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Essential Oil Composition of Different Parts of *Tetraclinis articulata*

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Abstract

The work evaluates the yield and the composition of the essential oil extracted from leafy and woody branches, cones, and seeds of a cultivated stand of *Tetraclinis articulata* (Vahl) Masters (Cupressaceae) growing in Malta. The main components were α-pinene, limonene, camphor, bornyl acetate, borneol and germacrene D. Our results show a number of differences in composition to a Moroccan study of the essential oil derived from homologous parts.

Key Word Index

Tetraclinis articulata, Cupressaceae, essential oil composition, chemotaxonomy, α-pinene, limonene, camphor, bornyl acetate.

Introduction

Tetraclinis articulata (Vahl) Masters is a coniferous species that has been known since antiquity. Its resistance to adverse environmental conditions including drought and fire, make it a useful tree species, especially for unfertile marginal land. But other attributes include its hard dense wood which is highly resistant to rot and worms. The wood and its veneer is also highly prized in the handicraft industry because of the smooth finish it can take. Gum sandarac, a resin exudate, was used for varnishes, pounce and in dental fillings. The wood is also used as fuel and to make a fine charcoal. In North Africa, different parts of the tree are used in traditional and veterinary medicine principally against intestinal and respiratory ailments as well as skin conditions. There are historical reports of its presence in Malta since antiquity and many places take their name from this tree, but none of the above uses are reported from this country.

Botany and Ecology: Tetraclinis articulata (syn. *Thuja articulata* Vahl (Basionym), *Callitris quadrivalvis* Vent., *Callitris articulata* (Vahl) Link.).

Common Names: Arabic: Arar or Ahrar, berbouch, Shagaret el-hayat, megloub, Abhel, Azuka; English: Sandarac Gum-Tree, Arar, Cartagena Cypress; French – Thuya du Barbarie.

Tetraclinis articulata is a small conifer in the Cupressaceae. The monospecific genus has a predominantly North African distribution (Morocco, Algeria and Tunisia) as well as a number of other stations namely the Sierra di Cartagena, southeast Spain, Malta, Cyprus and Libya (1).

Locally the tree attains an average height of 5-7 m when growing under favorable conditions.

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Table I. Percentage composition and yield of the oil from different parts of *Tetraclinis articulata*

Constituents	Terminal branches		Cones	Seeds
	Woody	Non-woody		
α-thujene	2.9	1.8		
α-pinene	31.0	46.4	68.2	46.3
camphene	2.3	1.9		
β-pinene		0.2		0.7
sabinene		0.9	1.7	1.7
myrcene	2.9	2.4	2.0	1.5
limonene	3.8	6.2	16.6	25.3
p-cymene		0.2		
camphor	18.1	7.3		1.1
linalool		0.5		
bornyl acetate	19.1	19.9	1.8	2.2
β-caryophyllene		0.6		1.1
terpinen-4-ol	0.7	0.6		
isoborneol		0.3		
α-terpineol		0.5	4.9	
borneol	2.8	3.6		0.4
verbenone	3.7	0.5	1.4	
germacrene D	1.6			5.0
Oil yield (% w/w)	1.53	1.20	0.31	0.55

Table II. Comparison of the major components (%) of woody terminal branches from Malta and Morocco

Constituents	Malta	Morocco Station E4
α-pinene	31.0	9.7
camphor	18.1	21.2
limonene	3.8	8.0
bornyl acetate	19.1	28.0
borneol	2.8	7.6
germacrene D	1.6	
α-terpineol		2.4

It is a slow growing tree with a pyramidal to rounded shape. Young branches are bright green, flattened, articulated and flexible. A considerable quantity of these branches are shed during the dry season giving the tree better chances of surviving the long harsh summer. The tree flowers in winter, cones mature in summer and seeds are shed in early autumn.

Further botanical details have been recorded elsewhere (1,2). Although no significant structural differences have been reported in specimens from different stations in Malta and abroad, one does not exclude numerous ecotypes.

Ecologically, *T. articulata* is described as a thermophilous and xerophytic species, characteristics manifested throughout its range. It prefers south facing slopes and a gentle semi-arid climate with a rainfall optimum at around 350 mm/year. Edaphically it is also not very demanding, the species tolerating a wide range of soils and soil depths. Locally it grows best on a type of clayey calcareous soil known as "terra rossa." In Malta, it is a protected species with natural populations limited to a few score individuals. It was adopted as the National Tree of Malta in January 1992.

Experimental

Non-woody and woody terminal branches, mature female cones and seeds were used. Non-woody terminal branches refer to the soft green terminal growth without woody twigs which, on drying, easily fragment when hand crushed. Woody terminal branches refer to the mature woody supporting branches between 0.5-1 cm diameter. These parts were collected from nine, 30 years old trees growing at the University Campus (Msida, Malta). Woody and non-woody terminal branches were collected in July and November respectively, mature cones and seeds in early October just before cones opened (voucher specimens are deposited at Argotti Herbarium (ARG), c/o University of Malta, Msida; numbers ARG 002.96/07/31, ARG 007.96/10/9, ARG 010.97/11/3 and ARG 011/97/11/3, respectively).

A representative sample of each of the four parts indicated was dried to constant weight in a cool dry room, shaded from direct light. Samples (200 g of each) were mechanically fragmented in a blender and subjected to a hydrodistillation process in a Clevenger type apparatus for 2 h as given in British Pharmacopoeia (3).

The GC analysis were accomplished with an HP-5890 Series II instrument equipped with an HP-5 and an HP-WAX capillary columns (30 m x 0.25 mm, 0.25 μm film thickness), working with the following temperature program: 60°C for 10 min, ramp of 5°C/min up to 220°C; injector and detector temperatures 250°C, carrier gas nitrogen (5 mL/min), detector dual FID, split ratio 1:30 injection of 0.5 μL aliquots. The identification of components was performed for both the columns by comparison of their retention times with those of pure authentic samples, by means of their relative retention times with respect to two internal standards (butyl cellosolve and cellosolve acetate) in combination with our reliability factors method (4), and by means of hydrocarbon retention indices and ethyl ester retention indices.

Results and Discussion

The principal constituents of the oil isolated from various parts of *T. articulata* are α-pinene, limonene, camphor, bornyl acetate, borneole and germacrene D (Table I). The α-pinene content progressively increases from the woody (more mature) terminal branches (31.0%) to the non-woody (juvenile) terminal branches (46.4%) and cones (68.2%). The seeds also have a high α-pinene contents at 46.3%. The same progressive increase is observed for limonene passing from 3.8% to 6.2% and to 16.6%, respectively. The highest limonene content was recorded in the seeds at 25.3%.

Conversely, the levels of camphor and bornyl acetate diminish progressively from 18.1% and 19.1% to 0% and 1.8%, respectively. Also noticeable is the fact that α-terpineol, which is practically absent in the woody and non-woody branches, is present in the cones at around 5%. In the seeds, α-terpineol disappears and it is replaced by 5% of germacrene D.

When comparing the results obtained from woody branches with homologous parts of plants grown in Morocco (5), there is a noticeable difference in composition and yield. In the above mentioned study, α-pinene had very low to moderate levels [(between 1.1% and 9.7%, while the main components reported were borneol, bornyl acetate and camphor (Table II)]. Their study reported the presence of α-terpineol in woody branches but no germacrene D, whereas with our samples the reverse situation was true. Additionally, the paper reported finding cedrene and cedrol, both absent in our analysis. We confirm also the absence of thujone.

In the previous study, the authors interpreted the rather constant composition in the parts investigated as evidence for the absence of chemical polymorphism due to low influence from environmental and ecological factors. This cannot be confirmed from our results. Moreover their investigation did not include a comparative study of the oil contents of seeds and cones.

The marked differences observed in our study for the various components are interesting and probably have important underlying ecological and chemotaxonomic implications. The progressive increase in α-pinene from woody to non-woody terminal branches as well as cones and seeds possibly confirm the ecological function of this chemical - as a deterrent to potential grazers and

other pests given its antifeedant attributes. Similar attributes are given for camphor (6). If such antifeedant functions are indeed operating, it would be interesting to compare the oil composition of juvenile and mature plants as well as the oil composition at various levels above the ground for a mature plant.

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