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Research Article

Chemical Variability and Antifungal Activity of *Tetraclinis articulata* (Vahl) Masters Woods and Leaves Essential Oils Against Wood Decaying Fungi

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ABSTRACT

The coniferous tree, *Tetraclinis articulata* (Vahl) Masters, commonly known as Barbary thuya is endemic to the western Mediterranean areas. Its woods, mainly root burl, were very appreciated by artisans for their natural beauty, homogeneity and good quality destined for marquetry and furniture. Use of essential oils (EO_s) of this species opens a second way for valorization in many fields as bioactive agents. EO_s were extracted, by hydrodistillation from sawdust and leaves samples originated from two thuya populations, and analyzed by GC-MS. Yields of EO_s varied greatly depending on biomass type and samples origin. EO_s of leaves are dominated by monoterpens, and contain α-pinene, camphor, and bornyl acetate as major compounds. Those of woods are however rich in sesquiterpens, and contain 3-tera-butyl-4-methoxyphenol, thymol, cedrol, and α-cedrene as major compounds. Antifungal bioassay, by direct contact in malt-agar medium, of these EO_s conducted on four wood decaying fungi, showed that root burl wood EO_s possess the best antifungal inhibitory power related to their richness in phenols (above 64%), followed by those of trunk wood; while leaves EO_s showed, however a less antifungal activity. Such antifungal potency of wood thuya EO_s allows us to recommend the use of these oils extracted from sawdust as preservative agents for less durable woods.

Keywords: *Tetraclinis articulata*, Essential oils, Chemical variability, GC-MS, Leaves, Woods, Wood decaying fungi.

INTRODUCTION

Thuya, *Tetraclinis articulata* (Vahl) Masters, belonging to the order of Pinales and family of Cupressaceae, is endemic to the western Mediterranean areas and it is par excellence a tree of the semi-arid temperate and hot bioclimate. In North Africa, natural stands of *T. articulata* cover a total area of 1 million hectares and grow at altitudes ranged from sea level up to 1800 m. It is found in Morocco, Algeria, Tunisia, and more rarely in Spain and Malta[1]. In Morocco, thuya populations occupy an area of approximately 566.000 ha and play an important socio-economic position in the satisfaction of the needs of the human riparian populations in terms of rangelands (for livestock), wood products (timber, fuelwood, wood of service and burl wood for crafts), sandarac gum, tannins and vegetal tar. However, in recent decades, this forest undergoes a significant degradation especially due to its overexploitation by the craft sector following a strong demand[2]. This cupressaceae was although placed by the UICN in the red listing as threatened species[3]. In the Iberian Peninsula, conservation and rehabilitation of this species began recently to receive more interest[4]. Thuya woods are very durable to durable against wood decaying fungi[5]. It is also known for its richness in extractable having antibacterial, antifungal and insecticidal properties[6,7]. Use of essential oils (EO_s) of this species, in other fields as bioactive agents, opens a second way for best valorization of second products of thuya. EO_s of leaves and twigs are dominated by monoterpenes, while woods are rich in sesquiterpenes and diterpenes, as reported by previous works in Morocco[8-13], in Algeria[14,15], and in Tunisia[16]. Bioassays of thuya EO_s were also conducted with success on bacterial and fungal strains[9,11,13,15]. A correlation between potential antioxidant activity and total phenolic level was also noted for thuya EO_s extracted from leaves[16,17]. The aims of this work is to study chemical variability of *T. articulata* EO_s, extracted from different parts of trees originated from two populations and to assess antifungal activity of these oils against four wood decaying fungi.

MATERIAL AND METHODS

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Plant material

Samples of leaves were collected from many trees in Oued Beht forest (Khemisset, Central plate of Morocco) and Smimou forest (Essaouira, south-west of Morocco) during April 2015. Trunk wood and root burl samples were collected from sweepings of craft processing workshops in the same regions. Leaves were carefully removed from twigs and dried at room temperature for one week. Woods samples were sawed to small pieces and then crashed to obtain sawdust of 2 mm mesh. Biomass humidity was determined after oven dried of 30 g biomass samples for 72 hours at temperature of 60°C.

Fungal strains

Three brown-rot fungi strains used in this study were *Gloeophyllum trabeum* BAM Ebw.109, *Oligoporus placenta* FPRL. 280, and *Coniophora puteana* BAM Ebw. 15, in addition to the white-rot fungus, *Trametes versicolor* CTB 863 A. Fungi strains were maintained in the mycological collection of the Laboratory of Botany, Mycology and Environment (Faculty of Sciences in Rabat) Morocco. They were chosen for the significant damages that they cause to wood and wood-based products.

Methodology

Extraction and chemical analysis of T. articulata essential oils

To analyze chemical composition of *T. articulata* EO_s, three extraction assays, from each type of biomass, were carried out by hydrodistillation in a Clevenger apparatus. During each assay, 200 g of biomass material were introduced into a one-liter flask containing water; the mixture was then boiled for 2 hours. Yield of obtained EO_s is expressed in mL per 100 g calculated on the basis of dry matter. Extracted EO_s was stored in a small dark glass bottle at 4°C until use.

Chemical analysis and identification of components were performed by an electronically controlled pressure gas chromatograph (GC) coupled with a mass spectrometer (MS). The GC is a Hewlett-Packard (6890) system, equipped with a capillary column HP-5 (30m x 0.25mm x 0.25µm film thickness) and a FID detector at 250°C. H_2/Air gas mixture was used in split-splitless injector heated at 250°C. The carrier gas is N_2 with 1.5 mL min^{-1}. The column temperature was programmed from 50 to 250°C by step of 4°C min^{-1}. The injected volume of essential oil was 1 µL diluted in *n*-hexane. The mass spectrometer used is a Hewlett-Packard (HP 5973 series); fragmentation is done by electron impact at 70 eV. The column is a capillary HP-5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column temperature is programmed from 50 to 200°C for 10 minutes by step of 4°C min^{-1}. The carrier gas was helium with a flow rate set at 1.5 mL min^{-1}. The injection mode is split (leakage ratio: 1/70). The device is connected to a computer system managing a mass spectrum library NIST 98. A standard solution of *n*-alkanes (C_8-C_{26}) was also used to obtain the retention indices. Individual volatile components were identified by comparison of their mass spectra (MS) and retention indices (RI) with those reported in the literature especially in the Adams Registry of Mass Spectral Data[18].

Assessment of anifungal activity of T. articulata essential oils against wood decaying fungi

Antifungal activity of *T. articulata* EO_s was determined by direct contact on agar medium according to the method reported by Remmal et al[19]. In order to effectuate a homogeneous distribution of oil in the medium, it was first emulsified in a sterile solution of water-agar at 0.2% (SA). To tubes containing 13.5 mL of malt-agar medium (20 g L^{-1} malt extract and 15 g L^{-1} agar) sterilized and kept at 45°C in a water bath, were added aseptically 1.5 mL of different dilutions prepared so as to obtain a range of ten final EO_s dilutions in the culture medium between 1/100 and 1/1700 v/v. The tubes were shaken vigorously and poured into Petri dishes. Similarly, control dishes containing 13.5 mL of culture medium and SA solution alone were prepared. All Petri dishes were inoculated by depositing two square fragments of 0.5 cm^2, taken from the margins of 10-day-old mycelia culture. Three replicates for each treatment and fungus were prepared and incubated in the dark for 7 days at temperature of 22°C. This bioassay allows us also to determine the minimum inhibitory concentration (MIC) of each combination of EO_s-fungus strain. The MIC is defined as the lowest concentration for which no visual growth of the fungus was observed[20].

RESULTS AND DISCUSSION

Chemical composition of T. articulata essential oils

Yields of EO_s varied greatly depending on biomass type and samples origin (Table 1). Root burl wood gave the best yield (2.62%), followed by those of trunk wood (1.52%). Only small amounts (0.23%) of EO_s were extracted from thuya leaves. Previous works reported similar amounts for leaves and trunk wood of this species in Morocco[9,11,12]. Thuya leaves originated from western Algerian forest gave more amounts (0.75%)[14]. Furthermore, yield of root burl wood EO_s reported by Akbli et al[13] for Essaouira population using the same distillation way, was however low (1.50%) compared to that obtained in this work (2.54%). Thuya twigs gave more amounts of EO_s (0.41%) than leaves[12]. Several factors influence yield of EO_s including, bio-climate, age, tree health conditions, biomass conditioning before EO_s extraction and distillation way[21,22].

A great variability was observed in chemical composition of EO_s and compounds amounts for studied populations and type of biomass considered. EO_s of leaves are very different from those of woods and they only shared six compounds with them. They are characterized by the predominance of monoterpenes group (29 to 39%), such as α-pinene (17 to 20%) and limonene (6 to 9%), followed by

Table 1: Yields (in %) of *T. articulata* essential oils.

Population	Type of biomass		
	Trunk wood	Root burl wood	Leaves
Khemisset	1.14	2.71	0.31
Essaouira	1.90	2.54	0.15
Average %	1.52	2.62	0.23

Table 2: The main components identified in essential oils of *T. articulata* originated from Khemisset (KH) and Essaouira (ESS) areas.

Component	KI	TW		RBW		LV	
		KH	ESS	KH	ESS	KH	ESS
borneol	1165	1.23	1.32	0.68	3.30	6.72	4.25
α-terpineol	1189	-	-	0.29	0.70	0.49	0.61
carvacrol	1278	0.81	0.80	1.40	2.07	-	-
thymol	1290	8.21	2.90	39.77	43.29	-	-
3-tera-butyl-4-methoxyphenol	1491	16.51	14.05	24.68	26.15	-	-
cedrol	1596	14.82	14.30	6.34	3.68	-	-
widdrol	1597	1.02	1.06	0.34	0.16	-	-
epi-cedrol	1611	1.23	0.10	0.77	0.23	-	-
α-acorenol	1633	4.21	17.73	1.12	0.40	-	-
β-acorenol	1637	4.15	6.65	1.27	0.87	-	-
himachalol	1647	0.00	0.00	0.32	0.11	-	-
α-cadinol	1653	0.00	0.00	0.00	0.00	0.20	0.78
totarol	2314	2.58	2.98	1.52	0.92	-	-
Total Alcohols		54.77	61.89	78.50	81.88	7.41	5.64
camphor	1143	0.11	0.43	-	-	21.00	10.55
verbenone	1204	-	-	-	-	1.17	0.21
9-iso-thujopsanone	1437	-	-	-	-	0.34	1.05
Z α-atlantone	1689	0.53	0.48	0.29	0.17	-	-
E α-atlantone	1773	0.74	0.61	-	-	-	-
Total Ketones		1.38	1.52	0.29	0.17	22.51	11.81
α-thujene	931	-	-	-	-	1.63	0.71
α-pinene	939	-	-	-	-	17.27	22.45
camphene	953	-	-	-	-	2.03	1.03
β-myrcene	991	-	-	-	-	1.68	2.42
limonene	1031	-	-	-	-	5.63	9.10
α-cedrene	1409	17.59	8.24	7.77	7.20	-	-
β-cedrene	1434	4.00	3.97	1.80	1.54	-	-
α-himachalene	1447	-	-	1.07	0.90	-	-
thujupsadiene	1462	-	-	1.10	0.93	-	-
α-acoradiene	1464	2.33	0.13	-	-	-	-
β-acoradiene	1465	2.26	3.56	-	-	-	-
β-alaskene	1495	0.67	0.54	-	-	0.31	1.49
cuparene	1498	0.79	1.40	-	-	-	-
β-himachalene	1499	-	-	1.25	1.27	-	-
γ-cadinene	1513	1.18	2.75	0.31	0.59	0.13	0.34
δ- cadinene	1524	0.47	0.46	0.33	0.19	0.18	1.00
α-humulene	1709	0.68	0.34	0.27	0.13	0.14	0.34
14 hydroxy δ- cadinene	1799	1.21	0.86	0.24	0.17	0.00	0.00
Total Terpens		31.18	22.25	14.14	12.92	29.00	38.88
bornyl acetate	1285	-	-	-	-	25.15	28.32
germacrene D	1480	-	-	-	-	0.53	0.87
italicene oxyde	1538	0.91	0.56	0.59	0.37	-	-
Others		0.91	0.56	0.59	0.37	25.68	29.19
Global		90.04	88.57	94.65	95.89	89.23	89.69

KI, Kovàts Index; TW, Trunk wood; RBW, Root burl wood; LV, Leaves.

esters group (26-29%), mainly as bornyl acetate (25 to 28%), and ketones group (12 to 22%), such as camphor (10 to 21%); while woods EO_s are rich in alcohols, 55 to 62% for trunk wood and 78 to 82% for root burl wood, as thymol (40 to 43% for burl wood and 3 to 8% for trunk wood), 3-tera-butyl-4-methoxyphenol **(**14 to 26%), cedrol (3 to 15%), and sesqueterpenes, 22 to 31% for trunk wood and 13 to 14% for root burl wood, as α-cedrene (7 to 18%).Trunk wood contained however more amounts of acorenols, acoradienes, γ-cadinene, and β-

Table 3: Minimal inhibitory concentrations (v/v) determined for *T. articulata* essential oils.

Essential oils Fungal strains	Trunc wood	Root burl wood	Leaves
T. versicolor	1/1000	1/4000	1/500
C. puteana	1/1000	1/4000	1/500
G. trabeum	1/1200	1/5000	1/800
O. placenta	1/800	1/5000	1/800

cedrene. Nineteen compounds were common for both trunk and root burl woods (Table 2).

Our results corroborate those of Moroccan literature, especially for major constituents with only minor differences in compounds amounts. EO_s leaves originated from Moroccan populations are dominated by bornyl acetate, α-pinene, and limonene, while those originated from Western Algeria thuya population of are therefore rich in camphor, bornyl acetate and borneol[14]. Another Algerian team reported, for the same EO_s originated from Tlemcen (Western Algeria), different major compounds, such as bornyl acetate, caryophyllene, caryophyllene oxide and germacrene D[15]. It seems that this second team had probably used leaves with twigs. It should be also noted that 3-tera-butyl-4-methoxyphenol was only reported by El Moussaouiti et al[23] for root burl wood oil.

Anifungal activity of T. articulata essential oils against wood decaying fungi

According to the bioassay conducted with *T. articulata* EO_s, a significant inhibitory effect on the four wood-decaying fungi was obtained, mainly for EO_s extracted from woods. Therefore, 1/500 v/v concentration of all EO_s was sufficient to inhibit the growth of all tested fungal strains. Root burl wood EO_s showed however the strong inhibitory potency against *G. trabeum* and *O. placenta* strains with dilutions over than 1/5000 v/v (Table 3).

Other studies conducted by our team showed that these fungal strains are also sensitive to the inhibitory action of *Cedrus atlantica* oils extracted from wood[24]. Antifungal activity of EO_s of other species, such as thyme, was recently tested against the same fungi. *Thymus bleicherianus* oil inhibited the growth of three fungi: *G. trabeum*, *C. puteana* and *O. placenta* at concentration of 1/3000 v/v, while growth of *T. versicolor* was inhibited by concentration up to 1/2000 v/v[25]. Furthermore, root burl wood EO_s showed an antibacterial activity two to six times greater compared to that of reference antibiotic, and were more effective on *Stapphylococcus aureus* ($Gram^+$) and *Escherichia coli* ($Gram^-$) with significant bacteriostatic and bactericidal effects[13]. Studies, already reported that natural durability of cupressaceae woods is related to their extractibles rich in tropolones and phenols[26,27].

In our investigation, the higher antifungal activity of thuya wood EO_s is probably related to their alcohols fraction, rich phenols as thymol and 3-tera-butyl-4-methoxyphenol, which probably conferred them this significant bioactivity. Combined action of two phenolic compounds, such as thymol and carvacrol was previously reported[25,28]. Action of phenolic compounds on fungi is primarily based on the inhibition of fungal enzymes containing SH group in their active site[29,30]. The synergistic effect between different compounds of EO_s could be also involved in the observed antifungal activity[31].

Furthermore, by splitting EO_s trunk wood of this species, on silica column, five fractions were previously identified: one hydrogenated fraction (FH) and four oxygenated fractions (FO). FH was dominated by hydrogenated sesquterpens, such as α-cedrene; FO_1, by totarol, FO_2 and FO_3, by α-acorenol and FO_4 by cedrol[10]. So, to optimize the use of thuya woods oils, a current work is focused, by our team, on splitting EO_s of root burl wood and remaking bioassay with each identified fraction. Extracted oil from sawdust, obtained from of wastes sawmills and craft workshops, can then be valorized in other fields including wood preservation for substitution petrochemical based products which are harmful to human health and environment.

CONCLUSION

This study allows us to conclude that *T. articulata* possess a great qualitative (EO_s composition) and quantitative (compounds amounts) variability depending on considered part of tree and thuya population. Yields of EO_s varied greatly from 0.23% for leaves to 2.62% for root burl wood. EO_s of leaves are very different from those of woods and they shared only six compounds, and they are dominated by bornyl acetate, α-pinene, camphor and limonene. Nineteen compounds were common for both trunk and root burl woods and their EO_s are rich in thymol, 3-tera-butyl-4-methoxyphenol, α-cedrene, and cedrol.

According to the bioassay conducted with *T. articulata* EO_s, a significant inhibitory activity on the four wood-decaying fungi was obtained, mainly for EO_s extracted from woods. Root burl wood EO_s showed however the strong inhibitory potency by dilutions over than 1/5000 v/v for *G. trabeum* and *O. placenta* strains. This strong antifungal activity is probably related to their alcohols fraction, rich in phenols.

These results will allow the recovery of EO_s extracted from sawmills and artisans workshops wastes, in other fields including wood preservation.

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