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Chemical Composition, Antioxidant and Antimicrobial Activities of Essential Oil of *Warionia saharae* from Oases of Morocco

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1. Introduction

The *Warionia saharae* which belongs to the important composite's family is an endemic species of North Africa, characterized by a discerning odour (Bonnet and Maurry, 1889). *Warionia saharae* was reported for the first time in the Oranais Sahara (Beni ounif in Algeria) by Dr. Warrion as a shrub of 1 to 3 m of height. The thick trunk, is covered of a gray peel, structural of very wavy terminal leaf bouquets, and of capitulate of yellow flowers (Photos 1 and 2). The picking of stems leafed of this bush, clear a very heady and spicy odour; the latex that flows out of injuries of the peel, glue to hands in a very tenacious way (Lebrun, 1979).

In Morocco, *Warionia saharae* is growing wild in various regions (Benabid and Fennane, 1994). The habitat is between schistose rocks (Watillon et al., 1988). *Warionia saharae* is known in Morocco by the Berber vernacular names of "âfessas" and "Tazart n-îfiss". In the Moroccan traditional medicine, the leaves of the plant are used to treat inflammatory diseases, such as rheumatoid arthritis, and for gastrointestinal disorders (Bellakhdar, 1997), inflammation of the womb, colds, Jaundice and cardiac pains (El Rhaffari et Zaid, 2002).

This work aims to study and characterise a new bioactive natural products from medicinal plants of Moroccan oases. Information concerning in vitro antioxidant, antimicrobial activities of the essential oil from the *Warionia saharae* has not been reported earlier.

2. Materials and methods

2.1 Plant material

The aerial part of *Warionia saharae* was collected in Er-rachidia (Morocco), during the flowering period (April/June, 2009). A duplicate specimen is held at the FST Er-rachidia. The dried plant material is stored in the laboratory at room temperature (25°C) and in the shade before the extraction.



Photo 1. *Warionia saharae* (Plant) (Taken y El Rhaffari L.)



Photo 2. *Warionia saharae* (Flower) (Taken y El Rhaffari L.)

2.2 Steam distillation apparatus and procedure

The extraction of essential oil of the aerial part of *Warionia saharae* was conducted by steam distillation in a Clevenger apparatus. The essential oil obtained was dried under anhydrous sodium sulfate and stored at 4°C in the dark before analysis.

2.3 Essential oil analysis

Analyses of volatile compounds were run on a Trace GC Ultra gas chromatography equipped with a VB-5 capillary column (Methylpolysiloxane with 5% phenyl; 25 m x 0.2 mm; film thickness 0.2µm) was directly coupled to the mass spectrometer (Polaris Q. MS). Helium (1 mL/min) was used as carrier gas. The program was 2 min isothermal at 40 °C, then the temperature increased by 4 °C/min to 180 °C and 2 min isothermal at 180 °C. The injection port temperature was 250 °C and that of the detector was 280 °C. Ionization of sample components was performed in the EI mode (70 eV).

Kovat's retention indices were calculated using cochromatographed standards hydrocarbons. The individual compounds were identified by MS and their identity was confirmed by comparing their retention indices relatives to C8-C32 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 (Adams, 1989 et 2001).

2.4 Antimicrobial activity

The antimicrobial activity was evaluated by paper disc diffusion and dilution methods against three selected Gram-positive and Gram-negative species: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and against the phytopathogenic fungi *Penicillium* sp. and *Candida albicans*.

The qualitative antimicrobial essay of the volatile fraction of *W. saharae* was carried out by the disc diffusion method (Anonymous, 1995).

The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of tested volatile fractions were determined using the Mueller Hinton broth (MHB) dilution method (Anonymous, 1995).

All samples were tested in triplicate. The MIC was defined as the lowest concentration preventing visible growth (May et al., 2000; Burt, 2004).

2.5 Antioxidant activity

The antioxidant activity was assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing ability (FRAP) and β-carotene bleaching method systems. Data collected for each assay was an average of three experiments.

The method is based on the radical scavenging activity of the antioxidant (Mensor et al., 2001; Tepe et al., 2004).

The FRAP assay was performed as described by Benzie and Nilsson (Benzie and Strain, 1996; Nilsson et al., 2005).

The β-carotene method was carried out according to Shahidi technique (Shahidi et al., 2001).

2.6 Statistical analysis

For antioxidant assays the results were expressed as value \pm error using statistical analysis formulas referring to the value of $p < 0.05$ of confidence interval for the Student's t-test law.

3. Results and discussion

3.1 Chemical composition of the essential oil

The essential oil was extracted by the hydrodistillation of the dried leaves of *Warionia saharae* from Errachidia region (Morocco), were analyzed by GC-MS.

The essential oil yields, calculated on a dry weight basis, was about 1.1% (w/w). The identified combinations in essential oil, retention time (RT) and quantitative percentage of the compounds are presented in table 1. A total of 32 compounds, amounting 92.5% of the oils, were identified.

Peak number	RT ^b	Compound ^a	Composition (%)
1	6.63	α -Thujene	0.9
2	6.75	α -Pinene	0.6
3	10.19	Camphene	0.99
4	10.68	Sabinene	2.22
5	11.79	α -Phellandrene	1.50
6	12.24	α -Carene	0.60
7	12.54	p-Cymene	3.77
8	12.77	1,8-Cineole	6.12
9	13.80	3-Carene	0.77
10	14.88	α -Terpinene	0.41
11	15.49	Linalool	16.79
12	18.12	Terpinen-4-ol	3.40
13	18.60	α -Fenchol	2.59
14	18.86	Ethyl 8-fluorooctanoate	0.55
15	20.84	Geraniol	1.14
16	22.56	Carvacrol	1.34
17	23.92	Ethyl 3-phenylpropionate	0.85
18	23.99	Ocimenyl acetate	0.81
19	28.51	Tridecan-1-ol	0.55
20	28.73	Longipinane	2.05
21	29.45	Allo-Aromadendrene	0.44
22	30.20	α -Farnesene	0.51
23	30.75	Trans nerolidol	17.95
24	31.19	Caryophyllene oxide	1.91
25	33.24	β -Eudesmol	23.74
Total			92.5

^a Compounds listed in order of elution from a DB-5 column.

^b Retention time (as minutes).

Table 1. Composition of essential oil of *Warionia saharae* (%).

The major components of *Warionia saharae* essential oils were β -Eudesmol (23.74%), Trans-Nerolidol (17.95%), Linalool (16.79%), 1,8 cineole (6.12%).

The high content of oxygenated identified compounds might explain the characteristic and fragrant odor of the oil (Figure 1 and Table 1).

RT: 0.00 - 45.02 SM: 7G

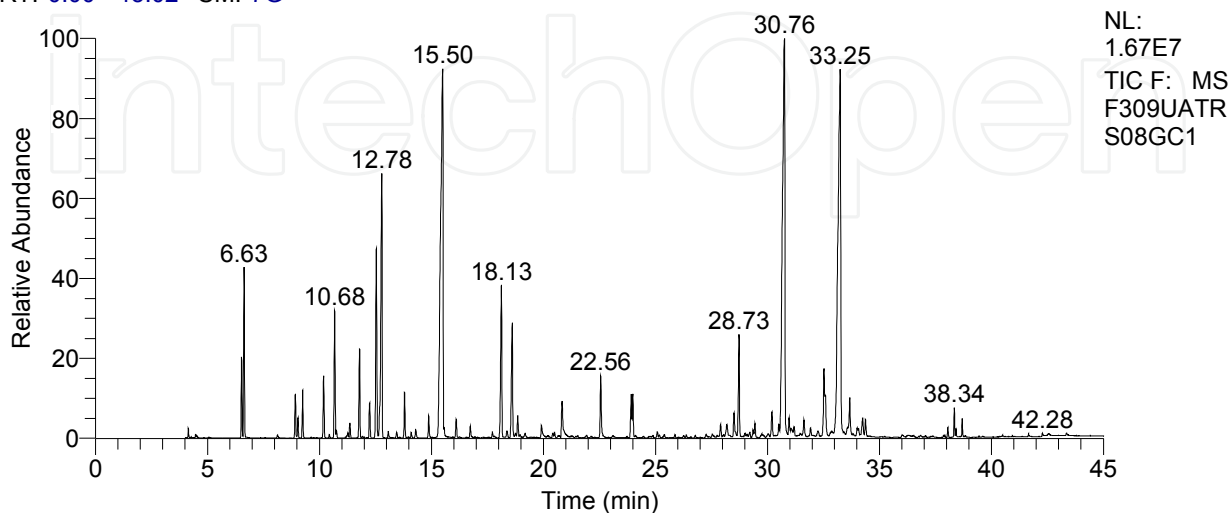


Fig. 1. Chromatogram *Warionia saharae* volatile fractions run on VB-5 capillary column.

3.2 Antimicrobial activity

Antimicrobial activities of *Warionia saharae* essential oil were evaluated by a paper disc diffusion method against bacterial strains including Gram+ and Gram-, and fungal strains (Table 2).

microorganism	source	Inhibition (mm) ^a					MIC ($\mu\text{g/ml}$)		
		Essential oil (EO)			STA ^b ($\mu\text{g/disk}$)		EO	Amp	Nys
		5 $\mu\text{g/disk}$	10 $\mu\text{g/disk}$	15 $\mu\text{g/disk}$	A10	N10			
<i>E. coli</i>	ATCC25922	12	13	16	16	Nd	20	25	Nd
<i>P. aeruginosa</i>	ATCC27853	16	20	22	18	Nd	10	5	Nd
<i>S. aureus</i>	ATCC25923	10	14	15	14	Nd	5	10	Nd
<i>Candida albicans</i>	BTCE	18	18	20	Nd	22	2.5	Nd	5
<i>Penicillium sp.</i>	BTCE	14	16	20	Nd	18	5	Nd	10

^a Diameter of the zone of inhibition (mm) including disk diameter of 6mm – average of three experiments.

^b standards antibiotic; A10 : Ampicillin (Amp), N10 : Nystatin (Nys).

MIC, minimum inhibitory concentration; values given as mg/ml for the essential oils and as $\mu\text{g/ml}$ for antibiotics.

BTCE, Blood Transfusion Center Errachidia (Morocco).

Nd, not determined.

Table 2. Antimicrobial activity of *Warionia saharae* essential oil.

The comparison between in vitro bacteriostatic activity of essential oil of *Warionia saharae*, and the inhibition zone formed by standard antibiotic disc (Ampicillin (A10) and Nystatin (N10)) showed that the essential oils were more active against the microorganisms tested. These results suggest that the volatile oil of *Warionia saharae* would probably be a good therapeutic agent against these bacteria and fungus.

The bacteriostatic properties of the oil are suspected to be associated with the high Eudesmol and Nerolidol content, which has been tested previously and was found to have a significant antibiotic activity (Brehm-Stecher and Johnson, 2003; Lattaoui and Tantaoui-Elaraki, 1994).

3.3 Antioxidant activity

The study of the antioxidant activities showed that the essential oil have a higher antioxidant capacity relative to the antioxidant of reference BHT and BHA ($94.2 \pm 0.4\%$).

In contrast to β -carotene/linoleic acid system, essential oil showed a moderate activity in this system ($65.2 \pm 0.4\%$).

The ferric reducing ability of plasma (FRAP) assay was used for assessing “antioxidant power” of *Warionia saharae* essential oil. The antioxidant power of the leaves oil samples were compared with BHA and BHT as reference antioxidants.

The results reported here can be considered as the first information on the antimicrobial and antioxidant properties of *Warionia saharae*. Further studies are needed to evaluate the in vivo potential of these oils in animal models.

4. Conclusion

This work interested *Warionia saharae* from Er-rachidia region (south eastern Morocco). It showed that the essential oil yield is about 1.1% (w/w) in dried leaves.

The essential oils is analysed by GC-MS. Thirty two compounds were identified, they are amounting 92.5% of the oils. The major components of essential oils were β -Eudesmol (23.74%), Trans-Nerolidol (17.95%), Linalool (16.79%), 1,8 cineole (6.12%), p-Cymene (3.77%) and terpinen 4-ol (3.40%).

Warionia oil exhibited a significant antimicrobial activity against *Staphylococcus aureus*, *Candida albicans* and *Bacillus cereus*. In each case the activity of essential oil was higher than those of the standard antibiotic.

The essential oil showed a relatively high radical scavenging ability and antioxidant activity determined by 1,1-diphenyl-1-picrylhydrazyl (DPPH) assay, ferric reducing (FRAP) assay and β -carotene bleaching test.

The GC and GC-MS analysis was useful in order to identify the components partially involved in antimicrobial and antioxidant activities of the essential oils obtained from *Warionia saharae* leaves.

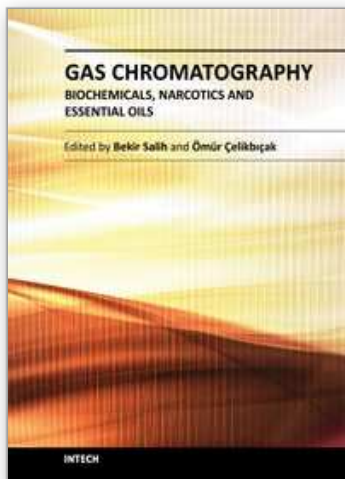
Therefore, *Warionia saharae* essential oil could be a source of pharmaceutical materials required for the preparation of new therapeutic and antimicrobial agents. This is the object of our future investigations.

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Gas Chromatography involves the study of various vaporizable molecules in chemistry and the other related research fields. This analytical method has a number of features and advantages that make it an extremely valuable tool for the identification, quantification and structural elucidation of organic molecules. This book provides detailed gas chromatography information to applications of biochemicals, narcotics and essential oils. The details of the applications were briefly handled by the authors to increase their comprehensibility and feasibility. This guide should be certainly valuable to the novice, as well as to the experienced gas chromatography user who may not have the enough experience about the specific applications covered in this book. We believe this book will prove useful in most laboratories where modern gas chromatography is practiced.

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