## Antioxidant activity of Thymus Kotschyanus Boiss. & Hohen. extracts and essential oil

Al-N. Aqeel, Narine A. Zakaryan\*, Vahagn S. Gevorgyan Eurasia International University Department of Pharmacy, \*narine.zakaryan@eiu.am

## Abstract

Medicinal plants are rich in therapeutically important secondary metabolites. In this paper, we are representing the phenolic content and the antioxidant properties of *Thymus kotschyanus* Boiss. & Hohen. decoction, ethanolic extract and different dilutions of essential oil. Based on the results we obtained from this study we concluded that decoction and 0.5% essential oil solution of *T. kotschyanus* have remarkable activity and can serve as a strong natural antioxidant.

Keywords: Thymus kotschyanus, essential oil, antioxidant activity, phenols.

**Introduction.** Medicinal plants are rich in therapeutically important secondary metabolites. The key advantages claimed for the therapeutic use of medicinal plants in various diseases are their economics, efficacy, and their safety, in addition to their availability. Because of these advantages, medicinal plants have been traditionally widely used in medicine. Among the various plants, *Thymus kotschyanus* Boiss. & Hohen. is traditionally used in Armenia by the vast majority of the population to treat numerous illnesses, however, it is not an officinal plant. The thyme has expectorant, antiseptic, antispasmodic and antifungal effects.

The contents and types of secondary metabolites differ depending on the locations of the plant. In general, essential oil (EO) of *T. kotschyanus* contains phenolic compounds such as thymol, carvacrol, linalool,  $\alpha$ -terpineol, and geraniol (Tohidi et al., 2018). Many *in vitro* pharmacological experiments conducted over the last decade have shown clear pharmacological activity in both thyme EO and

plant extracts (Baharfar et al., 2015; Zakaryan et al., 2018; Ghasemi et al., 2020; Golkar et al., 2020).

In Armenia, traditionally fresh or dried thyme is used to flavour tea, cheese, curd, bean and meat dishes, sausages, sauces, salads, pickles, bread, spirits, etc. Thyme is an excellent honey plant, producing abundant nectar. The essential oil is used in the confectionery and canning industry, as well as in perfumery. The branches and roots have tanning properties. In dry hay, in the form of a partial mixture, thyme has a beneficial effect on the digestion of cattle. The plant is ornamental, it is widely used to make flower carpets and rock gardens (Nanagulyan et al., 2020).

The aim of the research is the screening of antioxidant activities of the *T*. *kotschyanus* plant aerial part extracts and EO and the determination of its extracts' total phenolic contents by spectrophotometry method.

## Materials and Methods.

*Plant Material.* The species was collected from Vayots Dzor province of Armenia. Specimens were dried under natural conditions. Voucher specimens were deposited in the Herbarium of Yerevan State University (ERCB).

*Decoction.* Air-dried and powdered plant samples were heated with distilled water for 15 min in the water bath. The ratio of solvent to raw material is usually 20:1. After the incubation, the decoction was cooled for no less than 45 minutes at room temperature, filtered and added distilled water until the previous volume.

*Obtaining of Extracts.* Air dried samples (5.0g) were placed for 24 hours on magnetic stirrer with water-ethanol mixture (3:7(v/v), 50mL) for alcoholic extract preparation. After the incubation, extracts were filtered and used.

*Hydrodistillation*. Essential oils from aerial parts of *T. kotschyanus* were obtained by hydrodistillation using a Clevenger-type apparatus. The air-dried plant material (200.0 g) was placed in around-bottomed flask and was subjected to hydrodistillation for 3 h with 600 mL distilled water according to the European

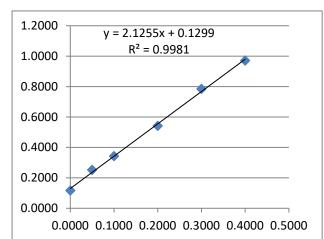
Pharmacopoeia. The obtained oils were dried over anhydrous sodium sulfate and stored at 4°C before the analysis.

Determination of Total Phenolic Content. The content of total phenols was determined by spectrophotometry, with some changes, using gallic acid as standard (Vamanu, Nita, 2012; Gevorgyan et al., 2017).

Determination of Antioxidant Activity. The antioxidant activity (AOA) was determined by potentiometric measurements of change of ORP of  $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$  mediatory system caused by antioxidants in extracts (Braynina et al., 2004; Gevorgyan et al., 2016).

**Results and Discussions.** EO was obtained by hydrodistillation using a Clevenger-type apparatus. The yield of EO of aerial parts in *T. kotschyanus* was 0.82% (w/w).

The total phenolic contents of the *T. kotschyanus* decoction and ethanolic extract were estimated through the Folin-Ciocalteu method. Gallic acid was used as standard for the calibration curve (Fig. 1). Total phenol content was expressed as milligrams Gallic Acid Equivalent (GAE) per mL of extract.



Total phenols of extracts ranged between  $3.31\pm0.72$  and  $5.75\pm2.02$  mg GAE/mL and decreased in the order of water>ethanol.

Fig. 1. Gallic acid calibration curve.

The AOA of *T. kotschyanus* was investigated for decoction and ethanolic extract and different dilutions of EO by potentiometric method of a change in Oxidation Reduction Potential (ORP) of  $[Fe(CN)_6]^{3-} / [Fe(CN)_6]^{4-}$  mediatory system. Results in Table 1 show the ORP and vitamin C equivalent values of *T. kotschyanus* decoction, ethanolic extracts and EO in different dilutions. Extracts show AOA as follows: 0.1 % essential oil > ethanolic extract > 0.25% essential oil > 0.5% essential oil > decoction.

Table 1.

Thymus kotschyanus essential oil and extracts redox potential and antioxidant activities equivalent in Vitamin C

Simple	Redox potential	<i>Vit.</i> $C \ge 10^{-4} g/l$
	mV	
Ethanolic extract	$234\pm2.36$	9
Decoction	$225\pm1.75$	112
0.1 % essential oil	$264.5\pm4.95$	-20
0.25% essential oil	$215.5 \pm 16.26$	26
0.5% essential oil	$191.5\pm2.12$	79
Buffer	271	

The antioxidant agent is considered to be active against free radicals if the Vitamin C equivalent is  $50 \times 10^{-4}$ g/l and more. So we can say that the ethanolic extract and 0.25% essential oil ethanolic solution have weak antioxidant activity and the 0.1% essential oil hasn't activity. The results indicate that *T. kotschyanus* 0.5% EO solution and decoction have higher activity and can serve as a strong natural antioxidant.

**Conclusion**. The yield of EO of aerial parts in *T. kotschyanus* was 0.82% (w/w). Our data showed that ethanolic extract of *T. kotschyanus* exhibited the highest amount of total phenols with values of  $5.75\pm4.02$  of GAE/mL. Based on the results of the determination of the AOA *T. kotschyanus* we concluded that decoction and 0.5% essential oil solution have remarkable activity and can serve as a strong natural antioxidant.

This work was partially supported by the RA MES State Committee of Science, in the frames of the research project № 21T-1F334.

## REFERENCES

Baharfar R., Azimi R., Mohseni M. Antioxidant and antibacterial activity of flavonoid-, polyphenol- and anthocyanin-rich extracts from Thymus kotschyanus Boiss & Hohen aerial parts // J Food Sci Technol. – 2015. - 52(10): 6777–6783. doi: 10.1007/s13197-015-1752-0

Braynina H.Z. et al. Assessment of Antioxidant Activity of Food Products via Potentiometric Method // News of Institutes of Higher Education. Food Technology. – 2004. – 4. - 73–75 (in Russian).

Doosti M.-H., Ahmadi K., Fasihi-Ramandi M. The effect of ethanolic extract of Thymus kotschyanus on cancer cell growth in vitro and depression-like behavior in the mouse // Journal of Traditional and Complementary Medicine. – 2018. – 8(1): 89-94. https://doi.org/10.1016/j.jtcme.2017.03.003

Gevorgyan V.S., Chantikyan A.A., Seferyan T.Ye. Potentiometric Method for Measuring Antioxidant Activities of Plant and Fungal Aquatic and Alcoholic Extracts // High Technologies, Basic and Applied Researches in Physiology and Medicine. – 2016. - 28–31 (in Russian).

Gevorgyan V.S., Nanagulyan S.G., Chantikyan A.A., SeferyanT.Ye. Assessment of antioxidant activities of some medicinal fungal extracts. Proc. of the Yerevan State Univ. Chemistry and Biology. – 2017. - 51(3): 163–165.

Ghasemi G., Alirezalu A., Ghosta Y., Jarrahi A., Safavi S.A., Abbas-Mohammadi M., Barba F.J., Munekata P.E.S., Domínguez R., Lorenzo J.M. Composition, Antifungal, Phytotoxic, and Insecticidal Activities of Thymus kotschyanus Essential Oil // Molecules. – 2020. - 25(5): 1152. https://doi.org/10.3390/molecules25051152

Golkar P., Mosavat N., Jalali S.A.H. Essential oils, chemical constituents, antioxidant, antibacterial and in vitro cytotoxic activity of different Thymus

species and Zataria multiflora collected from Iran // South African Journal of Botany. – 2020. - 130, 250-258.

Nanagulyan S., Zakaryan N., Kartashyan N., Piwowarczyk R., Łuczaj Ł. Wild plants and fungi sold in the markets of Yerevan (Armenia) // J Ethnobiol Ethnomed. – 2020. - 16(1): 26. https://doi.org/10.1186/s13002-020-00375-3.

Tohidi B., Rahimmalek M., Arzani A. Variations in Chemical Composition and Bioactive Compounds of Thymus kotschyanus Boiss. & Hohen Populations Originated from Different Collection Sites // Journal of Essential Oil Bearing Plants. – 2018. - 21(5): 1272-1283. https://doi.org/10.1080/0972060X.2018.1533435

Vamanu E., Nita S. Antioxidant capacity and the correlation with major phenolic compounds, anthocyanin, and tocopherol content in various extracts from the wild edible Boletus edulis mushroom // BioMed Research International. – 2012. - 2013. ID 313905. https://doi.org/10.1155/2013/313905

Zakaryan N.A., Adamyan R.G., Poghosyan A.V., Nanagulyan S.G. Assessment of antifungal activity of some species of the genus Thymus L. // Advances in medical mycology. – XVIII. - 2018. - 207-209. ISSN 2310-9467 (In Russian).