Study and Modeling the Influence of a Solvent on Extraction of Eugenol from *Laurus Nobilis* L. Leaves

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Abstract—**this paper presents the results of a study and modeling of the influence of the solvent's dielectric constant on the extraction of eugenol from the *Laurus nobilis* L leaves. The aim of this work is to study the effect of the solvent on the extraction of eugenol from the leaves of *Laurus nobilis* L., to propose a theoretical model for describing the results. Shredded plant material of *Laurus nobilis* L. leaves 0.1-0.5 mm. Aqueous solutions of ethanol (23, 39, 55, 73, 80, 96±1% v/v). The extracts were obtained by a ratio of plant materials - extractant 1:5 (w/v), insisting for 24 hours at a temperature of 24±1°C. Analysis of the extracts was carried out using reverse phase high performance liquid chromatography and a standard sample of eugenol. The dependence of the concentration of eugenol in alcohol-water extracts on the concentration of ethanol in solution was studied. It was found that the maximum concentration of eugenol is observed in solutions with ethanol concentrations of 85±15 % vol. This range of ethanol concentrations corresponds to a dielectric constant of the solvent of 33.5±3.5 units. The regression equation of the dependence of the concentration of eugenol in the extract on the dielectric constant of alcohol-water solutions is obtained. It was found that the dielectric constant of the solvent has the most significant effect on the process of isolating the phytocomponent from plant materials. A mathematical model is theoretically grounded and tested to describe the dependence of the concentration of eugenol on the dielectric constant of water-ethanol solutions. The optimal range of solvent dielectric constant was found to achieve the maximum concentration of eugenol in the extract.**

Keywords—*Laurus nobilis L. leaves, solvents, HPLC analysis, dielectric constant, eugenol*

I. INTRODUCTION

At the moment, there are very few works in the scientific literature in the field of phytochemistry that would be devoted to the development and creation of a mathematical model to describe the dependence of the concentration of biologically active substances in the extract on the physicochemical properties of the solvent.

Some of them focus only on an empirical study of the degree of transition of phytocomponents from plant materials to a particular solvent, without a theoretical explanation of the results [1]. Other studies are devoted to the mathematical description of the effect of ionic and hydrotropic liquids on the extraction of phytocomponents from plant materials [2, 3], which is certainly interesting, but not applicable to conventional types of solvents.

Another group of works is devoted to modeling the process of dissolution of phytocomponents in a solvent using thermodynamic principles [4]. However, the influence of the plant matrix on the distribution of phytocomponents between phases was not taken into account in the work.

It should be emphasized that the theoretical description of such a relationship provides the key to identifying the optimal solvent parameters to achieve the maximum degree of depletion of plant materials with minimal cost of solvent volume, energy and mass of processed plant materials.

Laurus nobilis L. is an interesting plant for scientific research, since this plant is widespread throughout the world, has a number of valuable pharmacological effects and contains several groups of biologically active substances

(flavonoids, essential oils, sesquilactones and several other groups of substances) [5-7].

The biologically active substances from the *Laurus nobilis* L. leaves exhibit some important pharmacological activities like following: antibacterial, antitumor, antioxidant, antinociceptive, etc. [7-10]. According to the literature, one of the important biologically active substances in the *Laurus nobilis* L. leaves is the eugenol.

The aim of this work is to experimentally study the effect of a solvent influence on the extraction of eugenol from the *Laurus nobilis* L. leaves and to propose a theoretical model to describe the results.

To achieve this goal it was necessary to solve a number of problems: to study the effect of a water-ethanol solvent on the concentration of eugenol in the extract; theoretically substantiate and test a mathematical model of the dependence of the concentration of eugenol on the dielectric constant of the solvent; to predict, experimentally verify the result and offer the optimal type of extractant for the isolation of eugenol *Laurus nobilis* L. leaves.

II. Experimental

Medicinal plant material

For research, we used plant raw materials, crushed to a homogeneous mass of *Laurus nobilis* L. leaves with particle range 0.1-0.5 mm purchased at the LLC Lavrusha, Krasnogorsk, Russia, series No. 530619, shelf life up to 07.2020.

Solvents and substances standards

Aqueous solutions of ethanol (23, 39, 55, 73, 80, 96±1% v/v), were used as extractant. All solvents except ethanol qualification "analytical grade", ethanol - pharmaceutical, produced in Russian Federation.

As a standard substance, we used a standard sample of eugenol (*Sigma-Aldrich*, Germany).

Getting extracts

Extracts for analysis were obtained according to the following procedure: two grams of raw material (exact weighed), filled with 10.0 ml of solvent (exact weighed), insisted for 24 hours, at a temperature of 24 ± 1°C.

The final extract was poured and centrifuged at a rotation speed of 13000 rpm for 5 min, and then analyzed using reverse phase high performance liquid chromatography (RP-HPLC).

The method of analysis of RP HPLC

The extracts were analyzed using an *Agilent Technologies* instrument of the *Agilent 1200 Infinity* series, manufactured in the USA. The analysis technique is described in detail in [6].

Suitability and validation parameters of the chromatographic method

The main parameters of the validation method of analysis and suitability of RP HPLC system for determination of eugenol are presented in Table I.

Theory

One of the key quantitative parameters of the solvent, which can significantly affect the distribution of the phytocomponent between the phases of the extraction system, is its dielectric constant [12, 13].

To build a mathematical model, the authors applied the energy approach, which is associated with a change in the chemical potential in the extraction system and the energy of intermolecular forces (that expressed through the Gibbs energy). For this, the authors used a number of assumptions: the molecules are polar; Debye's forces can be neglected; the matrix of plant materials is represented by cellulose; the dielectric constant of the impregnated matrix of plant materials (ε_y) is equal to the sum of the product of the volume fraction of components by their dielectric constant, i.e. cellulose (ε_1) and solvent (ε_x); the equilibrium constant (K) is equal to the equilibrium concentration of the phytocomponent in the extract (C).

Thus, using these assumptions and formulas from the source [14], one can compose a series of equations (1) - (5):

$$\ln\left(\frac{N_0}{N}-1\right)=\ln\left(\frac{m_0}{C \cdot V}-1\right)=\frac{\Delta\mu}{R \cdot T} \quad (1)$$

$$\Delta\mu = \Delta G_{solid} + \Delta G_{solv} + \Delta G_{unpred} \quad (2)$$

$$\Delta G_{soild} = N_A \cdot \left(\frac{\mu_1 \cdot \mu_2}{(4 \cdot \pi \cdot \varepsilon_0 \cdot \varepsilon_y) \cdot r^3} + \frac{3 \cdot \alpha_1 \cdot \alpha_2}{(4 \cdot \pi \cdot \varepsilon_0 \cdot \varepsilon_y)^2 \cdot 2 \cdot r^6} \cdot \left(\frac{I_1 \cdot I_2}{I_1 + I_2}\right) \right) \quad (3)$$

$$\Delta G_{solv} = -N_A \cdot \left(\frac{\mu_3 \cdot \mu_2}{(4 \cdot \pi \cdot \varepsilon_0 \cdot \varepsilon_x) \cdot r^3} + \frac{2 \cdot \mu_3^2 \cdot \mu_2^2}{(4 \cdot \pi \cdot \varepsilon_0 \cdot \varepsilon_x)^2 \cdot 3 \cdot k \cdot T \cdot r^6} + \frac{3 \cdot \alpha_3 \cdot \alpha_2}{(4 \cdot \pi \cdot \varepsilon_0 \cdot \varepsilon_x)^2 \cdot 2 \cdot r^6} \cdot \left(\frac{I_3 \cdot I_2}{I_3 + I_2}\right) \right) \quad (4)$$

TABLE I. Main Parameters Of The Validation Method Of Analysis And Suitability Of Rp Hplc System For Determination Of Eugenol

Parameter	Pharmacopoeia limitation [11]	Eugenol
1. Retention time (t_R), min	-	35.1±0.7
2. Asymmetry coefficient (T)	≤2.0	0.83
3. Separation coefficient (R_s)	≥1.5	2.1
4. Theoretical plates number (N)	≥1000	28,252
5. RSD of peak's area, %	≤2.0	1.3
6. LOD, g/ml	-	$4.8 \cdot 10^{-6}$
7. LOQ, g/ml	-	$1.5 \cdot 10^{-5}$
8. Determination coefficient, R^2	≥0.99	0.9991
9. Calibration linear equation, C(g/ml)=$f(S$(mAU·s))	-	$C=(4.7\pm0.2)\cdot10^{-7}\cdot S$

$$\varepsilon_y = \varphi_1 \cdot \varepsilon_1 + (1 - \varphi_1) \cdot \varepsilon_x \qquad (5)$$

where $\Delta\mu$ is the change of chemical potential in the extraction system, J/mol; N, N_0 are equilibrium quantity of the substance in the liquid phase and total quantity of the substance in the extraction system, mol; R is the gas constant, 8.314, J/(mol·K); T is the absolute temperature, K; C is the equilibrium concentration of the substance in the extract, mol/L or g/ml; ΔG_{solid} is the binding energy of the molecules of biologically active substances with the matrix of plant materials, J/mol; ΔG_{solv} is interaction energy of molecules of biologically active substances and solvent molecules, J/mol; ΔG_{unpred} is unaccounted energy processes, J/mol; N_A is the Avogadro number, $6.02 \cdot 10^{23}$ mol/L; π is the mathematical constant, 3.14; ε_0 is the electric constant, $8.85 \cdot 10^{-12}$ F/m; ε_1, ε_x are dielectric constant of the plant material matrix and solvent, respectively; μ_1 μ_2 μ_3 are molecule dipole moment of the plant material matrix, biologically active substances and solvent, respectively, C·m; α_1, α_2, α_3 are molecule polarizability of the plant materials matrix, biologically active substances and solvent, respectively, m³; I_1, I_2, I_3 are molecule ionization energy of the plant material matrix, biologically active substances and solvent, respectively, J; r is the distance between the molecules, m; φ_1 is the volume fraction of the plant material matrix.

After converting these equations and isolating the dielectric constant of the solvent from them, we can write the equation (6), which describe the relation between the equilibrium concentration of substances in the extract and the dielectric constant of the solvent:

$$\frac{R \cdot T}{N_A} \cdot \ln\left(\frac{m_0}{C \cdot V} - 1\right) = \frac{1}{\varepsilon_x^2} \cdot \left((D + E) - \frac{\varepsilon_x^2}{\varepsilon_y^2} \cdot B\right) + \frac{1}{\varepsilon_x} \cdot \left(F - \frac{\varepsilon_x}{\varepsilon_y} \cdot A\right) + \Delta G_{unpred} \qquad (6)$$

where

$$D = \frac{2 \cdot \mu_3^2 \cdot \mu_2^2}{(4 \cdot \pi \cdot \varepsilon_0)^2 \cdot 3 \cdot k \cdot T \cdot r^6}, \quad E = \frac{3 \cdot \alpha_3 \cdot \alpha_2}{(4 \cdot \pi \cdot \varepsilon_0)^2 \cdot 2 \cdot r^6} \cdot \left(\frac{I_3 \cdot I_2}{I_3 + I_2}\right), \quad B = \frac{3 \cdot \alpha_1 \cdot \alpha_2}{(4 \cdot \pi \cdot \varepsilon_0)^2 \cdot 2 \cdot r^6} \cdot \left(\frac{I_1 \cdot I_2}{I_1 + I_2}\right),$$

$$F = \frac{\mu_3 \cdot \mu_2}{(4 \cdot \pi \cdot \varepsilon_0) \cdot r^3}, \quad A = \frac{\mu_1 \cdot \mu_2}{(4 \cdot \pi \cdot \varepsilon_0) \cdot r^3}$$

Equation (6) is rather difficult for mathematical processing, therefore, to simplify it, the authors accepted another assumption that the ratios $\varepsilon_x^2 / \varepsilon_y^2$ and $\varepsilon_x / \varepsilon_y$ are constant. In this case, this dependence will be determined mainly by the balance of energy coefficients

$(D + E) - const \cdot B$ and $G - const \cdot A$, and can be analyzed even using the MS Excel tool, since when introducing a new variable $x = 1/\varepsilon_x$, equation (6) reduces to a quadratic equation (7):

$$\ln\left(\frac{m_0}{C \cdot V} - 1\right) = a \cdot x^2 + b \cdot x + c \qquad (7)$$

III. RESULTS AND DISCUSSION

The dependence of the concentration of eugenol in the extracts obtained using different concentrations of ethanol was studied. The results of the RP-HPLC analysis of the content of this substance in the extracts are given in table II.

As can be seen from the data in Table II, the highest concentration of eugenol is observed for an ethanol solution of 81% vol. However, it should be clarified that the maximum concentration of eugenol in the extract can be expected in the range of ethanol concentration of 85±15% v/v.

Then, using the data which was displayed in Table II and reference data on the dielectric constant for the ethanol – water mixture [15], we constructed the dependence in the coordinates $\ln\left(\frac{m_0}{C \cdot V} - 1\right) = f(1/\varepsilon)$ (see Fig. 1).

As can be seen from Fig.1, the experimental data are well described by the proposed mathematical model (7) within the range of the dielectric constant from 37 to 30 units ($1/\varepsilon = 0.027 \div 0.033$). Moreover, the regression equation has the form that was predicted by the theory $\ln\left(\frac{m_0}{C \cdot V} - 1\right) = 16 - 1279 \cdot \frac{1}{\varepsilon} + 21254 \cdot \frac{1}{\varepsilon^2}$, with the determination coefficient $R_e^2 = 0.993 > R_t^2 = 0.841$ ($P = 99\%$), which indicates the adequacy of the mathematical model.

According to the above equation, the maximum concentration of eugenol corresponds to a dielectric constant of the solvent of 33.5±3.5 units. The results can be used to improve some technological aspects of the selection of biologically active substances and, in particular, eugenol from *Laurus nobilis* L. leaves.

TABLE II. THE VALUE OF THE PEAK AREA AND THE CONCENTRATION OF EUGENOL IN EXTRACTS OBTAINED FROM HELICHRYSI ARENARII FLORES BASED ON ETHANOL OF VARIOUS CONCENTRATIONS

Parameter	Retention time, min	The concentration of ethanol,% v/v					
		23±1	39±1	55±1	73±1	80±1	96±1
1 Peak area, mAU·s *		44.9±2.3	126±6	213±11	237±12	259±13	227±11
2 The concentration of the eugenol, mg/ml	35,1±0,7	0.0211± 0.001	0.0592± 0.003	0.100± 0.005	0.111± 0.006	0.122± 0.006	0.107± 0.006
3. Dielectric constant		68±1	59±1	51±1	40±1	36±1	26±1

*Note. The average value and parameter error were calculated for three replicates $n = 3$ and significance level $P = 0.95$
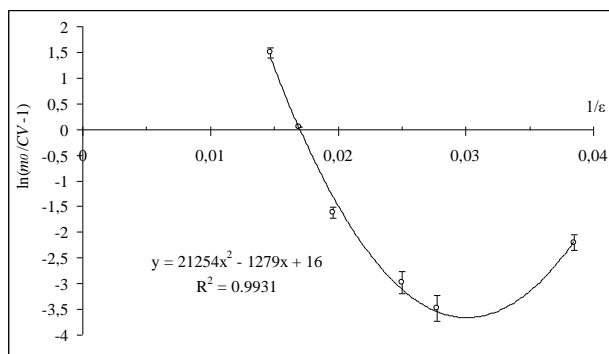
Fig.1. Dependence of the concentration of eugenol in extracts from the reciprocal value of the dielectric constant of the solvent.

IV. CONCLUSION

The influence of the type of solvent on the extraction of eugenol from the leaves of *Laurus nobilis* L. was studied. It was found that the dielectric constant of the solvent has the most significant effect on this process. A mathematical model has been theoretically substantiated and tested to describe the dependence of the concentration of eugenol on the dielectric constant of the solvent using water-ethanol solutions as an example. Range found the dielectric constant of the solvent is 33.5±3.5 units, at which the maximum concentration of eugenol in the extract is achieved. The results can be used in further studies to improve the technology for the isolation of eugenol from the *Laurus nobilis* L. leaves.

REFERENCES

[1] M. Fayaz, M.H. Bhat, A. Kumar, A.K. Jain, "Comparative Studies on Different Solvents Used for the Extraction of Phytochemicals from the Plant Parts of Arnebia benthamii. (Wall Ex. G. Don) Johnston", J. Chem. Pharm. Res, vol. 9, № 1. pp..220-224, 2017.

[2] N.R.A. Rahman, N.A. Yunus, A.A. Mustaffa, "Selection of optimum ionic liquid solvents for flavonoid and phenolic acids extraction", IOP Conference Series: Materials Science and Engineering, Sarawak: Malaysia, pp.1-12, 2017.

[3] J. Nagarajan, W.W. Heng, C.M. Galanakis, R.N. Ramanan, M.E. Raghunandan, J. Sun, A. Ismail, T. Beng-Ti, K.N. Prasad, "Extraction of phytochemicals using hydrotropic solvents", Separation Science and Technology, vol. 51, № 7, pp.1151-1165. 2016.

[4] W.L. Theo, A.A. Mustaffa, J.S. Lim, "Solubility modelling for phytochemicals of Misai Kucing in different solvents", Fluid Phase Equilibria, vol. 427, pp. 246-258, 2016.

[5] N.M. Nasuhova, O.M. Shevchuk, L.A. Logvinenko, "Investigation of phenolic compounds in extracts from the leaves of *Laurus nobilis* L.", Pharmacy & Pharmacology, vol. 5, № 2, pp.150-163, 2017. (In Russ.)

[6] E.T. Zhilyakova, O.O. Novikov, D.I. Pisarev, A.Y. Malyutina, N.N. Boyko, "Studying the polyphenolic structure of *Laurus Nobilis* L. leaves", Indo American Journal of Pharmaceutical Sciences, vol. 4, № 9, pp. 3066-3074, 2017.

[7] P. Ramling, M. Meera, P. Priyanka, "Phytochemical and pharmacological review on *Laurus Nobilis"*, Int. J. of Pharm. and Chem. Sci, vol. 1, № 2, pp.595-602, 2012.

[8] B. Ozcan, M. Esen, M.K. Sangun, A. Coleri, M. Caliskan. Effective antibacterial and antioxidant properties of methanolic extract of *Laurus nobilis* seed oil. Journal of Environmental Biology, vol. 31, pp. 637-641, 2010.

[9] F. Fang, Sh. Sang, K.Y. Chen, A. Gosslau, C.-T. Ho, R.T. Rosen, " Isolation and identification of cytotoxic compounds from bay leaf (*Laurus nobilis*)", Food Chemistry, vol. 93, pp. 497-501, 2005.

[10] R. Abu-Dahab, V. Kasabri, F. Afifi, "Evaluation of the volatile oil composition and antiproliferative activity of *Laurus nobilis* L. (*Lauraceae*) on breast cancer cell line models", Records of Natural Products, vol.8, pp. 136-147, 2014.

[11] State Pharmacopoeia of the Russian Federation. XIV ed. Vol. I. Moscow: Ministry of Healthcare of Russian Federation, 2018.

[12] P.C. Hiemenz, R. Rajagopalan. Principles of colloid and surface chemistry. 3rd rev. and expanded ed. New York: Marcel Dekker inc., 1997.

[13] N. Boyko, D. Pisarev, E. Zhilyakova, A. Pravlotskaya, O. Novikov, N. Makarevich, V. Kuznietsova, N. Sushchuk, "Study and modeling of the distribution process of some phenolic compounds between the solid and liquid phases", Journal of Advanced Pharmaceutical Technology & Research, vol.10, № 2, pp.56-62, 2019.

[14] N.N. Boyko, D. Pisarev, E. Zhilyakova, O. Novikov, V. Kuznietsova, N. Sushchuk, "Modeling of solvent effects on phytocompounds' extraction from glycyrrhizae radix", Journal of Advanced Pharmaceutical Technology & Research, vol.9, № 3, pp.66-72, 2018.

[15] Ch. Wohlfarth Static dielectric constants of pure liquids and binary liquid mixtures. Lechner MD, editor. Berlin: Springer-Verlag Berlin Heidelberg, 2015.