ORIGINAL ARTICLE

Study and modeling of the distribution process of some phenolic compounds between the solid and liquid phases

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ABSTRACT

The article presents the results related to the study of distribution of biologically active substances from the plant raw material between solid and liquid phases. The aim of this study is to develop theoretical bases of the extraction process in the equilibrium state by the example of study and modeling of the distribution process of biologically active substances from *Eucalyptus viminalis* leaves. In these studies, we used ground plant raw material of *E. viminalis* leaves with particle fraction of 0.1–0.5 mm; and ethanol with concentration 80% ±1% v/v was used as an extractant. Qualitative and quantitative analyses were carried out by reversed phase high-performance liquid chromatography with rutin, chlorogenic acid, and euglobal standards equivalent to spissum extract of chlorophyllipt of the State Pharmacopoeia of Ukraine. A hypothesis has been suggested that Henry's adsorption law and the law of conservation of matter play a fundamental role in this process. The experimental data are described well by the suggested equation with high value of determination coefficient R^2 =0.99. At the same time, F-test and the significance of coefficients in equations satisfy the statistic condition, which means that the current hypothesis about the adsorption mechanism of distribution of biologically active substances in the extraction system is not refuted. The results of these studies demonstrate good agreement of experimental data and theoretical model based on Henry's adsorption law and mass balance. The numerical values of constants in the model suggested have been calculated.

Key words: Distribution, equilibrium, *Eucalyptus viminalis* Labill, leaves, phenolic compounds

INTRODUCTION

The development of drugs including phytodrugs is still on top of its relevancy.[1-3] At present, among the drugs urgently needed, there are those having antimicrobial activity due to widespread of microorganisms with antibiotic resistance.[4] Herewith, active studies are carried out all over the world to solve this problem. One of the possible ways to solve this problem is combined use of antibiotics with other substances that improve their activity.[5]

A good alternative to drugs based on the synthetic substances or antibiotics used for local treatment is the

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use of phytodrugs that have additional useful properties, not only antimicrobial activity. In our previous works, we studied antimicrobial activity of extracts from plant raw materials and tinctures that contained phenolic compounds, for example, *Amorpha fruticosa* L. (fruits), *Centaurium erythraea* (herb), *Paeonia anomala* L. (root), *Cetraria islandica* (thallus), *Dryopteris filix-mas* L. (leaves and root), *Humulus lupulus* L. (cones), *Salix alba* L. (cortex), *Pentaphylloides fruticosa* L. (herb), and *Eucalyptus tincture*.[6-8] These results demonstrate that extracts from *H. lupulus* L., *D. filix-mas* L., and *Eucalyptus viminalis* L. on ethanol 70% v/v have a significant level of antimicrobial activity, which is confirmed by data from scientific literature.[9-11]

According to literary sources, phloroglucinol derivatives (flavaspidic acids, xanthohumol, and euglobals) are responsible for antimicrobial activity; moreover, these compounds have some other important activities, such as anthelmintic, antiviral, antitumor, anti-inflammatory, and antioxidant.[12-17]

Thus, plants that contain this group of biologically active substances are very promising for further study and development or improvement of phytodrugs technology, especially those having antibacterial activity. Plants of *Eucalyptus* genus are widely used in medicine all over the world.

The plants of *Eucalyptus* genus, *Myrtaceae* family, have been used for medicinal purposes for centuries due to their numerous useful activities (antiseptic, expectorant, anti-inflammatory, insecticide, repellent, etc.).[18,19] According to scientific sources, eucalyptus leaves contain different groups of substances: essential oil up to 6% (cineole), tannins up to 11%, triterpene saponins 2%–4% (ursolic acid derivatives), phenol carbonic acids (gallic, chlorogenic), flavonoids (rutin, hyperoside, and eucaliptine), and euglobals (acyl-phloroglucinol-monoterpenes and acyl-phloroglucinol-sesquiterpenes/macrocarpale).[20] At the same time, euglobals are a very important group of substances due to their antileishmanial, antiviral, and antimicrobial activities.[21,22] Therefore, the studies in the field of extraction of biologically active substances that have antimicrobial activities from plant raw material of *Eucalyptus* genus are an urgent task.

The stage of extraction of biologically active substances from the plant raw material is a necessary part in the technology of any phytodrug. A future technology of the drug and eventually its quality in many instances depend on how this process is organized. One of the important technological parameters for the extraction system is the equilibrium concentration of biologically active substances in the extractant under conditions of its dynamic equilibrium state onset. This parameter determines the expulsive force of mass exchange process of biologically active substances from the plant raw material particles into a free extractant and therefore determines the velocity of the extraction process. Moreover, this parameter determines the extract's therapeutic value and the number of drug's units per production lot and consequently the profit.

Therefore, forecast of this parameter is among important tasks in phytotechnology. We have not found any information in scientific sources on a possible distribution mechanism of biologically active substances from this plant raw material in the extraction system between the solid and liquid phases and, therefore, development of a mathematical model that describes this process seems to be an actual task.

Thus, the aim of this study is to develop theoretical bases of the extraction process in the equilibrium state by the example of study and modeling of the distribution process of biologically active substances from *E. viminalis* leaves.

MATERIALS AND METHODS

Plant raw material

For the study, we used pharmacopoeia plant raw material *E. viminalis* leaves from "Krasnogorskleksredstva" company, Krasnogorsk, Russia, batch No. 100917, best before 10/2020.

For extraction, we used the ground plant raw material with particle fraction between 0.1 and 0.5 mm, and we used ethanol with concentration 80% ±1% v/v as an extractant.

Chemicals and reagents

Qualitative and quantitative analyses of dominative biologically active substances were carried out by reversed phase high-performance liquid chromatography (RP HPLC) with rutin and chlorogenic acid standards; due to the fact that euglobal standard is not easily available, we carried out quantitative calculations in equivalent to spissum extract of chlorophyllipt of the State Pharmacopoeia of Ukraine. We used ethanol of pharmaceutical grade, manufactured in Russia.

Method of extracts obtaining

The equilibrium process in the extraction system was studied at 4, 20, 40, and 60°C ± 1°C; and a method of simple maceration for 24 h was used. Distribution of biologically active substances between the phases was studied at weight of plant raw material/volume of the extractant ratio of 1:5, 1:10, 1:20, and 1:40.

High-performance liquid chromatography analysis

RP HPLC analysis was carried out using a chromatograph by "Agilent Technologies," "Agilent 1200 Infinity" series, made in the USA. RP HPLC analysis was carried out under the following conditions: 1% water solution of formic acid was used as mobile phase (A); ethanol 96% v/v was used as second mobile phase (B); mobile phases were pumped in

a linear gradient elution regime; chromatographic column was Supelco Ascentis express C_{18} 100 mm × 4.6 mm with particle size of 2.7 μm; the velocity of the mobile phase was 0.5 ml/min; the temperature of chromatographic column was + 35°C; and the sample volume was 1 μl. A detailed description of chromatography conditions is presented in the article.[23]

RP HPLC analysis was carried out using a diode-array detector at the following wavelengths: 325 nm for chlorogenic acid, 350 nm for rutin, and 275 nm for euglobal.

Suitability and validation parameters for method of analysis

The main validation parameters of the analytical method and suitability of the HPLC system for determination of rutin, chlorogenic acid, and euglobal equivalent to spissum extract of chlorophyllipt are presented in Table 1.[24]

Theoretical part

To explain and determine the possibility of mathematical modeling of the distribution process of biologically active substance molecules in the extraction system, we hypothesized that Henry's adsorption law and the law of conservation of matter should make the background of this process.

Under this assumption, distribution of biologically active substances between the phases should have linear dependency of the reverse value of biologically active substances concentration in the extract from the volume of the extractant at constant temperature (1):

$$\frac{1}{C}=\frac{1}{m_0}\times V+\frac{M\times\phi\times K_H}{m_0}=\frac{1}{m_0}\times V+\frac{M\times\phi}{m_0}\times\exp\left(\frac{\Delta G}{R\cdot T}\right)=a\times V+b \quad (1)$$

where C is biologically active substance concentration, g/ml; m_0 is total quantity of biologically active substances in the extraction system, g; M is solid unsolved part of the plant raw material, g; V is volume of the extract, to simplify, we take it as volume of the extractant in the extraction system, ml; K_H is Henry's constant, which is $K_H=\exp\left(\frac{\Delta G}{R\times T}\right)$, ml/g; ΔG is energy constant of the distribution process of biologically active substances, J/mole; R is gas constant, which is equal 8.314 J/(mole·K); T is absolute temperature, K; φ is dimensionless constant; a is a constant equal to reverse value of total weight of biologically active substances in the extraction system, 1/g; b is constant, which is $(M\,\varphi/m_0)\cdot\exp[\Delta G/(R\,T)]$, ml/g.

The value of constants (ΔG) and ($M\cdot\varphi$) can be calculated using regression equation (2):

$$\ln\left(\frac{b}{a}\right)=\ln K_H=\frac{\Delta G}{R}\times\frac{1}{T}+\ln\left(M\times\phi\right) \quad (2)$$

Data analysis

Regression analysis of data was carried out in MS Office Excel 2010 with data analysis tool. The results were obtained at repeat count $n = 3$ and confidence coefficient $P = 0.95$.

RESULTS

Figure 1 presents a typical chromatogram of the extract (plant raw material/extractant ratio 1:10, at 20°C ±1°C) obtained by RP HPLC analysis using a diode-array detector at wavelength of 350 nm (for rutin).

Figure 2 presents a typical chromatogram of the extract (plant raw material/extractant ratio 1:10, at 20°C ±1°C) obtained by RP HPLC analysis at wavelength of 325 nm (for chlorogenic acid).

Figure 3 presents a typical chromatogram of extract (plant raw material/extractant ratio 1:10, at 20°C ±1°C) obtained by RP HPLC analysis at wavelength of 275 nm (for euglobals).

As it can be seen from chromatograms in Figures 1-3, in the extract on the basis of ethanol-water solution 80% v/v, we detected euglobals by RP HPLC analysis (at 275 nm, retention time from 40 to 51 min), which are dominant compared to all other compounds detected, as well as rutin (at 350 nm, retention time: Minute 17) and chlorogenic acid (at 325 nm, retention time: Minute 7), which agrees well with other sources mentioned above.

Figure 4 presents the results after experimental data processing in coordinates $1/C = f(V)$ for rutin at different temperature values and plant raw material/extractant ratios.

Figure 5 presents the results after experimental data processing in coordinates $1/C = f(V)$ for chlorogenic acid at different temperature values and plant raw material/extractant ratios.

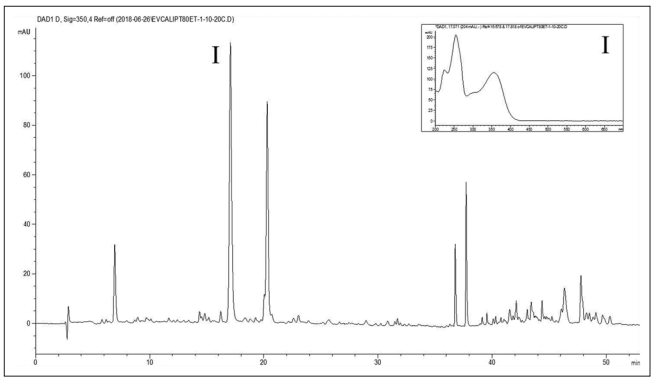

Figure 1: Reversed-phase high-performance liquid chromatography chromatogram of the extract at 350 nm. I is rutin with ultraviolet spectra

Figure 6 presents the results after experimental data processing in coordinates $1/C = f(V)$ for euglobal equivalent to spissum extract of chlorophyllipt at different temperature values and plant raw material/extractant ratios.

As it can be seen from Figures 3-6, experimental data are described well by equation (1) and have linear dependency with a high value of determination coefficient that is equal R^2 =0.99.

In addition, *F*-test satisfies the condition $F_{calc} \leq F_{table}$, and the significance of coefficients in regression equations satisfies the condition $p_{calc} \leq p_{table}$, which confirms the adequacy of regression equations. Therefore, the theoretical model suggested is probably significant.

Subsequently, we calculated Henry's constant (K_H=b/a), energy constant (ΔG), and dimensionless constant ($M \cdot \varphi$) using the regression equation constants obtained at different temperature values.

Figure 7 shows the regression equations between the logarithm of Henry's constant and reverse value of temperature for rutin, chlorogenic acid, and euglobal equivalent to spissum extract of chlorophyllipt.

As it can be seen from the data in Figure 7, experimental data are described well by equation (2) and have linear dependency with a high value of determination coefficient R^2 =0.99.

At the same time, F-test satisfies the condition $F_{calc} \leq F_{table}$, and the significance of coefficients in regression equations satisfies the condition $p_{calc} \leq p_{table}$, which confirms the adequacy of regression equations. Therefore, the theoretical model suggested is probably significant, and the suggested hypothesis about the distribution mechanism of biologically active substances in the extraction system is not refuted.

Table 2 presents the values of constants that were calculated: total quantity of biologically active substances in the extraction system (m_0) by equation (1); energy constant (ΔG) and dimensionless constant ($M \cdot \varphi$) by equation (2).

As it can be seen from Table 2, energy parameters (ΔG) for rutin, chlorogenic acid, and euglobal equivalent to spissum extract of chlorophyllipt have the same value; in general, it equals 23.3 kJ/mole, which is typical for energy of the process of physical adsorption.

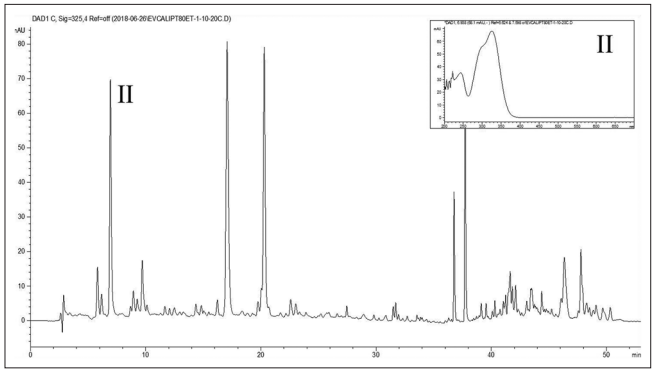

Figure 2: Reversed-phase high-performance liquid chromatography chromatogram of the extract at 325 nm. II is chlorogenic acid with ultraviolet spectra

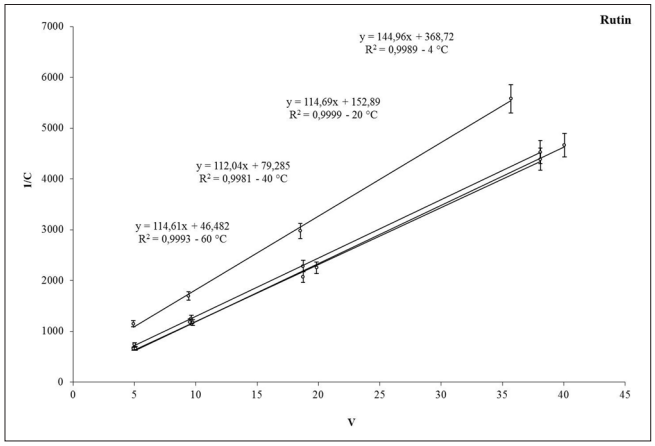

Figure 4: Experimental data and regression equations for rutin at different temperature values

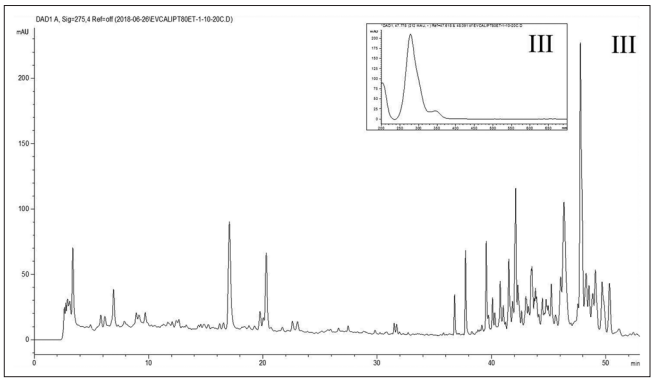

Figure 3: Reversed-phase high-performance liquid chromatography chromatogram of the extract at 275 nm for euglobals. III is the dominant euglobal with ultraviolet spectra

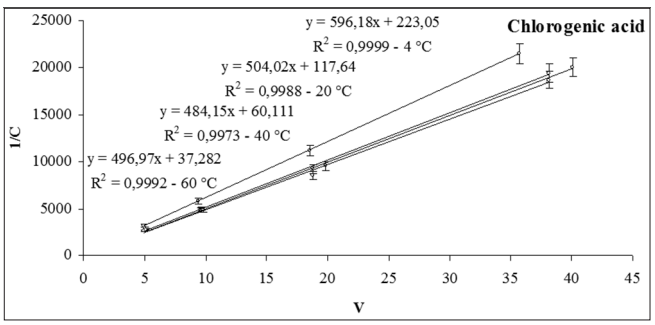

Figure 5: Experimental data and regression equations for chlorogenic acid at different temperature values

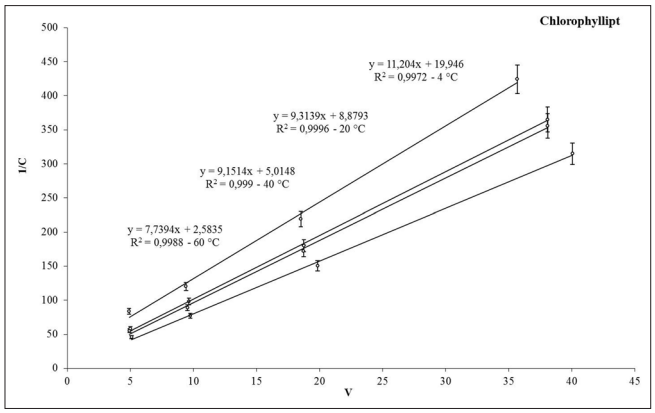

Figure 6: Experimental data and regression equations for euglobal equivalent to spissum extract of chlorophyllipt at different temperature values

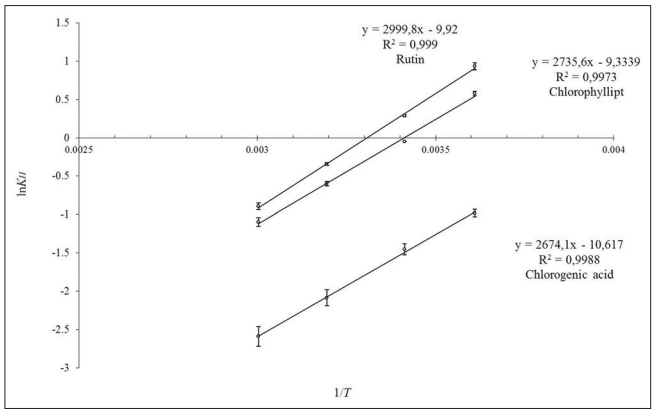

Figure 7: The dependency between the logarithm of Henry's constant and reverse value of temperature for rutin, chlorogenic acid, and euglobal equivalent to spissum extract of chlorophyllipt

Table 1: Main validation parameters of the analytical method and suitability of the HPLC system for determination of rutin, chlorogenic acid, and euglobal equivalent to spissum extract of Chlorophyllipt

Parameter	Pharmacopoeia limitation [12]	Compound		
		Rutin	Chlorogenic acid	Euglobal on an spissum extract
Retention time (t_R), min	-	16.9±0.2	7.0±0.2	46.7±0.5
Asymmetry coefficient (T)	≤2.0	0.79	0.70	0.48
Separation coefficient (R_s)	≥1.5	2.84	3.17	1.4
Theoretical plates number (N)	≥1000	36561	12037	480597
RSD of peak area, %	≤2.0	1.5	0.8	2.6
LOD, g/ml	-	2.3·10-5	2.2·10-5	5.0·10-4
LOQ, g/ml	-	6.8·10-5	6.5·10-5	1.5·10-3
Determination coefficient, r^2	≥0.99	0.9993	0.9999	0.9989
Calibration linear equation, C (g/ml)=f (S (mAU·s))	-	C=(4.90±0.18)·10^{-7}·S	C=(2.92±0.04)·10^{-7}·S	C=(4.49±0.28)·10^{-6}·S

*The mean value and its CI (mean±SEM) are calculated with repeat counts n=3 and significance level P=0.95. RSD: Relative standard deviation, CI: Confidence interval, SEM: Standard error of mean

Table 2: Values of theoretical constants for biologically active substances

No	BAS	Constant			
		ΔG, J/mole	m_0, g/g PRM	lnM·φ	M·φ
1	Rutin	24900±2400	(820±170)·10^{-5}	-9.9±1.0	(5.0±0.5)·10^{-5}
2	Chlorogenic acid	22200±2500	(190±30)·10^{-5}	-10.6±0.9	(2.5±0.2)·10^{-5}
3	Euglobal equivalent to spissum extract of Chlorophyllipt	22800±3600	(10700±2600)·10^{-5}	-9.3±1.4	(9.1±1.3)·10^{-5}

Note. * The mean value and its confidence interval (Mean±SEM). SEM: Standard error of mean

Table 3: Main pharmacognostic parameters of *Eucalypti viminalis* leaves

No	Parameters*	Experimental value**
1	Loss on drying, g/g PRM	0.067±0.002
2	Extractive substances, g/g PRM	0.37±0.01
3	Rutin, g/g PRM	(860±40)·10^{-5}
4	Chlorogenic acid, g/gPRM	(200±10)·10^{-5}
5	Euglobal equivalent to spissum extract of Chlorophyllipt, g/g PRM	(12400±1100)·10^{-5}

Note. * Parameters were found for wet plant raw material. The mean value and its confidence interval (Mean±SEM) are calculated with repeat counts n=3 and significance level P=0.95. SEM: Standard error of mean

The value of dimensionless constant ($M \cdot \varphi$) for rutin, chlorogenic acid, and euglobal equivalent to spissum extract of chlorophyllipt is equal to 0.000050, 0.000025, and 0.000091, respectively. When making a comparison of these values with those of total quantity of biologically active substances in the extraction system (m_0), it can be observed that they are <1% of the total quantity of the substance in the raw material, and probably, it is the limit value of the substance absorbed by the solid phase of the plant raw material.

Moreover, it is interesting to compare the values of total quantity of biologically active substances in the extraction

system (m_0) calculated with their respective experimental values. For this purpose, Table 3 shows the main parameters of plant raw material.

As it can be seen from data in Tables 2 and 3, the calculated value of total quantity of biologically active substances in the extraction system (m_0) coincides with its experimental value within inaccuracy range for rutin $(820 \pm 170)\cdot10^{-5} \approx (860 \pm 40)\cdot10^{-5}$, for chlorogenic acid $(190\pm30)\cdot10^{-5}\approx(200\pm10)\cdot10^{-5}$, and for euglobal equivalent to spissum extract of chlorophyllipt $(10700 \pm 2600)\cdot10^{-5} \approx (12400 \pm 1100)\cdot10^{-5}$ g/g plant raw material.

DISCUSSION

The results obtained in these studies are in good agreement with our previous work, where we used it for modeling of glycyram and licurosid distribution between *Glycyrrhizae* radix and 70% v/v ethanol.[25]

Thus, based on the mentioned above, we have concluded that experimental data are described well by the mathematical model suggested and the hypothesis about the adsorption mechanism of biologically active substances distribution in the extraction system from *E. viminalis* leaves and 80% v/v ethanol is not refuted.

The hypothesis and mathematical model suggested allow explaining and forecasting the distribution of biologically active substances between the phases under the equilibrium state.

Our next step will be using this model to forecast the concentration of biologically active substances for the method of fractional maceration and even for nonequilibrium filtration extraction method.

In the case of correspondence of experimental data and the model suggested, it will be possible to explain one of the two main sides of the extraction process of biologically active substances from the plant raw material, namely, the equilibrium state in the extraction system.

CONCLUSION

Distribution of rutin, chlorogenic acid, and euglobal equivalent to spissum extract of chlorophyllipt between the phases of the extraction system from *E. viminalis* leaves in ethanol with concentration 80% v/v has been studied.

A hypothesis about the adsorption mechanism of distribution of biologically active substances in the extraction system has been suggested and used for the development of a mathematical model to describe the experimental data obtained.

The results of our studies demonstrate good agreement of experimental data and mathematical model based on Henry's low and mass balance.

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Conflicts of interest

There are no conflicts of interest.

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