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Chemical composition and antioxidant activity of the essential oil of *Artemisia vulgaris* from Morocco.

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ABSTRACT

The focus of this study was to determine the chemical composition of Moroccan *Artemisia vulgaris* essential oil using a GC-MS method and its antioxidant activity in methanolic solutions by assessing the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The results of this work showed that the essential oil of Moroccan *Artemisia vulgaris* is mainly constituted of Camphor (39.9%), beta-Thujone (15.63%) and alpha-Thujone (5.42%). The radical scavenging activity assay gave a concentration of essential oil required to inhibit 50% of the DPPH free radical of IC_{50} = 42mg/ml demonstrating that this essential oil may be considered as an interesting source of natural antioxidants.

Keywords: antioxidant activity, essential oil, GC-MS, *Artemisia vulgaris.*

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INTRODUCTION

Plants are still considered to be a valuable source of natural bioactive compounds that can be used to provide a soft medication, without side effects, against human diseases. Among theses biomolecules, the presence of some important compounds like polyphenols, flavonoids or sesquiterpenes is often related to a marked antioxidant activity of the plant. These natural antioxidants have been shown to effectively reduce the oxidative stress and prevent or delay the damages caused by many conditions like inflammatory and rheumatic diseases, asthma or cancer [1-4].

Artemisia vulgaris (Asteraceae) is an aromatic plant used in traditional medicine for its therapeutic properties like the antibacterial and antifungal activity of its essential oil. *Artemisia vulgaris* essential oil, extracted from the aerial parts of the plant, has also been shown to be antioxidative but its chemical composition is variable according to the area of harvest, and the stage of development [5].

The aim of this work is to determine the chemical composition of Moroccan *Artemisia vulgaris* essential oil and to evaluate the in vitro antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazil) free radical scavenging assay.

MATERIALS AND METHODS

Plant material

The aerial parts (mainly leaves) of *Artemisia vulgaris* collected from different locations in Morocco and bought from herbalists were dried and ground to a fine powder before essential oil extraction.

Essential oil extraction

Essential oil was isolated by water distillation from the plant powder, using a Clevenger-type apparatus, according to the procedure described in the European Pharmacopoeia.

Gas chromatography – mass spectrometry

Essential oil profile was characterized by gas chromatography (GC) coupled to mass spectrometry (MS). The chromatographic conditions are shown in table 1.

Table 1 : GC-MS conditions for the essential oil analysis.

Apparatus name	Shimadzu – QP2010
Detection mode	Electronic impact
Ionization current	70 eV
Mass range (uma)	40-450
Capillary Column	BP5
Carrier gas	Helium
Interface temperature	300 °C
Injection temperature	250 °C
Source temperature	200 °C
Oven programming	60°C-200°C, 3°C/min
Injected volume	0,2 µl
Solvant	Pentane, dilution 1/100

Antioxidant activity assessment by DPPH free radical scavenging assay

The free radical scavenging capacity of the essential oil was determined using DPPH (2,2-diphenyl-1-picrylhydrazil) assay according to the method described by Koleva et al [6].

1ml of methanolic solutions of the essential oil at different concentrations (0- 400mg/ml) are mixed (in test tubes) with 2 ml of a 0.004% methanolic solution of DPPH.

The mixtures were shaken vigorously and incubated for 30 min, in the dark, at room temperature.

Absorbance of the resulting solutions was measured at 517nm using a VWR UV-1600 PC double-beam UV/Visible spectrophotometer against methanol.

As positive reference, methanolic solutions of ascorbic acid at different concentrations were used.

Percentage of DPPH scavenging activity was determined as follows:

DPPH Radical Scavenging Activity (%) = $[(A_0–A_1)/A_0]$,

where A_0 is the absorbance of control and A_1 is the absorbance of sample.

The IC_{50} value, which is the concentration of essential oil required to inhibit 50% of the DPPH free radical, was determined.

RESULTS AND DISCUSSION

Chemical composition of essential oil

Artemisia vulgaris essential oil yields only 0.64% (V/W) of essential oil. The essential oil was analyzed by GC-MS as described above. The chromatogram obtained from this analysis is shown in figure 1. The qualitative and quantitative composition are presented in table 2, where compounds are listed in order of their retention times (RT).

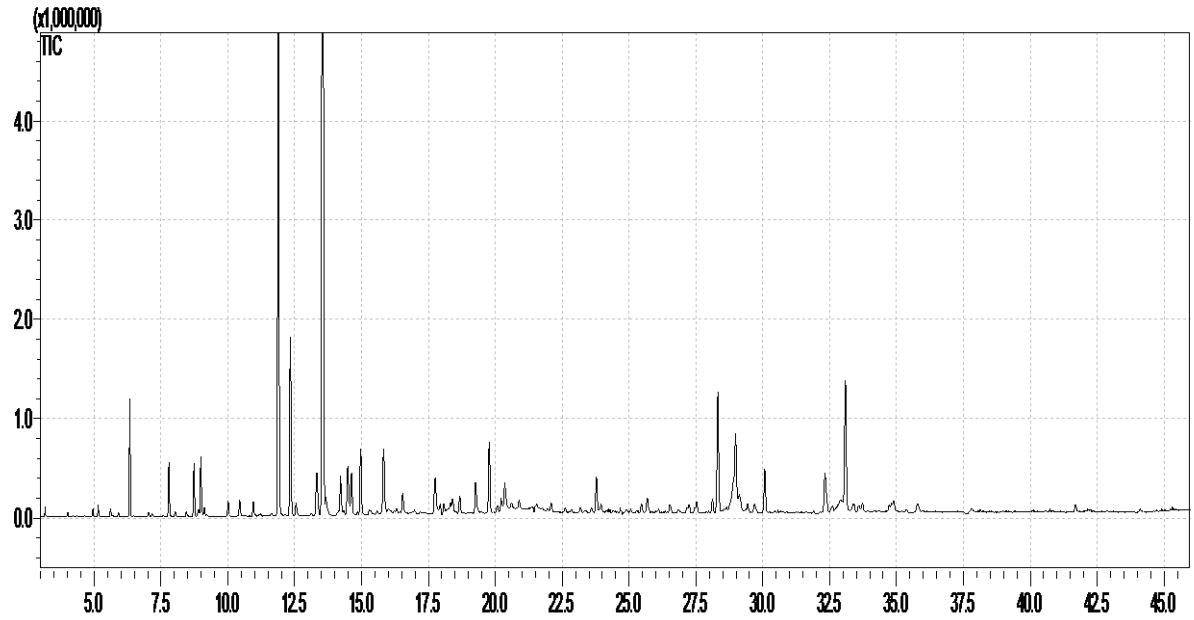

Figure 1: Chromatogram of the GC-MS analysis of *Artemisia vulgaris* essential oil.

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Table 2 : Chemical composition of *Artemisia vulgaris* essential oil by GC-MS.

RT (min)	Area %	Compounds
6.334	2.49	Camphene
7.796	1.25	3,3,6-Trimethyl-1,4-heptadien-6-ol
8.732	1.27	o-Cymol
8.988	1.43	Cineole
10.009	0.37	4-Carene
10.442	0.41	Butyric acid, 3-hexenyl ester
10.953	0.34	2,7-Dimethyl-2,6-octadien-4-ol
11.885	15.63	beta.-Thujone
12.334	5.42	alpha.-Thujone
12.548	0.34	Fenchene
13.322	1.66	trans-Pinocarveol
13.545	34.90	Camphor,
13.662	0.34	3-Pinanone
14.212	1.02	Santolina triene
14.483	1.77	Borneol
14.623	1.16	alpha.-Ionol
14.966	2.05	4-Terpineol
15.823	2.22	Myrtenol
16.534	0.61	Isopinocampheol
17.750	1.57	6-(1-Butenyl]-1,4-cycloheptadiene
17.942	0.44	alpha.-Limonene diepoxide
18.399	0.22	Piperitone
18.671	0.50	Chrysanthenyl Acetate
19.263	0.98	3-Carene, 2-(acetylmethyl)
19.772	2.31	Bornyl acetate
20.354	0.90	6-Hexadecen-4-yne,
23.780	1.12	Copaene
25.685	0.41	Caryophyllene
28.118	0.34	gamma.-Muurolene
28.323	4.34	Germacrene D
28.982	3.71	gamma.-Elemene
29.183	0.61	alpha.-Muurolene
30.073	1.42	delta.-Cadinene
32.323	1.80	Spathulenol
33.088	4.65	Carotol

35 compounds were identified. The main components were Camphor (39.9%), beta-Thujone (15.63%) and alpha-Thujone (5.42%). The other important components were: Germacrene D, gamma-Elemene, Myrtenol, 4-Terpineol and Camphene in different amounts.

In a similar study on *Artemisia vulgaris* essential oil from india, Camphor was also found to be a major constituent (10.7%) followed by ß-endesmol (8.95%) and Trans-Caryophyllene (6.525%) [5].

In an other study on the same essential oil from Nepal, the major components were sabinene (11.29%), beta-thujone (19.99%), chrysanthenone (4.48%), camphor (11.89%), borneol (4.44%) and germacrene D (8.42%) [7].

This variability of composition could be explained by the differences in genetic, geographic and climatic factors.

Free radical scavenging activity on DPPH of *Artemisia vulgaris* essential oil

Antioxidant compounds donate electrons to DPPH thus causing its reduction, and in reduced from its color changes from deep violet to yellow. The DPPH assay has been extensively used for screening antioxidants such as polyphenols [8].

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In our study, the DPPH free radical scavenging method was used to determine the concentration of methanolic solution of the essential oil at which it scavenges 50% of the DPPH solution termed as IC_{50} values. Ascorbic acid was used as standard for this purpose. The lower the IC_{50} value of an antioxidant the higher would be its free radical scavenging power. The scavenging abilities of different methanolic solutions (different concentrations) of *Artemisia vulgaris* essential oil were concentration-dependent as presented in figure 2.

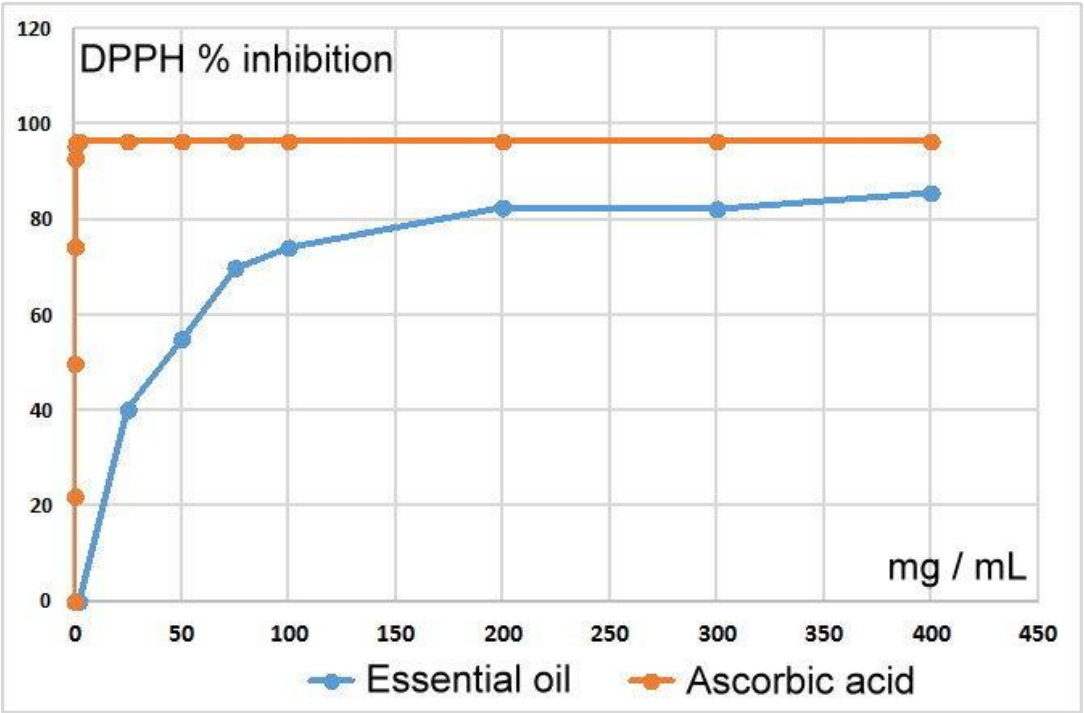

Figure 2: DPPH scavenging activity of *Artemisia vulgaris* essential oil.

The radical-scavenging activity expressed by the IC_{50} value was 42 mg/ml in our study. Better results were obtained in other works with an IC_{50} of 63.82 µg/ml for the essential oil from Nepal [7] and an IC50 of just 12.09 µg/ml for the essential oil from India [5]. This variability is mainly related to the differences in the molecular composition of the essential oils.

Meanwhile, the results we obtained confirm that *Artemisia vulgaris* essential is of great interest regarding its free radical scavenging potential.

CONCLUSION

In the present study, results from antioxidant activities reflected by DPPH assay have demonstrated that the Moroccan *Artemisia vulgaris* essential oil possesses an interesting antioxidant activity. This antioxidant capacity is mainly correlated to the presence of monoterpene and sesquiterpene compounds. The results showed that the aerial parts essential oil of this plant is a good source of natural antioxidants that may contribute to its therapeutic activities.

Further studies to evaluate the in vivo potential of this essential oil and the isolation and identification of the antioxidant principles should be carried out.

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