

## Chemical and microbiological characterization of the essential oil of *Artemisia mesatlantica* domesticated endemic species of Morocco

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**ABSTRACT:** *Artemisia mesatlantica* is an endemic species of Morocco, rare and endangered which is widely used in traditional medicine. This present work deals with the chemical composition and the antibacterial and antifungal efficacy of the EO of the domesticated *Artemisia mesatlantica*. The average yield of essential oil of the aerial part (stems, leaves and flowers) of this latter is 0.97%. Among the chemical constituents of the essential oil of *A. mesatlantica* are  $\beta$ -thujone (77.77%) which is predominant, followed by 1,8-cineol (6.31%), and camphor (3.52%) the other constituents are with small percentages. About the antimicrobial test, four bacterial strains (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*) and three fungal strains (*Aspergillus niger*, *Penicillium digitatum*, *Penicillium expansum*) were chosen for their pathogenicity and for their frequent involvement in food contamination. The bioassay shows that the minimum inhibitory concentration of the growth of the following microorganisms: *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus*, *Penicillium expansum*, *Penicillium digitatum* is 1/500 v/v whereas *Bacillus subtilis* and *Aspergillus niger* is 1/250 v / v.

**KEYWORDS:** *Artemisia mesatlantica*, yield, Essential oil, Antimicrobial test, Chemical composition, Morocco.

### 1 INTRODUCTION

*Artemisia mesatlantica* belongs to the genus *Compositae*, to the *Asteraceae* family. It was classified according to the IUCN (International Union for the Conservation of Nature in North Africa), as an endemic species of Morocco, rare and endangered [1]. It is located in High Atlas, Middle Atlas and Anti Atlas. The staging of *A. mesatlantica* occurs in the High Atlas on a poor, stony loamy soil. At an altitude of 1900 m, *Artemisia herba-alba* abounds with *A. mesatlantica* up to 2000 m altitude where *A. mesatlantica* forms a peppered stand of red juniper [2]. *A. mesatlantica* is widely used in traditional medicine. It is commonly used in traditional medicine in the care of diseases of the digestive and genital tract, dermatological problems and against cooling and diabetes [3].

Like the white sagebrush, and according to some rare works which have been done on the essential oil (EO) of *A. mesatlantica*, they show that the latter is composed of mostly oxygenated monoterpenes ( $\beta$ -thujone, camphor, camphene and  $\alpha$ -thujone) and flavonoids. Indeed, researches that have been done on the chemical composition of essential oils of *A. mesatlantica* have shown a variability of these volatile compounds including terpenoid (monoterpenes and sesquiterpenes) comprising terpene esters with terpinyl acetate, alcohols terpenics such as  $\alpha$ -terpineol. The especially oxygenated monoterpenes dominate this EO are  $\beta$ -thujone, camphene, myrcene, tricyclene and limonene. There are also oxides such as 1,8-cineole, sesquiterpene ketones including essentially piperitone and sesquiterpenes including  $\alpha$ -muroloene, and  $\delta$ -cadinene [4]. A similar study by sekkat et al. (2017) [3] also showed that the EO of *A. mesatlantica* is composed essentially of monoterpenes and sesquiterpenes, it is  $\beta$ -thujone, followed by camphor and  $\alpha$ -thujone.

In order to contribute to a better valorisation of this essence, this work deals with the chemical composition and the antibacterial and antifungal efficacy of the EO of the domesticated *Artemisia mesatlantica*.

## 2 MATERIALS AND METHODS

### 2.1 PLANT MATERIAL

Samples of the aerial part (stems, leaves and flowers) of *Artemisia mesatlantica* were collected from the forest nursery of Azrou (Regional Directorate of Waters and Forests and the fight against Desertification of the Middle Atlas - Meknes) in 2016 on plants taken at random and then dried for ten days in the shade before use.

### 2.2 EXTRACTION OF ESSENTIAL OIL

The extraction of the essential oil was carried out by hydrodistillation of ten samples in a Clevenger type apparatus (Clevenger, 1928) [5]. The distillation of 100 g of each sample cut into pieces of about 3 cm lasts three hours after the appearance of the first drop of distillate at the outlet of the condensation tube of the steam. The essential oil was stored at 4 ° C in the dark and dried with anhydrous sodium sulfate. The values of the yields are expressed in relation to the dry matter (in ml / 100 g of dry matter). The percentage of dry matter is estimated by drying 5 g of each sample for 4 hours in an oven at 102 ° C.

### 2.3 CHROMATOGRAPHIC ANALYSIS

For each sample, three chromatographic analyzes were performed. The chromatographic analyzes were carried out on a Hewlett-Packard-type electronic pressure-regulating gas chromatograph (HP 5890 series), equipped with a DB-5 fused silica capillary column 25 m in length, 0, 25 mm diameter and 0.25 µm film thickness, a flame ionization detector set at 260 ° C and powered by a mixture of H<sub>2</sub> gas / air and a split-splitless injector set to 240 ° C. The carrier gas is nitrogen at 1 ml / min. The injection mode is split (leak report 1/50, flow rate 66 ml / min). The temperature of the column is programmed at 50 ° to 250 ° C. at a rate of 4 ° C./min, and is then maintained at 250 ° C. for 20 minutes. The device is controlled by an "HP ChemStation" type computer system.

The identification of the constituents was performed based on their Kovats index and gas chromatography-mass spectrometry (GC / MS).

The latter is carried out on a Hewlett-Packard HP 5980 series gas chromatograph coupled to an HP 5772 series mass spectrometer. The fragmentation is performed by electronic impact under a field of 70 eV. The temperature of the column is as previously programmed from 50 to 250 ° C at a rate of 4 ° C/min. The carrier gas is helium at 2 ml / min. The injection mode is split (Leak report 1/70, flow rate 112 ml / min). The apparatus is connected to a computer system managing a library of NIST 98 mass spectra.

### 2.4 ANTIMICROBIAL TESTS

Seven microbial strains were chosen for their pathogenicity and for their frequent involvement in food contamination: four bacterial strains (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*) and three fungal strains (*Aspergillus niger*, *Penicillium digitatum*, *Penicillium expansum*). The bacterial strains are lots ATCC (American Type Culture Collection) maintained by subculture on nutrient agar favorable to their growth. The molds are grown on PDA (Potato Dextrose Agar). Antimicrobial tests are performed according to the method reported by Remmal and *al.* (1993) [6].

The essential oil is emulsified with a 0.2% agar solution in order to disperse the compounds and improve their contact with the germs tested, then diluted tenth in the agar solution. The final concentrations of essential oil are 1/100, 1/250, 1/500, 1/1000, 1/2000, 1/3000, and 1/5000 (v / v). Controls containing the culture medium and the 0.2% agar solution alone are also prepared.

The whole is striated using a platinum loop calibrated to collect the same volume of inoculum. The latter is in the form of a 24-hour culture broth for bacteria and a suspension in the physiological saline of spores from a seven-day culture in the PDA for molds. The Incubation takes place 24 h at 37 ° C for bacteria and seven days at 25 ° C for fungi. Each test is repeated three times.

## 3 RESULTS

### 3.1 YIELD AND CHEMICAL COMPOSITION

The average yield of essential oil of the aerial part (stems, leaves and flowers) of *Artemisia mesatlantica* is 0.97%. Eleven compounds were identified representing more than 99% of the total chemical composition of this oil (Table 1) The β-thujone

(77.77%) is the predominant compound. We note the presence in a lesser degree of 1,8-cineole (6.31%) and camphor (3.52%). The other compounds are present in very low quantities (Figure 1).

Table 1. Percentage of essential oil constituents of *Artemisia mesantlantica* domesticated

Constituents	Brute formula	Kovats index	%
Camphene	C <sub>10</sub> H <sub>16</sub>	943	1.44
1,8-cineol	C <sub>10</sub> H <sub>18</sub> O	1026	6.31
α- thuyone	C <sub>10</sub> H <sub>16</sub> O	1099	0.23
β- thuyone	C <sub>10</sub> H <sub>16</sub> O	1114	77.77
cis-β-dihydroterpineol	C <sub>10</sub> H <sub>20</sub> O	1136	2.94
camphor	C <sub>10</sub> H <sub>16</sub> O	1145	3.52
terpinen-4-ol	C <sub>10</sub> H <sub>18</sub> O	1172	2.10
davanone	C <sub>15</sub> H <sub>24</sub> O <sub>2</sub>	1587	0.13
humulene epoxide II	C <sub>15</sub> H <sub>24</sub> O	1608	2.68
10-Epi-γ-eudesmol	C <sub>15</sub> H <sub>26</sub> O	1623	0.09
α-eudesmol	C <sub>15</sub> H <sub>26</sub> O	1658	2.75
			99.96

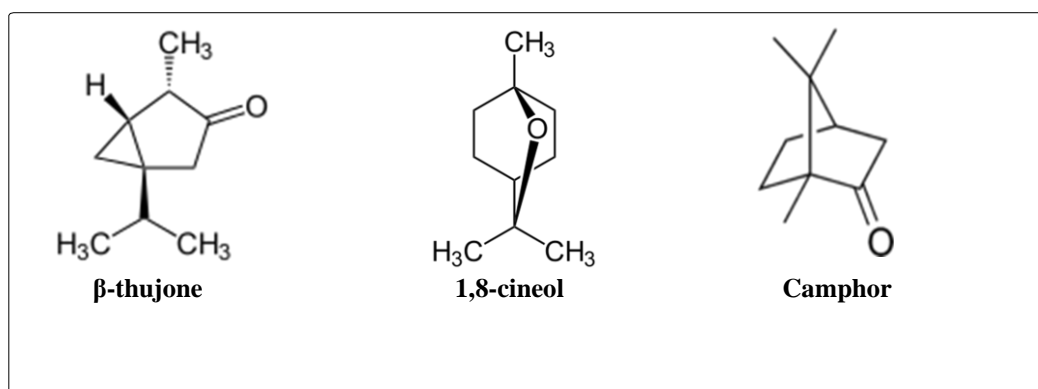


Fig. 1. Remarkable compounds of the essential oil of *Artemisia mesantlantica* domesticated

### 3.2 YIELD AND CHEMICAL COMPOSITION

The results differ according to the germs used (Table 2). *Bacillus subtilis* and *Aspergillus niger* are inhibited at 1/250 v / v while other strains have shown sensitivity to the action of EO of *Artemisia mesatlantica* domesticated at 1/500 v / v.

### 4 DISCUSSION AND CONCLUSION

The yield of EO obtained (0.97%) is comparatively high compared to that reported by Ghanmi et al., (2012) [4] ie 0.5% in the same species collected in March 2010 in the region of Ifrane. It can be considered as medium compared to some plants that are exploited industrially as a source of essential oils. However, it is higher than that of rose (0.1-0.35%), peppermint (0.5-1%), and neroli (0.5-1%), and lower than that of anise (1-3%), lavender (0.8-2.8%), rosemary (1-2.5%) and thyme (2-2.75%) [7].

Three chemical constituents of EO of *Artemisia mesatlantica* It is β-thujone (77.77%), followed by 1,8-cineol (6.31%), and camphor (3.52%), together with other constituents with small percentages: cis-β-dihydro-terpineol (2.94%), α-eudesmol (2.74%), Humulene epoxide II (2.68%), terpinen-4-ol (2.1%), and camphene (1.4%). We note the absence of chrysanthenone in the essential oil of *Artemisia mesatlantica* domesticated and this in agreement with the other works [8].

The chemical profile of the studied essential oil is relatively in agreement with what has been reported in the literature. Indeed, Ghanmi et al., (2012) [4] found that the majority constituents of the EO of this species taken from the Boulmane region are: β-thujone (56.33%) followed by camphene (7.48%) and camphor (4.17%). Similarly, Ouyahya et al. (1990) [2] revealed the presence

of a chemotype composed mainly of  $\beta$ -thujone and camphor (34% and 32%, respectively) in EO of *A. mesatlantica* from the Ifrane region. Another study by Holeman et al. (1991) [9] showed that the EO of *Artemisia mesatlantica* is dominated by  $\beta$ -thujone (60%). The EO of domesticated *Artemisia mesatlantica* may be a new source of  $\beta$ -thujone, a monoterpene ketone that is less toxic than  $\alpha$ -thujone.

The chemical composition of the domesticated *Artemisia mesatlantica* essential oil may be dependent on ecological and environmental factors and genetic factors, consistent with other work (Brada et al., 2007) [10].

**Table 2. Antifungal and antibacterial activities of the essential oil of *Artemisia mesatlantica* domesticated**

Concentration v/v	1/100	1/250	1/500	1/1000	1/2000	1/3000	1/5000	Control
<b>Bacteria</b>								
<i>Escherichia coli</i>	-	-	-	+	+	+	+	+
<i>Bacillus subtilis</i>	-	-	+	+	+	+	+	+
<i>Staphylococcus aureus</i>	-	-	-	+	+	+	+	+
<i>Micrococcus luteus</i>	-	-	-	+	+	+	+	+
<b>Fungi</b>								
<i>Aspergillus niger</i>	-	-	+	+	+	+	+	+
<i>Penicillium expansum</i>	-	-	-	+	+	+	+	+
<i>Penicillium digitatum</i>	-	-	-	+	+	+	+	+

The bioassay shows that the minimum inhibitory concentration of the growth of the following microorganisms: *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus*, *Penicillium expansum*, *Penicillium digitatum* is 1/500 v / v whereas *Bacillus subtilis* and *Aspergillus niger* is 1/250 v / v. This result is relatively consistent with the work of Satrani et al (2016) [11] on the antimicrobial activity of EO from the same species collected in the Boulmane region but with a higher antibacterial activity threshold (between 1/1000 and 1/3000 v/v). In addition, Lopes-Lutz et al. (2008) [12] showed that *E. coli* was less sensitive to essential oils extracted from seven wild species of the genus *Artemisia* harvested in the western region of Canada. In another study, C. Bouchra et al (2003) [13] showed that the antifungal activity of the essential oil of *A. herba-alba*, showed only low antifungal activity against *Penicillium digitatum*, ie a concentration of 250  $\mu$ g / ml.

The antimicrobial activity of EO of domesticated *Artemisia mesatlantica* can be explained by its chemical profile rich in oxygenated monoterpenes (thujone, camphor, and 1,8-cineole) characterized by the presence of the oxygen function responsible for increasing the antimicrobial properties of terpenoids [14], [15]. The antimicrobial activity of EO of this species could also be due to the synergistic interactions between the different constituents of EO.

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