehoue)	0.9

In table 2, were given the results relating to 3 samples collected in two areas of Benin, studied to control the influence of the mode of extraction on the chemical composition of the volatile components of the plant. A chromatographical analysis was also made by regulating the temperature of the injector at 100 °C instead of 200 °C (sample 4) to test the "thermostability" of the ascaridole. Essential oils are mainly constituted of metabolites volatile with p-menthanic structure, in accordance with the results of the literature. The comparative study of the chemical compositions of essential oils (1) to (3) show that α-terpinene is the main constituent (48.8-63.7%) in all the samples, followed by p-cymene, the total of the percentages relating of these 2 compounds representative from 67.9 to 82.7% of the mixture. Two other compounds "characteristics" of these essential oils are the ascaridole formed by addition of an oxygen molecule on α-terpinene (11.7-19.7%) and its derivative diepoxy, the isoascaridole (0.7-2.5%), always minority.

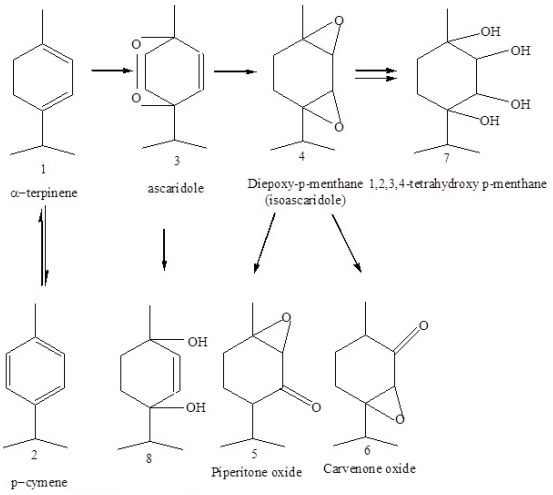

Fig. 1. Biogenetic and chemical filiation of the volatile components of Chenopodium ambrosioides L.

The ascaridole is described like a fragile molecule, sensitive to the thermal shocks and the chemical aggressions. These considerations agree badly with the chromatographic analyses which imply a volatilization of the samples at high temperatures; however, from recent work Cavalli *et al.* (2004) showed that the thermal decomposition of the ascaridole remains limited to the temperatures of usual injections. We wanted to verify this assertion and for this reason we carried out 2 types of experiments. We repeated several times the analysis of the same sample by maintaining the temperature

at 200 °C (according to same operating conditions as those applied to the whole of our samples). We observed a very good reproducibility of the results. We reinjected sample 5 by fixing the temperature at 100 °C instead of 200 °C and obtained results very comparable with those realized with 200 °C what validates the whole of our analyses. We notes in essential oil, beyond the low content of isoascaridole, the appearance of various oxygenated products: piperitone, oxide of piperitone (5), oxide of carvenone (6) and glycol ascaridole (8). These observations are coherent with the transformations clarified in Fig. 1. The plant generates initially α-terpinene (1) and this monoterpene are oxidized later in compounds (3) and (4); it can also lead to p-cymene (2) whch explains why the relative proportions of these compounds are directly dependent.

Table 2. Chemical composition (%w/w) of essential oils of leaves of *Chenopodium ambrosioides* L. collected in two areas of Benin.

Component	*RI	Percent Composition				Identification methods
		1	2	3	4	
myrcene	984	0.1	0.1	0.1	-	GC, MS, RI
α-terpinene	1027	48.8	60.0	63.7	67.7	GC, MS, RI
p-cymene	1030	19.1	15.4	19.0	17.0	GC, MS, RI
limonene	1034	0.6	0.6	0.6	0.6	GC, MS, RI
γ-terpinene	1058	0.7	0.8	0.9	0.9	GC, MS, RI
terpinolene	1086	0.1	0.1	0.1	-	GC, MS, RI
p-cymenene	1088	0.1	0.1	0.1	-	GC, MS, RI
terpinen-4-ol	1176	0.2	-	-	-	GC, MS, RI
p-cymen-8-ol	1188	0.2	0.2	0.1	-	GC, MS, RI
α-terpineol	1215	0.1	-	0.1	-	GC, MS, RI
ascaridole	1226	19.7	13.8	11.7	11.5	GC, MS, RI
oxyde de carvenone	1230	0.5	0.3	0.2	0.2	GC, MS, RI
cis-oxyde de piperitone	1235	0.6	0.4	0.2	0.7	GC, MS, RI
trans-oxyde de piperitone	1239	1.1	0.8	0.5	-	GC, MS, RI
trans-glycol ascaridole	1256	1.0	1.4	0.2	0.2	GC, MS, RI
cis-glycol ascaridole	1272	0.7	1.1	0.2	-	GC, MS, RI
thymol	1283	0.5	-	-	-	GC, MS, RI
carvacrol	1292	0.5	0.5	-	-	GC, MS, RI
isoascaridole	1299	2.5	1.6	0.7	0.4	GC, MS, RI
tiglate d'hexyle	1325	-	0.1	0.1	-	GC, MS, RI
périllaldehyde	1340	0.4	-	-	-	GC, MS, RI
acétate de 9-menthenyle	1405	-	-	-	-	GC, MS, RI
β-caryophyllene	1439	0.5	-	0.3	0.3	GC, MS, RI
Monoterpenes hydrocarbons		69.5	77.1	84.5	86.2	
Oxygenated monoterpenes		27.0	19.6	13.9	13.0	
Sesquiterpenes hydrocarbons		0.5	-	0.3	0.3	
Oxygenated sesquiterpenes		-	-	-	-	
Others compounds		1	0.6	0.1	-	
Total		98.0	97.3	98.8	99.5	

1 = Cotonou December 2003; 2 = Sehoue May 2004; 3 = Sehoue February 2006; 4 = Sample 3 collected in February 2006 injected at 100 °C instead of 200 °C who represents the temperature of the injector for all the others analyses. *RI on a column DB1

The conditions of extraction are also very important; the low contents of isoascaridole in essential oil let supposed that at the time of the hydrodistillation, the diepoxy-p-menthane (4) simultaneously is isomerized and hydrolized by the action of the protons of the medium (pH = 4 - 5) aqueous boiling. The first transformation gives oxides (5) and (6), the second leads finally to the tetrol (1, 2, 3, 4-tetrahydroxy p-menthane 7). We did not identify this compound in our samples; it is indeed very polar and is probably degraded on the level of the injector of the chromatograph in gas phase. It was however observed by other authors (Cavalli *et al.*, 2004; Ahmed, 2000) by study NMR, technique which preserves the integrality of structures. In our essential oils we had also identified the two glycols diastereoisomers (8a) and (8b); they are probably formed by reduction of the ascaridole (3). These products were already identified in other essential oils of *Chenopodium*.

Table 3. Potential antiradical of the essential oils samples of *Chenopodium ambrosioides*.

Chenopodium ambrosioides	SC_{50} (g/L)
1	6.00 ± 0.30 b
2	5.20 ± 0.26 c
3	8.60 ± 0.50 a

Data in the column followed by different letters are significantly different ($p < 0.05$). The values are means of three repetitions ± standard deviation.

Table 4. Anti-inflammatory Activities of the essential oils samples "fairly active" *Chenopodium ambrosioides*.

Samples	Concentrations (ppm)	Pourcentage d'inhibition (%)	IC_{50} (ppm)
1	100	20 ± 1.2 c	-
2	100	23 ± 1.4 b	-
3	100	13 ± 0.8 d	-
NDGA	0.75	91± 2.7 a	0.23

Data in the column followed by different letters are significantly different ($p < 0.05$). The values are means of three repetitions ± standard deviation

Antiradical activity

Three essential oils samples were tested for their properties antiradical. The results having been used for the determination of the SC_{50} of the various samples are given in table 3. The potential antiradical is relatively weak for four samples since the SC_{50} is between 5.2 ± 0.26 and 8.6 ± 0.5 g/L (either an antiradical activity approximately 1000 times weaker than that of the BHT).

Table 5. Antimicrobial activity (Minimal Inhibitory Concentration (MIC value) and Minimal Bactericide Concentration (MBC value), mg/mL of essential oil of leaves of *Chenopodium ambrosioides*.

Parameters	Microbial stock	Samples of essential oils		
		1	2	3
MIC	*Escherichia coli* ATCC 25922	6.86 ± 0.31 a	6.69 ± 0.30 a	6.77 ± 0.31 a
	Staphylococcus aureus ATCC 25923	1.71 ± 0.08 a	1.63 ± 0.08 a	1.56 ± 0.07 a
MBC	*Escherichia coli* ATCC 25922	27.45 ± 1.37 a	25.75 ± 1.80 a	26.35 ± 1.45 a
	Staphylococcus aureus ATCC 25923	-	-	-

-: not determined; Data in the line followed by different letters are significantly different ($p < 0.05$). The values are means of three repetitions ± standard deviation

Anti-inflammatory activity

The results obtained from the lipoxygenase tests performed on these essential oils were given in table 4. At 100 ppm (maximum concentration likely to be tested taking into account the solubility of the sample in the medium) we obtains (20 ± 1.2)%, (23 ± 1.4)% and (13 ± 0.8)% of inhibition respectively for samples 1, 2 and 3 of essential oils of *Chenopodium ambrosioides*.

At 100 ppm, all the samples mean an inhibition < 50%, a weak inhibiting action was observed which is 4 times weaker than that obtained for the reference drug NDGA (91± 2.7%) with a concentration more

than 100 times lower. These essential oils samples are characterized by a significant rate of hydrocarboned monoterpenes, in particular α-terpinene, accompanied by ascaridole; no correlation chemical composition/biological activity appears clearly with the examination of the results.

Antibacterial activity

Two microbial stocks were used in this present study. The Minimal Inhibitory Concentration (MIC) values were determined for all. The essential oils (Samples 1, 2 and 3) extracted from the leaves of *Chenopodium ambrosioides* almost have an antimicrobial activity interesting against *Staphylococcus aureus* ATCC 25923 with a Minimal Inhibitory Concentration (MIC) ranged from 1.56 ± 0.07 to 1.71 ± 0.08 mg/mL and average activity towards *Escherchia coli* ATCC 25922 (MIC ranged from 6.69 ± 0.03 to 6.86 ± 0.31mg/mL) (Table 5). These MIC values showed that essential oils of *Chenopodium ambrosioides* present an important inhibiting activity on *Staphylococcus aureus* than *Escherchia coli*. Nevertheless, essential oils tested presented bacteridal activity on *E. coli* with Minimal Bactericidal Concentration (MBC) ranged from 25.75 ± 1.80 and 27.45 ± 1.37 mg/mL and bacteriostatic activity against *S. aureus* (Table 5).

Conclusion

Essential oils compositions of *Chenopodium ambrosioides* from two different locations in Benin were investigated. α-terpinene, p-cymene and ascaridole contents oils were observed which were similar to the previous literature. The oils showed antiradical and anti-inflammatory activities weak. An interesting antimicrobial activity was observed on *E. coli* related to MBC determined on this strain. Considering their high content of hydrogenated monoterpene and oxygenated monoterpenes, the leaves of *Chenopodium ambrosioides* could be used for its many medicinal virtues.

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