Investigation of the Essential Oil of *Ormenis africana* from Morocco: Revision of Chemical Composition and Antibacterial Activity

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Abstract- The essential oil composition from the aerial parts of *Ormenis africana* (Asteraceae), an endemic species from Morocco, has been investigated by GC/MS. A total of 31 compounds were identified, representing 77%. After fractionation by column chromatography, the main compound was isolated and its structure elucidated by NMR spectroscopy. The essential oil was dominated by oxygenated compounds with spathulenol (45.8%) followed by camphor (7.1%), α-cadinol (5.9%) and α-bisabolol (5.9%) as the main compounds. This oil can be classified as spathulenol-type according to its spathulenol content. In vitro the antibacterial activity of the whole essential oil against three Gram positive (*Bacillus cereus, Enterococcus faecalis, Streptococcus C*) bacteria and three Gram negative (*Proteus vulgaris, Escherichia coli*, Pseudomonas aeroginosa) bacteria, showed significant results.

Keywords: Asteraceae, *Ormenis africana*, Essential oil, Spathulenol, Antibacterial activity.

Introduction

Northern Africa or Mediterranean Africa, comprises of Morocco, Algeria and Tunisia with a characteristic bioclimatic zone. In this sense, North African vegetation is a Mediterranean type, totally different in its botanical species and chemical composition with any other tropical African vegetation. In the arid bioclimatic zone, we can distinguish three series: the *Juniperus phoenicea* series, the field sage series with *Artemisia campestris* between the main shrubs and the white sage series. This last series is comprised of a complex group of plants, usually dominated by *Artemisia herba alba*. The main shrub spices are chamaephytes and between them grow *Ormenis africana* [1,2].

Ormenis africana (Jord & Four.) Litard. & Maire or *Chamaemelum africanum* Jord. & Four. (Family Asteraceae, Tribe Inuleae) is a perennial herb perennial herb with yellow flowers. The plant is endemic for North Africa (Morocco, Algeria and Tunisia). In Morocco, it's traditionally used for their spasmolytic properties[3]. The with yellow flowers. The aerial parts of this plant are used for their spasmolytic properties as well as for its stomacal pain[3]. A previous study have been reported on the antioxidant activity of ethanolic extract of inflorescence of *O. africana* found that hydroethanolic extract of this plant contain a considerable levels of antioxidant compound (polyphenols, flavonoids and anthocyanins...) and showed a high antioxidant activity in vitro and in vivo [4].

The chemical composition of the essential oil of *Ormenis africana* has been reported [3]. In the present study, the chemical composition of this specie was rechecked, and the major constituent was analysed by NMR technique after isolation by column chromatography. In addition, essential oils of *Chamaemelum* have been described to possess remarkable biological activities[5a,5b]. In this study, we investigated *in-vitro* the antibacterial activity by the disc diffusion method of the *O. africana* essential oil, against gram-positive and gram-negative microorganisms in comparison with Penicillin G.

Material and Method

Plant material: The plant material sample was collected in May, 2005 from plants growing in Errachidia (south of Morocco) at the flowering stage. Voucher specimens were authenticated by Pr. Ouyahya and deposited in the Department of Botany of the Scientific Institute, Rabat.

Isolation of the essential oil: Essential oil was obtained from the leaves and flowers of *O. africana* by hydrodistillation during five hours in a modified Clevenger type apparatus, yielding 0.8% (v/w) of a yellowish essential oil. This was dried over anhydrous sodium sulphate, diluted at 1% (v/v) in dichloromethane and stored at 4º C until the analysis by GC/MS.

Gas Chromatography-Mass Spectrometry: The GC-MS analysis was carried out with a thermomass spectrometer (model trio 1000); coupled to a thermo gas chromatograph (model 8000) (fusions Instruments). A Hewelt-Packard OV-17 capillary Column (25 m long × 0.25 mm) was employed for the analysis.

The column temperature program was 60 °C for 6 min, with 5 °C increase per min to 150 °C; which was maintained for 10 min. The carrier gas was Helium at a flow rate of 2 ml/min (splitless mode). The detector and Injector temperature were maintained at 250° and 225°C respectively. The quadruple mass spectrometer was scanned over the range 28-400 amu range at 1scan. s^{-1}, with an ionizing voltage of 70 eV, an ionization current of 150 µA.

The individual compounds were identified by MS and their identities was confirmed by comparing their retention indices relatives to C_8-C_{32} *n*-alkanes and by comparing their mass spectra and retention times with those of authentic samples or with data already available in the NIST library and literature [7].

Isolation of the major constituent: The essential oil (500 mg) was subjected to column chromatography over silica gel using n-hexane/diethyl ether (95:5) to give five fractions (F1-F5). Fraction F3 was further chromatographed to yield spathulenol (172 mg).

NMR analysis: The ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker ARX 400 (^{1}H 400 MHZ/^{13}C 100 MHz) spectrometer using deuterated chloroform as solvent. The chemical shift values are reported in parts per million with reference to internal TMS.

Antibacterial activity test: The essential oil was tested against 6 bacteria, three Gram-positive: *Bacillus cereus* (IPL 58605), *Enterococcus faecalis* (CIP 103214) and *Streptococcus* C (IPT 2-035), and three Gram-negative: *Proteus vulgaris* (CIP 58605), *Escherichia coli* (CIP 54127) and *Pseudomonas aeruginosa* (CIP A 22). The bacterial strains were supplied by the Pasteur Institute (Casablanca).

Preparation for microorganism culture: Screening of the essential oil was done by agar disc diffusion method[8]. It was performed using an 18 hours culture growth at 37°C. The cultures were adjusted to approximately 10^5 CFU/ml. Five hundred microliters of the suspensions were spread over plates containing Mueller-Hinton agar. Empty sterilised discs (6mm) impregnated with 5 or 10 µl of the essential oil was placed on the surface of the media. The plates were left for 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 calliper. Standard disc containing Penicillin G was used as reference control. Studies were performed in triplicate.

Results and Discussion

The hydrodistillation of the aerial parts from *Ormenis africana* produced 0.8% (v/w) of a yellowish essential oil. The components of the essential oil by GC-MS analysis are given in Table-1 in order of their elution on HP OV-17 column. Thirty-one constituents were identified representing 77% of the whole oil, which was characterized by oxygenated compounds (27 identified compounds), principally oxygenated sesquiterpenes that represented the most important group with spathulenol (45.82%), -bisabolol (5.92%) and -cadinol (5.87%) as the main compounds. Of the

oxygenated monoterpenes, it is interesting to note the large amount of camphor (7.10%) is followed by cis-chrysanthenol (2.52%), terpinen-4-ol (1.26%) and -terpineol (1.07%). The hydrocarbon fraction represented only by four sesquiterpenes hydrocarbons (-copaene, -caryophyllene, germacrene D and -cadinene) occurred in small amount (2.03% of the total identified essential oil).

These results differ so much with previous studies reported in literature about the same species[3]. Thus, Bellakhdar 1997, reported great differences in the essential oil composition of *O. africana*, characterized by high contents of the oxygenated monoterpene ocimenone (40%) and the sesquiterpene hydrocarbon-copaene (38%), this last compound represent only 0.19% in our study, whereas this main compound reported previously [3], was not found in the essential oil here analyzed. So this report can contribute to a better knowledge of this species.

Table-1 Essential oil composition of *Ormenis africana* (Jord & Fourr.)

Compound	**I.R**	**%**
Benzaldéhyde	964	0.03
Yomogi alcohol	999	0.10
1,8-Cinéole	1035	0.12
Cétone artémesia	1062	0.56
Trans-4-thujanol	1071	0.03
Artemesia alcohol	1084	0.13
Sabinene hydrate	1099	0.10
Chrysanthénone	1129	0.30
Trans-pinocarvéol	1139	0.27
Camphor	1149	7.10
Cis-chrysanthénol	1164	2.52
Bornéol	1170	0.92
Terpinen-4-ol	1177	1.26
p-Cymén-8-ol	1189	0.22
α-Terpineol	1192	1.07
Verbenone	1205	0.19
Pipéritone	1253	0.30
Chrysanthényl acetate	1265	0.13
p-Cymén-7-ol	1291	0.34
Carvacrol	1302	0.33
Eugenol	1361	0.17
α-Copaene	1380	0.19
Cis-jasmone	1401	0.14
β-Caryophyllene	1424	0.18
Germacrene D	1486	0.94
δ-Cadinene	1525	0.72
Nerolidol	1563	0.27
Spathulenol	1580	45.82
Caryophyllene oxide	1583	0.80
α-Cadinol	1654	5.87
5-(t-butyl)-4-méthoxy-1,2-dihydrobenzène	1667	2,83
α-Bisabolol	1686	5.92

Components listed in order of elution from a HP OV-17 column.
RI: Kovats indices calculated against C_8-C_{23} n-alkanes on the HP OV-17 column

To remove doubt, a sample of whole essential oil was subjected to column chromatography over silica gel using mixtures of n-hexane/Et_2O of increasing polarity as eluents, the major compound 1 (Figure- 1) was obtained as colourless oil. The mass spectra and ^{1}H and ^{13}C NMR data (Table-2) suggested that 1 is spathulenol[12]. Comparison of the spectroscopical data of 1 with those reported in literature of spathulenol[9] confirmed these assignments.

Although, this plant did not contain santolina alcohol, as identified previously in the oils of *Ormenis multicaulis*[10].

Table-2 Experimental and literature's ^{13}C NMR data for spathulenol (δ in ppm)

Carbon	1	2	3	4	5	6	7	8
Exp	54.4	26.8	41.8	81.0	53.3	29.9	27.5	24.8
Lit[9]	54.3	27.7	41.7	80.9	53.3	29.9	27.4	24.8
Carbon	**9**	**10**	**11**	**12**	**13**	**14**	**15**	
Exp	38.9	153.5	20.3	28.7	16.4	26.1	106.3	
Lit[9]	38.8	153.4	20.2	28.6	16.2	26.0	106.2	

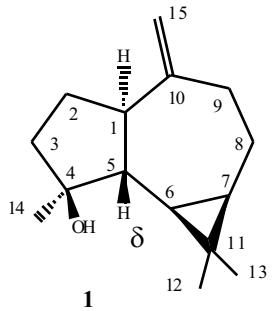

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Figure-1 Structure of purified compound, spathulenol

Table -3 shows in vitro bacteriostatic activity of essential oil from *O. africana* together the inhibition zones formed by standard penicillin G (10 unit) antibiotic discs.

Table-3 Antibacterial activity: Diameter of the inhibition zone[a] of essential oil of *Ormenis africana* and standard antibiotic penicillin G.

	EO *O. Africana*		**P10**
Microorganisms	5 µl	10 µl	
Bacillus cereus	20	23	13
Streptococcus C	17	21	19
Enterococcus faecalis	16	21	13
Escherichia coli	12	13	16
Proteus vulgaris	11	13	9
Pseudomonas aeroginosa	9	9	9

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

Although, at low quantities (5-10µl/disc), this oil exhibited a strong antibacterial activity against the most tested bacteria; in our study, *P. aeruginosa* was resistant at concentration at 5-10µl/disc of *O. africana*. The bacteriostatic properties of the oil are suspected to be associated with the high spathulenol content, which has been previously tested and was found to have significant antibiotic activity [11].

Conclusion

From this study, it can be concluded that essential oil of *Ormenis Africana* possesses a strong antibacterial activity against Gram-positive tested strains, and considerable activity against Gram-negative bacteria, this activity may be due of its high percentage of oxygenated sesquiterpenes, spathulenol (45,8%), α-cadinol (5.87%) and α-bisabolol (5.92%),the structure of the major compound was confirmed by NMR spectroscopy after purification using column chromatography. Our antimicrobial study justified the popular usage of this plant as traditional remedies for some infections.

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12. Principal pics in NMR ^{1}H spectra: NMR ^{1}H ($CDCl_3$): δ(ppm) 4,69 (s, 1 H), 4,66 (s, 1 H), 2,42 (dd, *J* = 13.4, 6.2 Hz, 1 H), 1,29 (s, 3 H), 1,06 (s, 3 H), 1,04 (s, 3H), 0,72 (ddd, *J* = 11,1; 9,5 and 6,1 Hz, 1 H), 0,47 (dd, *J* = 11.1, 9.5 Hz, 1 H).