Fingerprint Study of *Thymus spp*. by TLC

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Abstract

Background: Thymus is a widely used medicinal plant in food and pharmaceutical industries. Among different species of Thymus, *T. vulgaris* is used more than other species in therapeutic dosage forms.

Objective: This study was conducted to differentiate various Thymus species by TLC fingerprint.

Methods: In order to identify and differentiate various species of Thymus, TLC fingerprints of essential oil, dichloromethane, ethylacetate and *n*-butanol extracts of four available species named *T. vulgaris, T. pubescens, T. citriodorus* and *T. daenensis* were obtained and compared with each other.

Results: The results showed that the TLC chromatograms of essential oil and *n*-butanol extract can not be used as differential identification for the above-mentioned species; while the ethylacetate and dichloromethane extracts are more reliable to be used for TLC fingerprints. TLC chromatogram of ethylacetate extract is specific for identification of *T. pubescens* and *T. vulgaris* while *T. citriodorus* and *T. daenensis* can be best identified by their TLC fingerprints obtained from dichloromethane extract.

Conclusion: For best differentiation of various Thymus species, different extracts of the plants should be used.

Keywords: ***Thymus vulgaris, Thymus pubescens, Thymus citriodorus, Thymus daenensis,* TLC fingerprint**

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Introduction

Thymus is a well-known medicinal plant native to southern part of Europe, but nowadays it is cultivated worldwide. Thymus has approved expectorant, antitussive and spasmolitic activities. It's antiseptic property is estimated to be 25 times more effective than phenol, with less toxicity [1 - 3]. Different species of Thymus are different in content and type of components. Generally they contain thymol, carvacrol, flavonoids and phenolic compounds such as rosmarinic acid which may have antiedemic and macrophage-inhibiting effects [4 - 6]. Among different Thymus species, *T. vulgaris* is cultivated in many countries and is used more in pharmaceutical dosage forms because it contains more essential oil than other species with high amount of thymol which exhibits considerable antitussive and expectorant effects [7]. Considering the fact that *Thymus vulgaris* is less available and more expensive, some herbal industries tend to use other species of Thymus with different components. So it is crucial to develop a suitable and reliable identification method to confirm the quality of extracts and herbal drugs. Separation and detection of different constituents in plants have been always complicated. While conventional research mainly focuses on determination of the active components, fingerprinting can offer characterization of a complex system with a degree of quantitative reliability, so it has gained increasing attention for quality control systems over the past years [8]. Chromatography methods including TLC, HPLC, GC and electromigration techniques such as capillary electrophoresis are mainly used for fingerprinting [9 - 11]. TLC is a common rapid and cost-efficient method used for fingerprinting plant extracts. Moreover, several samples can be chromatographed simultaneously on a single plate and complicated instruments are not necessary [10, 12]. In this investigation, TLC chromatograms of essential oil and different extracts of four Thymus species growing in Iran, named *T. vulgaris, T. pubescens, T. citriodorus and T. daenensis*, were prepared and their patterns were compared with each other to specify the similarities and differences between them.

Materials and Methods

Plant Material

Aerial parts of *T. vulgaris, T. pubescens* and *T. daenensis* from Gorgan (Golestan province) and *T. citriodorus* from Zardband company farm (north-east of Tehran province) were collected in May 2006 and identified by M. Khatamsaz, Institute of forests and rangelands.

Preparation of essential oil

The air dried and powdered aerial parts of the plants were subjected to hydrodistillation for 4h using a Clevenger type apparatus. The obtained essential oil was dehydrated with anhydrous sodium sulphate and stored at +4°C before using.

Preparation of plant extracts

In order to prepare different plant extracts, 5 g of dried and milled aerial parts of each species were macerated with 50 ml dichloromethane, ethyl acetate and *n*-butanol, respectively (72 h with each solvent) and filtered.

Thin layer chromatography procedure

Thin layer chromatography was performed using CAMAG HPTLC silica gel 60 F_{245} plates. Table 1 shows other TLC requirements

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Table 1- Chromatography condition for different extracts of Thymus spp.

	Essential oil and DCM extract	EtAC extract	*n*-But extract
Developing solvent system	Toluene: ethylacetate 93:7	toluene: ethyl acetate: methanol 60:20:20	BAW 4:1:5
Spray reagent	Anisaldehyde/ sulfuric acid	NP/PEG 400	10% methanolic sulfuric acid

Camag Linomat IV was employed for spotting and photography was carried out by digital camera Hitachi HV-C20, camag.

Results and Discussion

Various constituents in plants make their quality control more complicated. Traditionally, some active components in herbal products were considered for evaluation of their quality. However, in most cases, it is difficult to specify the biologically active compounds and separate them from other components such as proteins and sugars which exist in large amounts but with insignificant therapeutic effects [9]. Nowadays, fingerprint analysis has been introduced by WHO as an acceptable strategy for assessment of herbal medicines [13]. Therefore, in this investigation in order to evaluate quality control of herbal products containing Thymus species, fingerprints of four Thymus species have been compared.

In figures 1-3, TLC chromatograms of dichloromethane, ethylacetate and *n*-butanol extracts of four species of Thymus are shown. In addition, the results of TLC chromatograms for three different extracts of Thymus spp. are summarized in tables 2-3. The chromatograms obtained from dichloromethane extract of *T. pubescens*, *T. vulgaris* and *T. daenensis* show the presence of thymol as an orange band in R_F 0.53 but this band is absent for *T. citriodorus*. The bands in R_F 0.14, 0.39, 0.48 are characteristic for *T. citriodorus* and the dark band in R_F 0.32 is characteristic for *T. daenensis*. The chromatograms obtained from essential oils are very similar to the ones related to dichloromethane extract.

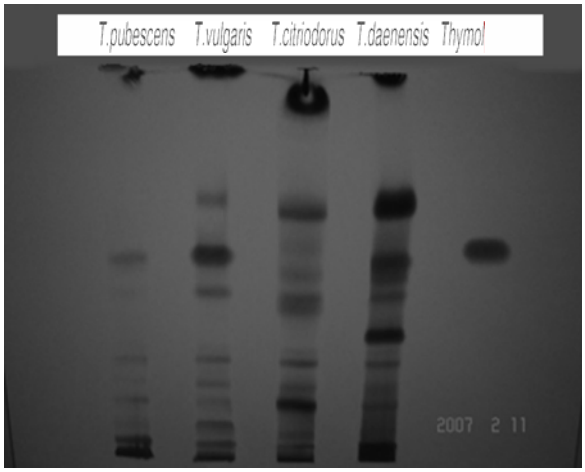

Fig. 1- TLC chromatogram of dichloromethane extract of Thymus spp.

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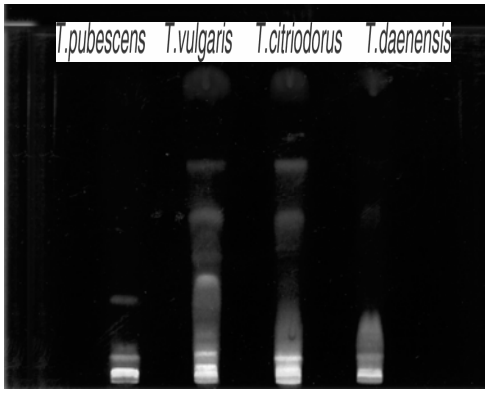

Fig. 2- TLC chromatogram of ethylacetate extract of Thymus spp.

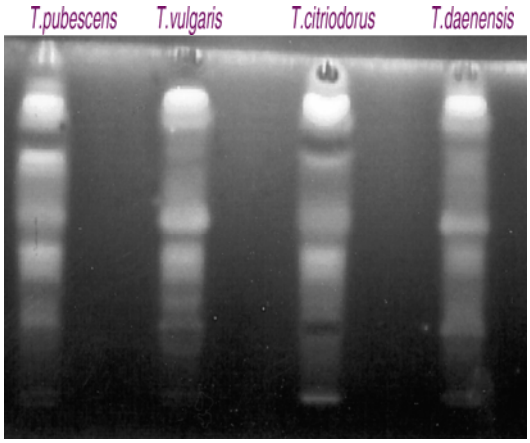

Fig. 3- TLC chromatogram of *n*-butanol extract of Thymus spp

Table 2- TLC pattern of dichloromethane extracts of Thymus spp.

Name	RF					
	0.14	0.32	0.39	0.48	0.53	0.68
T. pubescens	-	-	-	-	+	-
T. vulgaris	-	-	-	-	+	+
T. citriodorous	+	-	+	+	-	+
T. daenensis	-	+	-	-	+	+

Table 3- TLC pattern of ethylacetate extracts of Thymus spp.

Name	RF							
	0.18	0.22	0.26	0.30	0.36	0.41	0.46	0.61
T. pubescens	-	+	-	-	-	-	-	-
T. vulgaris	+	-	+	+	+	+	+	+
T. citriodorous	-	-	+	-	+	-	+	+
T. daenensis	-	-	-	-	-	-	+	+

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Notable differences between species can be observed in chromatograms of dichloromethane extract. Some similarities were found in ethylacetate extracts of *T. vulgaris*, *T. citriodorus* and *T. daenensis* but a yellow band in R_F 0.30 and an orange one in R_F 0.41 were found only in *T. vulgaris* chromatogram. A blue-purple band with intense florescence in R_F 0.22 only found in *T. pubescens* chromatogram. No significant difference was found in the chromatograms obtained from *n*-butanol extracts. The only exception was a blue fluorescence band for *T. pubescens* in R_F 0.54 while for the other species the colour was pink. It can be concluded that results obtained from the TLC chromatograms of essential oil and *n*-butanol extract can not be used for differential identification of the above-mentioned species; while the TLC fingerprints of ethylacetate and dichloromethane extracts are more reliable for comparative study. TLC chromatogram of ethylacetate extract is specific for identification of *T. pubescens* and *T. vulgaris* but *T. citriodorus* and *T. daenensis* can be best identified by their TLC fingerprint obtained from their dichloromethane extract. This experiment can be performed for all herbal products containing *T. vulgaris*, *T. pubescens*, *T. daenensis* and *T. citriodorous*, with previous preparation of different fractions using aforementioned solvents from herbal products.

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