Chapter

Durable Woods and Antifungal Activity of Their Essential Oils: Case of *Tetraclinis articulata* (Vahl) Masters and *Cedrus atlantica* Manetti

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Abstract

Cedrus atlantica Manetti and *Tetraclinis articulata* (Vahl) Masters are a resinous species originated from North Africa and well known for their durable and noble timbers. This work was conducted to assess the relationship between natural durability of their woods, assessed in previous works by European standards NF EN 350 and CEN/TS 15083-1, and the bioactivity of essential oils extracted from these woods by hydrodistillation and analyzed by GC-FID and GC-MS. Bioassay of sawdust essential oils, conducted by direct contact technique on agar medium on four wood-decaying fungi strains, revealed strong antifungal inhibition especially by *T. articulata* root burl oil due to its richness in phenols. Natural durability classes of *T. articulata* and *C. atlantica* woods were then positively correlated with antifungal activity levels of their oils.

Keywords: *Cedrus atlantica*, *Tetraclinis articulata*, wood durability, essential oils, wood-decaying fungi

1. Introduction

Tetraclinis articulata (Vahl) Masters (Cupressaceae) and *Cedrus atlantica* Manetti (Pinaceae) are threatened species [1] endemic to the western Mediterranean areas [2]. Moroccan *T. articulata* and *C. atlantica* populations occupy an area of about 500,000 and 130,000 ha, respectively, and satisfy many socioeconomic needs of the human riparian populations for various products. But in the last decades, forests of *T. articulata* were exposed to a significant degradation due to a strong demand by the craft sector for timbers [2, 3]. Even if *C. atlantica* forest provides approximately annually 90,000 m^3 of softwood logs intended for sawing and veneer, they also suffer the same fate [4]. Both species are famous for their durable and noble timbers. *T. articulata* root burl provides good quality woody material (hard, homogeneous, and fine grained) with remarkable flecks and an aesthetic aspect very appreciated in cabinetry and marquetry uses [3].

Laboratory tests showed that *T. articulata* trunk wood and its root burl are durable against wood-decaying fungi [5, 6] as well as *C. atlantica* heartwood [6–8].

A lot of waste as slabs and sawdust results from wood processing of those species timbers. However, only sawdust accounts for about 8% of *C. atlantica* sawn timber that contains appreciable amounts of extractives [9–12]. Many recent works highlighted that essential oils (EOs) extracted from *T. articulata* and *C. atlantica* woods possess numerous biocidal activities [12–15]. Nevertheless, only few attempts to investigate the capability of EOs to protect woods against fungi decay have been previously undertaken [16–18]. The relationship between natural durability of *T. articulata* and *C. atlantica* woods and the bioactivity of their EOs was not yet established. Therefore, the present study is devoted to investigate this relationship.

2. Material and methods

2.1 Material used for EOs extraction and chemical analysis

Trunk wood and root burl samples of *T. articulata* were collected from sweepings of craft processing workshops in Khemisset Region (central plate of Morocco), while samples of *C. atlantica* sawdust were collected from wood sawmill in the region of Azrou (Middle Atlas Mountains of Morocco). Sawdust was then sieved into particles of 1 mm size and triplicate samples of 250 g were subjected to hydrodistillation for 4 hours to obtain pure essential oils (EOs).

The chemical analysis and component identification of EOs were performed by gas chromatography (GC-FID) and by gas chromatography coupled with a mass spectroscopy (GC-MS). The identification of EO components was achieved by comparison of their retention indices (RI) relative to (C8-C22) *n*-alkanes with those of known compounds, and by comparison of similar mass spectra using Wiley/NBS mass spectral library of the GC-MS data system and other published mass spectra [19]. The percentage compositions of samples were calculated according to the area of the chromatographic peaks using the total ion current.

2.2 Material used for bioassay

Bioactivity of EOs extracted from *T. articulata* and *C. atlantica* woods was assessed in bioassay against the following wood-decaying basidiomycetes fungi: *Gloeophyllum trabeum* (BAM Ebw.109 strain), *Oligoporus placenta* (FPRL. 280 strain), *Coniophora puteana* (BAM Ebw. 15 strain), and *Trametes versicolor* (CTB 863 A strain). Fungi strains originated from the mycological collection of the Laboratory of Botany, Mycology and Environment, Faculty of Sciences in Rabat, Morocco.

2.3 Experimental

Bioassays of EOs were performed by direct contact of fungi strains on agar medium according to the method reported by Remmal et al. [20]. Oils were first diluted in a sterile solution of tap water-agar at 0.2% in order to obtain a homogeneous mixture, then distributed in test tubes containing 13.5 ml of sterilized malt-agar medium (20 g/l malt extract and 15 g/l agar), and kept at 45°C in a water bath. To obtain the final oil concentrations in the culture medium ranging from 1/250 to 1/5000 v/v, aseptic volumes of 1.5 ml of different dilutions were then added to those tubes before pouring EO-medium mixtures into Petri dishes. Additional control dishes containing only 13.5 ml of culture medium and agar solution at 0.2% (SA) alone were also prepared. Inoculation of Petri dishes was made by two 0.5 cm^2 fragments of 10 days old fungal culture in malt-agar. For each treatment, three repetitions were prepared and incubated in the dark for 7 days at 22°C. At the end of each bioassay, minimal inhibitory concentration (MIC) [21] was determined for each fungus.

2.4 Results and discussion

According to the bioassay conducted on oils extracted from *T. articulata* and *C. atlantica* woods, a significant inhibitory effect on the four tested wood-decaying fungi was observed (**Table 1**) with different levels of inhibition. *T. articulata* root burl wood EOs showed, however, a strong inhibitory action against those fungi strains with oil dilutions over 1/4000 v/v. *G. trabeum* fungus was the most sensitive to the inhibitory effect of this essential oil since it was inhibited by concentrations between 1/5000 for *T. articulata* root burl oil and 1/1000 v/v for Atlas cedar oil. *O. placenta* was the most resistant strain since its growth inhibition was not reached until 1/400 concentrations for *C. atlantica* oil and 1/800 for *T. articulata* trunk wood oil (**Table 1**).

Previous studies by our team [5, 8] showed that *T. articulata* and *C. atlantica* woods were classified as very durable to durable (DC 1 and 2) and means of mass loss of test specimens was below 5.20% compared to those of Scot pine wood (control) (40.70%). According to their durability indexes (*X*) determined by NF EN 350-1 and CEN/TS 15083-1 standards [22, 23] and the biological risks defined by EN 335-2 standard [24], natural durability levels of those woods against wood-decaying fungi allow them to access high-risk classes of biological attacks 4 and 5 for an end-use without preservative treatment regarding decay fungi [25, 26]. Compared to similar studies on Moroccan coniferous woods (**Figure 1**), the natural durability of native Atlas cedar wood is similar to that of *C. atlantica* heartwood (DC 1 and 2) originated from a south Italian plantation [7], whereas *Pinus halepensis* and *P. pinaster* woods were considered as less durable (DC 4) [6, 27]. Generally, pine woods contain less active extractives than those of *Cupressaceae*. Adamopoulos et al. [28] reported that the weakness of natural durability of both heartwood and sapwood of *Pinus leucodermis* is related to low presence of bioactive extractives that can inhibit the brown-rot fungus, *Coniophora puteana*.

In addition, other works by our team [8, 29] revealed that EOs of *C. atlantica* wood is dominated by ketones (52.05%) and alcohols (26.58%), while those of thuya are dominated by alcohols (about 55–78%) and sesquiterpenes (13–22%) (**Table 2**). Major components of *C. atlantica* oil are, respectively, E-γ-atlantone, E-α-atlantone, 5-isocedranol, 9-iso-thujopsanone, cedranone, Z-α-atlantone, cedroxyde, and 14-hydroxy-δ-cadinene [8] (**Table 3**).

	Essential oils fungal strains	Thuya trunk wood	Thuya root burl	Atlas cedar wood
Specific MIC	*T. versicolor*	1/1000	1/4000	1/800
	C. puteana	1/1000	1/4000	1/400
	G. trabeum	1/1200	1/5000	1/1000
	O. placenta	1/800	1/5000	1/400
Global MIC		1/800	1/4000	1/400

Table 1.
Minimal inhibitory concentrations (MIC) (v/v) determined for essential oils of thuya and Atlas cedar woods by bioassay conducted on malt-agar medium on wood-decaying fungi.

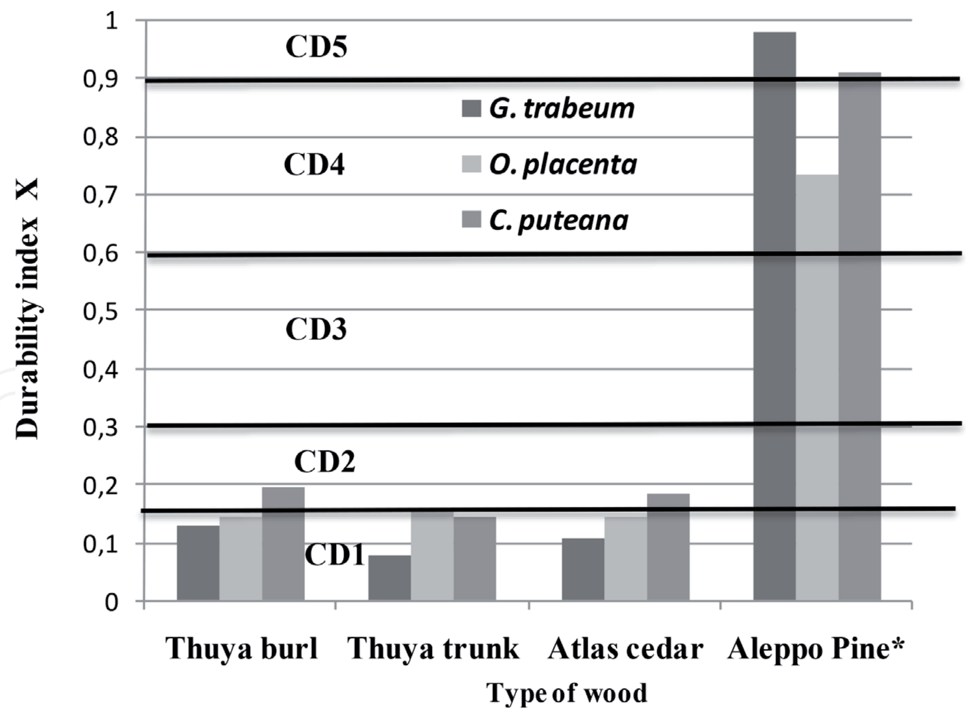

Figure 1.
Natural durability of studied woods against wood-decaying fungi.

Chemical group	Thuya trunk wood	Thuya root burl	Atlas cedar wood
Alcohols	54.80	78.50	26.60
Esters	—	—	5.70
Ketones	1.40	0.30	52.00
Oxydes	0.90	0.60	3.40
Terpenes	31.20	14.10	8.00
Others	1.75	1.15	—
Global	90.04	94.65	95.69

Table 2.
Chemical groups (in%) identified, by GC-MS, in essential oils of Tetraclinis articulata and Cedrus atlantica woods.

EOs of *T. articulata* woods are rich in thymol, 3-tert-butyl-4-methoxyphenol, cedrol, and α-cedrene (**Table 3**). The oil of *T. articulata* trunk wood contains phenols such as α- and β-acorenol, cedrol, and totarol, along with terpenes as α-cedrene that can protect this wood from fungi decay. The strong inhibitory effect of *T. articulata* root burl oil is probably due to its oxygenated fraction rich in phenols as thymol, 3-tert-butyl-4-methoxyphenol, and cedrol. A previous work already highlighted this significant antibacterial activity at low concentrations [30]. Abundance of tropolones and phenols in *Cupressaceae* woods may explain their high natural durability grades against wood-decaying fungi as reported by Haluk and Roussel [16] and by more recent works [13, 30, 31]. Early investigations found that thujaplicines (tropolones) of *Thuja plicata* possesses strong inhibitory effect against the wood blue stain fungi and many wood-decaying fungi [32].

Natural durability of *C. atlantica* wood can be correlated to bioactivity of its oils essentially rich in sesquiterpene ketones as atlantones [9, 14]. In the literature,

Chemical group	Component	KI	TTW	TRB	ACW
Alcohols	Thymol	1290	8.20	39.80	—
	3-Tert-butyl-4-methoxyphenol	1491	16.50	24.70	—
	Turmerol	1578	—	—	3.45
	Cedrol	1596	14.80	6.35	1.90
	α-Acorenol	1633	4.20	1.10	—
	β-Acorenol	1637	4.15	1.30	—
	Himachalol	1647	—	0.30	2.45
	5-Isocedranol	1669	—	—	11.70
	β-Santalol	1741	—	—	2.00
	E-Z-Farnesol	1742	—	—	1.10
	Totarol	2314	2.60	1.50	—
Esters	Hexyl isobutyrate	1150	—	—	1.38
	Benzyl benzoate	1762	—	—	1.16
	Z-β-Santalol acetate	1823	—	—	1.15
	Z-Ternine	1838	—	—	1.25
Ketones	Camphor	1143	0.11	—	1.28
	Cedranone	1620	—	—	4.13
	9-Iso-thujopsanone	1637	—	—	4.45
	Deodarone (Dihydro-2,2,6-trimethyl-6-(4-methyl-3-cyclohexen-1-yl)-2H-pyran-3(4H)-one)	1694	—	—	1.07
	E γ-Atlantone	1701	—	—	19.73
	Z α-Atlantone	1713	—	—	4.02
	E α-Atlantone	1773	0.74	—	16.86
Oxides	italicene oxide	1538	0.91	0.59	—
	Cedroxide	1704	—	—	2.38
Terpenes	α-Cedrene	1409	17.59	7.77	—
	β-Cedrene	1434	4.00	1.80	—
	α-Himachalene	1447	—	1.07	—
	Thujupsadiene	1462	—	1.10	—
	α-Acoradiene	1464	2.33	—	—
	β-Acoradiene	1465	2.26	—	—
	β-Himachalene	1499	—	1.25	0.79
	α-Dehydro-ar-himachalene	1511	—	—	1.13
	γ-Cadinene	1513	1.18	0.31	
	γ-Dehydro-ar-himachalene	1529	—	—	1.57
	14 Hydroxy-murolene	1775	—	—	1.00
	14 Hydroxy δ-cadinene	1799	1.21	0.24	1.94

KI, Kovàts Index; TTW, Thuya Trunk Wood; TRB, Thuya Root Burl; ACW, Atlas Cedar Wood.

Table 3.
The main components (in%) identified, by GC-MS, in essential oils of Tetraclinis articulata and Cedrus atlantica woods.

African padauk (*Pterocarpus soyauxii*) medium-heavy heartwood manifests a remarkable decay resistance attributed to its specific extractive compounds [33]. Moreover, extractive compounds obtained from black locust heartwood were able to increase the native durability of European beech against wood-decaying fungi from class 5 (not durable) to class 3 (moderately durable) [34].

The strong antifungal activity of *T. articulata* root burl wood EOs is probably related to their alcohol fraction, rich in thymol and 3-tert-butyl-4-methoxyphenol, which confer them this significant bioactivity. The combined action of two phenolic compounds, such as thymol and carvacrol, was previously reported [17, 35]. Action of phenolic compounds on fungi is primarily based on the inhibition of fungal enzymes containing SH group in their active site [36, 37]. The antifungal activity of the EOs of *C. atlantica* wood can be correlated to its sesquiterpene ketones, which are mainly atlantones (about 40.61%). Bioassays conducted with pure α-atlantones extracted from *Decalepis hamiltonii* revealed great inhibitory activity against pests and molds [38]. In our study, alcohols present in *C. atlantica* oil in significant amount (more than 26%), such as isocedranol, tumerol, himachalol, and cedrol, may also be involved in the inhibitory effect of this oil. Other compounds such as cadinenes (monoterpene hydrocarbon) could also have a great antimicrobial property [39]. However, synergistic action of two or more components of essential oils extracted from thuya and Atlas cedar woods can also be involved in the observed bioactivity reported in our study.

Antifungal activity of several thyme species oils was recently tested successfully against wood-decaying fungi especially those of *Thymus bleicherianus* [17]. Furthermore, EOs of *T. articulata* root burl showed an antibacterial activity two to six times greater compared to that of reference antibiotic and were more effective on *Staphylococcus aureus* (Gram$^+$) and *Escherichia coli* (Gram$^-$) with significant bacteriostatic and bactericidal effects [40]. Regarding inhibition mechanisms of active compounds of EOs, it was reported that volatile alcohols can act on both lytic and synthetic enzyme pathways and growth inhibition consequently occurred after breaking off natural extension hyphae of fungi [41, 42].

Active compounds contained in oils of *T. articulata* and *C. atlantica* woods, which were successfully tested against wood-decaying fungi, may protect and ensure good natural durability levels for these woods ranging from very durable (DC1) to durable (DC2) classes.

Findings of this study could allow us to consider recovering wastes from *T. articulata* and *C. atlantica* wood processing for the extraction of bioactive oils and use them as biocide especially in preservative treatment of less-durable woods such as those of pines. Also, these results are in favor of more protection for these threatened species and for the rehabilitation in their natural environment. Natural compounds of *T. articulata* and *C. atlantica* oils can then replace the use of current petrochemical compounds that are becoming more and more criticized for their harmful effect on human health and the environment.

3. Conclusion

The present study has clarified the relationship between the natural durability of *T. articulata* and *C. atlantica* woods and the antifungal activity of their EOs. According to the bioassay conducted on those oils, a significant inhibitory activity was obtained on the four wood-rotting fungi tested, mainly for oils extracted from *T. articulata* root burl. Stronger inhibitory effect was then reached by dilutions over 1/5000 v/v for this oil rich in phenols. The durability classes of *T. articulata* and *C. atlantica* woods positively correlated with bioactivity of their oils against the three wood-decaying fungi specified by the CEN/TS 15083-1 standard. Active EOs extracted from wastes of these woods can be valorized as wood-preservative treatment.

Acknowledgements

This project is supported by Forest Research Center in Rabat (High Commission for Water and Forests and Fight against Desertification) in collaboration with Faculty of Sciences in Rabat (Mohammed V University), Morocco.

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