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Comparative GC analyses of ripe fruits, leaves and floral buds essential oils of Tunisian *Myrtus communis* L.

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Abstract: The chemical composition of essential oils obtained by hydrodistillation from Tunisian wild growing myrtle ripe fruits, leaves and floral buds was examined by GC and GC-MS. The yields of hydrodistilled oils obtained from different plant parts were: leaves 0.5%, floral buds 0.2% and ripe fruits 0.02%. Significant differences were found in the concentration of main constituents of the oils: α-pinene [48.9% (floral buds), 34.3% (fruits), 23.7% (leaves)], 1,8-cineole [15.3% (floral buds), 26.6% (fruits), 61.0% (leaves)]. The leaves oil contained less linalool than floral buds and ripe fruits oils. Tunisian myrtle is characterized by the absence of myrtenyl acetate.

Keywords: Myrtus communis, Myrtaceae, essential oil composition, leaves, floral buds, fruits.

Introduction

Myrtus communis L. (commonly known as myrtle) is an evergreen shrub, belonging to the family of Myrtaceae, abundant in the Mediterranean region. In Tunisia, Myrtle tree is found growing in pine forests and riversides, particularly in the Ain Draham Mountains. Its leaves are commonly known due to the presence of essential oils and their compositions determine the specific aroma of plants and flavour of the condiment[1]. In folk medicines, leaves and fruits decoction or infusion of this plant are used as antiseptic, disinfectant and hypoglycaemic agent[2]. The oils extracted by steam distillation of fruits are used both in flavour and fragrance industries[3].

Until now, the majority of studies on myrtle have focussed on volatile compounds in leaves[4-8] and fruits[9,10]. To the best of our knowledge, a very little research has undertaken the chemical composition of floral buds essential oils[11]. In the present study, we have examined changes in essential oil composition of different myrtle organs namely: ripe fruits, leaves and floral buds harvested from wild growing plants in the north-western area of Tunisia, more precisely in the surroundings of 'Ain Draham'.

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Results and discussion

Essential oil yield

The essential oil yields obtained during this research were 0.5% for leaves, 0.2% for floral buds and 0.02% for ripe fruits. The highest yields were obtained for leaves and floral buds, but a very low yield of essential oils was obtained from mature myrtle fruits. The yield of extraction is linked to the harvest time, Jamoussi et al.[12] who studied the effect of harvest time on the yield and the composition of Tunisian myrtle oils and showed the existence of a correlation between the extraction yield and the vegetative cycle of the plant. The maximum and the minimum yields, respectively, were obtained in the middle (August) and at the end (September) of the flowering stage. These results were different to those of Pereira et al.[13] who showed that September seems to be the month with the best yields for all the parts of the plant. Bradesi et al.[14] recommend the period from June to November as the best harvest time for commercial production of essential oil. It may be suggested that these differences could be due to the effect of environmental conditions.

Chemical composition

The results obtained for the composition of the different essential oils extracted are shown in table 1. Thirty two, twenty three and thirty seven components were identified representing 93.9%, 97.8% and 98.7% as total composition from ripe fruits, leaves and floral buds, respectively.

All three parts of plant show a high content in monoterpenes, the floral buds exhibit a particularly high content in monoterpenes hydrocarbons (68.3%) with a large amount of α-pinene (48.9%). The leaves present high content in oxygenated monoterpenes (70.0%) with a large amount of 1,8-cineole (61.0%). The fruit essential oil composition additional to α-pinene and 1,8-cineole was characterized by the presence of aliphatic components (4.2%), benzenoid components (2.5%) and geranyl acetate (4.5%); there were, however, important amounts of α-Terpineol (4.4%) and linalool (5.9%).

Significant differences were found in the concentration of the main constituents of the oils: α-pinene [48.9% (floral buds)- 34.3% (fruits)- 23.7% (leaves)], 1-8,cineole [15.3% (floral buds)- 26.6% (fruits)- 61.0% (leaves)]. The α-pinene concentration decreased from 48.9% to 23.7% while 1,8-cineole increased from 15.3% to 61.0%. 1,8-cineole and α-pinene showed opposite variation, it's follow a cyclic evolution. According to a previous study[12-15] α-pinene and 1,8-cineole constituted the major fraction of Tunisian myrtle oils.

This variation in the distribution between the monoterpene hydrocarbons and the oxygenated monoterpenes in the plant materiel could be related to changes throughout the plant's vegetative cycle along with the environmental factors such as geography, temperature, day length and nutrients[16].

Myrtenyl acetate is one of the components that distinguish between myrtles of different origin. Its presence has been reported in the essential oils from Turkey[5], Croatia[10], Albania[9], Morocco[17], Spain[18] and Portugal[13]. In the present study, this compound is absent. This result

is in agreement with those obtained by Messaoud et al.[15] and Jamoussi et al.[12] who reported the absence of myrtenyl acetate in the Tunisian myrtle oils.

Table 1. Chemical composition of Tunisian myrtle oils

Constituents	**RI**	**Percentage composition[a]**			**Identification[b]**
		Ripe fruits	**leaves**	**Floral buds**	
Total aliphatic compounds		**4.2**	**1.1**	**0.70**	
Ethyl isobutyrate	761	-	-	0.2	RI, MS
Isobutyl isobutyrate	901	1.4	0.6	-	RI, MS
Isobutyl 2-methylbutyrate	1010	2.2	0.4	0.5	RI, MS
2-Methylbutyl isobutyrate	1014	0.6	-	-	RI, MS
Geranyl isobutyrate	1516	-	0.1	-	RI, MS
Total monoterpene hydrocarbons		**36.8**	**25.7**	**68.3**	
Tricyclene	924	-	-	0.1	RI, MS
α-Thujene	928	-	0.2	0.5	RI, MS, Co-GC
α-Pinene	938	34.3	23.7	48.9	RI, MS, Co-GC
α-Fenchene	948	-	-	0.2	RI, MS
Camphene	950	0.4	-	0.1	RI, MS, Co-GC
Sabinene	972	-	-	0.4	RI, MS
β-Pinene	980	0.5	0.5	0.1	RI, MS, Co-GC
Myrcene	991	0.2	0.2	0.1	RI, MS, Co-GC
δ-3-Carene	1012	-	0.5	1.6	RI, MS
α-Terpinene	1018	-	-	0.4	RI, MS, Co-GC
p-Cimene	1025	-	-	2.0	RI, MS
Limonene	1032	-	-	6.5	RI, MS, Co-GC
(Z)-β-Ocimene	1040	-	-	0.1	RI, MS
(E)-β-Ocimene	1048	-	-	2.1	RI, MS
γ-Terpinene	1063	0.6	0.4	2.5	RI, MS, Co-GC
Terpinolene	1093	0.8	0.2	2.7	RI, MS, Co-GC
Oxygen-containing monoterpenes		**46.6**	**70.0**	**27.0**	
1,8-Cineole	1033	26.6	61.0	15.3	RI, MS, Co-GC
Linalool	1101	5.9	1.7	3.1	RI, MS, Co-GC
trans-Pinocarveol	1155	0.2	0.3	-	RI, MS
p-Mentha-1,5-dien-8-ol	1181	-	0.2	-	RI, MS
Borneol	1163	0.2	-	0.2	RI, MS, Co-GC
Terpinen-4-ol	1179	0.5	0.8	0.3	RI, MS
α-Terpineol	1189	4.4	3.3	0.8	RI, MS
Myrtenol	1202	-	-	2.7	RI, MS
Nerol	1228	1.2	-	-	RI, MS
Carvone	1234	1.9	-	-	RI, MS
Citral	1237	0.3	-	-	RI, MS
Geraniol	1257	0.3	0.6	2.3	RI, MS
Linalyl acetate	1262	-	-	1.8	RI, MS, Co-GC
exo-2-Hydroxycineole acetate	1354	0.2	0.2	-	RI, MS
Neryl acetate	1368	0.4	-	0.2	RI, MS
Geranyl acetate	1384	4.5	1.9	0.3	RI, MS

Total benzenoid compounds		**2.5**	**0.3**	**1.7**	
Methyl chavicol	1215	0.3	-	-	RI, Ms
Eugenol	1357	-	-	1.1	RI, MS, Co-GC
Methyl eugenol	1404	2.2	0.3	0.6	RI, MS, Co-GC
Total sesquiterpenoid compounds		**3.8**	**0.7**	**1.0**	
β-Elemene	1392	-	-	0.1	RI, MS
β-Caryophyllene	1419	0.8	0.3	0.2	RI, MS
γ-Elemene	1433	-	-	0.1	RI, MS
α-Humulene	1454	0.5	0.1	-	RI, MS
allo-Aromadendrene	1460	-	-	0.1	RI, MS
α-Curcumene	1481	0.4	-	-	RI, MS
α-Zingiberene	1495	0.7	-	-	RI, MS
β-Bisabolene	1510	0.3	-	-	RI, MS
Spathulenol	1576	-	-	0.3	RI, MS
Caryophyllene oxide	1584	0.6	0.3	0.1	RI, MS
Humulene epoxide II	1603	-	-	0.1	RI, MS
β-Eudesmol	1648	0.2	-	-	RI, MS
a-Cadinol	1653	0.3	-	-	RI, MS

[a] Percentages (mean of three analyses) obtained by FID peak area normalization, all relative response factors being taken as one.
[b] RI: Relative retention indices to C_8-C_{24} *n*-alkanes on HP-5MS column, MS: mass spectrum, Co-GC: co-injection with authentic compounds.

Conclusion

Tunisian myrtle essential oils from different parts of the plant were obtained using Dean stark hydrodistillation. Yields are 0.5% for the leaves, 0.2% for the floral buds and 0.02% for the ripe fruits. 1,8-Cineole 61.0% (leaves) and α-pinene 48.9% (floral buds) constituted the major fraction of Tunisian myrtle oils. Linalool is also present at 5.9% in fruit. All three parts of the plant show approximately the same components, varying in proportions. The Tunisian myrtle essential oils are characterized by the absence of myrtenyl acetate.

Experimental Section

Chemicals

α-Thujene, α-pinene, camphene, β-pinene, myrcene, α-terpinene, limonene, γ-terpinene, terpinolene, 1,8-cineole, linalool, borneol, linalyl acetate, eugenol, methyleugenol, alkane standard solutions (C_8-C_{24}) were from Fluka Chemika.

Plant material

Leaves, fruit and floral buds were collected from plants grown in the region of Ain Draham over a period of time that covers the principal stages of the plant's vegetative cycle namely, flowering and ripe fruit. Thus, the months covered by this study were June (floral buds and leaves) and January (ripe fruit with dark blue colour).

After the botanical identification according to the Tunisian flora[19], the plant material was selected and cleaned of impurities in the laboratory.

Essential oil extraction

The essential oil was obtained by hydrodistillation using a Dean stark apparatus until there was not significant increase in the volume of oil collected to give the following yields (w/w). The oil were dried over anhydrous sodium sulphate and stored under N_2 at 4°C.

GC and GC-MS analysis

Analyses were performed on a Hewlett-Packard gas chromatograph, Model 6890, equipped with a flame ionization detector. Analytical conditions: HP-5 MS 5% phenylmethylsiloxane capillary column (30m × 0.25mm, film thickness 0.25 µm); carrier gas, helium; flow rate, 0.9ml/min; split, 1:10; injector temperature, 250°C; detector temperature, 280°C. The oven temperature was held for 1 min at 40°C, then programmed from 40°C, to 250°C at 2°C/min. GC-MS analysis was carried out on a HP 6890 instrument coupled to a Hewlett-Packard 5973N MS computerized system, ionization voltage 70eV, electron multiplier 1670V, ion source temperature 230°C, GC conditions as above. Individual components were identified by comparison of their GC retention indices[20] and MS spectra with those reported in the literature[21] and by computer matching with the Wiley 238.L library and, whenever possible, by co-injection with authentic compounds. The percentages of the compounds were calculated from the GC peak areas, using the normalization method.

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