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Antifungal activity of *Artemisia mesatlantica* essential oil of Morocco against wood rot fungi

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ABSTRACT

The purpose of the present work is to study the yield of the essential oil of an aromatic and endemic species Artemisia mesatlantica. Additionally, we sought to determine the chemical composition of the essential oil produced by this species, as well as the determination of its antifungal activity against four microorganisms. Our samples are collected from a rural and mountainous area located in Middle Atlas of eastern Morocco (between Ifrane and Boulmane cities). Forty four constituents were identified by chromatographic analysis (GC and GC/MS) in the essential oil of this species, among which four are preponderant: β–thujone, 1,8 cineole, camphene and camphor. The yield of the essential oil of Artemisia mesatlantica is 1% (mg/100g). The essential oil of Artemisia mesatlantica showed a significant antifungal activity against all four wood rot fungi. To our knowledge, this work is the first of its kind.

KEYWORDS

Artemisia mesatlantica;
Endemic;
Essential oil;
Chemical composition;
Wood rot fungi.

INTRODUCTION

Wood has many qualities; it is a renewable natural resource and it has both technical (mechanical strength, thermal materials…) and economical (cheap) properties. It is therefore suitable for many uses; in construction, for garden furniture, fencing and barriers (building, houses...). But, because of its biological nature, it is sensitive to environmental factors and biodegradation agents. It is more exposed to decay agents, some xylophages and last but not least, wood decay fungi[1].

Indeed, the potential risks to human health caused by the growth of this fungal decay in wooden residential and non residential structures have been a major concern for homeowners, contractors and insurance companies. The lawsuit claiming health problems caused by exposure to indoor mold exceeded 2.8 billion U.S. dollars in 2002[2].

The conventional chemical fungicides used to control the growth of mold on wood such as creosote, pentachlorophenol (PCP) and polycyclic aromatic bi-or hydrocarbons (PAH), are inappropriate for internal use and are considered harmful to the environment and the economy. These products have been subject to regulation by the directive "biocide", which is why they are or will be restricted or prohibited[3]. In fact, natural alternatives that are friendly and show a low toxicity to humans are desirable. In this context, new methods emerged to improve the durability and / or physicochemical properties of wood to meet the current requirements of quality and safety[4].

A first line of research is to better understand the behavior of some tropical essential oils that present a very good and natural durability (resistance to termites and fungi), and to encourage their use in place of chemical treatments[4].

This has led us to choose the essence of Artemisia mesatlantica for study. It belongs to Genera Compositae, to the daisy Family Asteraceae. It is distributed in the High Atlas, Middle Atlas and Anti Atlas of Morocco between 1900m and 2500m.

A. mesatlantica appears in the High Atlas on a loamy soil, poor and stony[5]. It has several properties used in traditional medicine: tonic, antipyretic, anti- spasmodic, anthelmintic and stomach relief remedy. It is also used to treat urinary tract infections and for post partum care. It has a bitter-sweet taste[6].

We have already demonstrated in a previous study that the essential oil of wormwood has an inhibitory effect against several bacterial microorganisms, i.e. Staphylococci, and against mold pathogens capable of causing toxicity to humans such as Penicillium[7]. Following the same line of research, this study aims to determine the chemical composition of the essential oil of Artemisia mesatlantica, and to evaluate its antifungal activity against four well known strains of fungi responsible for wood decay, in order to take advantage of its properties as a wood preservative.

To the best of our knowledge, no study has been published to date to evaluate the antifungal impact of essential oils of Artemisia mesatlantica against fungal wood decay.

MATERIALS AND METHODS

Plant material

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

Fungal strains

The four fungi used in this work are responsible for brown and white rot of wood. They are the most important wood-destroying fungi. They were chosen for the considerable damage they cause in buildings, wood in contact with the soil (poles and railways) or buildings (bridges)[8,9]. These are:

- ⇒ Coniophora puteana (Schumacher ex Fries) Karsten.
- ⇒ Gloeophyllum trabeum (Persoon ex Fries) Murril.
- ⇒ Poria placenta (Fries) Cooke sensu J. Eriksson.
- ⇒ Coriolus versicolor (L.) Quélet.

These four fungal strains belong to the collection of Mycotheque Laboratory of Microbiology of Forest Research Centre, Rabat, Morocco. They are regularly maintained by subculture on nutrient medium PDA (Potato Dextrose Agar).

Extraction of essential oils (EO)

The extraction of essential oils was performed by hydrodistillation in a Clevenger type apparatus [10]. Three distillations were carried out by boiling 200g of fresh plant material with 1 liter of water in a 2l 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[11].

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 ìm, 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 ìl. 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)[12] and on gas chromatography coupled with mass spectrometry electron impact (GC-SMIE)[13]. 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 ìm. 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.

IK are determined by injecting a mixture of C9 to C24 alkanes in the same operating conditions[12]. 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.

Antifungal 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.

Inoculation is done by depositing fragments of 1 cm2 in diameter, taken from the periphery of a mycelium cultured for 7 days in malt extract. Incubation is done in the dark for 7 days at 25°C. 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 & chemical composition

The yield

The average yield of essential oils (ml/100 g) is calculated from the dry plant material from the aerial part of the plant. The essential oil yield of Artemisia mesatlantica collected in June about 1%.It is relatively higher than other plants commonly used industrially as a source of essential oils: rose (from 0.1 to 0.35%), peppermint (0.5-1%), neroli (0.5-1%) and the laurel (from 0.1 to 0.35%)[16]. Comparing our species with other wormwood species, its yield exceeds that of Artemisia copa Philippi of Bolivia (0.37%)[17], Artemisia absinthium of Canada (0.5 %)[18], Artemisia absinthium of Morocco (0.57%) and Artemisia pontica of Morocco (0.31%)[19]. However, the yield obtained is relatively low compared to

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other species of Artemisia, such as Artemisia herba-alba of Morocco (1.23%)[20]. This difference in yield between the wormwoods can be explained with the type of species of Artemisia, the effect of the vegetative stage of the plant and the soil conditions of the region[20].

The chemical composition

The chromatographic analysis of essential oils has helped to identify forty-four compounds representing approximately 99.93% (see TABLE 1). ß-thujone is the main constituent (57.95%) followed by 1,8-cineole (6.96%), camphene (6.34%) and camphor (3.75%). They are accompanied by other minor components which are just as important: Cis-β-dihydro-terpineol (2.32%), Terpin-4-ol (2.02%), α thujone (1.53%), 1-epi cubénol (1.48%), germacrene-D (1.22%), and davanone (1.23%) totaling approximately 84.8%. Our result is consistent with an earlier study of Holeman which showed that ß-thujone represents 60% of the plant of Artemisia mesatlantica collected near Ifrane Boulmane[21].

The main components ß-thujone, 1,8-cineole, camphene and camphor represent 75% of the total chemical composition. This essential oil is dominated by oxygenated monoterpenes (81.16%), followed by hydrocarbon monoterpenes (9.32%), oxygenated sesquiterpenes (7.01%) and hydrocarbon sesquiterpenes (1.98%) (See Figure 1).

The chemical composition of mountain wormwood is different from the Moroccan white wormwood previously studied by our team[20]. It is generally known by its composition with chrysanthenone being the main component (48.45%) followed by camphor (24.85%) and α-thujone

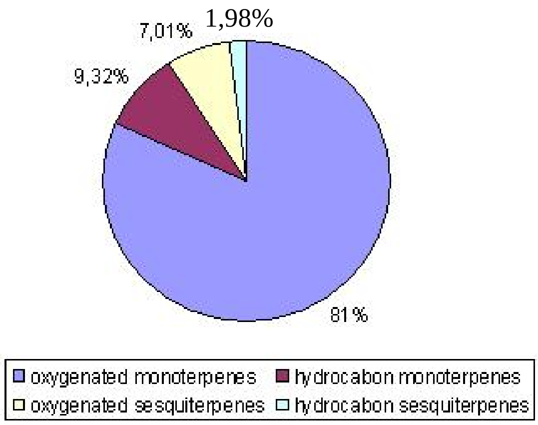

Figure 1 : Distribution of oxygenated monoterpenes, hydrocarbon monoterpenes, oxygenated sesquiterpenes and hydrocarbon sesquiterpenes in the essential oil of Artemisia mesatlantica.

TABLE 1 : Chemical composition of the essential oil of *A. mesatlantica* from Ifrane and Boulmane in Morocco,

N*	KI biblio	KI	Constituant	June
1	926	922	Tricyclene	0,19
2	931	928	α –thujene	0,84
3	939	943	α –Pinene	0,64
4	953	968	Camphene	6,34
5	976	972	Sabinene	0,36
6	1011	1016	Δ -3 -carene	0,47
7	1018	1024	α –terpinene	0,34
8	1031	1027	Limonene	0,14
9	1033	1031	1,8 -cineole	6,96
10	1062	1055	artemisia cetone	0,85
11	1062	1064	δ -terpinene	1,43
12	1083	1083	Artemisia alcool	0,17
13	1095	1095	α -oxide de pinene	1,02
14	1102	1101	α -thujone	1,53
15	1114	1114	β –thujone	57,95
16	1119	1118	Trans-pinan-2-ol	0,19
17	1133	1130	iso -3 -thujanol	0,59
18	1136	1135	Cis -β -dihydro-terpineol	2,32
19	1143	1140	Camphor	3,75
20	1163	1161	Trans -β –Terpineol	0,87
21	1177	1173	Terpin -4 –ol	2,02
22	1181	1181	Thuj -3 –en- 10-al	0,16
23	1189	1187	α –terpineol	0,50
24	1193	1193	Cis –piperitol	0,70
25	1204	1204	Verbenone	0,15
26	1229	1224	Nordavanone	0,30
27	1350	1350	α –acetate de terpinyl	0,16
28	1418	1412	E-Caryophyllene	0,14
29	1467	1465	9 –epi- E -caryophyllene	0,23
30	1480	1481	D -germacrene	1,22
31	1499	1497	α -muurolene	0,39
32	1581	1573	Oxyde de caryophyllene	0,57
33	1586	1580	Davanone	0,62
34	1600	1599	Trans -β –elemenone	0,35
35	1606	1603	Epoxide II d' Humulene	0,65
36	1614	1612	1,10 – di- epi- cubenol	0,43
37	1627	1625	1- epi- cubenol	1,48
38	1637	1631	3 – iso – thujopsanone	0,38
39	1649	1648	β –Eudesmol	0,14
40	1653	1655	α –cadinol	0,96
41	1674	1676	cadalene	0,63
42	1726	1723	Iso-longifolol	0,16
43	1741	1740	8- α - 11- elemodiol	0,43
44	1764	1769	14-oxy- α-muurolene	0,21

KI (*Kovats indices*)

(4.40%), α-pinene along with (2,16%), β-germacrene (0.77%) and β-elemene (1.78%)[20]. We note the absence of β-thujone in white wormwood and the absence of chrysanthenone in mountain wormwood. There is, therefore, a noticeable difference between the two wormwoods although both species are abundant at the same altitude (1900m)[5]. These major components, specific to each plant, can be considered as indicators to differentiate between the two species.

Also, Artemisia copa of Bolivia, which has the same ketonic composition as our wormwood and is located at the same altitudinal height (2500m), contain a totally different chemical profile. This wormwood from Bolivia is characterized by a dominance of β-thujone (42%), of chamazulene (6.5%), of homoterpene (6.0%), limonene (5%) and α-pinene (4.8%)[17].

All these results show that the chemical composition of essential oil from wormwoods is very variable depending on the species and its origin.

Antifungal activity of essential oils

The results of the essential oil antifungal activity of Artemisia mesatlantica are summarized in TABLE 2. In this study, we were interested in testing the antifungal activity of this oil against four wood destroying fungi in order to offer the possibility of their use in certain areas such as wood preservation in all its forms.

+: growth; - : inhibition

Generally, wood decay fungi showed good sensitivity to the essential oil of A. mesatlantica. The results of bioassays on crude essential oils of the species studied have shown that these oils are toxic and lead to death of fungi within seven days. The activity thresholds are between 1/250 and 1/500 v/v. Thus, the concentration of 1/500 v/v was sufficient to stop the growth of Gloeophyllum trabeum, Poria placenta and Coriolus versicolor. Coniophora puteana proved to be the most resistant to the essential oil at a concentration of 1/250 v/v.

TABLE 2 : Antifungal activity of *Artemisia mesatlantica* essential oil of Morocco

Dilutions v/v	CV Coriolus versicolor	CP Coniophora puteana	GT Gloeophyllum trabeum	PP Poria placenta
1/100	-	-	-	-
1/250	-	-	-	-
1/500	-	+	-	-
1/1000	+	+	+	+
1/2000	+	+	+	+
1/3000	+	+	+	+
1/5000	+	+	+	+
Negative control	+	+	+	+

We have established in our previous study of mountain wormwood that its essential oil is toxic against bacteria such as Staphylococcus aureus and Escherichia coli, and against fungi such as Aspergillus Niger, Penicillium digitatum and Penicillium expansum[7]. The antibacterial activity thresholds are between 1/1000 v/v and 1/3000 v/v and the antifungal activity threshold is between 1/100 and 1/250 v/v[7]. Note that the wood decay fungi are more vulnerable to A. mesatlantica EO (1/250 to 1/500 v/v) than mold, but they are less sensitive to it than bacteria[7].

With EO of thyme, Coriolus versicolor is the most resistant to phenolic compounds compared to others[22]. This can be attributed to the fact that this fungus produces laccase and extracellular enzymes that catalyze the oxidation of phenolic compounds and lead to their inactivation[23,24]. Usually, compounds such as geraniol, thymol and carvone are the most potent inhibitors of fungal growth (at a concentration between 1/1000v/v and 1/3000 v/v) and can serve as a broad-spectrum biocide against the common species of mold[25].

However, the EO of mountain wormwood dominated by ketone is more powerful than that of red Moroccan Juniper (Juniperus phoenicea) rich in terpene hydrocarbons, such as α-pinene and δ-3-carene. The essential oil of the fruits of this tree, which lies at an altitude of 1400m, has inhibited the growth of the same wood decay fungi at a concentration of 1/100v/v[26]. This has been proven by Channaoui and Elaraki[27] who showed that the antimicrobial activity of some terpene compounds is in the following increasing order: aldehydes> alcohols> ketones> hydrocarbons.

The fungicidal activity of essential oils from mountain wormwood can be explained by its chemical profile rich in oxides and ketones, including β-thujone and 1,8cineole. Ketones are physiologically highly active compounds and their use should be well controlled with fear to become toxic. At lower doses, ketones are engaged in neuroinhibition (act hypo). At higher or repeated doses, they are abortive in pregnant women, neurotoxic and narcotic[28]. The thujones, specifically, have demonstrated an anticoagulant action and a lytic action against mucus and lipids. Besides being slightly antiseptic and strongly immuno-stimulant, they have, in addition, anthelmintic and anti-fungal properties[28]. More over, 1,8cineole has several biological activities namely it is antibacterial, anti-inflammatory, antiviral, expectorant, sedative, herbicide and insect repellent[20].

However, it should be noted that the biological activities of Artemisia mesatlantica essential oil are not only caused by major compounds (ß-thujone, 1,8-cineole, camphene and camphor), but are due to all compounds in this oil. Indeed, it is already established that the essential oil of wormwood, rich in camphor and 1,8 cineole, exhibits antimicrobial activity in vitro[29]. In a comparative study of antimicrobial EO of several species of wormwood, tunisian researchers have determined that the type of essential oil characterized by a co-dominance of four main components (1,8-cineole, camphor, β-thujone and α -thujone) was the most active against yeast and bacterial germs to those with a dominance of thujone[30]. Similarly, a work study of A. sieberi essential oil 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. This mode of action is attributed to its potential to induce a state of oxidative stress through a cascade of free radicals which in turn are generated by the endoperoxide function that alkylates proteins and cause mitochondrial membrane depolarization[31]. Our positive results obtained in vitro support the eventual use of A. mesatlantica essential oil as a mean of preserving wood. Further studies of development of EO as an alternative to synthetic fungicides, are recommended to evaluate its efficacy and its long-term toxicity on wood.

CONCLUSION

This work is the first of its kind that studied the fungicidal effect of the mountain sagebrush wood rot fungi. We determined the yield, the chemical composition and antifungal properties of essential oil extracted from the leaves of Artemisia mesatlantica harvested in the region of Ifrane Boulmane (Morocco). The essential oil yield in June is 1%. Chemical analysis by GC and GC/MS has identified approximately 99.93% of total volatile components in this species. ß-thujone is the main constituent (57.95%) followed by 1, 8-cineole, camphene and camphor. A. mesatlantica essential oil showed a very significant inhibitory activity in vitro against four species of wood fungal decay. The fungicidal activity of this essential oil is mainly due to its richness in the following components: ß-thujone, α thujone, 1,8-cineole and camphor. Moreover, these compounds are well known for their antimicrobial properties. Antifungal performances highlighted deserve to be studied more vigorously in order to consider the prospects of application of this species in the area of wood.

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