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Comparative analysis, antibacterial and antiradical activities of essential oils in leaves and fruits of *Chenopodium ambrosioides* of Morocco

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ABSTRACT

In this study, the chemical composition, in-vitro antibacterial activity and antiradical potential of essential oils extracted from aerial parts of C ambrosioides has been investigated. The chemical characterization by GC-MS of essential oil from whole plant and their leaves showed a similar composition and the major components were α-terpinene (12.2-34.8%), p-cymene (19.3-41.7%), and ascaridole (10.8-41%).The chemical profile of essential oils from leaves and fruits differed markedly. In the fruits, α-terpinene was in trace, while ascaridole was the predominant compound (69.8%) followed by thymol (13%) and carvacrol (11%). In vitro antibacterial activity was investigated and the tested whole essential oil sample proved to be very active against Staphylococcus aureus, Bacillus cereus and Escherichia coli. Essential oils from leaves and fruits were also tested for their antiradical potential using DPPH radical. The IC 50 values were 75.07 µg/ml and 142. 45 µg/ml respectively.

Keywords: Chenopodium ambrosioides L., leaves, fruits, ascaridole, antibacterial activity, antiradical potential.

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INTRODUCTION

The genus *chenopodium* (chenopodiaceae) contains more than 200 species which are widely distributed in hot sub-tropical and sub-temperate regions. *Chenopodium ambrosioides* L. is widely distributed throughout Morocco; it is used in popular medicine as a vermifuge, diuretic, against oral abscesses, ulcers, and in the treatment of several gastrointestinal disorders such as typhoid and dysentery [1]. In other countries, *C. ambrosioides* L. is widely used as emmenagogue, abortifacient, vermifuge [2], vermicide [3] anti-parasitic [4] and to treat wounds, inflammatory processes and respiratory problems such as influenza, bronchitis and tuberculosis [4-5].

Previous works on *C. ambrosioides* L. extracts revealed corrosion inhibiting effect [6], molluscidal activity [7], antipyretic and analgesic properties [8]. Moreover, the essential oil of *C. ambrosioides* L. has been reported to have antimicrobial, antioxidant [9-11], insecticidal, nematocidal [10], larvicidal, allelopathic [12-13], anthelmintic [14], antileishmanial [15a] and   acetylcholinesterase inhibitory activities [16].

The composition of essential oils (EOs) of *C. ambrosioides* from various origins has been previously reported: China [17], France [18], Benin [10], Togo [19], Israel [20], Nigeria [21], Cameroun [12], Bresil [11], Cuba [15b-15c], Morocco [9, 22] and Yemen recently [16]. All the oils exhibited quite diverse compositions and high content of monoterpenes compounds such as α-terpinene, *p*-cymene, ascaridole, limonene and isoascaridole.

In Morocco, two recent studies [9, 22] were undertaken on EO of dried aerial parts of *C. ambrosioides* collected in five different areas of the country. However, there is some ambiguity on the chemotype of Moroccan oil. Indeed, some authors [22] reported that the major components of EO from three different areas were α-terpinene (35.15- 46.8%), terpinolene (17.42- 29.19%) and *p*-cymene (10.81-13.95%), while the EO from the fourth region was dominated by terpinolene (41.32%) and α-terpinene (35.17%). In contrast, other authors [9] did not find terpinolene, but α-terpinene (23, 77%) associated to ascaridole (14, 48%) and *p*-cymene (12.22%). These two reports suppose the existence of two different chemotypes of *C. ambrosioides* of Morocco.

In the present study, the chemical composition of EO of *C. ambrosioides* from Morocco is rechecked. To our knowledge, there are no published reports on the chemical composition of essential oil in leaves and fruits of *C. ambrosioides* from Morocco. Additionally, we investigated *in-vitro*, antibacterial activity by the disc diffusion method and antioxidant potential by the DPPH assay of   *C. ambrosioides* essential oils.

MATERIALS AND METHODS

Plant Material

The plant material containing stems, leaves and fruits was collected in February 2015, in "Sidi Bennour", El Jadida, Morocco. Voucher specimen (N°102638) was authenticated by Pr. M. Fennane and deposited in the Department of Botany of the Scientific Institute -Rabat.

Isolation of the essential oils

Immediately after collection, the plant material was divided in three parts: whole plant (S), leaves of S (S_L) and fruits of S (S_F). All these samples were allowed to dry at room temperature. The essential oil from each sample was obtained by hydro-distillation for 2 h using a Clevenger apparatus. The Essential oil was dried over anhydrous sodium sulfate and stored at 4° C in a sample vial in the dark until analysis.

Gas chromatography

GC analyses were performed on a Perkin Elmer 8500 gas chromatograph with FID, fitted with a fused silica DB-5 MS capillary column (30 m x 0.25 mm (i.d.), film thickness: 0.25μm). The temperature of the column was programmed from 60ºC to 250ºC at a rate of 3ºC /min. The injector and detector temperatures were programmed at 230ºC and 280ºC, respectively.

Gas chromatography - mass spectrometry

GC-MS analyses were performed on a Hewlett-Packard 5973-6890 system operating in EI mode (70 eV) equipped with a split/splitless injector (220ºC), a split ratio 1/10, using 2 different columns: a fused silica HP-5 MS capillary column (30 m x 0.25 mm (i.d.), film thickness: 0.25 μm) and a HP-Innowax capillary column (30 m x 0.25 mm (i.d.), film thickness: 0.50 μm). The temperature program for the HP-5 MS column was from 60ºC (5min) to 280ºC at a rate of 4ºC/min and for the HP-Innowax column from 60ºC to 260ºC at a rate of 3ºC/min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The injection volume of the sample was 2 μL. Retention indices for all compounds were determined according to the Van der Dool approach [23], using n-alkanes as standards. The identification of the components was based on comparison of their mass spectra with those of Wiley and NBS Libraries and those described by Adams [24], as well as by comparison of their retention indices with literature data [25]. Optical rotation was measured on a Perkin Elmer 341 Polarimeter.

Antibacterial activity test

The agar disc diffusion method was used for the determination of antibacterial activity of the essential oil [26]. The following Gram-negative bacteria were used: *Citrobacter freundii* (ATCC 8090), *Escherichia coli* (ATCC 25922) and *Salmonella sp*, as well as the Gram-positive bacteria: *Bacillus cereus* (IPL 58605), *Listeria ivanovii* (ATCC 19119) and *Staphylococcus aureus* (ATCC 25923). The bacterial strains were supplied by the Pasteur Institute (Casablanca, Morocco).

Preparation for microorganism culture

Screening of the essential oil was done by agar disc diffusion method. It was performed using an 18 hours culture growth at 37 °C. The cultures were adjusted to approximately 105 CFU/ml. Five hundred microliters of the suspensions were spread over plates containing Mueller-Hinton agar. Empty sterilized discs (6mm) impregnated with 10 μl of the essential oil were placed on the surface of the media. The plates were left 30 min at room temperature to allow the diffusion of the oil, and then they were incubated at 37 °C for 24 hours. At the end of the period, the inhibition zone around the disc was measured with a caliper. Standard disc containing Penicillin G was used as reference control. Studies were performed in triplicate.

DPPH radical-scavenging activity

The essential oils from leaves (S_L) and fruits (S_F) were tested for their radical- scavenging activity (RSA) by 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) method [27]: 2 ml of a 60 μM DPPH methanolic solution was mixed with 50μl of diluted essential oil. The mixture was vortexed and left in the dark at room temperature for 30 mn. The absorbance of the control (A_c) and samples (A_s) was measured spectrophotometrically at 517 nm, and the RSA of the tested essential oils expressed in percentage was calculated as follow:

$$RSA\ (\%) = \frac{Ac - As}{Ac} \times 100$$

The RSA was expressed as IC_{50}, which is the antiradical concentration required to cause 50% of inhibition. Butylated hydroxytoluene (BHT) was used as positive control. All the spectrophotometric measures were repeated three times.

RESULTS AND DISCUSSIONS

The yellowish EOs were obtained by hydro-distillation of the samples of *C. ambrosioides* with yields of 0.5-0.8% (w / w) on a dry-weight basis. The identified volatile components are listed according to their retention indices in Table 1. Thirteen constituents were identified representing 90.8 to 97.5% of the volatile components (Figures 1-3).

Table 1: Chemical composition of different essential oils samples from *Chenopodium Ambrosioides*.

| Compounds	RI[a]	S[b]	S$_L$[c]	S$_F$[d]
α-Terpinene	1018	12.2	34.8	tr
p-Cymene	1029	21.9	41.7	1.5
Limonene	1033	tr[e]	-	0.2
γ-Terpinene	1058	tr	0.6	tr
p-Cymen-8-ol	1184	tr	0.4	tr
Ascaridole	1239	41	10.8	69.8
cis-Piperitone oxide	1256	0.9	0.6	tr
trans-Piperitone oxide	1261	tr	0.9	tr
trans-Ascaridolglycol	1272	1.4	1.3	tr
Thymol	1298	8.7	3.5	13
Carvacrol	1304	6.8	1.6	11
Isoascaridole	1307	0.9	1.3	0.4
β-Ionone	1487	-	-	-
Total		93.8	97.5	95.9

[a]RI: retention indices calculated against C9-C24 *n*-alkanes on the HP-5 column.
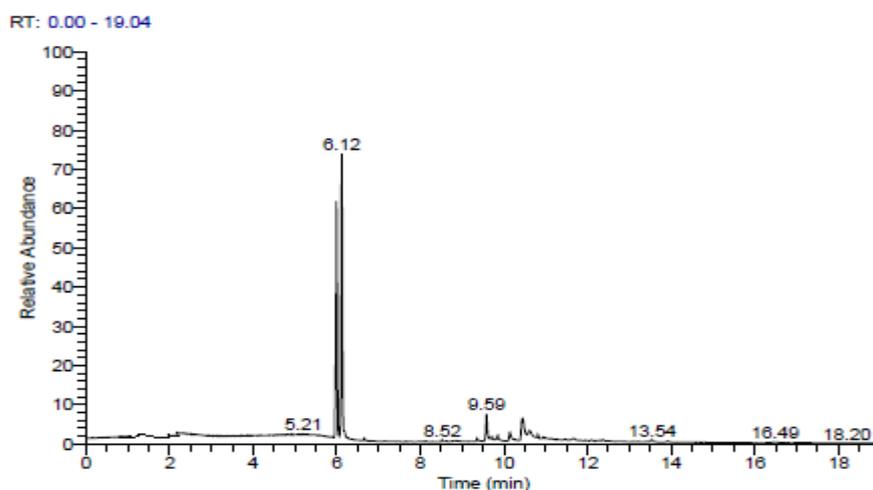[b] S: whole plant
[c]S$_L$: leaves
[d]S$_F$: fruits
[e]tr: trace (< 0.05%)
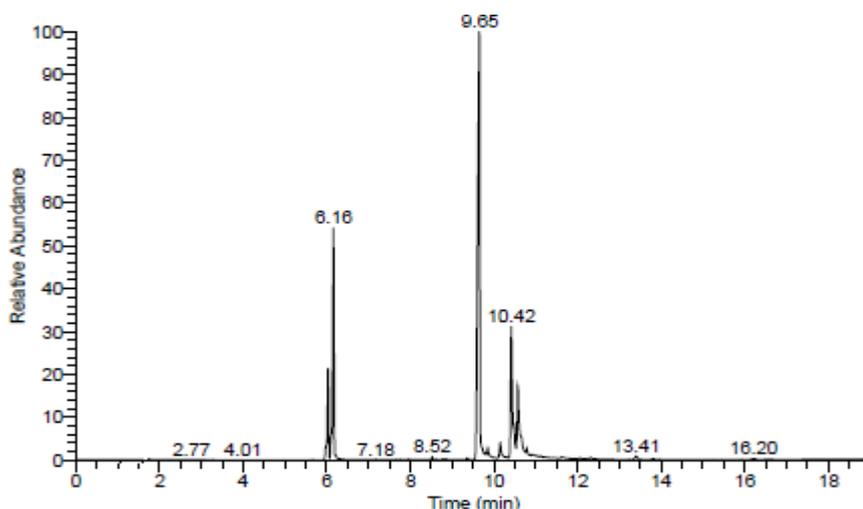
Figure 1: Chromatogram of essential oil in the whole plant (S).

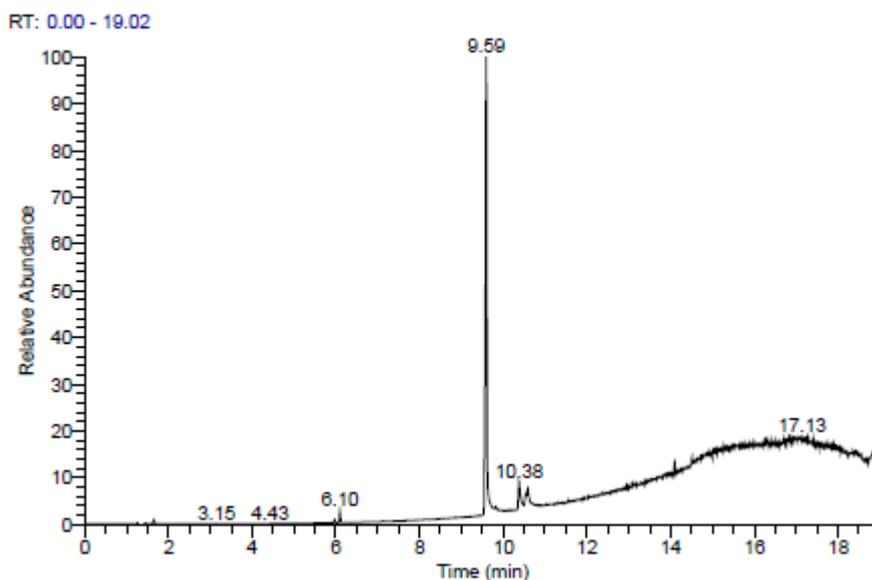**Figure 2: Chromatogram of essential oil in the leaves (S$_L$).**

Figure 3: Chromatogram of essential oil in the fruits (S$_F$).

The main compounds in samples S and S$_L$ were ascaridole (10.8-41%), α-terpinene (12.2-34.8%) *p*-cymene (21.9-41.7%), thymol (3.5-8.7%) and carvacrol (1.6-6.8%). The chemical composition of EOs from leaves and fruits differed markedly. The main components in the leaves were α-terpinene (34.8%) *p*-Cymene (41.7%) and ascaridole (10.8%). In the fruits, α-terpinene was in trace, while ascaridole was the predominant compound (69.8%), associated to thymol (13%) and carvacrol (11%). The chemical profiles of the EOs from S and S$_L$ therefore were somewhat similar to the chemical compositions described previously on *C. ambrosioides* from three areas of Benin [10] with α-terpinene (48.8-67.7%), *p*-Cymene (15.4-19.1%), ascaridole (11.5-19.7%) and isoascaridole (0.4-2.5%) and from Cuba [15b] with α-terpinene (19.7-20.7%), *p*-cymene (20.2-21.3%), ascaridole (35.1-47.1%) and isoascaridole (0.5-5%), but differed from those described recently in Yemen [16] with α-terpinene (0.7%), *p*-cymene (8.1%), ascaridole (54.2%) and isoascaridole (27.7%), in Cuba [15c] with ascaridole (22.54%) and carvacrol (62.36%), and in the French commercial sample of *C. ambrosioides* [18] with α-terpinene (9.7%), *p*-cymene (16.2%), ascaridole (41.8%) and isoascaridole (18.1%). If compared to EOs of *C. ambrosioides* described previously from Morocco, our EOs (S and S$_L$) differed slightly from that obtained by Ait Sidi Brahim et al. [9] from *C. ambrosioides* var. *ambrosioides* with some quantitative and qualitative differences. In fact, the chemical profile of the studied volatile oils was characterized by the presence of high content of ascaridole if compared to α-terpinene and high content of thymol and carvacrol (Table 1). However, when the chemical profiles of the analyzed EOs (S, S$_L$ and S$_F$) were compared with those of *C. ambrosioides* recently studied in Morocco [22], they appeared different, since ascaridole (10.8-69.8%, our oils) was not found, while terpinolene, identified at high content (17.42-41.32%) in four samples of *C. ambrosioides* from various regions of Morocco [22], is totally absent in our oils. To our knowledge, terpinolene has never been found as major component in any of the EOs of *C. ambrosioides* studied until now.

The fruits EO was characterized by high content of ascaridole (69.8%), these results differ so much with those of leaves (Table 1). The chemical profile of fruits EO was somewhat similar to that obtained in Cuba [15c] with ascaridole (22.54%) and carvacrol (62.36%).

These differences of essential oils chemical compositions were probably related to geographical origin, seasonal collection, plant material and genetic factors.

Table 2 shows *in vitro* bacteriostatic activity of EO (sample S) from *C. ambrosioides* together with the inhibition zones formed by standard penicillin G (10 unit) antibiotic discs. As can be seen, the EO of *C. ambrosioides* (S, 10µl/disc), exhibited a strong antibacterial activity against the most tested bacteria. Furthermore, the zone of inhibition produced by volatile oil is much higher than that of the antibiotic Penicillin G (10 unit) especially against *Bacillus cereus, Staphylococcus aureus and Escherichia coli.* Ait Sidi Brahim et al. [9] reached similar results to those given in the current study and revealed that the volatile oil of *C. ambrosioides* var. *ambrosioides* from Morocco possessed potent antibacterial properties against *Escherichia*

coli, Bacillus cereus, Micrococcus luteus and *Staphylococcus aureus.* Previous reports showed that EOs of *C. ambrosioides* present an important inhibiting effect on *Staphylococcus aureus* than *Escherichia coli* [10].

Table 2: Antibacterial activity: Diameter of the inhibition zone[a] of essential oil (S) of *C. ambrosioides* and standard antibiotic penicillin G.

Microorganisms	S (10μl)	P10[b]
B. cereus	22	13
L. ivanovii	15	11
S. aureus	23	11
C. freundii	12	11
E. coli	20	12
Salmonella sp	11	8

[a]**:** Includes diameter of disc (6mm);
[b] P10: penicillin G (10 unit).

On the other hand, two EOs of *C. ambrosioides* (S_L and S_F) were tested for their antiradical properties using DPPH essay. EOs from leaves and fruits present a free radical scavenging with IC_{50} of 75.07 μg/ml and 142.45 μg/ml respectively. The antiradical potential of leaves EO was weaker than that of the BHT (37.42 μg/ml) but better than that of EO of fruits. These results were probably due to the high content, diversity and synergistic effect of oxygenated monoterpenoids (95.9%) in leaves EO. Comparing with previous reports on antiradical activity of *C. ambrosioides* EOs obtained for DPPH essay, the IC_{50} values of leaves and fruits were more efficient than those of *C. ambrosioides* var. *ambrosioides* of Morocco [9] and *C. ambrosioides* of Benin [10] but less effective than that of Yemen EO [16].

CONCLUSION

In this study, the chromatographic analysis of the studied EOs places *C. ambrosioides* from Morocco in the ascaridole chemotype. The essential oil of the fruits is richer in ascaridole than the leaves. Essential oils are composed of chemical molecules that determine their properties, fields of action, toxicities and use precautions. Considering the properties of ascaridole and the other identified major compounds, EO of *C. ambrosioides* has enormous potential in various fields including food, pharmaceutical and industry.

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