herbmed.skums.ac.ir

Chemical composition and antioxidant activity of Shirazi *Thymus vulgaris* essential oil

Zahra Nemati[1*], Rasoul Barzegar[1], Maryam Khosravinezhad[1], Ebrahim Talebi[2*], Hamid Reza Safaei[1]

[1]*Applied Chemistry Dept., Shiraz Branch, Islamic Azad University, Shiraz, Fars, I.R. Iran;*
[2]*Darab Branch, Islamic Azad University, Darab, Fars, I.R. Iran.*

Received: 10/May/2017 Accepted: 24/Jul/2017

ABSTRACT

Background and aims: Coinciding with the rise of human population, the use of medicinal plants began by ancient peoples as medicine, poison, detergent, food and paint upon it.

Methods: In present study, the chemical composition and antioxidant activity of Shirazi thyme, *T. vulgaris*, essential oil was analyzed by three methods (DPPH, potassium ferricyanide reaction and CUPRAC methods) and comparison with TBHQ method for determination of reducing power of *T. vulgaris* essential oil by potassium ferricyanide method. Phytochemical composition of *T. vulgaris* essential oil was identified by GC/Mass device.

Results: The results showed that *T. vulgaris* essential oil has a good potential against oxidants even near to TBHQ. The essential oils of this plant are Thymol (40.02%) and Carvacrol (18.31%).

Conclusion: The results of antioxidant activity of Thymus vulgaris using three methods and comparison with TBHQ (Tertiary butyl hydroquinone) showed Thymus vulgaris essential oil have a good potential for scavenging of free radicals similar to TBHQ

Keywords: *Thymus vulgaris*, Antioxidant activity, Essential oil, GC/mass, TBHQ.

Original article

INTRODUCTION

The pharmaceutical properties of aromatic plants exist in essential oils. They are used to treat diseases traditionally.[1,2] Thyme (*T. vulgaris*) plant grows in throughout of Iran, especially in Chahar Mahal and Bakhtiari and Fars provinces.[3] The "Avishan or Azorbe," (Persian name of Thyme) consists 14 species which grow in many regions of Iran.[4] Thyme (*T. vulgaris*) is especially well known for its aromatic and therapeutic properties due to the essential oil of its leaves.[5] Thyme (*Thymus vulgaris*), belonging to the lamiaceae family, is a pleasant smelling perennial shrub, which

[*]**Corresponding author:** Zahra Nemati. Applied Chemistry Dpet., Shiraz Branch, Islamic Azad University, Shiraz, Fars, I.R. Iran; Tel: 00989174844255, E-mail: nemati_232000@yahoo.com; Ebrahim Talebi. Darab Branch, Islamic Azad University, Darab, Fars, I.R. Iran; Tel: 00989177312823, E-mail: talebi226@iaudarab.ac.ir

grows in several regions in the world. Thyme is used for marine feed, poultry, soups and vegetables, for flavoring liqueur in herbal teas prepared for colds and flues, as well. Thyme and its oil have been used as fumigants, antiseptics, antioxidant and mouth washes. The main constituents of *Thymus vulgaris* include Thymol, carvacrol and flavonoids to have antibacterial, anti-flatulent and anti-worm properties.[6] Plants belonging to the Lamiaceae family are known to be rich in compounds possessing strong antioxidant activity.[7] The essential oils isolated from some species of thymus are characterized by different analytical methods that show of a high concentration of Thymol and carvacrol.[8]

Based on research report, the essential oils with high proportions of the phenolic components Thymol and/or carvacrol exhibited the highest antioxidant activity.[9] Based on investigation, the principle percentage of component in the essential oils of common thyme from different geographical sources (Estonia and in other European country) are Thymol (0.9-75.7), carvacrol (1.5-83.5), p-cymene (4.3, 34.4) γ-terpinene (0.9-19.7), linalool (0.4-4.8), (E)-β-caryophyllene (0.5-9.3) and terpinen-4-ol (tr-3.8).[10] Data on the essential oil composition of *Thymus vulgaris* of other continents have been presented in numerous publications. Depending on the geographical origin and the specific ecological sites from which it is collected, its quantitative and qualitative composition vary greatly between countries and the diversity of essential oils arises from the existence of many chemotypes.[11] The condition of Iran is suitable for growth medicinal plants and these condition influences to chemical composition of them. Iranian habitats support about 8000 species of flowering plants (belonging to 167 families and 1200 genera), of which almost 1700 are endemic.[12] The antioxidant activity of *Thymus vulgaris* essential oil from some areas in the world such as Bosnia and Herzogovin, Romain, Yemen, Tunisia, Jordan, Eastern Austria, Northeastern Mexico was evaluated.[7,9,11,13-16] In this study antioxidant activity of *Thymus vulgaris* essential oil that grown in Shiraz, Iran was evaluated by DPPH, potassium ferricyanide reaction and CUPRAC (Cupric Reducing Antioxidant Capacity) methods and these results were compared with Tertiary Butyl hydroquinone (TBHQ) which is a Synthetic antioxidant. Also, chemical composition of *Thymus vulgaris* essential oil that grown in Shiraz, Iran was analyzed by gas chromatography-mass spectrometry (GC/mass) device.

METHODS

Thymus vulgaris (*Zataria multiflora bioss* with herbarium code 35314) was prepared and dried in spring season from Shiraz, Iran. GC/Mass instrument in model of 7890A, Agilent Technologies and Micro plate reader instrument in model of Beijing Beifen-Ruili, VIS-7220G/UV-9200 were used for analysis. TBHQ, DPPH, methanol, copper (II) chloride were purchased from Sigma-Aldrich Company and potassium ferricyanide, trichloro acetic acid, phosphate buffer from Merck Company.

For extraction of *Thymus vulgaris* essential oil, 50 g of dried *Thymus vulgaris* was added with 500 ml distilled water after

that was extracted by Clevenger device. Finally, the essential oil was kept in a glass bottle at 18 ºC until research.

GC/Mass system condition of the column was HP-5MS (30 m ×0.25 mm, film thickness 0.25 μm). The column temperature was programmed at 60 to 210ºC with rate of 3 ºC for 1 min, and then temperature changed at 210 to 240 ºC with rate of 20 ºC for 1min after that for 8 minute staying in this temperature. The carrier gas was helium. The injection port and the detector temperature was 280 ºC (split ratio: 1/100). DPPH method

First of all, added 20 µl of samples (essential oil of *Thymus vulgaris* and TBHQ) with 6.25 to 800 concentrations into cells 1 to 8 of micro plate rows. Then, 200 µl of DPPH solution was added to the cells of row. Then, 20 µl of samples with 6.25 to 800 concentrations was added into cells 1 to 8 of other row of micro plate with 200 µl of methanol as blank. Also, 20 µl of methanol with 200 µl of DPPH solution was added into last cell of each row as control. This experiment was performed in three replicates. After that, the micro plate was kept in dark place for 30 minute. Finally, micro plate was placed into micro-plate reader instrument.

The antioxidant activity of samples was calculated using the following equation and applied the graph pad prism software:

$$IC50=100 - [\frac{ABsample-ABblunk}{ABcontrol}] \times 100$$

IC50 values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.[17]

For determination of reducing power of *Thymus vulgaris* essential oils by potassium ferricyanide method, at first, 1 ml of *Thymus vulgaris* essential oils was mixed with 2.5 ml of phosphate buffer (0.2 M, pH=6.6) and 2.5 ml of 1% potassium ferricyanide in 10 ml test tubes. The mixtures were incubated for 20 min at 50 ºC. At the end of the incubation, 2.5 ml of 10% trichloroacetic acid was added to the mixtures, followed by centrifuging at 5000 rpm for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride and the absorbance was measured at 700 nm.[18]

CUPRAC method is based on reduction of Cu^{+2} to Cu^{+1} by reductants (antioxidants) present in a sample.[18] This method should be advantageous over the ferric reducing antioxidant power method because the redox chemistry of copper (II) as opposed to that of ferric ions involves faster kinetics. The method comprises mixing of the antioxidant solution with a copper (II) chloride solution, a neocuproine alcoholic solution, and an ammonium acetate aqueous buffer at pH=7 and subsequent measurement of the developed absorbance at 450 nm after 30 min.[19] Finally, the cupric ions (Cu^{+2}) reducing capacity of samples was expressed as trolox equivalent (µg/ml).

RESULTS

The results of GC/mass chromatography analysis of *Thymus vulgaris* essential oil were shown in Table 1 that contains twenty six compounds. The important percentages of compounds were carvacrol (18.31), Thymol (40.02).

Table 1: Chemical composition of *Thymus vulgaris* essential oil using gas chromatography-mass spectrometry (GC/Mass)

6	component	Retention time	Area%
1	α-Thujene	5.7	0.27
2	α-pinene	5.9	0.97
3	Camphene	6.3	0.60
4	β-pinene	6.6	0.16
5	Myrcene	7.4	0.89
6	α-Phellandrene	7.9	0.14
7	α-Terpinene	8.3	1.10
8	Cymene(p)	8.8	16.78
9	Limonene	8.8	0.46
10	ɣ-Terpinen-7-al	9.9	4.16
11	Sabinene hydrate	10.1	0.22
12	Trans-Linalool oxide(furanoid)	10.3	0.14
13	Linalool	11.5	4.84
14	Bornrol	14.0	2.67
15	Terpinen-4-ol	14.5	0.65
16	α-Terpineol	15.0	0.15
17	Thymol	19.4	40.02
18	Carvacrol	19.9	18.31
19	Piperitone	21.5	0.10
20	Thymol acetate	22.0	1.47
21	Carvacrol acetate	22.7	0.40
22	Caryophyllene(E)	24.6	1.23
23	Aromadendrene	25.4	0.10
24	α-Humulene	26.0	0.18
25	Caryophyllene	31.1	2.10
26	Caryophylla-4-(12),8(13)-dien-5-β-ol	33.0	0.31

The results of antioxidant activity of *Thymus vulgaris* essential oil and TBHQ by DPPH method showed that IC50 for *Thymus vulgaris* essential oil and TBHQ are 45.21±0.34 µg/ml and 23.83±2.44 µg/ml, respectively (Table 2).

Table 2: Antioxidant activity of *Thymus vulgaris* essential oil and TBHQ using DPPH method in µg/ml

Sample	IC50
TBHQ	23.83±2.44
Thymus vulgaris	45.21±0.34

The result of antioxidant activates of samples by potassium ferricyanide method revealed that the amounts of *Thymus vulgaris* essential oil and TBHQ by reducing of Fe^{+3} to Fe^{+2} is 509.48±18.35 µg/ml and 610.24±12.67 µg/ml, respectively (Table 3).

Table 3: Total antioxidant potential assay and reducing power of *Thymus vulgaris* essential oil and TBHQ using potassium ferricyanide method in µg/ml

Sample	µg/ml	µg/ml
TBHQ	907.54±16.74	610.24±12.67
Thymus vulgaris	524.76±8.47	509.48±18.35

DISCUSSION

The IC50 of *Thymus vulgar* about essential oil in comparison to TBHQ is similar. Based on my results and reported cases, *Thymus vulgaris* essential oils exhibited significant in vitro antioxidant activity.[7]

Also, the results of antioxidant activities of samples by total CUPRAC antioxidant capacity was shown that the amount of *Thymus vulgaris* essential oil and TBHQ by reducing Cu^{+2} to Cu^{+1} are 524.76±8.477 µg/ml and 907.54±16.74 µg/ml, respectively (Table 3). According to the results by total CUPRAC methods and potassium ferricyanide indicated that *Thymus vulgaris* essential oils have a good potential by reducing of Fe^{+3} to Fe^{+2} and also Cu^{+2} to Cu^{+1} similar to TBHQ. The result of antioxidant activity of *Thymus vulgaris* by three methods and comparison with TBHQ showed that this plant has a good potential of against oxidants similar to TBHQ. The based on report, the essential oil of *Thymus vulgaris* has a potential antioxidant activity and a protective effect against AFs toxicity and this protection was dependent dose.[20] Previous studies have shown that oregano essential oil that contains of high content of Thymol and carvacrol has a strong antioxidant activity.[7] *Thymus vulgaris* which grown in Shiraz, Iran contains a high concentration of Thymol (40.02%) and carvacrol (18.31%). So, the high antioxidant activity of Shirazi *Thymus vulgaris* essential oil related to concentration of carvacrol and Thymol.

CONCLUSIONS

The results showed that the essential oil of *Thymus vulgaris* grown in Shiraz contains twenty six compounds. The important compounds were Thymol (40.02%) and Carvacrol (18.31%). Thymol and Carvacrol were the main constituents of *Thymus vulgaris* essential oil. The results of antioxidant activity of *Thymus vulgaris* using three methods and comparison with TBHQ revealed that *Thymus vulgaris* essential oil has a good potential for

scavenging of free radicals similar to TBHQ. These activates related to concentration of Thymol and carvacrol. In conclusion, *Thymus vulgaris* that grown in Shiraz can be used in the wide range of foods and pharmaceutical applications.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENT

The authors would like express their since to thank Research Center of Shiraz branch, Islamic Azad University for partial supporting of this research.

REFERENCES

1. Ratajac R, Stojanovic D, Petrovic J, Milanov D, Vasic R, Stojanov I, et al. Antibacterial activity of the essential oil of Mountain Savory (Satureja montana) against Arcanobacterium pyogenes. Planta Med. 2011; 77(12): 5-7.
2. Miladi H, Ben Slama R, Mili D, Zouari S, Bakhrouf A, Ammar E. Chemical composition and cytotoxic and antioxidant activities of *Satureja montana* L. essential oil and its antibacterial potential against Salmonella spp. strains. J Chem. 2013; 4(2): 9-11.
3. Ashnagar A, Gharib Naseri N, Ramazani M. Characterization of the major chemical compounds found in *Thymus vulgaris* plant grown wildly in Chaharmahal and Bakhtiari province of Iran. Int J PharmTech Res. 2011; 3: 1-4.
4. Pirbalouti AG, Hashemi M, Ghahfarokhi FT. Essential oil and chemical compositions of wild and cultivated Thymus daenensis Celak and *Thymus vulgaris* L. Ind Crops Prod. 2013; 48: 43-8.
5. Razzaghi-Abyaneh M, Shams-Ghahfarokhi M, Rezaee M-B, Jaimand K, Alinezhad S, Saberi R, et al. Chemical composition and antiaflatoxigenic activity of Carum carvi L. *Thymus vulgaris* and Citrus aurantifolia essential oils. Food Control. 2009; 20(11): 1018-24.
6. Ozcan M, Chalchat J-C. Aroma profile of *Thymus vulgaris* L. growing wild in Turkey. Bulg J Plant Physiol. 2004; 30(3-4): 68-73.
7. Kulisic T, Radonic A, Milos M. Antioxidant properties of thyme (*Thymus vulgaris* L.) and wild thyme (*Thymus serpyllum* L.) essential oils. ITAL J Neurol Sci. 2005; 17(3): 315.
8. Al Hashmi LS, Hossain MA, Weli AM, Al Riyami Q, Al Sabahi JN. Gas chromatography-mass spectrometry analysis of different organic crude extracts from the local medicinal plant of *Thymus vulgaris* L. Asian Pac J Trop Biomed. 2013; 3(1): 69-73.
9. Hudaib M, Aburjai T. Volatile components of *Thymus vulgaris* L. from wild-growing and cultivated plants in Jordan. Flavour Frag J. 2007; 22(4): 322-7.
10. Raal A, Arak E, Orav A. Comparative chemical composition of the essential oil of *Thymus vulgaris* L. from different geographical sources. Herba Pol. 2005; 1(2): 10-7.
11. Marzouk B, Edziri H, Haloui I, Issawi M, Chraief I, El-Ouni M, et al. Chemical composition, antibacterial and antioxidant activities of a new chemotype of Tunisian *Thymus vulgaris* oils growing in Sayada. J Food Agric Environ. 2009; 7(2): 263-7.
12. Heshmati G. Vegetation characteristics of four ecological zones of Iran. Int J Plant. 2012; 1(2): 215-24.
13. Jianu C, Mihail R, Muntean SG, Pop G, Daliborca CV, Horhat FG, et al. Composition and antioxidant capacity of essential oils obtained from *Thymus vulgaris*, Thymus pannonicus and Satureja montana grown in Western Romania. Rev Chim-Bucharest. 2015; 66: 2157-60.
14. Al-Maqtari M, Alghalibi SM, Alhamzy EH. Chemical composition y antimicrobial activity of essential oil of *Thymus vulgaris*

from Yemen. Turkish J Biochem. 2011; 36: 342-9.
15. Chizzola R, Michitsch H, Franz C. Antioxidative properties of *Thymus vulgaris* leaves: Comparison of different extracts and essential oil chemotypes. J Agric Food Chem. 2008; 56(16): 6897-904.
16. Guerra-Boone L, Alvarez-Román R, Salazar-Aranda R, Torres-Cirio A, Rivas-Galindo VM, Waksman de Torres N, et al. Antimicrobial and antioxidant activities and chemical characterization of essential oils of *Thymus vulgaris*, Rosmarinus officinalis, and Origanum majorana from northeastern México. Pak J Pharm Sci. 2015; 28(1): 363-9.
17. Pourmorad F, Hosseinimehr S, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr J Biotechnol. 2006; 5(11): 1142-5.
18. Jayaprakasha G, Singh R, Sakariah K. Antioxidant activity of grape seed (Vitis vinifera) extracts on peroxidation models *in vitro*. Food Chem. 2001; 73(3): 285-90.
19. Apak R, Guclu K, Ozyurek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. J Agric Food Chem. 2004; 52(26): 7970-81.
20. El-Nekeety AA, Mohamed SR, Hathout AS, Hassan NS, Aly SE, Abdel-Wahhab MA. Antioxidant properties of *Thymus vulgaris* oil against aflatoxin-induce oxidative stress in male rats. Toxicon. 2011; 57(7): 984-91.

How to cite the article: Nemati Z, Barzegar R, Khosravinezhad M, Talebi E, Safaei H. Chemical composition and antioxidant Activity of Shirazi *Thymus vulgaris* essential oil. Adv Herb Med. 2018; 4(2):26-32