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MOLECULAR DIFFUSION COEFFICIENT OF TANNINS IN ETHANOL EXTRACTS OF WHITE OREGANO (*ORIGANUM HERACLEOTICUM* L.) CULTIVATED IN BULGARIA

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Abstract: *White oregano (Origanum heracleoticum L.) is a Herbaceous Perennial of Lamiaceae family. It is usually wild but nowadays it is cultivated in different regions of the country. It contains various biologically active substances as polyphenolic acids, flavonoids, tannins, proteins, minerals, vitamins, etc., which is why it is increasingly used as a herb and in the form of extracts. The aim of this study is to determine the molecular diffusion coefficient of tannins in ethanol extracts of white oregano cultivated in Bulgaria. A static extraction was carried out with two solvents – 50 and 70% ethanol at a hydromodule of 1:10 and three temperatures (20, 40 and 60°C) and duration of the process of 1 h as well. The data show that the values are highest at a temperature of 60°C, with the 50% ethanol extracts having a molecular diffusion coefficient of* $5.26.10^{-6}$ m_2/s *and 70% ethanol extracts having a molecular diffusion coefficient of* $4.5.10^{-6}$ m_2/s*.*

Keywords: *Origanum heracleoticum L., coefficient of diffusion, tannins, extracts.*

INTRODUCTION

White oregano (Origanum heracleoticum L.) is a Herbaceous Perennial that belongs to the Lamiaceae family.

The genus *Origanum* includes up to 43 species that are distributed in different countries of the world. They differ in morphological features as coloration of flowers, flowering time, arrangement of leaves, etc. (Petkov, V., 1982).

In our country, white oregano is found growing wild but in recent years, due to its wider use in the food industry, its cultivation began in Ruse, Pleven, Momchilgrad and Plovdiv regions (Stanev, S., 2015).

White oregano is a rich source of minerals, vitamins, fibres, amino acids (including essential), tannins and other biologically active substances with proven healing properties. A number of studies have shown various therapeutic properties of oregano: antimicrobial (Akrayi, R., Salih, P., & Hamad, P., 2015), antioxidant (Zhang, X., Guo, S., Wang, H., Li, Q., Xu, J., Chung, Y., Ye, W., Li, Y., & Wang, G., 2014), anti-inflammatory (Pezzanir R., Vitalini, S., & Iriti, M., 2017) and others.

When white oregano was treated with ethanol at different concentrations, extracts with a high content of phenolic acids and flavonoids were obtained (Baycheva, S., 2020; Oreopoulou, A., Goussias, G., Tsimogiannis, D., & Oreopoulou, V., 2020). Due to the content of these biologically active substances, extracts have been found to have high antioxidant activity (Pezzani R., Vitalini, S., & Iriti, M., 2017; Tsibranska, I., Tylkowski, B., Kochanov, R., & Alipieva, K., 2011; Zhang, X., Guo, S., Wang, H., Li, Q., Xu, J., Chung, Y., Ye, W., Li, Y., & Wang, G., 2014) and antimicrobial activity (Brđjanin S., Bogdanovic, N., Kolundžic, M., Milenkovic, M., Golic, N., Kojic, M., & Kundakovi, T., 2015).

Tannins are high-molecular polyphenolic compounds, contained in almost all plants. Their amount depends on the period of vegetation, age, climatic conditions and other factors. They are extracted with hot water or with water-alcohol solutions. Extracts containing tannins are increasingly used in medicine and cosmetics, where their astringent and antimicrobial properties are used (Aires, A., 2020).

It is known that the main process until the moment of extraction of the various biologically active substances on the surface of the plant raw material is diffusion, which is described by Fick's law of diffusion. The transfer of the extracted substances is a result of the driving force of the process and is expressed by the concentration difference of the substances in the particle and on its surface and of the diffusion resistance as well. The latter depends mainly on the structure and composition of the cell envelopes and protoplasm, solvent type and temperature. A generalized expression of the diffusive properties of the extractable material is the molecular diffusion coefficient, which is different for the individual plant raw materials (Stoyanova, E., & Georgiev, E., 2007).

There is no data available in the literature to determine the content of tannins in extracts of white oregano as well as to calculate the molecular diffusion coefficients, which is the aim of the present study. The obtained data will enrich the studies on white oregano, which will expand its scope of application in the food industry, cosmetics, pharmacy.

MATERIALS AND METHODS

Plant material

An epigeous mass of white oregano (Origanum heracleoticum L.) cultivated in Bulgaria, purchased from the sales network of company "BulgarLuk OOD" in the village of Katunitsa, harvest 2021, attached by Quality Certificate No. 1000010383 as of 12.04.2022 was used, which includes physical, chemical and microbiological parameters.

Prior to analyses, the raw material was ground on a laboratory mill to particle sizes of 0.002 × 0.003 m.

Methods

In the raw material, moisture content (%) was determined by azeotropic distillation in a Dean and Stark laboratory apparatus (Stoyanova, A., Georgiev, E., & Atanasova, T., 2007) and of tannins (%) by extraction of the raw material with hot water under reflux condenser, and the extractant being changed in every 30 min. The resulting pooled aqueous extract was titrated with 0.1 N potassium permanganate under indigo carmine indicator (State Pharmacopoeia of the USSR, 1990).

The content of tannins in the obtained extracts was determined according to the method described for the extraction of tannins from starting raw material.

Determination of molecular diffusion coefficient

The extraction of the plant material was carried out in a periodic static mode by soaking under the following conditions: extractants of 50 and 70% ethanol; ratio of raw material: extractant = 1:10; temperature 20, 40 and 60°C; extraction duration: 1 h. The values of the studied technological parameters were chosen based on literature data and on our preliminary unpublished studies.

The molecular diffusion coefficient was calculated using the Minosian formula (Beloborodov, V., Dementii, V., & Voronenkov, B., 1971):

$$D = \frac{l^2\ .\ 2{,}3\ .\ \lg(E_1 - E_2)}{\pi^2(\tau_{1-}\tau_2)}, \quad (1)$$

where: D is the internal molecular diffusion coefficient, m^2/s; l – linear size of the raw material, m; $E1$, $E2$ – content of tannins at times $\tau 1$, $\tau 2$,, %; $\tau 1$, $\tau 2$, which are moments of time of the extraction process, s.

All experiments were performed in triplicate, and the data are arithmetic mean with their respective error.

The information presented in the figures is processed with Micro Soft's Mircocal Origin program.

RESULTS AND DISCUSSIONS

The resulting liquid extracts are light green in colour and have a specific odour.

The moisture content of the analysed white oregano was 12.97 ± 0.10%, and the content of tannins – 17.12 ± 0.12%. The value of tannins is higher than that published in a study by Baycheva, S. (2020), which is 13.84%. It can be explained by the different origin of the cultivated white oregano - in our study it is from Plovdiv region, and in the study of Baycheva, S. (2020) - from Ivaylovgrad region.

The change in the amount of extracted tannins is shown in Table 1.

Table 1. Concentration of tannins in extracts of white oregano extracted with 50 and 70% ethanol.

Temperature, ℃	Duration, min	Tannins, %	
		50 % Ethanol	70 % Ethanol
20	10	7,6 ± 0,07	6,2 ± 0,05
20	20	5,7 ± 0,05	5,2 ± 0,04
20	30	4,2 ± 0,04	3,2 ± 0,02
20	40	2,5 ± 0,02	2,1 ± 0,02
20	50	1,4 ± 0,01	0,8 ± 0,01
20	60	0,6 ± 0,0	0,4 ± 0,03
40	10	8,5 ± 0,06	7,5 ± 0,06
40	20	7,6 ± 0,07	4,8 ± 0,03
40	30	5,5 ± 0,05	3,6 ± 0,03
40	40	4,2 ± 0,04	2,8 ± 0,02
40	50	2,2 ± 0,02	1,1 ± 0,01
40	60	1,2 ± 0,09	0,6 ± 0,0
60	10	9,6 ± 0,06	8,6 ± 0,07
60	20	7,5 ± 0,03	6,5 ± 0,05
60	30	5,2 ± 0,04	3,4 ± 0,02
60	40	3,9 ± 0,03	2,1 ± 0,01
60	50	2,3 ± 0,02	1,2 ± 0,01
60	60	0,6 ± 0,03	0,4 ± 0,02

The analysis of the experimental data shows that the yield of tannins depends on the temperature and the duration of the extraction. It can be seen that the amount of extracted tannins in 10 min decreases with time, regardless of the process temperature and solvent concentration. A maximum amount of tannins is extracted at 60℃, which is due to the positive influence of temperature on the ability of the solvent to extract them.

Based on the experimental data, the molecular diffusion coefficients of the tannins were calculated, and their values are shown in Fig.1 and Fig. 2.

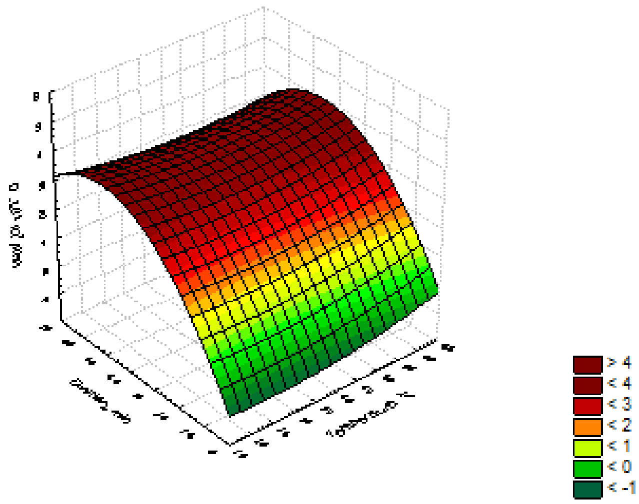

Fig.1. Molecular diffusion coefficients of tannins in extracts of white oregano with 50% ethanol.

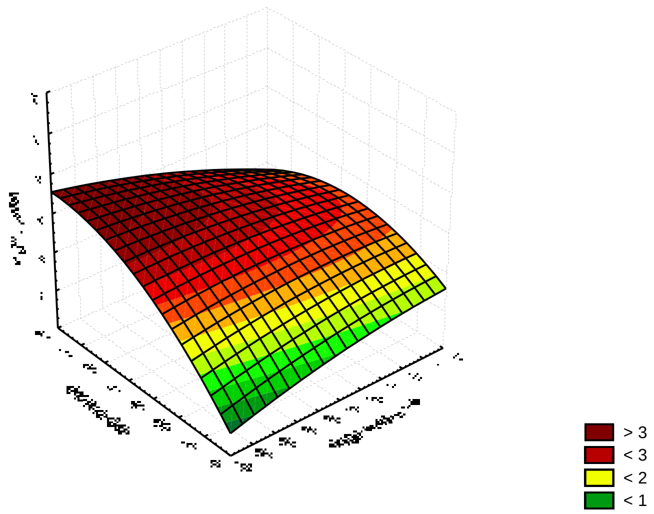

Fig.2. Molecular diffusion coefficients of tannins in extracts of white oregano with 70% ethanol.

The data show that as the temperature increases, the values of the molecular diffusion coefficient also increase, being the highest at a temperature of 60°C.

The values of the molecular diffusion coefficient at 50% ethanol are the highest - $5.26.10^{-6}$ m^2/s and $4.5.10^{-6}$ m^2/s for 70% ethanol. The differences in the values of the molecular diffusion coefficients depending on the solvent concentration are small. The coefficient values are slightly higher when extracting tannins with 50% ethanol, which confirms the assumption that this concentration is suitable for extracting tannins.

The molecular diffusion coefficient has higher values at the end of the process, which can be explained by the higher concentration of the extracted molecules, due to the overcome diffusion resistance.

Compared to the data from the literature for other leaf and grass essential oil and medicinal raw materials, the obtained values for molecular diffusion coefficients, compared to tanning substances, are different, for example, Basil grass - 25.10^{-11} cm^2/s (Damianova, S., Stoyanova, A., & Damianov, D., 2004), Thyme (51.10^{-11} cm^2/s) (Damianova, S., Tasheva, S., Stoyanova, A., &

Damianov, D., 2008), Tobacco leaves Burley type ($22.19.10^{-7}$ cm^2/s) and Oriental type ($10.50.10^{-7}$ cm^2/s) (Ivanova T., Popova, V., Damyanova, S., Tasheva, S., Atanasova, T. & Damyanov, D., 2009), Laurel leaves ($2.05.10^{-9}$ m^2/s) (Stefanova, G., Tasheva, S., Damyanova, S. & Stoyanova, A., 2017)., Sage leaves ($16{,}61.10^{-9}$ m^2/s) (Damyanova, S., Tasheva, S., Mollova, S., Korolkova, N., & Stoyanova A., 2016), Hawthorn leaves ($9{,}82.10^{-9}$ – 548, 10^{-9} cm^2/s) (Damianova, S., Tasheva S., Ergezen M., Stoyanova, A., & Birka, A., 2011). These results reflect both the importance of the structure of the plant material (cellular structure, porosity, dimensions, etc.), which can hinder the extraction of the biologically active substances and the entry of the extractant, and the specific conditions of the process – polarity of the solvent and its concentration, temperature.

CONCLUSION

For the first time, the coefficients of molecular diffusion in relation to tannins were calculated when obtaining extracts with 50% and 70% ethanol from white oregano cultivated in Bulgaria. Its value is highest in the extract obtained with 50% ethanol at 60°C - $5.26.10^{-6}$ m^2/s.

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