

Chemical Composition and Antioxidant Properties of Essential Oil of *Thymus kotschyanus* L. Collected from East Azarbaijan Province, Iran

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ABSTRACT

Background and Objectives: Due to their antimicrobial, anticancer and antioxidant properties (due to the presence of free radical scavengers), essential oils and extracts of medicinal plants are of great importance as natural medicinal compounds in public health, treatment of diseases, and protection of raw and processed foods .

Methods: Chemical composition and content of essential oil of *Thymus kotschyanus* was determined by gas chromatography–mass spectrometry. The amount of phenolic and flavonoid compounds in the essential oil was determined by spectrophotometry using gallic acid and quercetin as standards. The antioxidant properties of the essential oil were evaluated by the DPPH method.

Results: The analysis of essential oil with gas chromatography–mass spectrometry showed that thymol (51.1%), p-cymene (13.78%) and α -pinene (7.42%) are the main components. The amount of phenolic compounds was 82 ± 6.43 μ g gallic acid/ml essential oil, while the flavonoid content was 30.79 ± 0.5 μ g quercetin/ml essential oil. In terms of antioxidant activity, the IC₅₀ value of *T. kotschyanus* essential oil was determined as 32.35 μ g/ml, which is weaker than synthetic antioxidant butylated hydroxytoluen.

Conclusion: The results indicate that the essential oil of *T. kotschyanus* has good antioxidant activity and can be used in combination with other preservatives to protect food against a variety of oxidative systems.

Keywords: Essential Oil, Antioxidant Activity, *Thymus Kotschyanus*.

INTRODUCTION

Lipid oxidation is one of the most important known causes of deterioration in the quality of food during storage or processing. Most edible vegetable oils that contain high levels of unsaturated fatty acids are highly susceptible to oxidation. This process not only involves the rapid development of pungent taste, unpleasant odor, and paleness, but also reduces the nutritional value of oils and lipids and exerts adverse effects on human health by producing some toxic and dangerous compounds. In order to delay or slow down the oxidation reaction process, synthetic antioxidants including butylated hydroxytoluene (BHT) and tertiary butylhydroquinone are widely used in many countries. These cheap and available compounds are highly efficient oxidation inhibitors, but they are known to increase the risk of developing cancer and liver and cardiovascular diseases (1). Today, due to emergence of drug-resistance in various pathogenic microorganisms and raised awareness of consumers and healthcare providers about the harmful effects of chemical and synthetic preservatives used in foodstuff, there is a need for identification of new natural compounds with antimicrobial and food-preserving properties. Such compounds not only increase the shelf life of food, but also protect the consumers from the harmful effects of chemical and synthetic preservatives (2-5). Since essential oils and extracts from medicinal herbs have antimicrobial, anticancer, antioxidant and free radicals scavenging properties, they have the potential to be used as new and natural preservatives (6, 7). Among herbal compounds that have antioxidant properties, phenolic compounds are widely distributed. The antioxidant properties of phenolic compounds are mainly due to their reduction capacity and chemical structure, which enable them to neutralize free radicals, form complex with metal ions, and eliminate singlet and triplet oxygen molecules. In addition to their anticancer and hypoglycemic effects, phenolic compounds can inhibit lipid oxidation reactions through electron donation to free radicals (8). *Thymus* (thyme) is a genus of plants from the mint family Lamiaceae. *Thymus kotschyanus* is one of the 14 native thyme species in Iran (9). Phytochemical study of different species of thyme indicates the presence of phenolic

compounds such as thymol, carvacrol, caffeic acid, terpenoids, saponin, and flavonoids that are widely used in the food and cosmetic industries because of their antibacterial, antifungal, analgesic, antioxidant and insecticidal activities (10-13).

Considering the importance of medicinal plants, especially native medicinal plants of our country, we studied the functional properties of essential oil from *T. kotschyanus*, as an affordable and available source of food preservative that could prevent food spoilage and its resulting economic impact as a step towards improved public health and food safety.

MATERIAL AND METHODS

Fully dried *T. kotschyanus* plant was obtained from herb shops in city of Tabriz, Iran. The scientific name was confirmed by the Herbarium Center of the Faculty of Pharmacy, Tabriz University of Medical Sciences, Iran. In order to prepare the essential oil, the plant was crushed and grinded. Then, the essential oil was extracted by water distillation using a Clevenger apparatus (14). After drying using sodium sulfate, the essential oil was passed through 0.45 μm microfilter, and stored in a dark glass container at 4 $^{\circ}\text{C}$ away from sunlight. Analysis of essential oil composition using Gas chromatography mass spectrometry (GC-MS) Identification of essential oil components was done using retention indices by evaluating mass spectra of compounds and comparing them with standard mass spectra and valid references. For this purpose, the prepared essential oil was injected into the GC system, and the optimum column temperature was calculated for the complete separation of the essential oil's components. The essential oil was then injected into the mass spectrometer attached to the gas chromatograph to study the mass spectra of the compounds. The GC-MS device Agilent 6890 with a HP-5MS capillary column (0.25 mm internal diameter, 30m length, film thickness 0.25 μm) was used in this study. The column temperature program started with 70 $^{\circ}\text{C}$ heating for 2 min. Then, the temperature was increased to 220 $^{\circ}\text{C}$ at rate of 15 $^{\circ}\text{C}$ per minute, and continued to 300 $^{\circ}\text{C}$ for 2 min (14). Evaluation of phenolic compounds was done using Folin-Ciocalteu reagent and gallic acid as standard (15). First, 0.1 ml of different

concentrations from essential oil was added to an Erlenmeyer flask. Then, 46 ml of distilled water and 1 ml of the Folin–Ciocalteu reagent were added to the flask. The contents of the flask were mixed thoroughly for 3 min. After adding 3 ml of 2% sodium carbonate solution, the mixture was stirred for 2 hours. Absorbance at 760nm was measured using a spectrophotometer. Same steps were performed for the standard gallic acid solutions (0-1000 µg/0.1 ml). Standard curve was plotted to calculate the concentration of phenolic compounds based on µg gallic acid/ml essential oil. Various concentrations were prepared from the essential oil to measure the flavonoid content. Then, 0.5 ml of each concentration was poured into a test tube. The tube was filled with 500 µl of 2% aluminum sodium chloride solution dissolved in ethanol. After placing the tube at room temperature for 1 hour, the absorbance of the solution was read at 420 nm by a spectrophotometer (16). The calibration curve of quercetin was plotted in the 5 to 60 mg/ml range using the same method. The total amount of flavonoids was calculated based on µg quercetin/ml essential oil. The hydrogen atoms donation ability of the essential oil was measured by bleaching the purple colored methanolic solution of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) (17). The stable radical DPPH was used as reagent in this spectroscopic evaluation. Briefly, 50 µl of different concentrations of essential oil were added to 5 ml of 0.004% DPPH methanolic solution. After 30 min incubation at room temperature, the absorbance was read at 517 nm, and then compared with the control.

The DPPH free radical scavenging activity was calculated as percent (I%) using the following formula:

$$I\% = (A \text{ blank} - A \text{ sample} / A \text{ blank}) \times 100$$

In the formula above, A blank is absorbance of the control solution (containing all the reagents except the essential oil). A sample is absorbance of the solution containing various concentrations of the essential oil. The synthetic antioxidant BHA was also used as positive control. Each experiment was repeated three times and the mean value was calculated. The antioxidant activity of the essential oil was expressed as half-maximal inhibitory concentration (IC50) value, which indicates the concentration of essential oil that inhibits 50% of the radicals.

RESULTS

Efficiency of the essential oil of *T. kotschyanus* is 0.2% based on the dry weight of the sample. The main constituents of the essential oil and the percentage of their inhibitory activity are presented in Table 1. The 18 compounds identified are accounted for 97.44% of the total essential oil. The main constituents of the essential oil were alpha-pinene (7.42%), gamma-terpinene (9.03%), p-cymene (13.78%), and thymol (51.1%).

RT: Retention time

The antioxidant activity of the essential oil was evaluated using the DPPH method. The IC50 value for the essential oil was determined as 32.35 µg/ml, which is weaker than that of BHT(7.06 µg/ml). In addition, the results showed that increasing the concentration of the essential oil increases the free radical scavenging activity.

Level of phenolic compounds

The phenol content of the essential oil was 82 ± 6.43 µg Gallic acid/ml essential oil in the Folin–Ciocalteu method.

The flavonoid content of the essential oil was 30.79 ± 0.5 µg quercetin/ml essential oil.

Table 1- Analysis of chemical composition of the essential oil of *T. kotschyanus* by GC-MS

No	Compounds	RT (min)	Percentage	No	Compounds	RT (min)	Percentage
1	Alpha-Thujene	4.38	0.65	10	Camphor	8.14	0.58
2	Alpha-Pinene	4.52	7.42	11	Cyclohexane	8.72	1.58
3	Camphene	4.78	0.95	12	Isoborneol propionate	9.11	0.93
4	Beta-myrcene	5.24	0.75	13	Carvacrol	11.06	1.41
5	Alpha-Terpinene	5.92	1.87	14	Thymol	11.6	51.1
6	p-Cymene	6.08	13.78	15	Trans-Caryophyllene	13.60	1.93
7	Beta-Phellandrene	6.15	1.05	16	D Germacrene	14.88	0.29
8	1,8-cineole	6.19	3.35	17	Delta-Cadinene	15.79	0.38
9	Gama-Terpinene	6.67	9.03	18	Cis-alpha-bisabolene	16.17	0.39
Sum			97.44				

DISCUSSION

The proper use of medicinal plants requires thorough scientific knowledge and identification of plants' chemical composition, which is responsible for their therapeutic properties. This study confirms the presence of flavonoids and phenolic compounds in the essential oil of *T. kotschyanus*. Analyzing the chemical composition of the essential oil of *T. kotschyanus* with GC-MS indicated the presence of high levels of monotropic compounds, which have high antibacterial and antioxidant activities. Our results are partially consistent with other studies in this regard (18, 19). In study of Bagci et al. on two species of thyme in Turkey, the main components of *T. kotschyanus* at flowering stage were carvacrol (11.7%), thymol (35.5%), p-cymene (17.7%), alpha-pinene (8.8%) and alpha-terpineol (6.5%), which are similar to the results of our study. However, there are some differences in the percentage of the constituents (19).

Study of Semnani et al. on *T. kotschyanus* collected from Behshahr (Iran) found the following compounds: pulegone (18.7%), isomenthol (17.8%), thymol (14.9%), 1, 8-cineole (9%) and carvacrol (5.5%). Although their results are similar with our results in terms of the chemical composition, the percentage of the components is different (20). In chemical analysis of the essential oil of *T. kotschyanus* and *T. Persicus* by Rasooli and Mirmostafa, moderate levels of carvacrol (22.7% and 27%) and thymol (16.5% and 27.07%) were found. The total carvacrol and thymol content found in the mentioned study is less than that of the main compounds in our study (18). Study of Mehdizadeh et al. evaluated the antibacterial, antioxidant, and optical properties of starch-chitosan based edible film containing *T. Kotschyanus* essential oil. They found that increasing the concentration of essential oil significantly increases the concentration of phenolic compounds in the film ($P < 0.05$), so that at concentration of 1 and 2% essential oil, the level of phenolic compounds is 10 and 13.3 mg Gallic acid/gram film, respectively.

Moreover, increasing the concentration of essential oil significantly increases the antioxidant property ($P < 0.05$). Both results reported by the mentioned study are in agreement with our findings (21).

Study of Hosseini et al. investigated the free-

radical scavenging activity of different essential oils and methanolic extracts of *Zataria multiflora*, *Salvia officinalis*, rosemary, *Mentha pulegium* and cinnamon. They found that the essential oil of *Z. multiflora* has the highest antioxidant effect with IC₅₀ of 667 µg/ml. However, the essential oil of *Z. multiflora* has lower antioxidant effect compared to the essential oil of *T. Kotschyanus* analyzed in the present study (22). IC₅₀ is inversely associated with the antiradical activity of the compounds i.e. lower IC₅₀ value indicates higher antiradical activity. Zhang et al. evaluated the antioxidant activity of *Petroselinum crispum* essential oil by the DPPH method. The IC₅₀ value of the essential oil was found as 80.81 mg/ml, which indicates a very weak antioxidant activity (23). Fazel et al. determined the antioxidant activity of essential oils of thyme and *Satureja* by the DPPH method. The IC₅₀ values of these essential oils were 8.9 and 5.8 mg/ml, respectively. Considering the IC₅₀ values, it can be concluded that the essential oil of *T. kotschyanus* has higher antioxidant activity compared to these two essential oils (24). In another study by Gulluce et al., the antioxidant activity of essential oil and methanolic extract of *Mentha piperita* was determined by the DPPH method as 10700 µg/ml and 74.4 µg/ml, respectively (25). Comparison of the results of this study with the present study shows that the essential oil of *T. kotschyanus* has stronger antioxidant effect. Folin-Ciocalteu method of spectrophotometry is based on chemical reduction of a tungsten and molybdenum oxide mixture. In this method, phenolic compounds make complex reactions with the phosphotungstic and phosphomolybdenum acids in the Folin-Ciocalteu reagent. Studies show that increasing the amount of essential oils increases the amount of phenolic compounds and absorbance rate at the desired optical spectrum, which is consistent with our findings (26). The differences observed in the antioxidant properties of medicinal plants in various studies can be due to differences in the composition of the plants (due to genetic, climate, harvesting season, and other factors), especially in their phenolic and polyphenolic content, in a way that there is a direct relationship between the phenol content and antioxidant activity of medicinal herbs (27).

CONCLUSION

According to the results of our study and the need for natural preservatives, the essential oil of *T. kotschyanus* can be used in the food industry as an antioxidant. In addition, this essential oil could be simultaneously used with other preservatives to achieve higher efficacy and economic efficiency in the production of safe foodstuff. The synergistic effect of these factors in foodstuff could be the subject of

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future studies.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding this manuscript.

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