

Influence of extraction solvents on total phenolic content and antioxidant activity of Tunisian *Thymbra capitata* (L.) Cav. aerial parts extracts

Ines Taieb¹, Amel Azaza², Farah Zidi³, Abdallah Fraj³, Ramzi Amari³, Monia Bendhifi-Zarroug⁴, Sondes Stambouli-Essassi⁴, and Kheiria Hcini^{3, 4*}

1. Laboratory of Analysis, Treatment and Valorization of the Pollutants of the Environment and Products, Faculty of Pharmacy, University of Monastir. Avicenne Street, Monastir 5000, Tunisia.
2. Laboratory of Biotechnology and Biomonitoring of the Environment and Oasis Ecosystems (LR21ES26), University of Gafsa, Faculty of Sciences of Gafsa, Department of Life Sciences, University Campus, Sidi Ahmed Zarroug, Gafsa, Tunisia.
3. Department of Life Sciences, Faculty of Sciences of Gafsa, University Campus Sidi Ahmed Zarroug, University of Gafsa, Gafsa 2112, Tunisia.
4. Biodiversity, Biotechnology and Climate Change Laboratory (LR11ES09), Department of Life Sciences, Faculty of Science of Tunis, University of Tunis El Manar, 2092, Tunisia.

Abstract

Natural compounds from the Lamiaceae family (thyme, sage and rosemary) have been reported in several studies for their antioxidant, anti-inflammatory, antimicrobial, antidiabetic, antiaging and anti-carcinogenic properties. Among the sources of these bioactive molecules, the *Thymus* genus is particularly noted for its long history of use for different food, pharmaceutical and medicinal purposes, which could be attributed to its polyphenolic compounds. The objective of this study is to evaluate the effect of solvents (water, ethanol, methanol) on extract yields, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant potential of Tunisian *Thymbra capitata* (L.) Cav. aerial parts. The highest extract yield was obtained from hydroethanolic extract (9.4 mg dry extract per gram dry plant weight, mg DE/g DW) followed by hydromethanolic (8.2 mg DE /g DW) and aqueous extract (7.4 mg DE/g DW). The TPC, evaluated by the Folin-Ciocalteu method, reached the values of 30.20 mg gallic acid equivalent per gram dry extract (mg GAE/g DE), 32.14 mg GAE/g DE and 30.31 mg GAE/g DE of aqueous, hydroethanolic and hydromethanolic thyme extracts, respectively. The highest TFC (16.20 mg quercetin equivalent per gram dry extract, mg QE/g DE) was recorded in hydroethanolic extract, followed by hydromethanolic (11.29 mg QE/g DE) and aqueous extract (4.18 mg QE/g DE). The thyme extract showed strong antioxidant activity with the methanolic extract showing the highest activity reflected by IC₅₀ value of 15.44 µg/mL. The results reveal that thyme aerial parts extracts have proven to be an effective potential source of polyphenols, as natural antioxidants, which is beneficial to human health, and could be useful in replacing or even decreasing synthetic antioxidants in foods, cosmetics and pharmaceutical products.

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Corresponding Author

Dr. Kheiria Hcini
E-mail: hcini kheiria@yahoo.fr
Tel: +00216 49491686

Keywords

Thymbra capitata (L.) Cav., total phenolic content, total flavonoid content, antioxidant activity, solvent effect.



1. Introduction

Aromatic and medicinal plants have been of great interest, as they have been the sources of natural products, commonly named as bioactive compounds with antioxidant activity and other beneficial properties to human health [1]. Specifically, the natural compounds from the Lamiaceae family (thyme, sage and rosemary) have been reported in several studies for their antioxidant, anti-inflammatory, antimicrobial, antidiabetic, antiaging and anti-carcinogenic activities [2-5]. Among the sources of these bioactive molecules, the *Thymus* genus is particularly noted for its long history of use for different food, pharmaceutical and medicinal purposes, which could be attributed to its polyphenolic compounds. It is also beneficial in treating respiratory and gastrointestinal tract disorders [6-10]. This genus, belonging to the Lamiaceae family, includes 350 species widespread around the world and their phytochemical composition has been studied earlier [11-13].

In Tunisian flora, the *Thymus* genus is mainly represented by *Thymbra capitata* L. Cav. (syn. *Thymus capitatus* (L.) Hoffman and Link) which has been commonly used as a spicy herb and is locally known under the common name "zaâtar" [14]. Tunisian *Thymus capitatus* (L.) Cav. is known to be an effective potential source of natural antioxidants, which confer benefits to human health [9, 15]. Their bioactive properties are mainly due to their polyphenolic compounds. The aerial parts of *Thymus capitatus* (L.) Cav. plants have a long history of use in food and traditional medicine. Because of its flavoring and seasoning properties, this plant has been widely used in the preparation of many foods and for the treatment of different kinds of disorders [16]. To the best of our knowledge, no previous research has investigated the effect of solvents on the variation of the phenolic content and antioxidant activity of Thyme aerial parts extracts. In this context, this research aims to determine the impact of different solvents (water, ethanol, and methanol) on the total polyphenolic and flavonoid contents and *in vitro* antioxidant activity of *Thymus capitatus* (L.) Cav, in order to promote this plant as a potential source of bioactive molecules with beneficial effects for human health.

2. Materials and methods

2.1. Plant material and preparation of thyme extracts

The plant materials consisted of the aerial parts of thyme were acquired from a local supermarket (Gafsa, Tunisia). The dried aerial parts of the plant were crushed into a fine powder using an electric milling machine. Dried sample (1g) was macerated in 10 mL of distilled water, hydroethanolic (70%), and hydromethanolic (70%) solvents for 24 hours at room temperature [5]. The obtained thyme extracts were filtered using Whatman filter paper and dried in a forced-air dryer at 37 °C. The residue was redissolved in the same solvent and made up to 5 mL. The yield of the extracts was expressed in terms of milligrams of dry extract per gram of dry weight (mg DE/g DW). The final extract was kept in vials at 4°C until the corresponding analyses were conducted.

2.2. Estimation of total polyphenol content

The total phenolic content (TPC) in the thyme extracts was determined by the Folin-Ciocalteu reagent method [17] with slight modification. 20 µL of the different thyme extracts were added to 1155 µL of distilled water and 100 µL of Folin-Ciocalteu reagent (10%). A vigorous stirring was performed and 225 µL of sodium carbonate (10%) was added. After 30 min of incubation at 25 °C, the absorbance of the resulting blue-colored solution was measured at 765 nm. A standard curve was prepared by using different concentrations ranging from 0.1 to 1 mg/mL of gallic acid. The TPC was expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g DE). All experiments were performed in triplicate.

2.3. Estimation of total flavonoid content

The total flavonoid content (TFC) was measured using an aluminum chloride colorimetric assay [18]. Briefly, 100 µL of the different thyme extracts were mixed with 900 µL of distilled water in a test tube. After 3 min, 500 µL of AlCl₃ (2%) was added and allowed to stand for another 15 min. The absorbance was measured against the blank at 510 nm. Quercetin was used to prepare a standard calibration curve with different concentrations ranging from 0.1 to 1 mg/mL of quercetin. The results were expressed as mg of quercetin equivalent per gram of dry extract (mg QE/g DE). All measurements were performed in triplicate.

2.4. Radical scavenging activity by DPPH

The scavenging activity of different thyme extracts was measured according to the method described by Brand-Williams et al. [19]. 500 μ L of thyme extracts, at different concentrations (10, 20, 30, 40, 50 μ L from the solution that has been diluted), were added to 1000 μ L of DPPH \cdot solution (0.1 mM) and kept in the dark at room temperature for 30 min. The absorbance was measured at 517 nm against a control (500 μ L of solvent and 1000 μ L of DPPH solution). All the assays were conducted in triplicate. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration, using the following equation:

$$\%I = [(Ab_{\text{control}} - Ab_{\text{sample}}) / Ab_{\text{control}}] \times 100$$

The results were expressed as the inhibitory concentration of the extract needed to decrease DPPH \cdot absorbance by 50% (IC₅₀). Concentrations are expressed in micrograms of dry extract per milliliter of solvent (IC₅₀, μ g/mL).

2.5. Statistical analysis

All experiments were performed in triplicate (n = 3) and data were reported as means \pm standard deviation (SD). A General Linear Model procedure was carried out to assess for significant differences (significant model was accepted for a p-value < 0.05) using the Variance analysis (ANOVA)

3. Results and discussion

3.1. Extraction yield

Thyme extract yields varied depending on the type of solvent used (Fig. 1). The highest yield was obtained from hydroethanolic extract with a value of 9.4 mg DE/ g DW, followed by hydromethanolic extracts (8.2 mg DE/ g DW) and aqueous extract (7.42 mg DE/g DW). Extract yields were considerably impacted by the extraction solvents. In fact, the variation in extraction yield is due to the polar ability of solvents to dissolve extractable components, resulting in varied chemical compositions of plants [10, 20]. Indeed, the combined use of water and organic solvent improved the extraction of phenolic compounds. Variations in thyme extract yields based on the plant's geographical origin, extraction methods and type of solvent have been documented in the scientific literature [9, 21, 22].

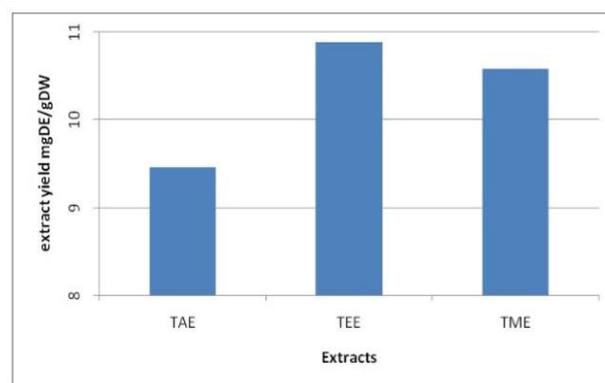


Figure 1. Thyme aerial parts extract yields (mg DE/g DW). (TAE: Thyme aqueous extract, TEE: Thyme hydroethanolic extract, TME: Thyme hydromethanolic extract, mg DE/ g DW: milligrams of dry extract per grams of dry weight).

3.2. Total phenolic and total flavonoid contents

The total phenolic content (TPC) of the aqueous, hydroethanolic and hydromethanolic extracts of thyme reached the values of 30.20, 32.14 and 30.31 mg gallic acid equivalent/g dry extract (mg GAE/g DE), respectively. On the other hand, the total flavonoid content ranged from 4.18 to 16.20 mg quercetin equivalent/gram dry extract (mg QE/g DE) (Table 1).

Table 1. Total phenolic and flavonoid contents of thyme aqueous, ethanolic, and methanolic extracts.

Samples	Total phenolic Content (TPC, mg GAE/g DW)	Total flavonoids content (TFC, mg QE/g DW)
TAE	30.20 \pm 0.57 ^b	4.18 \pm 0.75 ^c
TEE	32.14 \pm 0.138 ^a	16.20 \pm 0.72 ^a
TME	30.31 \pm 0.21 ^b	11.29 \pm 0.45 ^b

Values are expressed as means \pm Standard Deviations (SD) of triplicate determinations. Means with different letters within the same column are significantly different at 5% using the *Duncan* test ($P < 0.05$). TAE: Thyme aqueous extract, TEE: Thyme hydroethanolic extract and TME: Thyme hydromethanolic extract.

These values demonstrate that *Thymbra capitata* (L.) Cav. is rich in polyphenolic compounds. When comparing our results with previous studies, notable differences emerged. In fact, the total phenolic content reported by Msaada et al. [9] in methanolic extract of Tunisian thyme (18.40 mg GAE/g DE) was lower than our values. Phenolic compounds are well-known bioactive agents that have been extensively reviewed and have attracted considerable attention owing to their benefits to human health and in curing and averting numerous illnesses [23, 24]. In this context,

several studies prove the richness of thyme in total polyphenolic content and variation of its amount depending on the solvent used for extraction [12, 25]. In addition, the amount of TPC and TFC depends on the part, species of plant, method, solvent extraction, region of collection. Several studies showed that solvents significantly affect the yield of thyme crude extract and bioactive compound solubility and proved that methanol was the most efficient solvent to recover thyme polyphenol, followed by ethanol [15, 22, 24, 26, 27]. Similarly, Tamma et al. [28] proved the richness of thyme in flavonoid. They have been reported to be effective antioxidants, antibacterial, anticancer, cardioprotective, anti-inflammation, immune system promoting agents, skin protectors, and are therefore outstanding candidates for pharmaceutical and medical purposes [13, 29].

3.3. Antiradical scavenging activity

The antiradical activity of aqueous, ethanolic, and methanolic extracts of thyme, assessed through the DPPH assay, is presented in Fig. 2.

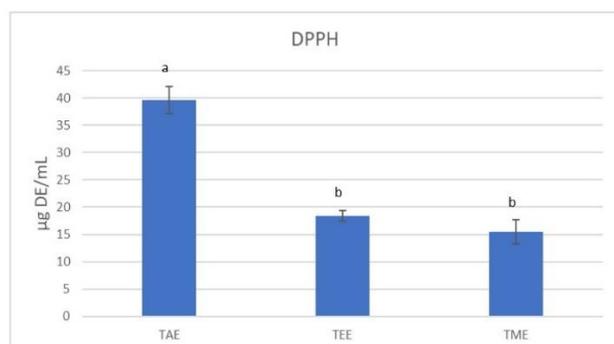


Figure 2. DPPH free radical scavenging capacity (IC₅₀ value) of Thyme aerial parts extracts.

(Values are expressed as means \pm standard deviations (SD) of triplicate experiments. Means with different letters within the same column are significantly different at 5% using the *Duncan* test ($P < 0.05$). TAE: Thyme aqueous extract, TEE: Thyme hydroethanolic extract and TME: Thyme hydromethanolic extract).

All extracts of thyme have strong antioxidant activity. The hydromethanolic extract is the richest in antioxidants with IC₅₀ value of 15.44 $\mu\text{g}/\text{mL}$ followed by the hydroethanolic extract with a value of 18.43 $\mu\text{g}/\text{mL}$ followed by the aqueous extract (15.44 $\mu\text{g}/\text{mL}$). These results show that plants with high antioxidant capacity are characterized by high levels of total polyphenolic content [5, 15, 26, 30]. In fact,

polyphenolic extracts of different parts of various plant sources reveal significant antioxidant activity, playing an essential role in mitigating oxidative stress-related diseases. Due to their potent bioactive molecules, these extracts are increasingly utilized as natural preservatives and functional ingredients in food products [26, 31]. The results obtained in different tests of antioxidant activity confirmed that the phenolic constituents are responsible for the antioxidant activity of thyme.

4. Conclusions

The present study has investigated the effect of solvents on extract yield, total phenolic content, total flavonoid content and antioxidant activity of Tunisian *Thymbra capitata* (L.) Cav. aerial parts. The thyme aqueous, hydroethanolic and hydromethanolic extracts showed a high amount of total phenolic and flavonoid contents. The evaluation of the antioxidant activity of different solvents of thyme extract showed a strong antioxidant activity. This highlight confirmed that thyme aerial parts have the potential effect as natural antioxidants due to their significant antioxidant activity and seem to be useful in pharmaceutical, cosmetics, and food industries with beneficial properties for human health. Therefore, supplementing a balanced diet with herbs may have beneficial health effects. Further, *in vitro* and *in vivo* investigations are needed to fully understand its biological properties.

Authors' contributions

Conceptualization, I.T., K.H.; Methodology, I.T., K.H., A.A., M.B.Z.; Formal analyses, I.T., K.H., F.Z., A.F., R.A., A.A.; Investigation, I.T., K.H., A.A.; Resources, K.H.; Writing-original draft preparation, I.T., K.H.; Writing-review and editing, Supervision, K.H., S.S.E.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare that they have no financial and commercial conflicts of interest.

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