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Effect of temperature and extraction process on antioxidant activity of various leaves crude extracts of *Thymus vulgaris*

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PEER REVIEW

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Comments
This paper studies on the effect of temperature and extraction process on antioxidant activity of various leaves crude extracts of T. vulgaris and give the valuable and scientific information about this plant.
Details on Page 133

ABSTRACT

Objective: To observe the effect of temperature and extraction process on the estimation of antioxidant activity of various organic crude extracts from the leaves of *Thymus vulgaris* (*T. vulgaris*) species native to Sultanate of Oman.
Methods: The dry powder samples of *T. vulgaris* were extracted with methanol using two different extraction methods. Both methanol crude extracts from the leaves of *T. vulgaris* were defatted with water and extracted successively with different polarities of solvents with increasing polarities, *e.g.*, hexane, ethyl acetate, chloroform and butanol.
Results: The yield of methanol crude extract by Soxhlet extraction method is better than maceration method. The yield of extraction was increasing with increasing temperature. The antioxidant activity of different crude extracts from both extraction methods was measured by DPPH with modification. By Soxhlet extraction method, the activity result found in butanol crude extracts was highest and the lowest in hexane crude extract as the following order of butanol>methanol>ethyl acetate extract>chloroform>hexane extract. However, by maceration method, the activity was highest in ethyl acetate and lowest in chloroform as the order of ethyl aectate>methanol extract>butanol>hexane >chloroform.
Conclusions: In conclusion, the maceration method is the best method for the evaluation of antioxidant activity.

KEYWORDS
Thymus vulgaris, Lamiaceae, Soxhlet extractor, Maceration extraction, Antioxidant activity

1. Introduction

All kinds of plants have been used as different medicines for centuries in most of the ethnic community throughout the world. The potential source of natural antioxidants is plants, fruits and vegetables. Secondary metabolites components from plants are considered as natural antioxidants or phytochemical antioxidants[1].

Food ingredients are also the most important source of natural antioxidants. Food processing and heat will contribute to loss of nutritional content or components. Thus it is important to control temperature and time of extraction to reduce the loss of nutritional content or components.

Thymus vulgaris (*T. vulgaris*) belonging to the family of *Lamiaceae* is one of the most popular hybrid plant used worldwide. Locally, *T. vulgaris* species native to the Sultanate of Oman known as "zaater" and their dried whole parts are used in herbal tea, condiments, and folk medicine[2]. Since ancient times, this aromatic plant has been used for the preparation of different aliments to cure various curable and chronic diseases.

The plant is pruned regularly. Spring is usually the best time, because it will inspire good air circulation through the plant. Normal dose of thyme medicine is generally regarded

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as safe. But high doses may cause intestinal problems such as diarrhea and bloating[3]. The use of thyme medicine is not always safe during pregnancy. Strong medicinal doses of this plant should be avoided if there is possibility of pregnance.

The main chemical constituents of crude extracts and essential oil of this plant are thymol, carvacrol and phenols [3-6]. The percentage of main chemical constituents varies drastically in its essential oil and crude extracts with weather, harvesting time, rainfall, storage conditions and extraction process. The maximum variation of the main chemical constituents is about 0.75% and 6.5%. Normally, the concentration of phenol content in the crude extracts and essential oil is found lower in winter season, but in summer season, the concentration of phenol increases drastically about 70%, with significant amounts of carvacrol. In addition, some other major non-phenolic chemical components are also found in the essential oil and crude extracts, such as thymol methyl ether, cineol, cymene, α-pinene, borneol and esters.

Thyme plant can be asexually propagated with ease. Thymol is an organic chemical compound which is commercially used as an antiseptic. One of the main chemical constituents in this plant is thymol. So this plant is also used as an antiseptic. Thymol is used to kill various fungi that commonly infect toenails and cure coughs and bronchitis [7-14]. Traditionally, this plant is used in this country for the treatment of coughs and bronchitis[7-14]. In addition, this plant also contains some other active ingredient which is commercially used to produced various mouthwashes like Listerine[7-11]. In nineteen century, the essential oil from thyme was used to prepare medicate bandages[12,13]. Nowadays different pharmaceutical antibiotics are discovered and the use of essential oil from this plant is declined. Due to its medicinal importance, scientists sand researchers are showing interests in this plant and screening exclusively on different parts as well as different parameters. However, scientific data on this plant are still lacking. Various *Thymus* species are available here and *T. vulgaris* is one of them. Literature search reveals that there is no work on Omani *T. vulgaris*. Therefore, the aim of this work is to observe the effect of temperature and extraction process on the estimation of antioxidant activity of various organic crude extracts from the leaves of *T. vulgaris* species native to Sultanate of Oman.

2.Materials and methods

2.1. Chemicals and reagents

Hexane, chloroform, ethyl acetate, butanol and methanol solvents were purchased from BDH, UK. DPPH (2, 2-diphenyl-1-picrylhydrazyl) and ascorbic acid were obtained from Sigma-Aldrich, Germany. The other chemicals were analytical reagent.

2.2. Instrument and apparatus

Soxhlet extractor, grinder (Super deluxe, India) and rotary evaporator (Yamato, rotary evaporator and Model-RE 801) were used for sample preparation. UV spectroscopy (Therom scientific, Genesys 10vis, Japan) was used to measure the absorbance of the samples.

2.3. Plant Sample

Fresh leaves of *T. vulgaris* were collected on October 2012 in the afternoon at 3 pm from AL-Jabal AL-Akhdar, Sultanate of Oman. The leaves samples were packed in a polyethylene bag. The samples were transported to my house for cleaning, dying and grinding.

2.4. Preparation of samples

The leaves samples of *T. vulgaris* were washed carefully with tap water to remove dust and insects. The washed leaves samples were dried under shade at room temperature for 3 d. After completing dry, about 500 g of leaves of *T. vulgaris* were ground using a kitchen grinder for 20 seconds. Finally, the leaves samples were prepared as a powder form by using blender machine.

2.5. Extraction by Soxhlet method

The dry powder samples (107 g) were extracted with methanol solvent (250 mL) at 68 °C by using Soxhlet extractor for 72 h[14]. After completing extraction, it was filtered and the methanol solvent was evaporated by rotary evaporator to give gammy solid crude extract (16.55 g). The crude extract (16.29 g) was defatted with water and then extracted successively with different organic solvents increasing polarities followed by hexane, chloroform, ethyl acetate and butanol to give: hexane (2.08 g), chloroform (0.52 g), ethyl acetate (3.83 g), butanol (1.85 g) and residual methanol fractions (3.93 g).

2.6. Extraction by maceration method

The dry leaves samples (107 g) were extracted with methanol solvent (250 mL) at 29 °C by using maceration method for 3 d. After extraction, the sample was filter with filter paper. The methanol solvent was evaporated by rotary evaporator under pressure for 30 min to give amorphous solid crude extract (13.12 g). About 0.34 g of methanol crude extract was transferred in a test tube for evaluating antioxidant activity. The remaining methanol crude extract (12.09 g) was defatted with water and then extracted successively separately with 30 mL and 20 mL of hexane, chloroform, ethyl acetate and butanol. After extraction, all crude extracts were put inside the fume hood for dry. After solvent evaporation, the hexane crude extracts (2.68 g), ethyl acetate (1.32 g), chloroform (3.11 g), butanol (2.29 g) and residual methanol fractions (3.21 g) were obtained.

2.7. Antioxidant activity by DPPH method

Antioxidant activity of different organic dry crude extracts from the leaves of *T. vulgaris* by both methods was estimated as described by Blois with minor modification[15]. Different concentrations, *e.g.*, 12.5, 25, 50, 100 and 200 mg/L were prepared from different crude extracts obtained by two

extraction methods. From each crude extracts of *T. vulgaris* (4 mL) at different concentrations were taken in separate test tubes. DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution was dissolved in methanol, then DPPH solution (1 mL) was added to the tubes and mixed together by hand. Then, all the test tubes were kept in the dark place for 45 min. The blank and positive controls were prepared as the same way without extract. Ascorbic acid was used as standard at the concentration of 50 mg/L. The absorbance of the prepared samples was measured using UV spectroscopy of the wavelength at 517 nm. Finally, calculated the antioxidant activity by using the following formula:

Measurement of antioxidant activity (%)

$$\% \text{ Inhibition} = \frac{A_{control} - A_{extract}}{A_{control}} \times 100$$

3. Results

The results of our present study showed that the variation of temperature and extraction process affect the activity of the extract as presented in the Figure 1 and Figure 2. The obtained results from our study were almost similar with the results of Herodez *et al.*[16] and Rahim *et al.*[17] that the temperature of extraction, sample particle size and the ration of solvent to sample will increase extraction yield. Dry samples of *T. vulgaris* were extracted with methanol solvent by Soxhlet extractor and maceration method. The methanol free crude extract was dissolved in water and extracted successively with increasing polarities, *e.g.*, hexane, chloroform, ethyl acetate, and butanol. The highest yield of extraction 15.42% was obtained from Soxhlet method and the lowest 12.26% from maceration method. The absorbance was gradually increasing with increasing concentration of various organic crude extracts from dry samples. The determination of activity of various organic crude extracts was obtained from both extraction methods by DPPH. The results of antioxidant activity for ethyl acetate, chloroform, butanol and methanol crude extracts from the leaves dry samples of *T. vulgaris* by Soxhlet method at different concentrations (12.5, 25, 50, 100 and 200 mg/L) showed that activity ranged between 76-98% (Figure 1). The highest activity was obtained in butanol crude extract and the lowest activity was found in hexane crude extract. The result of antioxidant activity order of different crude extracts was followed butanol>methanol>ethyl acetate extract>chloroform>hexane extract.

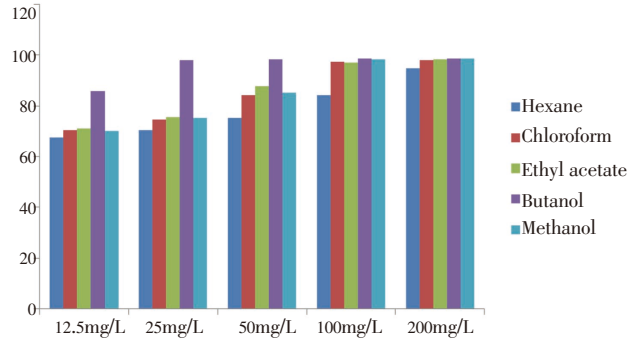

Figure 1. Antioxidant capacity of various crude extracts by Soxhlet extractor method from dry samples of *T. vulgaris*.

However, the results of antioxidant activity from maceration method at different concentration showed the activity range of 78-100% (Figure 2). The result of activity was obtained highest in ethyl acetate and lowest in chloroform crude extract and followed by the order of ethyl aectate>methanol extract>butanol>hexane>chloroform. The above results from both methods showed that Soxhlet extractor method is the good method for extraction and maceration method is the best method for the evaluation of antioxidant activity.

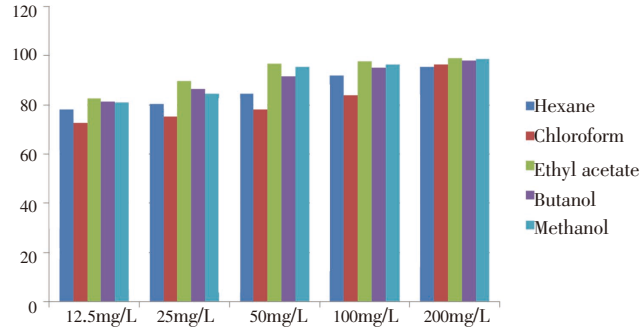

Figure 2. Antioxidant capacity of various crude extracts by maceration methods from dry samples of *T. vulgaris*.

4. Discussion

Thermal treatments are the main cause of reduction or breakdown in natural antioxidants[18-20]. In our present study, increasing the extraction temperature seemed to cause reduction and breakdown in the antioxidant content (Figure 2). Due to temperature increasing, the active ingredients in the sources may be decomposed. All the plants and fruits contain antioxidants such as ascorbic acid, which is the vital compound to maintain the nutrient content. So it is essential to control the extraction temperature. The ascorbic acid antioxidant in tomato almost lost 38% during the extraction time[21]. The other report was also available on oxidative heat damage by Zanoni *et al.*[22]. His report showed that the ascorbic acid loss was largely dependent on temperature. However, the heat treatment can change or damage the chemical constituents of vegetables, fruits and plants[23-25].

Antioxidants are widely available in nature which are different in chemical composition, chemical and biological properties and their mechanism of action[26]. The antioxidant activity is determined on different plant materials and extracts by various popular and established *in vitro* models, such as DPPH method[27], nitric oxide method[28], DMPD method[29], ABTS methods[30], *etc*. Antioxidants are widely used in diet and have been investigated for the prevention of various curable and incurable diseases, such as cancer, heart disease and even altitude of sickness[27-30]. The antioxidant activity of any pure substance or chemical constituents which is present in low concentrations in the samples (substance or extracts) can significantly delay or prevent antioxidant activity of cell content like protein, lipids, *etc*[28-30]. The role of antioxidant is a molecule that

inhibits the oxidation reaction of other molecules. Oxidation is a chemical process that produces electron or hydrogen from a substance (substance or extracts) to an oxidizing agent. The oxidation reactions can also produce free radicals. The antioxidant activity of the leaves extracts of *T. vulgaris* by different methods were tested through DPPH method and the results were presented in the (Figure 1 and Figure 2). The role of antioxidants is their interaction with oxidative stable free radicals. The stable free radical DPPH was widely used for the determination of antioxidant activity with decolorizing its colour. The degree of discoloration indicated the scavenging potentials of the sample antioxidant. In the present study, different crude extracts of *T. vulgaris* obtained from two extraction methods were able to decolourise DPPH. The antioxidant potentials of various crude extracts from Soxhlet method were found to be in the order of butanol> methanol>ethyl acetate>chloroform> hexane extract. However, the antioxidant potentials of various crude extracts from maceration method were found to be in the order of ethyl aectate>methanol extract>butanol>hexane> chloroform. Literature search reveals that some main chemical compounds, such as cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds and aromaticamines reduce and decolourise α, α-diphenyl-β-picrylhydrazyl by their hydrogen donating ability[5,27-30]. However, it is concluded that the five extracts of each extraction from the leaves of *T. vulgaris* possess hydrogen donating capabilities to act as antioxidant. The results of our experiments is not similar with other reported results on antioxidant activity [13,16,17,26]. Previous studies have mostly focused on single solvent extraction system. While this study clearly indicates that solvent systems involving polar organic solvents are more effective towards recovering optimal amount of antioxidant components from *T. vulgaris*[5,13,15]. Therefore, proper temperature and extraction method employed prior to extraction of the samples, can also significantly enhance the recovery of antioxidants from *T. vulgaris*. The reduction of antioxidant components in fruits, plants and vegetables is a great loss of nutritional value. These antioxidant compounds are able to fight heart disease, cancer, neuronal disease, diabetes, *etc*[31].

The antioxidant results of the present investigation can be concluded that maceration method is the best method for recovering antioxidant components. The effect of temperature and extraction process has a significant role on the antioxidant activity from *T. vulgaris*. However, further research work is needed to optimize the two variables, so that the best combination of temperature and extraction method could be determined.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

T. vulgaris is the most popular hybrid plant used worldwide, which belongs to the family of Lamiaceae. Locally it is known as "zaater" and their dried whole parts are used in herbal tea, condiments, and folk medicine. Literature search reveals that there is no work on Omani *T. vulgaris* by researchers.

Research frontiers

The aim of this work is to observe the effect of temperature and extraction on the estimation of antioxidant activity of various organic crude extracts of *T. vulgaris* native to the Sultanate of Oman.

Related reports

Literature search reveals that there is no work on Omani *T. vulgaris* by researchers. The other parameters of this plant has been done by other researchers.

Innovations and breakthroughs

Although the experimental work done by the author is routine work, but it gives the new information and data to the scientific community.

Applications

This plant is used worldwide as a medicine. According to the paper, there are so many bioactive compounds that can be used to prepare medicine.

Peer review

This paper studies on the effect of temperature and extraction process on antioxidant activity of various leaves

crude extracts of *T. vulgaris* and give the valuable and scientific information about this plant.

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