P 9: Identification of volatile components in two *Thymus* species from Iran and their antioxidant properties

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DOI 10.5073/jka.2016.453.042

Abstract

Thymus species are well known to have significant amount of phenolic compounds and exhibit strong antioxidant activities.

This study is designed to analyze the essential oils of two Iranian *Thymus* species, (*T. kotschyanus* Boiss. et Hohen and *T. pubescense* Boiss. et Kotschy ex Celak) obtained by hydrodistillation of aerial part of this plants, using GC-FID and GC/MS and evaluate the *in-vitro* antioxidant activities in two quantitative methods (namely $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ assay) to determine the total phenolic content of the species (assayed by colorimetric techniques) and to study the possible composition-antioxidant activity relationship.

The major aroma constitutes in the essential oil of *T. pubescense* were found to be thymol (38.7 %), γ-terpinene (7.5 %), *p*-cymene (5.5 %), α-terpenyl acetate (3.8 %) and β-bisabolene (3.7 %) while in the essential oil of *T. kotschyanus*, α-terpineol (16.9 %), 1,8-cineol (14.4 %), linalool (9.6 %), thymol (7.2 %) and geranyl acetate (5.4 %) were the main compounds.

Both of the tested essential oils exhibited concentration-dependent antioxidant activity. *T. pubescense* showed more activity in both $DPPH^{\cdot}$ [IC_{50}= 285.2 (236.5-344.0) µg/mL] and $ABTS^{\cdot+}$ methods [IC_{50}= 1.956 (1.810-2.113) µg/mL], as well as total phenolic content of *T. pubescence* [70254 ± 0.0049 µg/mg] was found to be slightly higher than *T. kotschyanus* [62933 ± 0.0026 µg/mg].

Keywords: *T. Pubescense; T. kotschyanus*; Antioxidant activity; Essential oil; Chemical composition; Labiateae.

Introduction

Thymus is an important genus of the Labiateae family, originated from the Mediterranean region. Among 300 to 400 species of this genus grown in the World, 14 species are distributed in the Iranian flora. Leaves and flowering part of *Thymus* species are commonly used in traditional medicine, as tonic and herbal tea, antiseptic, antitussive and carminative. In addition, *Thymus* essential oils are widely used in pharmaceutical, cosmetics and perfume industry, also for flavoring and preservation of several food products. Indeed, many species of this genus are well known for their health-benefit effects, including antioxidant, anti-inflammatory, antibacterial, antifungal, antiviral, antiparasitical and antispasmodical activity (Stahl-Biskup and Saez, 2002). In turn, these properties have been associated to phenolic composition of these plants.

The aim of the present work is to evaluate and compare the antioxidant activities of essential oils obtained from two Iranian *Thymus* species (*T. kotschyanus* and *T. pubescense*) by different methods. Because of the important role of the phenolics as potent antioxidants, the total amounts of the compounds are also determined.

Materials and Methods

Plant materials: The aerial parts of *T. kotschyanus* and *T. pubescense* were collected respectively from Azerbaijan and Khorasan provinces, in Iran, June 2013.

Isolation of the essential oils: The dried aerial parts of the plants were subjected to hydrodistillation for 3h using Clevenger-type apparatus to obtain the essential oils.

Quantitative Antioxidant assays

DPPH· Assay: The free radical scavenging abilities of the samples were measured using the stable radical DPPH· and IC_{50} were calculated for both essential oils and standards (NICKAVAR et al., 2007)

$ABTS^{\cdot+}$ assay: The antioxidant capacity of the samples were evaluated by a method based on the decolonization of radical cation of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$) and IC_{50} were calculated for both essential oils and standards (NICKAVAR et al., 2008)

Qualitative Antioxidant assay: Bioautographical analysis (Both of essences were loaded on silica gel plate (TLC); after running, the plates were observed under UV254 light and then sprayed by DPPH· and $ABTS^{\cdot+}$ solutions and the results were compared)

Total phenolic content: The total phenolic contents (TPCs) of the extracts were determined spectrophotometrically by using Folin-Ciocalteu reagent and then calculated as gallic acid equivalents (NICKAVAR et al., 2008)

GC-FID and GC/MS analysis condition: Quantitative data were obtained from the electronic integration of the FID peak areas. The components of the essential oils were identified by comparision of their mass spectra and retention indices published in the literature (ADAMS, 1995) and presented in the MS computer library (WILY275.L).

Results

Tab. 1 Antioxidant potency of the studied Thymus species, *(p>0.05)

Plant species/standard	IC_{50} (DPPH·) * (µg/mL)	IC_{50} ($ABTS^{\cdot+}$) * (µg/mL)
T. pubescens	246.7 (210.7-288.9)	1.859 (1.709-2.022)
T. kotschyanus	599.1 (570.3-629.3)	9.017 (8.197-9.919)
Vitamin C	2.016 (1.743-2.331)	0.5044 (0.4762-0.5343)
Thymol	38.76 (33.19-45.28)	1.178 (1.091-1.272)

Tab. 2 Total phenolic contents of the studied Thymus species, *(p>0.05)

Plant species	Essential oil density*(g/mL)	Total phenolic content* (µg gallic acid/mL essential oil)
T. pubescens	0.9508±0.0032	70254 (69920-70588)
T. kotschyanus	0.9224±0.0086	62933.25 (62636-63230)

Tab. 3 Top ten chemical composition of T. pubescens and T. kotschyanus essential oils, *RI (retention index) measured relative to n-alkanes (C9–C18) on the non-polar HP-5-DB-5 column

	T. pubescens			*T. kotschyanus*		
	Compound	**RI***	**Content (rel. %)**	**Compound**	**RI***	**Content (rel. %)**
1	Thymol	1302	38.67	α-Terpineol	1199	16.94
2	γ-Terpineol	1057	7.46	1,8-Cineol	1032	14.37
3	*p*-Cymene	1024	5.54	Linalool	1096	9.65
4	α-Terpinyl acetate	1352	3.78	Thymol	1293	7.16
5	β-Bisabolene	1508	3.71	Geranyl acetate	1382	5.36
6	Linalyl acetate	1276	3.54	Geraniol	1256	3.71
7	α-Pinene	935	3.54	Borneol	1166	3.59
8	Carvacrol	1311	3.07	Spatulenol	1586	3.40
9	α-Terpineol	1204	2.99	Terpinen-4-ol	1178	2.60
10	Linalool	1093	2.84	Carvacrol	1302	2.29

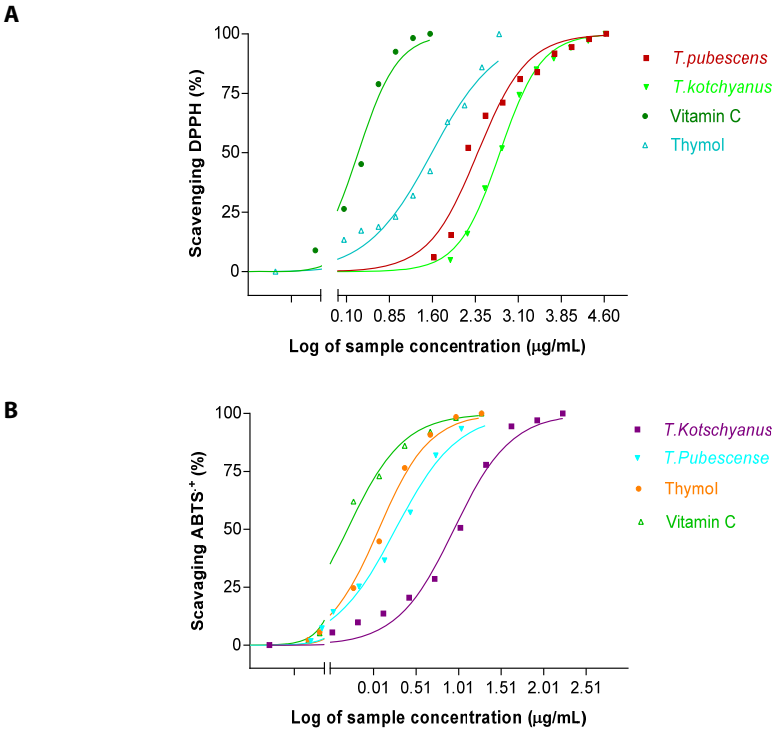

Fig. 1 Dose-dependent antioxidant activities of the studied Thymus extracts measured by using (A) the DPPH. Assay and (B) the ABTS.+ assay. Each point represents the mean of three experiments.

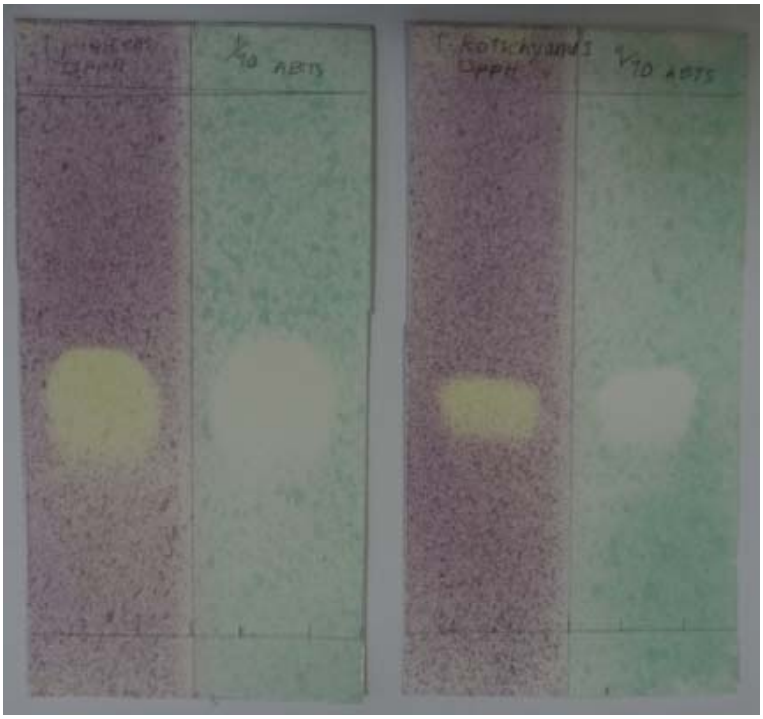

Fig. 2 Bioautographical analysis of T. pubescens and T. kotschyanus sprayed by DPPH. and ABTS.+ solutions.

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