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Research Article
Antioxidant Activities of Thyme Extracts

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Abstract

Background and Objective: Many herbs are known to contain large amounts of phenolic antioxidants other than the well-known compounds like vitamin C, vitamin E and carotenoids. Phenolic antioxidants in herbs primarily consist of phenolic acids, flavonoids and catechins. The antioxidant activity of phenolic compounds is primarily due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. This study was conducted to determine the antioxidant activities of aqueous and ethanolic extracts of thyme using three different methods. **Materials and Methods:** The thyme seeds were locally obtained, cleaned and ground. About 20 g of ground material was extracted with 250 mL of distilled water or 95% ethanol at the boiling point under reflux for 1 h. The extract was filtered and evaporated at 50°C to compete dryness. Phenolic contents, antioxidant activities and flavonoids were determined. **Results:** Ethanolic and aqueous extracts of *Thymus vulgaris* leaves were analyzed for their phenolic and flavonoid contents, which were 20.31, 13.44, 11.39 and 10.31% for ethanolic and aqueous extracts, respectively. The reducing power of aqueous and ethanolic thyme leaves was also determined. The reducing power was enhanced by increasing sample concentration, it was 92.54% for an aqueous extract at 10 mg mL^{-1} concentration and 94.51% for an ethanolic extract at the same concentration. The aqueous extract showed low chelating capacity compared with the ethanolic extract (50.75 and 66.03, respectively) using the reducing power of EDTA as a reference (93.00) at 10 mg mL^{-1}. **Conclusion:** *Thymus vulgaris* is rich in total phenols. The phenolic contents were determined to be much higher in the ethanolic extract than in the aqueous extract, which may be correlated to the solvent used for extraction.

Key words: *Thymus vulgaris*, thyme, ethanolic extract, aqueous extracts, antioxidants activities, phenolic compounds, flavonoids

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bioactive compounds commonly observed in fruits, vegetables, herbs and other plants have exhibited possible health benefits, such as antioxidative, anticarcinogenic, atherosclerosis, antimutagenic and angiogenesis inhibitory activities[1,2]. Interestingly, many herbs are known to contain large amounts of phenolic antioxidants other than the well-known compounds vitamin C, vitamin E and carotenoids. Phenolic antioxidants in herbs are mainly composed of phenolic acids[1], flavonoids[3] and catechins[4]. The antioxidant activities of phenolic compounds are mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides[5].

Thymus vulgaris L. (thyme), locally known "zaatar" or "zaitra', a member of the Lamiaceae family, is widely used in medicine for its expectorant, antitussive, antibroncholitic, antispasmodic, anthelmintic, carminative and diuretic properties. The aromatic and medicinal properties of the genus *Thymus* have made it one of the most popular plants worldwide. *Thymus* species are commonly used as herbal tea, flavoring agents (condiment and spice) and medicinal plants[6] and have high levels of antioxidant activity and phenolic substance contents[7,8]. Thyme contains phenolic and flavonoids[9,10]. The flavonoids have anti-inflammatory effects, they reduce the peroxidation of lipids[11] and they have anticarcinogenic effects[12].

The objective of the present study is to determine the antioxidant activities of aqueous and ethanolic extracts of thyme using three different methods.

MATERIALS AND METHODS

The thyme seeds were locally obtained, cleaned and ground. About 20 g of ground material was extracted using 250 mL of distilled water or 95% ethanol at boiling point, under reflux for 1 h. The extract was filtered and evaporated at 50°C to compete dryness.

Determination of total phenolic compounds: The Folin-ciocalteu calorimetric method was used as described by Biglari *et al.*[13]. In 0.5 mL of (1 mg mL^{-1}) extract, 2.5 mL of a ten-fold-diluted folin-ciocalteu reagent and 2 mL of 7.5% sodium carbonate solution were added before the reaction was allowed to stand for 30 min at room temperature. The absorbance was recorded at 760 nm using a Pye unicam spectrophotometer. The total phenolic compounds were determined according to a gallic acid standard curve (Fig. 1).

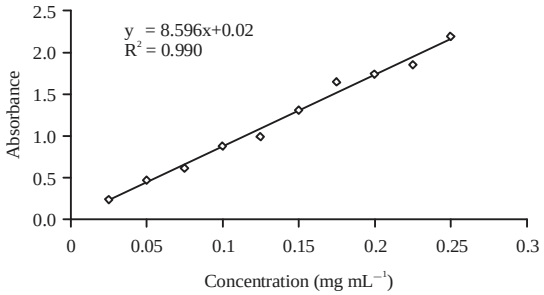

Fig. 1: Concentration-response curve for gallic acid at 760 nm

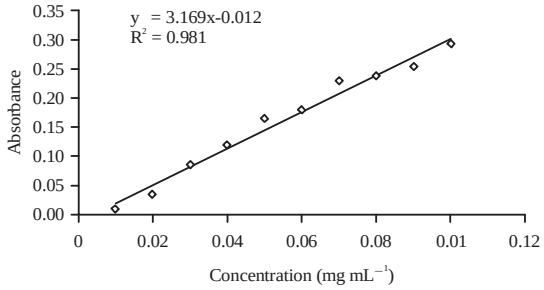

Fig. 2: Concentration-response curve for catechin at 510 nm

Determination of flavonoids: The total flavonoids in the aqueous and ethanolic extracts were determined according to the method described by Zhisben *et al.*[14]. About 1 mL of extract solution (1 mg mL^{-1}) was placed in a 10 mL volumetric flask, to which 5 mL of distilled water and 0.3 mL of 5% $NaNO_2$ solution were added. After 5 min, 0.6 mL of 10% $AlCl_3$ was added. About 2 mL of 1 M NaOH solution was added after another 5 min and the volume was brought up to 10 mL with distilled water.

The mixture was mixed thoroughly and the absorbance was measured at 510 nm. The flavonoid compounds were determined according to a catechin standard curve (Fig. 2).

Antioxidant activity assay

Reducing power: The reducing power was estimated as described by Chou *et al.*[15]. A 1 mL extract (2-10 mg mL^{-1}) was mixed with 2.5 mL of 1% potassium ferric cyanide and 2.5 mL of 0.2 M (pH 6.6) sodium phosphate buffer and incubated at 50°C for 20 min. To stop the reaction, 2.5 mL of 1% trichloroacetic acid (TCA) was added to the mixture, which was centrifuged for 10 min at 3000 rpm. One-half milliliter of the supernatant was mixed with 1 mL of 1% ferric chloride and the mixture was allowed to stand for 10 min. The absorbance was measured at 700 nm. Tert-butyl-4-hydroxytoluene (BHT) (0.02%) used as a reference.

Table 1: Phenolic contents in the thyme extracts

Plant	Extractions	Phenolic contents (%)
Thyme	Ethanolic	20.31
	Aqueous	13.44

Table 2: Flavonoid contents in the thyme extracts

Plant	Extractions	Flavonoid contents (%)
Thyme	Ethanolic	11.39
	Aqueous	10.31

Chelating capacity: Chelating capacity was determined as described by Su *et al.*[16], with some modifications. About 1 mL (2-10 mg mL^{-1}) of extract was mixed with 0.2 mL of 2 mM ferric chloride and 0.2 mL of 5 mM 8-Hydroxyquinoline. After 10 min at room temperature, the absorbance was determined at 562 nm. The EDTA-Na_2 was used as a reference.

RESULTS AND DISCUSSION

Aqueous and ethanolic extracts of *Thymus vulgaris* were analyzed for their phytoconstituents. The quantitative estimations of the total phenolic contents were 20.31 and 13.44% for the ethanolic and aqueous extracts, respectively (Table 1). The phenolic contents were determined to be much higher in the ethanolic extract than in the aqueous extract, which may be correlated to the solvent used for extraction. These results revealed that *Thymus vulgaris* is rich in total phenols[8,17].

The quantitative estimations of the total flavonoid contents were 11.39 and 10.31% for the ethanolic and aqueous extracts, respectively (Table 2).

Reducing power indicates compounds that are electron donors that can act as primary and secondary antioxidants[18]. The levels of reducing power of the aqueous extract of thyme were 60.14, 63.01, 65.61, 77.31 and 92.54% at concentrations of 2-10 mg mL^{-1}, respectively (Fig. 3), while the levels of reducing power of the ethanolic extract were 62.20, 64.09, 70.50, 80.25 and 94.51% at the same concentrations (Fig. 4). From these results, it is observed that the reducing power was enhanced by increasing the sample concentration. Higher reducing power might be attributed to higher amounts of total phenolic and flavonoid compounds and the reducing power of a compound may reflect its antioxidant potential[19].

The phenolic compounds have been recognized as antioxidant agents that can act as free radical oxidation terminators[20], the reducing properties of which are generally associated with the presence of reductions[21]. The results showed that the differences between two extraction methods may be due to the extract solvent and the compounds can be produced in the reducing reaction.

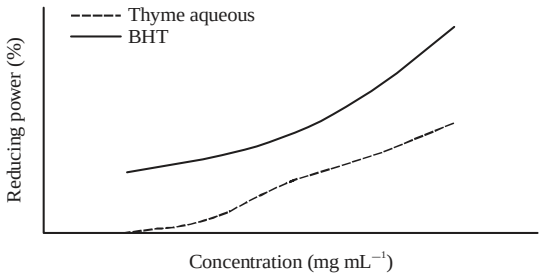

Fig. 3: Reducing power of the aqueous extract of thyme leaves compared with BHT at the same concentration

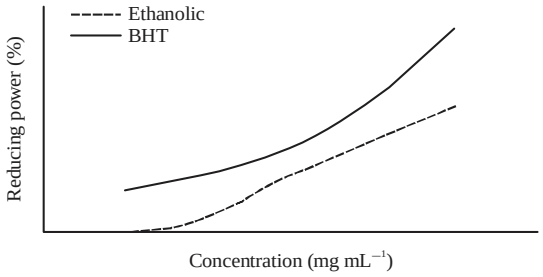

Fig. 4: Reducing power of the ethanolic extract of thyme leaves compared with BHT at the same concentration

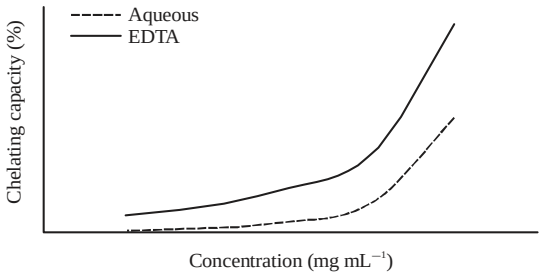

Fig. 5: Chelating capacity of an aqueous extract of thyme leaves compared with EDTA at the same concentration

Water was selected as the extraction solvent since it is commonly used in the food industry in a variety of ways. Aqueous and ethanolic extracts were subjected to screening for their possible antioxidant activities, with a decrease in absorption taken as a measure of the chelating capacities of the extract. Flavonoids have been demonstrated to display a wide range of pharmacological and biochemical actions, such as antimicrobial, antithrombotic, antimutagenic and anticarcinogenic activities[22].

The aqueous extract showed low chelating capacities in comparison with an EDTA compound, with absorbances of 50.75 and 93.00, respectively, at 10 mg mL^{-1} (Fig. 5) and lower than those of the ethanolic extract, which had an absorbance of 66.03 (Fig. 6). These results agreed with many studies that have reported that the chelating capacities

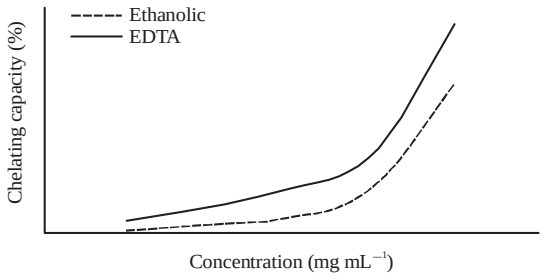

Fig. 6: Chelating capacity of an ethanolic extract of thyme leaves compared with EDTA at the same concentration

for aqueous extracts of dates ranged from 62.87-81.30% at 5 and 10 mg mL^{-1} concentrations[23], with a capacity of 3.15% at 0.25-2 mg mL^{-1} concentrations, while others found that the chelating capacity for ethanolic extracts of dates was 1.06% at 0.25-2 mg mL^{-1} concentration[24]. In contrast, the chelating capacities for alcoholic extracts were 47.19% for pomegranates, 57.23% for fig and 48.58% for black grape at 5 mg mL^{-1} concentrations[25]. Extracts of dates (Deglet noor) contained antioxidants that included a wide range of phenolic compounds[26]. It was found that binding compounds form bonds with metals that act as secondary antioxidants[27].

CONCLUSION

Thymus vulgaris is rich in total phenols and flavonoids. The herb's antioxidant activity and reducing power might be attributed to higher amounts of total phenols and flavonoids. The antioxidant activities of phenolic compounds are primarily attributable to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

SIGNIFICANCE STATEMENT

This study reveals that *Thymus vulgaris* L. (thyme), which is widely used as an herbal tea, a flavoring agent (condiment and spice) and a medicinal plant, has antioxidant activity. This study will help to uncover properties of *Thymus vulgaris* that many researchers have not been able to explore previously. Thus, a new theory on these properties and other possible benefits of thyme may be formulated.

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