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Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*

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PEER REVIEW

Peer reviewer

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Comments

The present study on phytochemical screening, toal phenol and total flavonoids of various leaves crude extracts of *T. vulgaris* is giving the valuable brief and scientific information about this plant.
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ABSTRACT

Objective: To prepare various crude extracts using different polarities of solvent and to quantitatively evaluate their total phenol, flavonoids contents and phytochemical screening of *Thymus vulgaris* collected from Al Jabal Al Akhdar, Nizwa, Sultanate of Oman.
Methods: The leave sample was extracted with methanol and evaporated. Then it was defatted with water and extracted with different polarities organic solvents with increasing polarities. The prepare hexane, chloroform, ethyl acetate, butanol and methanol crude extracts were used for their evaluation of total phenol, flavonoids contents and phytochemical screening study. The established conventional methods were used for quantitative determination of total phenol, flavonoids contents and phytochemical screening.
Results: Phytochemical screening for various crude extracts were tested and shown positive result for flavonoids, saponins and steroids compounds. The result for total phenol content was the highest in butanol and the lowest in methanol crude extract whereas the total flavonoids contents was the highest in methanol and the lowest hexane crude extract.
Conclusions: The crude extracts from locally grown *Thymus vulgaris* showed high concentration of flavonoids and it could be used as antibiotics for different curable and uncurable diseases.

KEYWORDS

Thymus vulgaris L.,Various crude extracts, Phytochemical screening, Soxhlet extractor, Total phenol, Flavonoids contents.

1. Introduction

Medicinal plants have been used from ancient time for their medicinal values as well as to impart flavor to food. Nowadays, the crude extracts and dry powder samples from medicinal and aromatic plants and their specieshas have been shown interest for the development and preparation of alternative traditional medicine and food additives[1-5]. *Thymus* has approximately 150 species abundantly found mainly Asia, Africa and North America. Recently, it is widely extended in the Iberian Peninsula, most species being endemic. *Thymus vulgaris* L (*T. vulgaris*) is a medicinal plant belonging to the Lamiaceae family. There are many species that are available in Sultanate of Oman. Locally it is called "kekik". This plant is small shrub about 25 cm high. Its stem and branches are quadrangular. The leaves of this plant are 6-12 mm long, oval, blunt, entire, margin revolute, thick, smooth, dotted with many oil-glands, paler, pubescent beneath. It blooms flowers from June to July. The entire plant is aromatic and attractive to bees, flies *etc*. The identified main chemical constituents in the essential oil collected from *T. vulgaris* and their species are shown to be

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presence of high concentration of phenolic monoterpenes, thymol and carvacrol[6-9]. The essential oil from locally grown *T. vulgaris* also contains high concentration of thymol about 20%-54%. Except thymol, the essential oil from this plantis also contains some other additional active chemical constituents such as p-cymene, myrcene, borneol and linalool. Therapeutically, thymol is widely used as an antiseptic. The other active chemical ingredient is listerine, commercially used to produce mouthwashes[7]. Thyme oil was also used to medicate bandages before the discovery of modern pharmaceutical medicine antibiotics[1]. Recently, thymol has also been shown to be active ingredient against various bacteria and fungi that commonly infect toenails[8]. More recently, it can also be found as the active ingredient in some natural commercial products and hand sanitizers.

The identified chemical constituents in the essential oil from this plantspecies and their antimicrobial activity have been reported earlier[8,9]. The essential oil was useful to prepare various commercial products mainly as antimicrobial, and antioxidant agents have also been reported[8,9]. Extensive clinical studies have been done and reported by several scientists on this plant species and showed that they have very strong antibacterial, antioxidant, antifungal, insecticidal and anti platelet activities[10,11]. The researchers showed that they also have anti infectious, gynaecological effect and contractile activities of the smooth muscles[12]. Generally, the activities of crude extracts obtained from medicinal plants may be subjected to change based on the variations of chemical composition. Also, the activities difference may be due to the geographical origin, locality, climate conditions, and the harvest time of the collected plant material[4,8,13]. However, according to our knowledge on phytochemical screening, total phenol and flavonoids contents of different crude extracts obtained from Omani, *T. vulgaris* and other *Thyme* species available here have never been studied before. For this reason, the present work is design to investigate the phytochemical screening and to evaluate total phenol and flavonoids contents of different polarities crude extracts from the leaves of *T. vulgaris* species native to Oman.

2. Materials and methods

2.1. Chemicals and reagents

All kinds of solvents and acid used in this present study were purchased analytical grade from BDH, UK. The quercetin and gallic acid standard for total phenol and flavonoids content were from E. Merck, Germany. The other chemical reagents were from BDH, UK. UV spectra were recorded on UV-1800 Shimadzu spectrophotometer Ultrospeck in methanol (λ max in nm).

2.2. Sample collection

The leave sample of *T. vulgaris* was collected on the month of October 2012 from Al Jabal Al Akhdar, Nizwa, Sultanate of Oman. The fresh and healthy leaves were separated instantly and packed in a polyethylene bag. The samples were transported to the laboratory and kept at room temperature until processing.

2.3. Preparation of samples

The whole leaves samples of *T. vulgaris* were washed with natural water twice and dried under shade at room temperature for 3 d. The dry leave samples (100 g) were ground using a grinder (Jaipan, Super Deluxe, India) for 30 seconds. The semi power samples were powder form by grinder for 3 min. The whole leave powder samples of *T. vulgaris* were packed in a sealed plastic bottle until extraction.

2.4. Extraction procedure

The dried powder samples of *T. vulgaris* (92.22 g) were extracted with methanol (360 mL) using Soxhlet extractor for 3 d until complete extraction. After extraction, it was filtered and evaporated by rotary evaporator (Yamato, Rotary Evaporator, model-RE 801, Japan) to give amophorous solid masses. The crude extract (49.12 g) was defatted with water (75 mL). The crude extracts was transferred into a separatory funnel and finally extracted by different solvents with increasing polarities followed the sequence of hexane, chloroform, ethyl acetate and butanol to give hexane (2.43 g), ethyl acetate (0.53 g), chloroform (0.14 g), butanol (0.21 g) and residual methanol fractions (7.0 g), respectively. The extraction procedures were followed twice and combined.

2.5. Preliminary phytochemicals screening

The hexane, ethyl acetate, chloroform, butanol and methanol crude extracts (1 g) was completely dissolved in 100 mL of its own mother solvents. It was prepared the stock solution. The obtained stock solution was used for phytochemical screening following the methodology of Harborne and Kokate[14,15].

2.5.1. Test for alkaloids

One gram powder samples of *T. vulgaris* were taken in a conical flask and added ammonia solution (3 mL). It was allowed to stand for few minutes to evaluated free alkaloids. Chloroform (10 mL) was added to the conical flask shaken by hand and then filtered. The chloroform was evaporated from the crude extract by water bath and added Mayer's reagent (3 mL). A cream colour precipitation was obtained immediately that showed the presence of alkaloids.

2.5.2. Test for flavonoids

The stock solution (1 mL) was taken in a test tube and added few drop of dilute NaOH solution. An intense yellow colour was appeared in the test tube. It became colourless when on addition of a few drop of dilute acid that indicated the presence of flavonoids.

2.5.3. Test for saponins

The stock solution (1 mL) was taken in a test tube and diluted with 20 mL of distilled water. It was shaken by hand for 15 min. A foam layer was obtained on the top of the test tube. This foam layer indicated the presence of saponins.

2.5.4. Test for steroids

The crude plant extracts (1 mg) was taken in a test tube and dissolved with chloroform (10 mL), then added equal volume of concentrated sulphuric acid to the test tube by sides. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

2.5.5. Test for tannins

The stock solution (3 mL) was taken in a test tube and dilutedwith chloroform and added acetic anhydride (1 mL). Finally, sulphuric acid (1 mL) was added carefully by the side of test tube to the solution. A green colour was formed which showed the presence of tannins.

2.5.6. Test for triterpenoids

The dry crude plant extract (5 mg) was dissolved in chloroform (2 mL) and then acetic anhydride (1 mL) was added to it. Concentrated sulphuric acid (1 mL) was added to the solution. Formation of reddish violet colour shows the presence of triterpenoids.

2.6. Determination of total phenol by Folin-reagent method

Total phenol content was determined by Folin-Ciocalteu reagent method with modification. From each crude extracts (1 mg) was dissolved in methanol (1 mL). A total of 10% Folin-Ciocalteu reagent was prepared by adding Folin-Ciocalteu reagent (10 mL) in water (90 mL). Then, 5% Na_2CO_3 (3 g) was prepared by dissolving Na_2CO_3 (3 g) in water (50 mL). Each crude sample (200 μL) was taken in a test tube and added 10% Folin-Ciocalteu reagent (1.5 mL). Then all the test tube was kept in a dark place for 5 min. Finally, 5% Na_2CO_3 (1.5 mL) was added to the solution and mixed well by hand. Again all the test tube was kept in the dark for 2 h. The absorbance was measured for all solution by using UV-spectrophotometer at constant wavelength 750 nm.

2.7. Gallic acid standard curve

Gallic acid calibration crude was prepared by Folin-Ciocalteu reagent method with modification. Gallic acid (3 mg) was dissolved in methanol (10 mL). It was the concentration of 300 mg/L. It was diluted by adding methanol to prepared serial concentration 200, 100, 50 and 25 mg/L. The above same procedure was followed forgallic acid standard. The absorbance was measured for all standard solutions by using UV-spectrophotometer at constant wavelength 750 nm.

2.8. Determination of total flavonoids by colorimetric method

The methanol crude extract and its fractions hexane, ethyl acetate, chloroform and butanol crude extract were used to determine the total flavonoids contents. The total flavonoids contents of different crude extracts were estimated by aluminium chloride colorimetric method as described by Hossain *et al*[16]. Sodium nitrate (2.5 g) was taken in a volumetric flash (50 mL) and added water upto the mark that was 5% sodium nitrate. Sodium hydroxide (2.5 g) was taken in another volumetric flash (50 mL) and added water upto the mark that was 4% sodium hydroxide. Then 10% aluminium chloride solution was prepare the same procedure. The different crude extracts (0.25 mg) were taken in a test tube and added water (1.25 mL) and sodium nitrate (0.75 μL) then mixed together. All the test tubes were kept in the dark place for 6 min. Then 10% aluminium chloride (0.150 μL) was added into the test tube and wait for 5 min in the dark for complete reaction. Finally, 5% sodium hydroxide (0.5 mL) and water (0.275 mL) were added to the test tube. The absorbance was measured of all samples at a fixed wavelength 510 nm using UV Biomate 5 spectrophotometer. Quercetin standard was used for

the calibration curve. The estimation of total flavonoids contents in the crude extracts was carried out in triplicate and the results were averaged. The total flavonoids was calculated by the following formula:

X=(A. mo)/(Ao. m)

Where "X" is the flavonoid content, mg/g plant extract, "A" is the absorption of plant crude extract solution, "Ao" is the absorption of standard quercetin solution, "m" is the weight of crude drug extract in mg and "mo" is the weight of quercetin in the solution in mg.

3. Results

The amorphous solid masses were obtained by evaporation of methanol. The methanol dry crude extract was defatted with water and then extracted with different organic solvent with increasing polarities such as hexane, chloroform, ethyl acetate and butanol. The result for phytochemical screening of hexane, ethyl acetate, chloroform, butanol and methanol extracts were from the leaves of *T. vulgaris* showed the presence of flavonoids, saponins and steroids, but alkaloids, tannins, and triterpenoids not present in the crude extract (Table 1). All the groups were absent in hexane crude extract except steroids. However, steroids and saponins were present and the other groups such as alkaloids, flavonoids, tannins and triterpenoids were absent in ethyl acetate crude extract. Methanol crude extracts contained only the flavonoids and butanol crude extracts also contained flavonoids and saponins. None of groups presented in the chloroform crude extract.

Table 1
Phytochemical analysis of different extracts of *T. vulgaris*.

Biochemicals	Inference				
	Methanol	Hexane	Ethyl acetate	Chloroform	Butanol
Alkaloids	−	−	−	−	−
Steroids	−	+	+	−	−
Flavonoids	+	−	−	−	+
Saponins	−	−	+	−	+
Tannins	−	−	−	−	−
Triterpenoids	−	−	−	−	−

+: Presence; −: Absence.

The total phenol contents of five crude extracts determined by Folin-Ciocalteu method were reported as gallic acid equivalents (Figure 1). Among the five crude extracts, butanol extract contained the highest (245.26 mg/g) amount of phenol compounds followed by hexane extract (160.35 mg/g), chloroform extract (158.5 mg/g), ethyl acetate extract (84.85 mg/g), and methanol (49.43 mg/g).

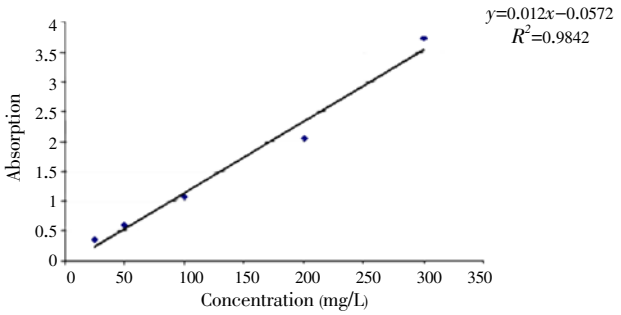

Figure1. Gallic acid standard curve.

The result of total flavonoid contents of the five crude extracts of *T. vulgaris* is given in Figure 2. The total flavonoid contents in the different crude extracts varied from 3.44 to 53.28 mg quercetin/g weight (Figure 2). Among the five crude extracts, methanol extract contained the highest (1.71 mg/g) amount of flavonoids content compounds followed by butanol (1.55 mg/g), chloroform (1.37 mg/g), ethyl acetate (1.29 mg/g) and hexane (1.18 mg/g). The variation may be due environmental conditions, which can modify the constituents of the plant.

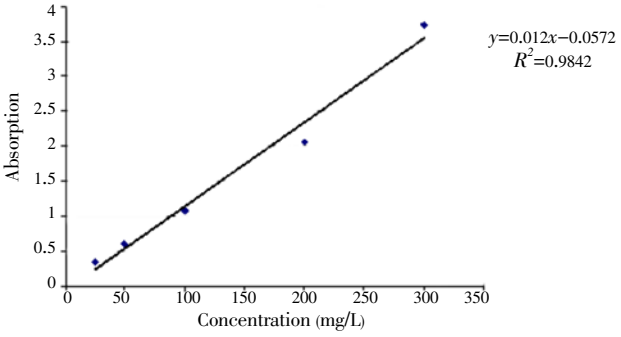

Figure 2. Calibration cruve of quercetin standard.

4. Discussion

The chemical constituents in the plants or crude extracts are known to be biologically active ingredients. Some chemical constituents are considered as secondary metabolites components. They are directly responsible for different activity such as antioxidant, antimicrobial, antifungal and anticancer[13-15]. All these secondary metabolites components showed antioxidant and antimicrobial properties through different mechanism. Normally these secondary metabolites components were isolated from the polar plant extract[16]. The phytochemical screening of hexane, ethyl acetate, chloroform, butanol,

methanol crude extracts from dry powder leaves samples of *T. vulgaris* used in this present study. The crude extracts revealed that the crude extracts contained flavonoids, saponins and steroids compounds. The screening of the methanol and butanol dry leaves crude extracts studied were showed the presence of active constituent flavonoids. The other groups of compounds were not present in methanol and butanolcrude extracts. Alkaloids, triterpenoids and tannins were not present in all crude extracts. Saponins were present in butanol and ethyl acetate crude extracts of *T. vulgaris*. The most important bioactive compounds flavonoids were found in methanol and butanol crude extracts. Therefore, the detected different bioactive compounds in the different crude may be responsible for the antibacterial activity of the plant crude extracts. Several authors already reported on flavonoids groups exhibited a wide range of biological activities such as antioxidant, anti-inflammatory, antimicrobial, anti-angionic, anticancer and anti-alergic[15-21]. Saponins are other type bioactive chemical constituents which are involved in plant disease resistance because of their antimicrobial activity[17]. Tannins are phenolic compound and their derivatives are also considered as primary antioxidants or free radical scavengers[18].

The total phenol contents of the crude extracts as determined by established method are reported as gallic acid equivalents. Among the five extracts, butanol extract contained the highest amount of phenol compounds followed by the order hexane>chloroform extract>ethyl acetate extract>methanol. The literature search reveals that there is no work that has been done on total phenol contents of this Omani *Thyme* plant species. Comparison our results with other reported results on total phenol are not similar[13-15].

The present study, the total flavonoids contents of the different organic crude plant extracts were determined by Hossain *et al.* method that are reported as quercetin equivalents[16]. Among the five crude extracts from *T. vulgaris*, methanol extract was containing highest amount of flavonoids content compounds followed by butanol>chloroform>ethyl acetate>hexane crude extract. In previous several studies on flavonoids contents, their findings has been reported similar trained[5,7,9]. The difference of results obtained might possibly be due to the different method of extraction and solvents polarities. During the samples processing and drying, may be some volatiles active compounds destroyed or evaporated from the samples.

Medicinal plants are the best sources for chemical ingredients, antimicrobial and antioxidant agents for cure of different diseases. The butanol and methanol crude extracts from *T. vulgaris* showed good amounts of total phenol and flavonoids contents and these crude extracts could be used as antibiotics or different aliments.

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 belonging 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. According to the literature search, there is no work that has been done on Omani *T. vulgaris* by the researcher.

Research frontiers

The aim of this study is to prepare various crude extracts using different polarities of solvent and to quantitatively evaluate their total phenol, flavonoids contents and phytochemical screening of *T. vulgaris* collected from Al Jabal Al Akhdar, Nizwa, Sultanate of Oman.

Related reports

According to the literature search, there is no work that has been done on Omani *T. vulgaris* by the researcher. The other parameters of this plant has been done by other

researchers.

Innovations & breakthroughs

Although the experimental work done by the author is routine work, 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

The present study on phytochemical screening, toal phenol and total flavonoids of various leaves crude extracts of *T. vulgaris* is giving the valuable brief and scientific information about this plant.

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