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Effect of phenological stages on yield, chemical composition and bioactiviy of *Artemisia mesatlantica* essential oil of Morocco

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ABSTRACT

Artemisia mesatlantica is an aromatic plant, spontaneous and widespread in Morocco. It is used by the local populations for its medicinal virtues. The purpose of this present work is the comparative study of the yield according to the date of harvest. Additionally, we sought to determine the chemical composition of the essential oil stemming from this species, as well as the determination of its antimicrobial activities towards eleven pathogens. Our samples result from a rural area in eastern Morocco, namely a region between Ifrane and Boulmane. Seventy eight constituents were identified by chromatographic analysis (GC and GC/SM) in essential oil of this plant among which four are preponderant: β–thujone, 1,8 cineole, camphene and camphor. The best yield in essential oil of the Artemisia mesatlantica is in June and it is in the order of 1 % (mg/100g). The essential oil of Artemisia mesatlantica showed a significant antimicrobial activity against four species of bacteria, three species of fungus and all four tested fungi that cause wood decay. To the best of our knowledge, this work is the first of its kind.

KEYWORDS

Artemisia mesatlantica;
Essential oil;
Wood decay fungi;
Phenological stages.

INTRODUCTION

The sector of Medicinal and Aromatic Plants (MAP) is an important business in Morocco. The country exports the equivalent of 250 million dirhams in MAP to the United States and the European Union[1]. Essential oils exportations only are estimate to 165 million dirhams and this estimate can potentially double[1]. Wormwood, which dominate the landscape of medicinal and aromatic regions of eastern Morocco, is among the species that are the subject of significant commercial transactions[1].

The genus Artemisia includes some 400 species spread over five continents[2]. In Morocco, it is represented by twelve species[3]. Artemisia mesatlantica (generally called wormwood, sagebrush or in arabic Chih) belongs to the Genus Compositae, Family Asteraceae. It was classified, according to IUCN (International Union for Conserva-

tion of Nature in North Africa), as an endemic species to Morocco, rare and endangered[4]. It is situated in the High Atlas, Middle Atlas and Anti Atlas (1400-3000m). The staging of A. mesatlantica appears in the High Atlas on a loamy soil poor and stony; at an altitude of 1900m, A. herba-alba is abundant with A. mesatlantica up to 2000m where A. mesatlantica form a settlement dotted with red juniper[5].

Wormwood species grows naturally in Morocco and is described as a plant of 20-40cm, with numerous stems, covered with short intertwined leaves. The inflorescences are erect and very dense[6]. The flowers are grouped in small erect stems; flower heads are covered with a woolly pubescence and whitish. Each plant can produce between 20,000 and 70,000 seeds, which germinate in the spring from March to April[7].

The species of wormwood is proven effective in combating obesity, hyperglycemia, hypertriglyceridemia, hypercholesterolemia and oxidative stress in particular[8]. In 1990, the scientific community in WHO finally recognized that wormwood extract is as good as chloroqine for the treatment of malaria[9,10]. It is also used to treat urinary tract infections.

We have already established in a previous study that A. mesatlantica essential oil (EO) has antimicrobial properties[11]. To the best of our knowledge, there is no previous report on the seasonal variations of the essential oil analysis and antibacterial activity of A.mesatlantica. Thus, in this paper, it was considered appropriate to study the effect of age of the plant and harvest date on the content and chemical composition of this species. The composition and antimicrobial activity of the essential oils of this plant at different stages of its development are also reported.

MATERIALS

Plant material

Samples of aerial parts (stems, leaves and flowers) of Artemisia mesatlantica were collected in March, June and September 2010 in a region between Ifrane and Boulmane Morocco. The species was identified by Dr. A. AAFI, a botanist at the Forest Research Centre, Rabat, Morocco.

Microorganisms studied

The antimicrobial activity of the A. mesatlantica oils was evaluated on eleven isolated strains. The microorganisms used were as follows:

- **Bacteria**: Escherichia coli, Bacillus subtilis, Staphylococcus aureus and Micrococcus luteus.
- **Fungi**: Aspergillus Niger, Penicillium digitatum and Penicillium expansum.
- **Wood decay fungi**: Gloeophyllum trabeum, Poria placenta, Coriolus versicolor and Coniophora puteana.

The four pathogenic bacteria are chosen for their high antibiotic resistance and toxicity in humans. They are frequently encountered in many infections in Morocco and pose a clinical and therapeutic problem. The three selected fungi are agents of decay in common food and fruits and can be toxic and pathogenic for humans and animals. The four wood decay fungi used in this work are the most important wood-destroying fungi in buildings, wood in contact with the soil (poles and railways) or buildings (bridges). They were chosen for the considerable damage they cause to wood and related products[12,13]. Bacterial strains are lots of "American Type Culture Collection" ATCC, they are deposited by subculture on nutrient agar for 24 h in the dark at 37 ˚C. Mold and wood decay fungi belong to the collection of Mycothèque of Microbiology Laboratory in Forestry Research Centre, Rabat, Morocco. They are regularly maintained by subculture on nutrient medium PDA (Potato Dextrose Agar) for seven days in the dark at 25 ˚C.

The technique used is the dispersion of EO in the 0.2% agar solution. The minimum inhibitory concentrations (MIC) of the EO of Artemisia are determined using the method reported by Remmal et al. and Satrani et al.[14,15].

METHODS

Extraction of essential oils

The extraction of essential oils was performed by hydrodistillation in a Clevenger type apparatus[16]. Three distillations were carried out by boiling 200g of fresh plant material with 1 liter of water

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in a 2 l flask for two hours surmounted by a column of 60cm in length connected to a condenser. The essential oil yield is determined from a dry matter, estimated from three samples of 30g dried to constant weight for 48 to 60 hours in an oven at 60 °C.

The EO was stored at 4 °C in the dark in the presence of anhydrous sodium sulphate. Then it was diluted in methanol (1/20 v/v) prior to analysis by GC and GC/MS according to AFNOR standard[17].

Chromatographic analysis

Chromatographic analysis of the Artemisia mesatlantica EO was performed on a gas chromatograph with electronic pressure control, type Hewlett Packard (HP series 6890) equipped with a capillary column HP-5 (5% diphenyl, 95 % dimethylpolysiloxane) (30 m x 0.25 mm) with a film thickness of 0.25 µm, with an FID detector set at 250 °C and fed by a gas mixture and a H2/ Air split-splitless injector set at 250 °C. The volume injected is 1 µl. The injection mode was split (split ratio: 1/50 flow: 66 ml/min). The gas used is nitrogen with a flow rate of 1.7 ml/min. The column temperature is programmed to increase from 50 to 200°C at a rate of 4 °C/min and held for 5 minutes at the final temperature. The detection limit is less than 1ppm. The device is controlled by a computer system type "HP ChemStation", managing the operation of the device and monitoring the changes in chromatographic analysis.

Identification of components was performed based on their Kovats indices (KI)[18] and on gas chromatography coupled with mass spectrometry electron impact (GC-SMIE)[19]. The latter is performed on a gas chromatograph, type Hewlett-Packard (HP series 6890) coupled with a mass spectrometer (HP 5973 series). Fragmentation is performed by electron impact at 70 eV. The column used was a HP-5MS capillary column (30m x 0.25mm); the film thickness is 0.25 µm. The column temperature is programmed from 50 to 200 °C at 4 °C/min. The carrier gas is helium with a flow rate set at 1.5 ml/min. The injection method is the split mode (split ratio: 1/70). The device is connected to a computer system that manages a library of mass spectra NIST 98. Indeed, the index system is based on a notion of relative retention. It compares the retention of any product to that of a linear alkane. This system is applicable in gas chromatography to any compound on any column. By definition, it assigns an index of 800 in the linear alkane C8 (n-octane), 1000 to C10 linear alkane (n-decane), and this, whatever the stationary phase, the length of the column, the temperature or flow rate.

Kovat indices are determined by injecting a mixture of C9 to C24 alkanes in the same operating conditions[18]. In general, the technique of IK is widely used to identify compounds typical of EO, but it is insufficient to determine the total chemical composition. IK tables specific to each product are proposed in the literature. They were developed using analysis of different types of columns. These indices are compared with those calculated from our samples.

Antimicrobial assays

Minimum inhibitory concentrations (MIC) of essential oils were determined according to the method reported by Remmal & al. and Satrani & al.[14,15]. Due to the immiscibility of essential oils in water and therefore in the medium culture, the emulsification is carried out with a solution of 0.2% agar to promote contact germ/compound. Dilutions are prepared to 1/10th, 1/25th, 1/50th, 1/100th, 1/200th, 1/300th and 1/500th in the agar solution. Each test tube contains 9 ml of agar medium in the malt extract (2%), autoclaved (20 min at 121°C) and cooled to 45 °C. To that, we added 1 ml of each dilution to obtain final concentrations of 1/100, 1/250, 1/500, 1/1.000, 1/2.000, 1/3.000 1/5.000 (v/v), followed by stirring the tubes properly before pouring them into Petri dishes. Negative controls, containing the culture medium and the agar solution at 0.2% only, were also prepared.

For bacteria and mold, inoculation is done by streaking with a platinum loop calibrated to collect the same volume of inoculum. The latter is a culture broth of 24 hours for bacteria and a suspension in physiological saline of spores from a culture of seven days in the PDA.

For wood-decay fungi, inoculation is done by depositing fragments of 1 cm2 in diameter, taken from the periphery of a mycelium cultured for 7 days in PDA. Incubation is done in the dark for

24 h at 37°C for bacteria and for seven days at 25°C for mold and wood decay fungi. Each test was repeated three times. The MIC is determined as the lowest concentration of oil able to inhibit the visible growth of each micro organism on the agar plate.

RESULTS AND DISCUSSION

The yield and chemical composition

The yield

Average yields of essential oils were calculated based on the dry plant material from the aerial part of the plant.

The essential oil yield (w/w %) of Artemisia mesatlantica change according to phenological stages. The samples provided a rate of 0.5% in March (Pre-flowering stage), reaching a maximum of 1% in June (Flowering stage) and then back to 0.69% in September (Post-flowering stage). The low value of yield in September can be attributed to the senescence of leaves collected for the period after flowering.

The best acceptable yield obtained of Artemisia mesatlantica essential oil (EO) was in June during the flowering period, which can be profitable for industrial production. Indeed, wormwood blooms from June to September which is the harvest period. Vegetative growth occurs in the fall (large leaves) and then at the end of winter and spring (smaller leaves). Seasonal dimorphism of foliage allows it to reduce the sweaty surface and avoid water loss[20]. This might explain the high yield obtained in June.

The June performance can be considered average in comparison to some plants that are used industrially as a source of essential oils: higher than that of peppermint (0.5-1%), rose (0.1 to 0, 35%) and neroli (0.5-1%) and lower than that of anise (1-3%), lavender (0.8 to 2.8%), rosemary (1 -2.5%) and thyme (2 to 2.75%)[21].

This result is similar to previous work of wormwood (Artemisia herba alba) of our team where its essential oil yield was better in June with 1.23% compared to those obtained during the months of April and September with 0.86% and 0.56% respectively[22].

In a study conducted in Italy, the yield of Artemisia verlotiorum recorded a maximum in April (0.6%) and a minimum of 0.1% in the month of January[23]. The yield of EO obtained by steam distillation of three provenances of A. frigida (Kazakhstan, Russia and Mongolia) varies between 0.1% and 0.7%[24]. For Kazakhstan, the yield of different parts of the plant was also determined: Flowers (0.6%), leaves (0.1%), stems (0.001%) and roots (0.1%).

This difference in yield between the wormwoods depends on many factors: phenological stage, provenance, soil type and climate, the part of the plant distilled, the extraction technique, etc…[22].

Chemical composition

The volatile components percentages of the EO analyzed by GC and GC/MS of A. mesatlantica collected at different phenological stages in the Boulmane Ifrane region of Morocco are reported in Table I. Sixty volatile compounds were found in March against forty four components in June and seventy-two in September. These components account for approximately 99.77%, 99.93% and 99.03% of total EO of harvested wormwood during the month of March, June and September respectively.

A comparison among the composition of the essential oils revealed both quantitative and qualitative differences. However, the overall chemical profile of essential oils is not altered during the different growing seasons. The most abundant component in all three species is βthujone.

We can distinguish three chemotypes depending on the harvesting season:

- (β thujone, camphene, camphor) in the pre-flowering stage on March.
- (β thujone, 1, 8 cineole, camphene) in the volatile of flowering stage on June.
- (β thujone, cubitene, camphene) in the oil obtained from post-flowering stage on September.

In another study, it is striking to know that the steam distillation affected the composition of A. mesatlantica, and revealed a chemotype of ß-thujone and camphor predominantly (34 and 32%, respectively)[3]. This suggests that the chemical polymorphism, virtually inexistent in endemic plants, begins to manifest with the species having a larger area[25].

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TABLE 1 : Effect of phenological stage on chemical composition of A rtemisia mesatlantica essential oil from Ifrane and Boulmane in Morocco

NO.	IK Biblio graphie	IK	Constituant	March	June	Sept
1	926	922	Tricyclene	0,09	0,19	0,37
2	931	928	α –thujene	0,83	0,84	0,40
3	939	943	α –Pinene	0,87	0,64	0,28
4	967	962	Verbenene	--	--	0,72
5	953	968	Camphene	7,48	6,34	3,25
6	976	972	Sabinene	0,25	0,36	0,35
7	980	975	β –pinene	0,08	--	--
8	991	986	Myrcene	0,19	--	--
9	1011	1016	Δ -3 -carene	0,22	0,47	0,35
10	1018	1024	α –terpinene	0,47	0,34	0,54
11	1031	1027	Limonene	0,15	0,14	0,11
12	1033	1031	1,8 -cineole	2,63	6,96	2,85
13	1062	1055	artemisia cetone	0,50	0,85	0,83
14	1062	1064	δ -terpinene	0,88	1,43	0,81
15	1068	1072	Cis-hydrate de sabinene	--	--	0,07
16	1083	1083	Artemisia Alcool	0,08	0,17	0,14
17	1095	1095	α -oxide de pinene	0,52	1,02	0,67
18	1102	1101	α –thujone	1,38	1,53	1,38
19	1109	1109	6- camphenol	--	--	0,71
20	1114	1114	β –thujone	56,33	57,95	41,87
21	1119	1118	Trans-pinan-2-ol	0,10	0,19	0,26
22	1123	1122	Chrysanthenone	--	--	0,09
23	1133	1130	iso -3 –thujanol	0,24	0,59	0,15
24	1136	1135	Cis -β -dihydro-terpineol	1,93	2,32	1,31
25	1143	1140	Camphre	4,17	3,75	1,26
26	1156	1152	β -oxyde de pinene	--	--	0,14
27	1158	1157	Trans -β -dihydro- terpineol	0,30	--	0,17
28	1163	1161	Trans -β –Terpineol	0,63	0,87	0,92
29	1177	1173	Terpin -4 –ol	0,80	2,02	1,91
30	1181	1181	Thuj -3 –en- 10-al	0,09	0,16	0,23
31	1189	1187	α –terpineol	0,26	0,50	0,24
32	1193	1193	Cis –piperitol	0,61	0,70	0,13
33	1204	1204	Verbenone	0,16	0,15	0,17
34	1205	1208	Trans –piperitol	--	--	0,84
35	1229	1224	Nordavanone	0,30	0,30	0,59
36	1235	1236	Trans-acetate de chrysanthenyl	--	--	0,20
37	1258	1256	Trans myrtanol	0,77	--	--
38	1287	1287	δ –terpinene-7- al	0,23	--	0,17
39	1315	1314	Trans-dihydro-α-acetate de terpinyl	--	--	2,50
40	1350	1350	α –acetate de terpinyl	0,17	0,16	0,18
41	1373	1368	longicyclene	0,11	--	--
42	1387	1372	Iso-longifolene	0,26	--	--
43	1398	1406	β –longipinene	0,12	--	1,90
44	1418	1412	E-Caryophyllene	0,61	0,14	0,83
45	1467	1465	9 –épi- E -caryophyllene	0,44	0,23	0,24
46	1477	1469	γ muurolene	--	--	0,32
47	1480	1481	D -germacrene	2,29	1,22	1,09
48	1499	1497	α -muurolene	0,66	0,39	2,00
49	1503	1502	A -germacrene	0,27	--	0,15
50	1514	1514	Cubebol	0,30	--	--
51	1524	1522	Δ- cadinene	0,17	--	0,17
52	1549	1551	Elemol	0,16	--	0,25
53	1556	1556	Germacrene B	--	--	0,20
54	1566	1566	Longipinanol	--	--	0,14
55	1581	1573	Caryophyllene oxyde	0,89	0,57	0,58
56	1586	1580	Davanone	1,23	0,62	1,01
57	1595	1587	Cis alcool artenuuique	0,31	--	--
58	1594	1594	Cis –β- elemenone	--	--	3,13
59	1600	1599	Trans -β – elemenone	0,51	0,35	0,55
60	1606	1603	Epoxide II d' Humulene	0,92	0,65	0,72
61	1614	1612	1,10 – di- epi- cubenol	0,46	0,43	0,46
62	1618	1619	Trans-iso-longifolanone	--	--	0,24
63	1627	1625	1- epi- cubenol	2,16	1,48	1,54
64	1637	1631	3 – iso – thujopsanone	0,18	0,38	0,27
65	1640	1636	Epi –α- cadinol	0,19	--	0,69
66	1649	1648	β –Eudesmol	0,30	0,14	0,13
67	1653	1655	α –cadinol	0,71	0,96	0,79
68	1664	1664	14-hydroxy-9-epi-E-caryophyllene	--	--	0,17
69	1674	1676	cadalene	0,56	0,63	0,26
70	1693	1684	Germacrone	0,30	--	2,58
71	1717	1713	β –davanone-2-ol	--	--	1,07
72	1726	1723	Iso-longifolol	0,13	0,16	0,18
73	1741	1740	8- α – 11- elemodiol	1,39	0,43	0,45
74	1764	1769	14-oxy- α-muurolene	0,31	0,21	1,81
75	1799	1794	14-hydroxy – Δ –cadinene	--	--	0,25
76	1814	1816	Iso-acetate de longifolol	0,12	--	0,40
77	1872	1874	cubitene	--	--	3,58
78	1900	1880	n-nonadecane	--	--	0,60
79	1959	1958	Neocembrene	--	--	3,12
			Total	99,77%,	99,93%	99,03%

IK : Kovats Indices

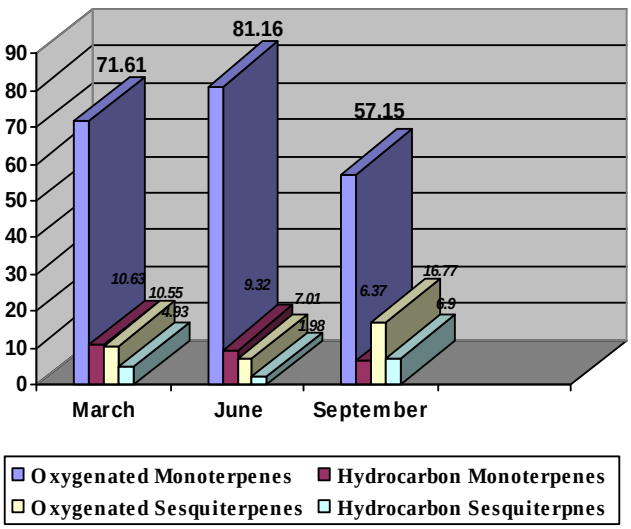

Figure 1 : Distribution of oxygenated monoterpenes, hydrocarbon monoterpenes, sesquiterpenes and oxygenated hydrocarbon sesquiterpenes in the essential oil of *Artemisia mesatlantica* of Morocco

The combination of volatile compounds of this species of wormwood is variable in terms of diversity and concentration of compounds. Classification of components identified based on functional groups is shown in Figure 1.

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Asteraceae family is widespread with a composition of monoterpenoids, especially oxygenated[26]. The GC and GC–MS analysis of Artemisia mesatlantica showed that the distribution of oxygenated monoterpenes of A. mesatlantica is dominant in all three seasons but remarkably different in all three developmental stages. It is relatively high in March (71.61%), peaked in June (81.16%) and decreased in September (57.15%). Therefore, this result shows that oxygenated monoterpenes are present in large quantities during the Flowering stage. June seems the month in which the major components are dominant. ß-thujone is the main constituent (57.95%) followed by 1.8 cineole (6.96%). They are accompanied by other minor components which are just as important; Terpin-4-ol (2.02%) and α thujone (1.53%), whereas camphor is high in March (4,17 %).

The hydrocarbon monoterpenes are distributed in March and June in larger quantities (10.63%, 9.32%, respectively), whereas in September, they decrease to 6.37%. These hydrocarbon monoterpenes are characterized mainly by camphene with 7.48% and 6.34% respectively in March and June followed by 3.25% in September. Camphor is high in March (4.17%) and decreases in September to 1.26%. Camphene and camphor follow the same biosynthesis path which gradually decreases according to the developmental stages.

The plant in September seems to be richer in sesquiterpenes (23.67%) against 15.48% in March and 8.99% in June. The sesquiterpene hydrocarbons are less abundant than the oxygenated sesquiterpenes. Among the most abundant hydrocarbon sesquiterpenes, D-germacrene (2.9%) in March and α-muurolene (2%) in September that grow opposite according to phenological stages. There are other specific components of September and relatively interesting: trans-dihydro-α terpinyl acetate (2.5%), Cis-β-elemenone (3.13%), Germacrone (2.58%) and benzene compounds cubitene (3.58%) and neocembrene (3.12%). This composition can be used for chemotaxonomic purposes to differentiate between collections and to distinguish an essential oil quality.

Note that the chrysanthenone, a major constituent in most wormwood, is inexistent in the mesatlantica. The presence or absence of this component can also be used as a taxonomic marker to characterize our species[22].

Phenological behavior

The typical annual cycle of wormwood follows a standard phenological model that can be described as follows[27]:

- A period of autumnal bud;
- A period of winter dormancy;
- An active growth in spring;
- The appearance of flower buds in May;
- An optimum fruiting in December;
- A maximum spread in mid-February.

Furthermore, the chemical variability observed may be related with the climate fluctuations during the year[28,29]. It appears that the heavy precipitations and low temperatures favor the biosynthesis of sesquiterpenes and derivatives while high temperatures and low rainfall appear to be favorable for the biosynthesis of oxygenated terpenes and their derivatives[30]. High temperatures could limit photosynthesis in the plant and change absorption of nutrients from soil that would sway organic matter, sugar and aminoacids production. In this situation, plant feels stress and with reduction in activity of primary metabolites cycles, it activates secondary metabolites (essence) to resist against stress which increases essence as a result[29,31].

The correlation between the chemotypes and the weather is not limited to our species but is of a more general case. Indeed, similar correlations were found in the wormwood and in other genera belonging to other families such as Satureja, Euphorbia and Valeriana[30].

Therefore, all these results show the diversity of the chemical composition that vary with environmental parameters (phenological stage of the plant, biotic and abiotic stress, etc..) which direct the biosynthesis towards the preferential formation of specific compounds.

Antimicrobial activity of essential oils

The results of the antibacterial and antifungal activity of Artemisia mesatlantica essential oils are summarized in Table 2 below:

The results of bioassays on crude oils of the three vegetative stages of Artemisia mesatlantica

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TABLE 2 : Antimicrobial activity of *Artemisia mesatlantica* essential oil of Morocco collected in March, June and September from Ifrane Boulmane region

Concentration v/v	1/100			1/250			1/500			1/1000			1/2000			1/3000			1/5000			Témoin		
Microorganism	**M**	**J**	**S**	**M**	**J**	**S**	**M**	**J**	**S**	**M**	**J**	**S**	**M**	**J**	**S**	**M**	**J**	**S**	**M**	**J**	**S**	**M**	**J**	**S**
Bacteria																								
E. coli	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
B. subtilis	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+
M. luteus	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S. aureus	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Mold																								
A. niger	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P. expansum	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P. digitatum	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Wood decay fungi																								
C. versicolor	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C. puteana	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
G. trabeum	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P. placenta	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

study showed that these oils are toxic and lead to death of bacteria, mold and wood decay fungi. We notice that the wood decay fungi are more vulnerable to A. mesatlantica EO (1/250v/v to 1/1000 v/v) than mold (1/100 and 1/500 v/v), but they are less sensitive than bacteria (1/1000 v/v and 1/3000 v/v).

The essential oil of the preflowering stage in March is the most active with a pronounced sensitivity against bacteria. Thus, the concentration of 1/3000 v/v was sufficient to stop the growth of Bacillus subtilis against 1/2000 v/v for both June and September collects. Bacillus subtilis is the most sensitive, followed by Staphylococcus aureus and Escherichia coli and at last, Micrococcus luteus The EO showed a more pronounced effect against bacteria Gram (+). The resistance of bacteria Gram (-) is attributed to the low solubility of thujones and 1,8-cineole and their ability to form hydrogen bonds, which limits their entry into Gram (–) that have inoperable channels in the external hydrophobic membrane[32,33,34].

Our results are comparable to those of Amarti who obtained a complete inhibition of bacterial strains at a concentration between 1/2000 v/v and 1/3000 v/v with the essential oil of Thyme[35,36]. Thymus capitatus contains carvacrol (70.92%) as major constituent and Thymus zygis is composed of thymol (33.02%), o-cymene (32.02%) and β-E-ocimene (11.90%). The EO of the mountain wormwood has shown to be even more active on Bacillus subtilis than Thymus bleicherianus that's dominated by the α-terpinene (42.20%) and thymol (23.90%) and inhibited all bacteria studied at a concentration of 1/2000v/v. Generally, phenolic compounds such as thymol, carvacrol and geraniol, are identified as potent inhibitors of bacterial growth (at a concentration between 1/1000v/v and 1/3000v/v) depending on the concentration of inoculum[37]. In our case, it seems that the β thujone of A. mesatlantica EO exerted a major similar bactericidal effect.

Indeed, the stereochemistry influences the antibacterial activity. It was observed that ß-isomers are relatively more active isomers than α isomers [38]. The β thujone present several interesting biological activities with insecticidal, anthelmintic and anti-fungal properties[22,39]. Ketones, in general, are physiologically highly active compounds and their use should be well controlled with fear to become quickly toxic. The thujones, specifically, have demonstrated a lytic action against mucus and lipids, an anticoagulant action and finally a healing action. They are, specifically, abortive for pregnant women, neurotoxic, narcotic, and strongly immuno-stimulant[39].

The presence of oxygen in the structure of EO increases the bacteriostatic and fungistatic effect of terpenoids. Indeed, the high content of oxygenated monoterpenes (thujones, camphene, camphor

and 1,8-cineole) in the essential oil of Artemisia mesatlantica is responsible for its pronounced activity against Staphylococcus aureus and its high activity against Bacillus subtilis. It has been demonstrated that Staphylococcus aureus is the most affected by the monoterpene ketones such as thujones only[38,40].

The variation in percentages of the other major components such as 1,8 cineole and camphor may also be responsible for the differentiation of the antimicrobial activity observed. It has already been established that the essential oil of wormwood, rich in camphor and 1,8 cineole, exhibits antimicrobial activity in vitro[41]. The eucalyptol (1,8cineole) has an antibacterial, anti-inflammatory, antiviral, expectorant, sedative, herbicide and insect repellent properties. Camphor has been demonstrated as an inhibitor of bacteria and fungi[42]. The percentage of camphor in March (4.17%) is higher than other collections, which could potentially be a reason for this hypersensitivity.

The EO of A. mesatlantica shows fungitoxic activity against all tested fungi. Penicillium expansum was the most sensitive to EO of June and September (1/500v/v). On the other hand, Penicillium digitatum is the most resistant pathogen studied, with the inhibitory concentration of 1/100 v/v. Our EO is as active as wormwood of Guercif Artemisia herba alba that inhibited the same mold species at a concentration between 1/250 and 1/500v/v[22]. The richness of the mountain wormwood EO in β thujone procures an interesting antifungal activity[22,39]. The antifungal effect was shown by Tantawi-Elaraki with the essential oil of Artemisia where many of its constituents were toxic to pathogens. The EO of wormwood inhibits all three stages of asexual fungi: spore germination, sporulation and mycelial development[43]. For wood decay fungi, they are more vulnerable to the essence of mountain wormwood than mold. Concentration of 1/1000 v/v in March was sufficient to stop the growth of Gloeophyllum trabeum. It was the most sensitive, followed by Coriolus versicolor and Poria placenta. Coniophora puteana proved the most resistant at a concentration of essential oil 1/250 v/v.

In a study done by El ajjouri et al.[44], it was Coriolus versicolor that showed resistance to phenolic compounds of thyme essential oil compared to other wood-destroying fungi. This was attributed to the fact that this fungus produces laccase and extracellular enzymes that catalyze the oxidation of phenolic compounds and leads to their inactivation[45]. The EO of the mountain wormwood is more powerful than that of Eastern Red Cedar (Juniperus phoenicea) rich in terpene hydrocarbons, such as α-pinene and δ-3-carene. It inhibited the growth of wood decay fungi at a concentration of 1/250v/v to 1/500v/v[46]. This has been proven by Channaoui and Elaraki which showed that the antimicrobial activity of some terpene compounds is in the following increasing order: aldehydes> alcohols> ketones> hydrocarbons[47].

In a study of EO antimicrobial activity of several species of wormwoods, tunisian researchers have determined that the type of essential oil characterized by a codominance of four main components (1,8-cineole, camphor, α and β-thujone) was the most active against yeast and bacterial germs than the EO with dominance of thujone only[48]. Similarly, a study of the A. sieberi EO suggests that its high antifungal activity is associated with the presence of terpenoids (α and β-thujone, camphor and 1.8-cineole) as main constituents. The antifungal mechanism of action is attributed to its potential to induce a state of oxidative stress through a cascade of free radicals generated by the function which endoperoxide alkyl proteins and causes mitochondrial membrane depolarization[49].

Therefore, it is difficult to attribute the activity of a complex mixture to a particular constituent. The major components or even those in trace such as cis β dihydroterpineol may have a synergistic or antagonistic effect on biological activity.

In conclusion, the virtues of essential oils differ depending on the harvest period, altitude and sun exposure. Thus, the same type of plant does not give an essential oil with the same properties [50,51,52].

CONCLUSION

The study of a plant as a source of aromatic and flavoring compounds requires the analysis of not only the whole plant but also its composition at different developmental stages. Chemical characterization and antibacterial screening studies on the plant-based essential oils could also lead to

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the discovery of new natural antimicrobial agents.

The present work is the first of its kind that's devoted to determining the yield and chemical composition of mountain wormwood according to harvest date and the variation of antibacterial and antifungal properties of essential oil extracted from the leaves of Artemisia mesatlantica harvested in the region of Ifrane Boulmane (Morocco), depending on a phenological stage. The best activity of EO occurred with the harvest of March (up to 1/3000 v/v) but the best yield was obtained in the month of June (1%). The oils showed promising antibacterial activity against resistant bacteria (B.subtilis, E.coli and S.aureus, pathogenic mold especially P.expansum and wood decay fungi mainly G.trabeum.

Chemical analysis by GC and GC / MS in June, has identified approximately 99.93% of total volatiles in this species. ß-thujone is the major constituent of the forty four characterized followed by 1,8 cineole and camphene. The results obtained in this study show that the essential oil of leaves of A.. mesatlantica showed a very significant inhibitory activity in vitro on the eleven microbial species. The bactericidal and fungicidal property of the essential oil is mainly due to its high content of oxygenated monoterpenes (thujone, 1,8-cineole and camphor). These antibacterial and antifungal performances highlighted deserve to be studied more vigorously in order to consider the prospects of application of this species in the pharmaceutical field. Also, our in vitro results with wood-rot fungi must be supplemented by detailed studies for possible use of these essential oils as a method for preserving wood. Further studies of development of EO as an alternative to synthetic fungicides, are recommended to evaluate the efficacy and toxicity of its long-term application on wood.

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