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Chemical composition and antiproliferative activity of essential oil from aerial parts of a medicinal herb *Artemisia herba-alba*

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Abstract: *Artemisia herba-alba* Asso., Asteraceae, is widely used in Morrocan folk medicine for the treatment of different health disorders. However, no scientific or medical studies were carried out to assess the cytotoxicity of *A. herba-alba* essential oil against cancer cell lines. In this study, eighteen volatile compounds were identified by GC-MS analysis of the essential oil obtained from the plant's aerial parts. The main volatile constituent in *A. herba-alba* was found to be a monoterpene, Verbenol, contributing to about 22% of the total volatile components. The essential oil showed significant antiproliferative activity against the acute lymphoblastic leukaemia (CEM) cell line, with 3 µg/mL as IC50 value. The anticancer bioactivity of Moroccan *A. herba-alba* essential oil is described here for the first time.

Short communication

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Introduction

The *Asteraceae* family comprises many aromatic and medicinal plants. Some important genera of this family are *Artemisia, Santolina, Centaurea, Chrysanthemum* etc. The genus Artemisia belongs to the *Anthemideae* tribe which is one of the largest *asteraceae* family and contains more than 300 species of small herbs and shrubs (Dob & Benabdelkader, 2006).

Plants of the genus *Artemisia* (Asteraceae family, *Anthemideae* tribe) have been used in folk medicine by many cultures since ancient times. Herbal infusions from these species have been used as analgesic, antibacterial, antispasmodic, and haemostatic agents (Abou El-Hamd et al., 2010).

Historically, *Artemisia* has been a productive genus in the search for new biologically active compounds. Phytochemical investigations have proven that this genus is rich in sesquiterpenes and monoterpenes (Akrout et al., 2010; Houari & Ferchichi, 2009; Tang et al., 2000).

Artemisia herba-alba Asso., Asteraceae, is a medicinal and aromatic dwarf shrub that grows wild in arid areas of the Mediterranean region, extending into north-western Himalayas (Vernin et al., 1995). It is used in folk medicine for the treatment of colds, diabetes mellitus, coughing, intestinal disturbances, including intestinal worms, and for the treatment of human and livestock wounds (Bailey & Danin, 1981; Yashphe et al., 1979).

So far, to the best of our knowledge, there is no known studies on the antitumor activity of *Artemisia herba-alba* essential oil. In the present paper we carry out a detailed investigation on the chemical composition and antiproliferative activity of the essential oil of *A. herba-alba.*

Material and Methods

Aerial parts of *Artemisia herba-alba* Asso., Asteraceae, were collected in July 2007 from a population located at Imilchil in Er-Rachidia province, central eastern Morocco. The species was identified and stored as a voucher specimen in the plant collection of Sultan Moulay Slimene University "Herbier FSTBM,LT0020".

Determination of essential oil composition

Samples of dried and ground leaves (200 g each sample) of *A. herba-alba* were hydrodistilled for 4 h in a modified Clevenger-type apparatus to obtain the volatile constituents. The essential oil of *A. herba-alba* was analysed by GC on a Trace GC ULTRA with FID detector gas chromatograph equipped with a column (30 m x 0.25 mm x 0.25 µm) type VB-5 (methylpolysiloxane

with 5% of phenyl) and split injection. Mass spectrometry (MS) analysis were performed on a Polaris Q MS mass spectrometer (with an ion-trap at 70 eV). The temperature program was 40 °C for 2 min, then raised to 180 °C at 4 °C/min. The carrier gas was helium (1.4 mL/min). The volatile constituents of the essential oil were identified by automated comparison of their mass spectra with that of the NIST (National Institute of Standards and Technology) library.

Cell culture

Acute lymphocytic leukaemia (CEM) cells, were grown in Eagle's Minimum Essential Medium (MEM) (Gibco) supplemented with 5% heat-inactivated foetal bovine serum (FBS) (Gibco), 1% penicillin-streptomycin-neomycin, and 0.2% sodium bicarbonate (Sigma), under a fully humidified atmosphere of 95% air and 5% CO_2 at 37 °C.

Cytotoxicity assay

Cellular viability was determined by the MTT reduction assay using a tetrazolium salt (3- [4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide, MTT) (Mosmann, 1983, Hussain et al., 1993). In brief, growing concentrations of the tested essential oil (solubilised in DMSO), starting from 0.2 µg/mL were applied to the wells of a 96-well plate containing the confluent cell monolayer (104 cells per well) in triplicate. Methotrexat as positive control drug was added in the same concentrations and conditions. After 48 h of incubation, 12 µL of the MTT solution [5 mg/mL in phosphate buffered saline (PBS)] was added. After incubation in the same conditions for 4 h, the plates were treated with a mixture of HCl/isopropanol (24:1) to dissolve the blue intracellular formazan product. One hour later, the plates were read on a MicroELISA reader using two wavelengths (540 and 630 nm). DMSO was used as negative control. The median inhibitory concentration (IC50) was calculated as the concentration of the sample that leads to 50% of cell lysis comparatively to the negative control.

Results and Discussion

The colorless aromated essential oil obtained by hydrodistillation yielded 0.22% of *Artemisia herba-alba* dry weight. A total of eighteen compounds have been identified representing around 98.8% of the total oil (Table 1). Among the identified compounds, ten molecules have not been previously reported in *A. herba-alba* essential oils.

As shown in Table 1, the monoterpenes: verbenol (21.83%), bisabolone oxide (17.55%), farnesene epoxide (17.08%) and β-thujone (6.14%) were the major constituents in the volatile oil of *A. herba-alba*. Other representative compounds were identified as camphor (5.12%), myrtenol (4.19%), fenchol (3.86%), and α-bisabolol oxide (2.99%).

Table 1. Chemical composition of the essential oil of aerial parts of *A. herba-alba*.

Component	Percentage (%)
Terpinen-4-ol	2.43
Piperitonea	2.69
3,5-Heptadienal, 2-ethylidene-6-methyl[a]	1.74
β-Thujone	6.14
cis-Sabinol	2.40
Thujon	1.01
trans-Sabinene hydrate	1.24
Camphor	5.12
Fenchol[a]	3.86
Verbenol[a]	21.83
Myrtenola	4.19
Caryophyllene oxide[a]	1.76
Farnesene epoxide, E[a]	17.08
Bisabolone oxide[a]	17.55
Eucalyptol (1,8-Cineole)	2.27
α-bisabolol oxide A[a]	2.26
Bergamotol, Z-α-*trans*[a]	2.24
α-Bisabolol oxide[a]	2.99
Yield of essential oil (%)	0.22

[a]components reported for the first time in *A. herba-alba* oils.

In essential oils of the genus *Artemisia*, thujane and camphane derivatives as well as 1,8-cineole are the major and most widely spread structural types. Cineole was cited as the major component in the essential oil of *A. herba-alba* from Spain (Salido et al., 2004), from Palestine (Feuerstein et al., 1986), from Egypt (Soliman, 2007) and from Morocco (Lamiri et al., 1997). In our present study, cineol is not the main component since it represents only 2.27% of detected compounds.

Other studies from Spain showed that camphor constitutes the major component. This chemical is one of the most encountered components in *A. herba-alba* essential oil. In Morocco, five chemotypes were defined as camphor type oils (Lamiri et al., 1997). In Jordan, regular monoterpenes were predominant and the principal components were α- and β-thujones, qualifying the plant as being a thujone chemotype (Hudaib & Aburjai, 2006).

The Spanish *A. herba-alba* oil fits this pattern by containing large amounts of l,8-cineole and bornane derivatives (Feuerstein, 1988; Salido, 2004). In contrast,

Palestinian and Morroccan chemotypes, were defined as producing camphor type oils (Lamiri et al., 1997).These same chemotypes lack significant quantities of thujane derivatives and contain large amounts of sesquiterpens. Some of these molecules were also found in the oil of a Spanish population from Valencia region and in the Sinai chemotype (Feuerstein et al., 1986).

A thujone-camphor oil was also observed in Morocco (Lamiri et al., 1997) and cineole-camphor-borneol oil was observed in Palestine (Feuerstein et al., 1986). The most frequent chemotype is composed of cineol and camphor and was observed in samples from Morocco, Spain and Palestine (Feuerstein et al., 1988; Feuerstein et al., 1986; Lamiri et al., 1997). In the present study, we identified for the first time a new chemothype (Verbenol- Bisabolone oxide- Farnesene epoxide) in Moroccan (Imilchil, Er-Rachidia Province) *Artemisia herba-alba.*

The main components observed in these oils: verbenol (21.83%), bisabolone oxide (17.55%), farnesene epoxide, (17.08%) were not observed in *A. herba-alba* essential oil composition published elsewhere.

The differences in the chemical profile of the populations are ascribed to the environmental conditions such as soil, climatic conditions and interactions between flora and fauna.

Our experimental results of the in vitro antiproliferative activity using the MTT assay indicated that the essential oil of *A. herba-alba* possessed significant antiproliferative activity against CEM

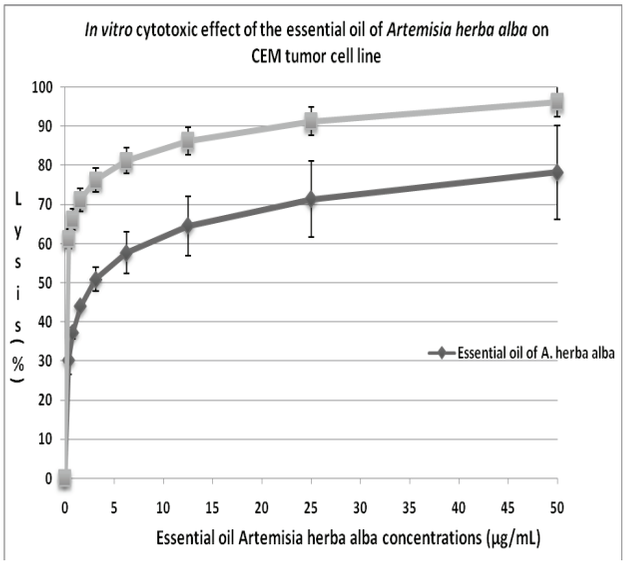

Figure 1. Cytotoxic effect of *Artemisia herba-alba* essential oil against CEM tumor cell line. (mean±SEM in triplicate).

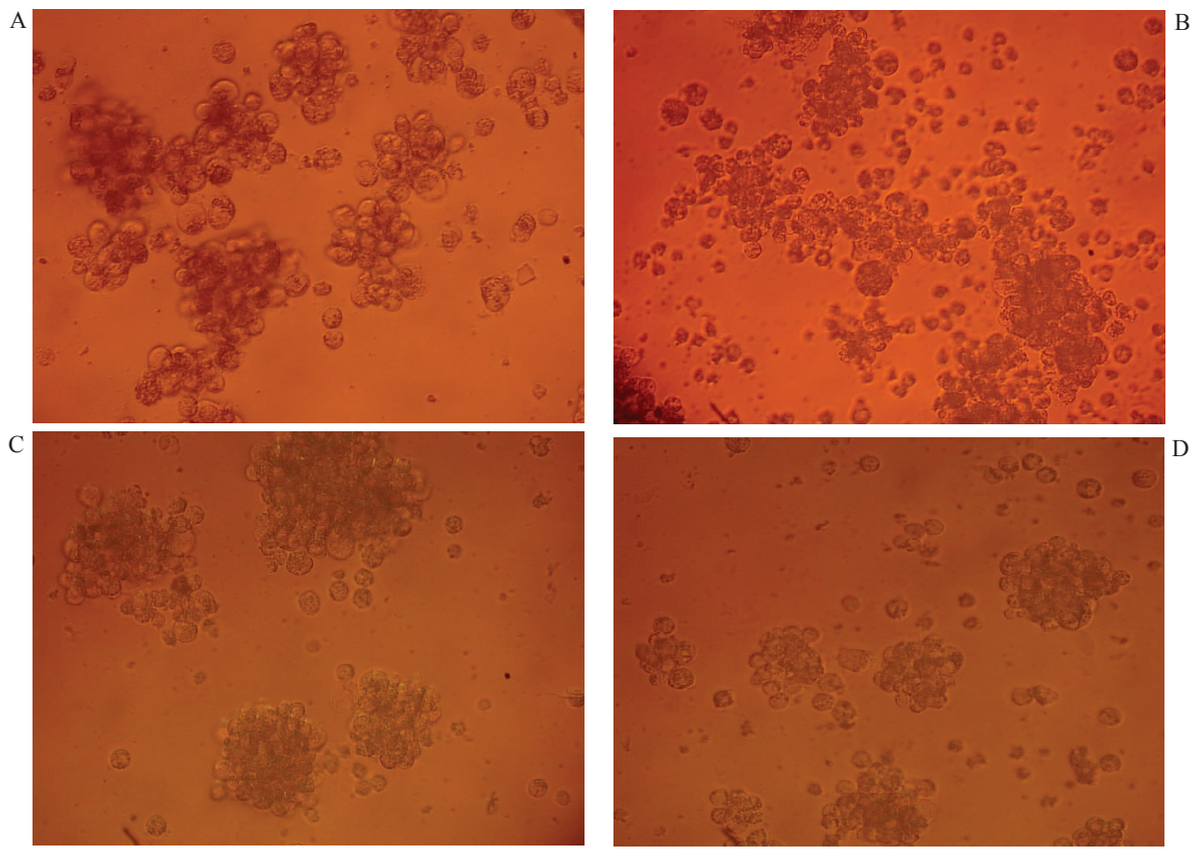

Figure 2. Photographs of CEM cells at x 400 magnification. A: un treated CEM cells. B, C, and D: CEM cells treated respectively with 50, 6.25 and 0.4 µg/mL essential oil of *Artemisia herba-alba.*

cancer cell line. It is shown in Figure 1 and Figure 2, that essential oil have an important dose dependant cytotoxic effect. This cytotoxicity starts at small concentrations (less than 0.5 µg/mL) of oil. Indeed, at high concentration (50 µg/mL), the percentage of cell lysis is about 80%. Figure 2 shows the appearance of CEM cells, untreated (A) and treated (B, C, D) with different concentrations of the essential oil. In fact, the concentration leading to 50% cytotoxicity (IC50) is about 3 µg/mL. This low IC50 value confirms the strong anticancer properties of *A. herba. alba* aerial parts essential oil. It is well known that the plant essential oil containing predominantly monoterpens have significant antimicrobial activity (Akrout et al., 2010; Yashfe et al., 1979). Furthermore, the plants such as *Nigella sativa* and *Thymus* sp. containing essential oils exhibited effective cytototoxic effects on various tumor cell lines in a dose dependent and differential manner (Ait M'Barek et al., 2007; Jaâfari et al., 2007). On the other hand, the essential oils are not cytotoxic against normal cells (Ait M'Barek et al., 2007), and usually non genotoxic (Bakkali et al., 2008). The anti cancer cytotoxic effect of *Artemisia herba-alba* essential oil is also demonstrated by the present study. These findings are confirmed by the results of other authors with the essential oil of *Artemisia annua* L. witch could induce apoptosis of cultured SMMC-7721 hepatocarcinoma cell line (Li et al., 2004), and with the essential oil of *Artemisia iwayomogi* (Cha et al.,2009). The results on cytotoxic assays against cancer using the human oral epidermoid carcinoma cell line, KB cells, strongly suggest that essential oil from *Artemisia iwayomogi* have cancer chemo-preventive and therapeutic potential. This activity is closely related to the ability of the essential oil to induce apoptosis and to activate the MAPK-mediated signaling pathways with the subsequent induction of a mitochondria- and caspase-dependent mechanism (Cha et al., 2009). The molecular mechanisms involved in the antitumor activity induced by essential oil of Moroccan *A. herba-alba* which showed a differential chemical composition needs to be investigated.

Conclusions

This paper presents an interesting analysis of the chemical composition of essential oil of *Artemisia herba-alba* from central eastern Morocco. It presents also for the first time a cytotoxic activity of the essential oil against a tumor cell line. The obtained results are now in development with the aim to understand the activities on other cell lines and the molecular mechanisms of these activities.

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