Biochemical Systematics and Ecology 80 (2018) 63–69

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Essential oil variation of *Thymbra spicata* L. (Lamiaceae), an East Mediterranean "oregano" herb

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ARTICLE INFO

Keywords:
Aromatic plants
Chios Island
Oregano
Thyme
Carvacrol
Thymol

ABSTRACT

This is the first account of the essential oils of wild Greek plants of *Thymbra spicata*, an East Mediterranean species used as a culinary herb and herbal tea. In particular, the yield and composition of the essential oil of *Th. spicata* from Chios Island is recorded and compared between two seasons. Considering that Chios provides the westernmost studied material of *Th. spicata*, the geographical variation in the main essential oil compounds in the total natural range of the species in relation to its systematics is also discussed. The essential oil yield of the Chios plants ranged from 2.4 to 4.8 mL 100 g^{-1} dry weight (aerial parts without stems). Carvacrol (responsible for the "oregano" scent) was dominant in considerably high amounts (67.5%–88.3% of the total oil) and without significant seasonal differences. Other main compounds were *p*-cymene (0.7%–17.4%), γ-terpinene (0.1%–6.9%) and caryophyllene oxide (0.9%–2.5%). Thymol (responsible for the "thyme" scent) was always very low (0.3%–0.7%). Throughout the East Mediterranean, carvacrol is dominantly the major essential oil compound of *Th. spicata*. *p*-Cymene, γ-terpinene and other, more minor, compounds contribute to local differences in the species' essential oils in its total range. The thymol-chemotype is scarce throughout the species' range and not related to the local Turkish subspecies *intricata* as previously suggested. Despite the closer taxonomic affinities with the genus *Thymus* and the thyme-related vernacular names attributed to *Th. spicata*, this species can be characterized as an "oregano" with regard to its essential oil composition and herbal use.

1. Introduction

Thymbra L. is a small genus in the Lamiaceae family comprising four species distributed in the Mediterranean region. Among them, *Th. spicata* L. is an East Mediterranean species growing wild from Iran westwards to Greece (Morales, 1987). It is an essential oil-rich species, sometimes with considerably high essential oil content (Müller-Riebau et al., 1997). The main essential oil compound is carvacrol, an oxygen-containing monoterpene with phenol-like behaviour, which is responsible for the "oregano" scent and has significant antioxidant, antibacterial and other activities (Baser, 2008), In *Th. spicata* carvacrol sometimes reaches more than 90% of the total oil (Kizil, 2010). Commonly known among others as "kekik", "kara kekik" or "zatar", *Th. spicata* is used as a culinary herb, herbal tea and folk medicine in East Mediterranean countries (Ravid and Putievsky, 1985; Kizil, 2010).

The essential oils of *Th. spicata* plants from Turkey have been extensively studied (Tümen et al., 1994; Müller-Riebau et al., 1997; Akgül et al., 1999; Baydar et al., 2004; Fleischer and Fleischer. 2005; Ili et al., 2007; Sarac et al., 2009; Ünlü et al., 2009; Jamil et al., 2010; Kizil, 2010; Saidi et al., 2012; Barakat et al., 2013), while some other studies concern plants from Israel (Ravid and Putievsky, 1985; Fleisher and Fleisher, 2005) Lebanon (Barakat et al., 2013), Iraq (Jamil et al., 2010) and Iran (Saidi et al., 2012). In these studies, the plant parts which have been used are predominantly the aerial parts including stems, or the leaves. In most cases the essential oils were isolated using hydrodistillation for a duration of 2.5 h (Kizil, 2010), 3 h (Tümen et al., 1994; Müller-Riebau et al., 1997; Akgül et al., 1999; Baydar et al., 2004; Ünlü et al., 2009; Barakat et al., 2013) or 4 h (Sarac et al., 2009; Saidi et al., 2012). The essential oil yields range from 0.3 to 6.6 mL 100 g^{-1} dry weight (DW) for aerial parts including stems (Tümen et al., 1994; Akgül et al., 1999; Baydar et al., 2004; Fleisher and Fleisher, 2005; Sarac et al., 2009; Ünlü et al., 2009; Kizil, 2010; Saidi et al., 2012; Barakat

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https://doi.org/10.1016/j.bse.2018.06.006
Received 16 April 2018; Received in revised form 31 May 2018; Accepted 24 June 2018
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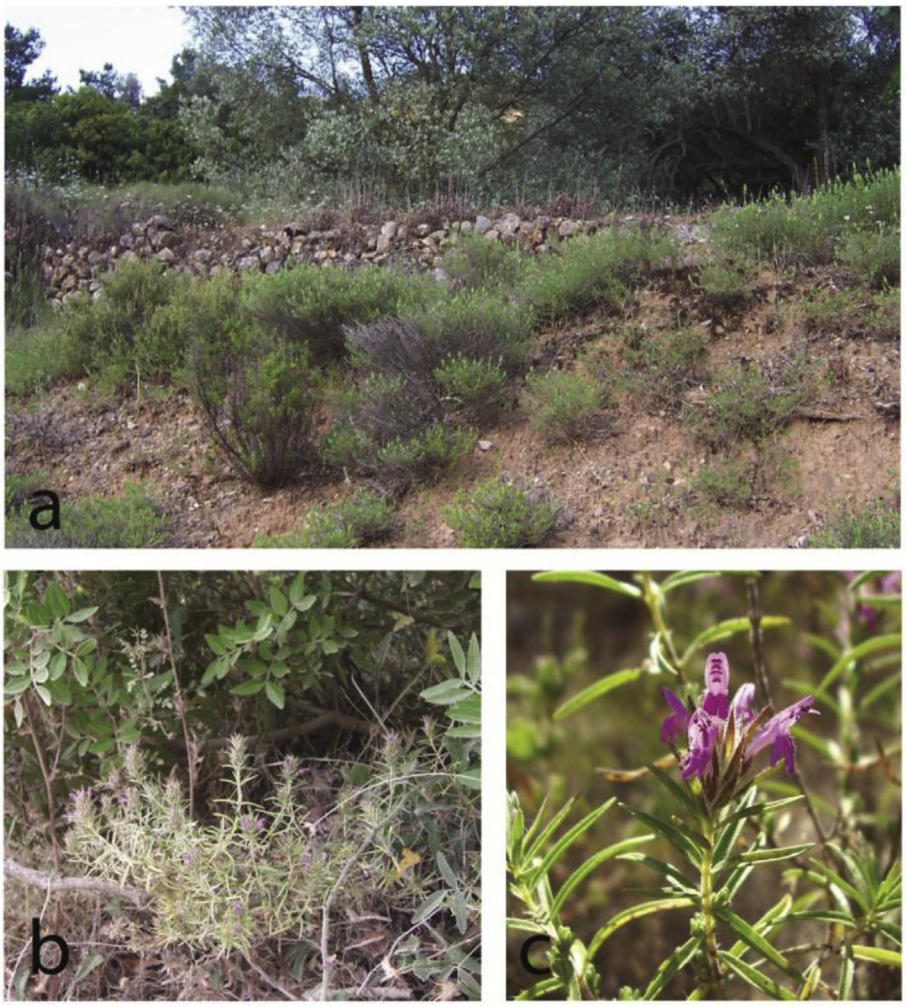

Fig. 1. *Thymbra spicata* plants growing wild at the edges of olive- (a) and mastic-groves (b) in Chios Island, Greece, and detail of a flowering individual (c).

et al., 2013) and from 2.9 to 10.8 mL 100 g^{-1} DW for the leaves (Tümen et al., 1994; Müller-Riebau et al., 1997; Ili et al., 2007). The main essential oil component of *Th. spicata* in the majority of cases was carvacrol while in a few cases it was thymol (Ravid and Putievsky, 1985; Tümen et al., 1994; Fleisher and Fleisher, 2005). Regarding Greece, the westernmost limit of the species' natural range, there is only one study on cultivated plants of unspecified origin (Marković et al., 2011). The essential oils of wild Greek *Th. spicata* plants have not been examined up to now.

In this study the essential oils of *Th. spicata* growing wild on Chios Island (East Aegean Islands, Greece) are examined. *Th. spicata* is locally common in the southern part of Chios, where it grows on the edges of traditional orchards (mastic, olive), pine forests, shrubland and occasionally on the banks of seasonal rivulets, from sea level up to 400 m (Fig. 1) (Stefanaki, 2012; Stefanaki and Kokkini, 2015). It is collected and used by local people but is less popular than the other carvacrol-rich plants growing wild on Chios, i.e. *Th. capitata* and *Origanum onites*, which are more abundant on the island (Stefanaki et al., 2010, 2016; Stefanaki and Kokkini, 2015). In particular, we present the yield and composition of essential oils of *Th. spicata* in Chios and assess whether the main essential oil quality characteristics of *Th. spicata* in Chios, i.e. the yield and content of main compounds, vary between different seasons (spring, summer). Chios, which lies at the phytogeographical border between Asia and Europe (Stefanaki and Kokkini, 2015), provides the westernmost studied material of *Th. spicata*, we therefore explore also the geographical patterns of the main essential oil compounds throughout the species' total natural range in relation to its systematics.

2. Materials and methods

2.1. Plant material

The aerial parts of *Thymbra spicata* plants were collected from 20 locations on Chios Island (Fig. 2; Table S1, Supporting information), ten of which during May and ten in July. Each sample contained at least ten randomly selected individuals. The plant material was air-dried in the shade. Voucher specimens were deposited in the Herbarium of Aristotle University of Thessaloniki (TAU).

2.2. Essential oil isolation

The dried plant material was hydrodistilled for 2 h using a Clevenger type apparatus. Only leaves and inflorescences of the dried material were distilled in order to avoid inconsistences in the results deriving from the different amounts of woody stems that have a negligible contribution to the essential oil yield. The essential oil was kept until analysis at 4 °C in the dark. The oil yield was expressed as mL 100 g^{-1} DW.

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