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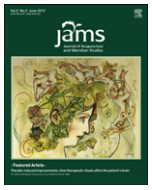

RESEARCH ARTICLE

Evaluation of the Antioxidant Capacity and Phenolic Content of Three *Thymus* Species

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Abstract
Thymus species are known to have significant amounts of phenolic and flavonoid compounds and exhibit strong antioxidant activities. This work was designed to evaluate the antioxidant activities of three endemic Iranian *Thymus* species (including *T. daenensis*, *T. kotschyanus*, and *T. pubescens*) in different test systems [namely DPPH$^{\bullet}$ (2,2′-diphenyl-1-picrylhydrazyl), ABTS$^{\bullet+}$ [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid], and linoleic acid/β-carotene bleaching assays] to determine the total phenolic and flavonoid contents of the species (assayed by colorimetric techniques) and to study the possible composition-activity relationship. All the tested plants exhibited concentration-dependent antioxidant and free radical scavenging activities. *T. pubescens* showed the highest free radical scavenging activities in both DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ methods, while *T. daenensis* and *T. kotschyanus* were the most active species in the β-carotene bleaching inhibition test. Alternatively, *T. pubescens* exhibited a significantly higher level of the total flavonoid content compared with those of the other species, while no significant statistically differences were found among the tested plants regarding the total phenolic content. In addition, significant correlations were found between the flavonoid content and DPPH$^{\bullet}$/ABTS$^{\bullet+}$ radical scavenging activities, but not between the β-carotene bleaching inhibition system and the flavonoid content.

1. Introduction

Plants are good sources of active natural products that differ widely in terms of structure and biological properties so; they can be used for various applications, especially as food additives and health promoting ingredients. For the reason, during last few decades, they have been become a subject for study of bioactive compounds [1].

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A growing amount of evidence has shown that the oxidative stress and free radicals play the important roles in the etiology of some chronic diseases. Epidemiological studies have revealed that the consumption of antioxidants is positively associated with the reduced risk of developing chronic and ageing related diseases. On the other hand, synthetic antioxidants have been shown to be potentially toxic. Therefore, there is a growing interest in searching for antioxidants naturally present in plants [2]. Numerous crude extracts and pure natural compounds have been reported to have antioxidant and radical scavenging activities [3]. Among the various kinds of plant constituents, phenolic compounds and flavonoids have received particular attention as potential natural antioxidants [4,5]. They can delay or inhibit the oxidation of molecules by inhibiting the initiation or propagation of oxidative chain reactions [6].

A great number of aromatic and spicy plants, especially from Labiatae family, contain chemical compounds with antioxidant properties. For example, rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis* L.) have been reported to be rich in phenolic compounds and possess antioxidant properties comparable with synthetic antioxidants [7]. Among the Labiatae members, various *Thymus* species are among the most popular plants throughout the world. They are commonly used as herbal teas, flavoring agents, and aromatic and medicinal plants. These species have been used as carminative, antitussive, and expectorant agents. They are also reported to possess several and potent biological and pharmacological properties such as anti-inflammatory, antispasmodic, among others. Recent studies on some *Thymus* species indicate that they possess antioxidative and hepatoprotective activities [8].

The genus of *Thymus* consists of about 215 species which particularly prevalent in the Mediterranean area [8]. The genus is represented in the flora of Iran by 14 species which some of them are endemic such as *T. daenensis*, *T. pubescencs,* among others [9]. They are widely used as herbal tea, condiment, spice, and digestive and to treat whooping cough, bronchitis, inflammation and rheumatism in Iranian folk medicine [10—12]. Despite the considerable antioxidant activities shown by a lot of *Thymus* species investigated, there are few studies that evaluate Iranian *Thymus* species. However, these studies have been focused on the essential oils obtained from the plants. According to the study of Alavi and colleagues [13], the essential oil from *T. daenensis* was found to be active in some of antioxidant assays. Asbaghian and colleagues [14] have shown that the essential oil of *T. kotschyanus* was an effective scavenger of the DPPH$^{\bullet}$ free radicals. Based on the study of Nazemiyeh and his co-workers [15], the essential oil of *T. pubescens* had an anti-2,2'—diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) activity. The aim of the present work was to evaluate and compare the antioxidant activities of the hydroalcoholic extracts obtained from three Iranian *Thymus* species (including *T. pubescence* Boiss. & Kotschy ex Celak, *T. daenensis* Celak, and *T. kotschyanus* Boiss. & Hohen) by different methods. Because of the important roles of the phenolics and flavonoids as potent antioxidants, the total amounts of the compounds were also determined.

2. Materials and methods

2.1. Plant materials

The aerial parts of *T. daenensis* and *T. kotschyanus* were collected in Hamedan province and *T. pubescens* was collected in Tehran province, Hamedan and Tehran, Iran, during their flowering period in June 2008. The species were identified by Dr. Amin at Tehran University of Medical Sciences and Eng. Kamalinejad at Shahid Beheshti University of Medical Sciences. The voucher specimens have been deposited at the Herbarium of School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

2.2. Chemicals and instruments

All of the chemicals used in this study were purchased from Sigma-Aldrich Chemical Co. (France) and/or Merck Company (Germany). A Shimadzu Multispect-1501 spectrophotometer (Kyoto, Japan) was used for absorbance measurements.

2.3. Preparation of extracts

The dried and ground aerial parts of thyme samples (200 g) were extracted with 90% EtOH (2000 mL) at 25 °C for 72 hours. The extracts were filtered and concentrated under reduced pressure at 35 °C. The dried extracts were kept in the dark at 4 °C until tested.

2.4. Antioxidant assays

2.4.1. DPPH$^{\bullet}$ assay

The free radical scavenging abilities of the samples were measured using the stable radical DPPH$^{\bullet}$ [5]. A total of 1 mL of DPPH$^{\bullet}$ solution (0.3 mM) was added to 2.5 mL of each sample (at concentrations 800, 400, 200, 100, 50, 25, 12.5, and 6.25 μg/mL in 90% ethanol). The tubes were incubated at room temperature for 30 minutes, then the absorbance values were determined at 518 nm. Rutin and gallic acid were used as positive controls. Inhibition percentage of DPPH$^{\bullet}$ ($I_{\text{DPPH}^{\bullet}}$ %) was calculated by the following formula:

$$I_{\text{DPPH}^{\bullet}}(\%) = 100 \cdot \left[\frac{A_{\text{control}} - \left(A_{\text{sample}} - A_{\text{blank}}\right)}{A_{\text{control}}}\right],$$

where A_{sample}, A_{blank} and A_{control} were the absorbance of sample, blank sample, and control, respectively.

2.4.2. ABTS$^{\bullet+}$ assay

The antioxidant capacities 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$^{\bullet+}$) [4]. The ABTS$^{\bullet+}$ radical cation was prepared by the reaction of 7 mM ABTS with 2.54 mM potassium persulfate, after incubation at room temperature for 12—16 hours. Prior the assay, the ABTS$^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. A total of 5 mL of the diluted ABTS$^{\bullet+}$ solution was added to 50 μL of each sample (at concentrations 800, 400, 200, 100, 50, 25, 12.5, and 6.25 μg/mL in 90% ethanol). The