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Variation of phenolic constituents of Tunisian *Thymus capitatus* (L.) Hoff. et Link. populations

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ABSTRACT

Nine Tunisian *Thymus capitatus* (L.) Hoff. et Link. populations harvested in six bioclimatic zones extending from the sub humid (550 mm rainfall/year) to the lower arid (96.3 mm rainfall/year), and grown at altitudes ranging from 60 to 600 m were assessed for their phenolic composition. The identification of phenolic compounds was revealed by UHPLC-DAD-ESI/MS^n. 11 phenolic compounds including three phenolic acids (rosmarinic acid, salvianolic acid A, salvianolic acid E), three flavanones (hesperidin, eriodictyol and naringenin), two C-glycoside isomers of the flavone apigenin, one flavanol gallocatechine, one flavanonol taxifolin and one phenolic monoterpen (carvacrol) were identified. Differences in phenolic composition among populations, in relationship with altitudes and/or annual rainfall were shown. Higher amounts of total phenols, total flavonoids, rosmarinic acid and carvacrol were detected in samples from the most arid zones (96.3–167 mm rainfall/year) in comparison with populations located in wet lands (450–660 mm rainfall/year). The altitude within or between contiguous climates is a main factor influencing phenol compounds content. The chemical variation among the populations should lead to the selection of plants with high potential in order to use them in health care and food industry.

1. Introduction

Phenolic compounds including, among others, flavonoids, phenolic acids, flavones, lignanes and coumarines, are presently the focus of intense research to assess their usefulness to treat chronic diseases related to oxidative stress and free radical induced cell damage (e.g. cardiovascular, neurodegenerative, inflammatory, cancer and diabetes). These compounds, can act as free radical scavenging, reducing agent chelating, transition metals and regulating defense enzymes. They are also used in cosmetic and food industries as natural antioxidants (Mitjavila and Moreno, 2012; Saxena et al., 2012).

Among numerous plants, the genus *Thymus* (Lamiacea) with 200–350 perenial herbaceous diploids (2n = 2x = 24, 26, 28, 30, 32), tetraploids (2n = 4x = 52, 56, 58, 60) and hexaploids (2n = 6x = 42, 48, 84, 90) was more and more assessed for its polyphenols (Cronquist, 1988; Morales, 1996; Zaidi and Crow, 2005; Ben El Hadj Ali et al., 2010). Thymes due to its phenolic composition various properties have been described like antibacterial (Varga et al., 2015), antifungical (Alizadeh et al., 2013; Pinto et al., 2013), anti-inflammatory (Fachini-Queiroz et al., 2012), antioxidant (Safaei-Ghomi et al., 2009; Galasso et al., 2014; Parveen and Kyunn, 2016) and antidegenerative (Mata et al., 2007; Albano et al., 2011; Kindl et al., 2015; Miguel et al., 2015) activities. Flavonoid aglycones, flavonoid glycosides, phenolic acids and phenol terpenoids (i.e. carvacrol and thymol) were known as main phenolic compounds involved in these properties (Morimitsu et al., 1995; Stahl-Biskup and Sáez, 2002; Boros et al., 2010; Fachini-Queiroz et al., 2012). However, quantitative and qualitative phenol diversity within and among taxa linked to taxonomic ranks, genetic, environmental and biological traits have been reported (Dajic Stevanovic et al., 2008; Ben El Hadj Ali et al., 2012a; Delgado et al., 2014; Saija et al., 2016).

Thymus capitatus (L.) Hoff. et Link. [syn. *Thymbra capitata* (L.) Cav., syn. *Coridothymus capitatus* (L.) Reichb.fil., syn. *Satureja capitata* L.], a diploid species (2n = 30), belongs to the subgenus

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Coridothymus [(Reichb.f) Borbas], and is characterized by erect spearing branches (20–40 cm high), small linear and glandulous spotted leaves, ovoid terminal inflorescences with pink flower corolla (Jalas, 1971). It is well represented in the Mediterranean garrigues, dry slopes and is typical of degraded *Pinus halepensis* and *Juniperus phoenicea* forests. In Tunisia, the species is widespread in the North, the dorsal ridge, the Cap Bon and becomes rarest in the southern part. In traditional medicine, *T. capitatus* is used to treat intermittent fever and rheumatism, as antihypertensive agent, expectant and stimulant for the blood circulation (Megdiche-Ksouri et al., 2015).

In spite of its wide range of distribution and the heterogeneity of its habitats, *T. capitatus* showed a relative homogeneity of the composition of its essential oils dominated by carvacrol (Ben El Hadj Ali, 2010; Salas and Téllez, 2013; Miguel et al., 2015). The most occurring phenols in extracts of aerial parts are flavonoids in the form of aglycones (luteolin, apigenin), phenolic acids (i.e. ferulic, isoferulic, chlorogenic, cinnamic and rosmarinic acids), flavones and phenol terpenoids such as carvacrol and thymol (Jabri-Karoui et al., 2012; Msaada et al., 2015).

Both, oils and phenols, of *T. capitatus* were endowed with antimicrobial (Faleiro et al., 2005), antioxidant (Salas and Téllez, 2013; Msaada et al., 2015; Saija et al., 2016), anti-proliferative (Miguel et al., 2010), antifungal (Salgueiro et al., 2004; Palmeira-De-Oliveira et al., 2013), neurodegenerative (Albano et al., 2011) and parasitical (Machado et al., 2010) activities. Marked differences among these biological activities associated to the chemical composition of extracts were reported (Faria et al., 2013). However, few studies have evaluated the variation of phenols and their biological activities among populations in relationships with ecological conditions.

The aim of this study is to assess the infraspecific variation of phenol composition among nine Tunisian *T. capitatus* populations distributed throughout its natural area in Tunisia. This work, conducted jointly with those performed on the genetic diversity of the species and several of its biological properties (i.e. antioxidant, antiacetylcholinesterase, antidiabetic effects…) that we presently analyze constitutes a first step to ensure the rational use of the natural populations, and should allow the detection of plants with high potential for medicinal and industrial uses.

2. Materials and methods

2.1. Plant material

Nine populations of *Thymus capitatus*, growing wild in different geographic regions, were assessed for their phenols. Samples were determined by Pr. BOUSSAID Mohamed. Voucher specimens were deposited at the Herbarium of the National Institute of Applied Science and Technology of Tunis (ref. T.A.15–3.17). The populations belong to six bioclimatic zones: sub-humid (sh), upper semi-arid (usa), means semi-arid (msa), lower semi-arid (lsa), upper arid (ua) and lower arid (la), according to Emberger's Q_2 pluviothermic coefficient [$Q_2 = 2000P/(M^2\text{-}m^2)$ where P is the mean annual rainfall (mm), M is the average maximum temperatures (°K) in the warmest month (June) and m (K) is the mean minimum temperatures in the coldest month (February)]. The altitude of sites ranged from 60 to 600 m (Table 1). In each population, 10 individuals at the flowering phase were sampled at random in an area exceeding 2 ha. From each plant leaves were taken and kept for drying during 7 days at room temperature, then ground to powder before analysis.

2.2. Quantification and identification of phenolic compounds of methanolic extracts

Methanolic extracts were prepared using 1 g of dry leaves. After maceration in 10 mL of methanol for 24 h at room temperature, the samples were filtered and stored in a cooling room (+4 °C) until analysis.

2.2.1. Total phenolic contents

The total phenols for each individual were determined using a spectrophotometric method (Singleton et al., 1999). An aliquot of each diluted sample extract (0.5 ml) was mixed with 2 ml Folin-Ciocalteu reagent. After 5 min, 2.5 ml of sodium carbonate solution (7.5%) was added. After incubation (90 min) in dark, the absorbance of samples versus that of the blank was read at 760 nm. Total phenols were expressed as Gallic acid equivalents (mg l^{-1} of GAE).

2.2.2. Total flavonoid contents

The total flavonoid content was determined according to Chetoui et al. (2013). One milliliter of the sample diluted was mixed with 1 mL of 2% $AlCl_3$. After incubation for 15 min, the absorbance was measured at 430 nm. The percentage contents of flavonoids were expressed as mg Rutin equivalent/g DW (mg ER/g DW), using the calibration curve of Rutin (0–400 μg/ml range).

2.2.3. Identification and quantification of phenolic compounds by UHPLC–DAD–ESI/MS^n

The LC-DAD-ESI/MS^n analysis was performed on an Ultimate 3000 (Dionex Co., USA) apparatus equipped with an ultimate 3000 Diode Array Detector (Dionex Co., USA) and coupled to a mass spectrometer. Analysis was run on a Hypersil Gold (Thermo Scientific, USA) C18 column (100 mm length; 2.1 mm i.d.; 1.9 μm particle diameter, end-capped) and its temperature was

Table 1
Main ecological traits of the nine analysed Tunisian *Thymus capitatus* populations.

Populations	Code	Bioclimatic zone[a]	Q_2	Rainfall[b] (mm/year)	Latitude	Longitude	Altitude(m)
Korbous	1	sub humid (sh)	$70 < Q_2 < 120$	550	36°50′	10°35′	280
Essabahia	2	upper semi-arid (usa)	$45 < Q_2 < 70$	450	36° 36′	10°10′	112
Jendouba	3	upper semi-arid (usa)		660	36°25′	8°44′	150
Sers	4	mean semi-arid (msa)	$37 < Q_2 < 45$	245	36°6′	9°40′	474
Gbollat	5	mean semi-arid (msa)		245	36°22′	9°52′	150
Siliana	6	mean semi-arid (msa)		293	35°51′	9°12′	450
Sousse	7	lower semi-arid (lsa)	$35 < Q_2 < 37$	167	35°30′	10°50′	70
Gabes	8	lower arid (la)	$28 < Q_2 < 35$	96.3	33°53′	10°70′	60
Toujene	9	upper arid (ua)	$10 < Q_2 < 28$	134	33°27′	10°08′	600

Q_2: Emberger's pluviothermic coefficient.
[a] Bioclimatic zones were defined according to Emberger's (1966) pluviothermic coefficient.
[b] Values are average established on five years (2010–2015).