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## Conventional and unconventional extraction methods applied to the plant, *Thymus serpyllum* L

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**Abstract.** This study deals with the application of two conventional and three non-conventional extraction approaches for isolation of bioactive compounds from the plant *Thymus serpyllum* L. The extracts obtained were tested regarding their chemical profile (content of phenolics, flavonoids, condensed tannins, gallotannins and anthocyanins) and antioxidant activities. Subcritical water extract of *Thymus serpyllum* L. generally had the highest concentrations of the chemical bioactive compounds examined and the best antioxidant properties.

### 1. Introduction

Phenolic compounds, which are the product of secondary metabolism of plants, are one of the most investigated class of natural products due to their wide range of biological activities, such as antioxidant, cytotoxic, antimicrobial, anti-inflammatory, antiulcer, antispasmodic, antiviral and many other activities [1, 2]. There are over 8000 compounds which belong to one of the following groups, among others: simple phenolics, phenolic acids, stilbenes, flavonoids, coumarins and tannins [3, 4].

For isolation of these and other compounds from their natural sources, many different approaches can be applied. Among them are conventional extraction techniques such as maceration and Soxhlet extraction and non-conventional techniques such as ultrasound-assisted, microwave-assisted and subcritical water extraction techniques [5, 6]. Every technique possesses certain advantages and disadvantages, and they are usually combined to obtain improved results.

There are only two studies which reported the useful properties of the plant *Thymus serpyllum* L. [7, 8]. The aim of this study was to apply conventional and non-conventional extraction techniques for isolation of biologically active compounds from *Thymus serpyllum* L., to investigate the biological activity of the extracts obtained and to establish their chemical profiles. Results were compared in order to evaluate the efficiency of the applied extraction techniques and the activities of the extracts obtained.



## 2. Materials and methods

### 2.1. *Thymus serpyllum* L

*Thymus serpyllum* L. (wild thyme) is a perennial shrub, native to areas of northern and central Europe, belongs to the family *Lamiaceae*. Due to its pharmacological properties, the essential oil of wild thyme, a plant used in traditional medicine, is an important natural resource for the pharmaceutical industry. In addition, it can be a source of natural antioxidants, nutritional supplements, or components of functional foods in the food industry. The chemical composition and yield of the essential oil of *Thymus serpyllum* are considered to be affected by geographic region, the development stage of the plant, the harvest season, habitat, and climatic conditions [9].

The aim of this study was to examine the use of different extraction methods, one conventional and one non-conventional, on the composition and quality of essential oil from *Thymus serpyllum* L. collected in Central Serbia.

### 2.2. Extractions

**2.2.1. Conventional method.** Soxhlet extraction was conducted in the following manner: plant material (75.0 g) was crushed and homogenized into small 3–5mm pieces by a cylinder crusher and placed in the Soxhlet apparatus. Extraction was carried out for eight hours using 96% ethanol as a solvent (600 mL). The Soxhlet extract (SE) obtained was filtered through filter paper (Whatman – qualitative filter paper Grade 1, Sigma-Aldrich) and evaporated by a rotary evaporator (Devarot, Slovenia) under vacuum and dried at 60°C to the constant weight. The dried extracts were stored in a dark glass bottle at 4°C to prevent oxidative damage.

Maceration was conducted using the following procedure: plant samples (10.0 g) were extracted using 96% ethanol (300 mL) as a solvent. The extraction process was carried out under laboratory conditions at 22°C in a sheltered, dry place for seven days, with occasional shaking to improve the maceration process. After seven days, maceration extract (ME) was filtered through filter paper and concentrated to dry mass by a rotary evaporator under vacuum and dried at 60°C to the constant weight. The dried extracts were stored in a dark glass bottle at 4°C to prevent oxidative damage.

**2.2.2. Non-conventional extractions.** Ultrasound-assisted extraction (UAE) was performed in ultrasonic water bath (EUP540A, Eustruments, France). A sample (5.0 g) was placed in volumetric flask and 100 mL of solvent (96% ethanol) was added. The mixture was sonicated for 30 min at a frequency of 40 kHz and ultrasound power of 90% (216 W).

Microwave-assisted extraction (MAE) was performed in a domestic microwave oven, which was previously modified for this purpose. Extraction was conducted using the same sample weight, solvent volume and extraction time. The extraction procedure program was as follows: one min pre-heating at 160 W; one min pre-heating at 320 W and 30 min extraction at 600 W.

Subcritical water extraction (SWE) was performed in a previously described home-made extractor system [10]. In all experimental runs, 5.0 g of plant material was mixed with 100 mL of double-distilled water. Extraction was performed at a pressure of 40 bars and at 140°C for 30 min. Agitation was assured by vibrational movements of vessel platform at a frequency of 3 Hz. After extractions, process vessels were immediately cooled in a flow-through water-bath at 20°C.

### 2.3. Spectrophotometric assays

Total phenolics (TPC) and total flavonoids (TFC) contents were determined using the previously described methods [11]. Condensed tannins (CT) were determined according to a previously described method which relies on the precipitation of proanthocyanidins with formaldehyde, while gallotannins (GA) were determined using the described potassium iodate assay [12]. Anthocyanins were determined according the previously described procedure [13] using pH single and differential methods. Antioxidant activities of the obtained extracts were determined using the following,

previously described assays: total antioxidant capacity [14], lipid peroxidation assay [15], hydroxyl radical scavenging activity [16] and DPPH radical scavenging activity [17] with slight modification [18].

#### 2.4. Statistical analysis

Statistical analysis was carried out using Statistica 6.0. (StatSoft Inc, Tulsa, US). All extractions were performed at least in triplicate unless specified otherwise. Results are presented as a value  $\pm$  standard deviation (SD). Significance levels were defined at  $p < 0.05$ .

### 3. Results and Discussion

#### 3.1. Chemical profile of *Thymus serpyllum* L. extracts

Results for TPC, TFC, CT, GA and TAC obtained using spectrophotometric assays are presented in table 1. According to the results, the highest contents of all compound classes was observed in SCW extract, while the lowest level of compounds was in SE extract. The amount of TPC obtained in SCW was about 50% higher than the corresponding value obtained by SE, while this percentage was slightly lower in the case of TFC, CT, GA and TAC. Results obtained for MAE and UAE extracts were slightly lower than those for SCW (about 10–20%), while MAC extract in some cases also achieved satisfactory results. The reasons for such diversity among the investigated extracts could be different mechanisms of thermal and mass transfers, as well as different solubility of compounds in the medium.

Antioxidant activity was established using four different assays: total antioxidant capacity, inhibition of lipid peroxidation, hydroxy radical scavenging and DPPH scavenging activities, the results of which are presented in table 2.

**Table 1.** Chemical profile of *Thymus serpyllum* L. extracts obtained by spectrophotometric assays.

Extract	TPC (mg GAE/g) <sup>a</sup>	TFC (mg RU/g)	CT (mg GAE/g)	GA (mg GAE/g)	TAC (mg C <sub>3</sub> G/g)
SE	95.86 $\pm$ 0.49	16.18 $\pm$ 0.37	53.85 $\pm$ 0.44	16.13 $\pm$ 0.94	102.05 $\pm$ 0.40
MAC	115.14 $\pm$ 0.26	19.57 $\pm$ 0.58	55.19 $\pm$ 0.37	18.74 $\pm$ 0.96	111.63 $\pm$ 0.73
UAE	124.42 $\pm$ 0.87	20.55 $\pm$ 0.17	58.65 $\pm$ 0.54	19.19 $\pm$ 0.42	122.56 $\pm$ 0.19
MAE	133.56 $\pm$ 0.19	21.81 $\pm$ 0.60	59.70 $\pm$ 0.19	20.36 $\pm$ 0.77	129.72 $\pm$ 0.54
SWE	141.12 $\pm$ 0.23	23.24 $\pm$ 0.18	61.53 $\pm$ 0.81	23.56 $\pm$ 0.64	130.32 $\pm$ 0.28

<sup>a</sup> Results are mean values  $\pm$  SD from three extractions.

SE – Soxhlet Extraction.

MAC – Macerate Extraction.

UAE – Ultrasound-Assisted Extraction.

MAE – Microwave-Assisted Extraction.

SWE – Subcritical Water Extraction.

TPC – total phenolics.

TFC – total flavonoids

CT – condensed tannins.

GA – gallotannins.

TAC – total anthocyanins.

**Table 2.** Antioxidant activity of *Thymus serpyllum* L. extracts obtained by spectrophotometric assays.

Extract	TA (mg AA/G) <sup>a</sup>	ILP <sub>50</sub> <sup>b</sup> (mg/mL)	OH <sub>50</sub> <sup>b</sup> (mg/mL)	IC <sub>50</sub> <sup>b</sup> (mg/mL)
SE	111.34 ± 0.43	30.35±0.42	32.51±0.88	45.68±1.01
MAC	118.92±0.48	29.73±0.08	29.15±0.93	36.83±0.98
UAE	133.71±0.29	27.57±0.44	25.83±0.77	35.47±0.69
MAE	158.09±0.82	26.56±0.81	23.41±0.81	29.60±0.99
SWE	170.32±0.87	20.71±0.45	19.63±0.94	22.73±0.53

<sup>a</sup> Results are mean values ± SD from three extractions.

<sup>b</sup> ILP<sub>50</sub>, OH<sub>50</sub> and IC<sub>50</sub> values were determined by non linear regression analysis.

SE – Soholot Extraction.

MAC – Macerated Extraction.

UAE – Ultrasound-Assisted Extraction.

MAE – Microwave-Assisted Extraction.

SWE – Subcritical Water Extraction.

TA – Total antioxidant activity.

ILP<sub>50</sub> – lipid peroxidation activity.

OH<sub>50</sub> – hydroxy radical scavenging activity.

IC<sub>50</sub> – DPPH radical scavenging activity.

The highest antioxidant activity of all four tested antioxidant types was observed in SWE, while the lowest was detected in SE. MAE and UAE expressed similar lipid peroxidation activities, hydroxy radical scavenging and DPPH scavenging activities, while these extracts differed in total antioxidant capacity (MAE had more TA capacity). On the other hand, similar activity between MAC and SE was observed in the case of total antioxidant capacity, lipid peroxidation test and hydroxy radical scavenging, while DPPH activity was higher in SE.

#### 4. Conclusion

The present study showed that *Thymus serpyllum* L. possesses good potential to be used as a source of biologically active compounds. Based on the presented results, the prepared extracts exhibited high antioxidant activity. Subcritical water extract generally showed the best properties, but other non-conventional techniques demonstrated satisfactory results. All in all, the results obtained in this study encourage further and deeper investigation of this plant together with the new fields and possibilities for its application.

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