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EXPERIMENTAL PAPER

Actual solubility ($S_{|real.|}$), level of hydrophilic-lipophilic balance ($HLB_{Requ.}$, HLB_D , HLB_G) and partition coefficient ($\log P$) of phytochemicals contained in *Ext. Camellia sinensis* L. *aqu. siccum* in the light of general Hildebrand-Scatchard-Fedors theory of solubility

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Summary

Introduction: Using the general Hildebrand-Scatchard-Fedors theory of solubility, the mole fraction (x_2) of solubility of phytochemicals contained in the dry green tea leaves was calculated which determines the profile of pharmacological activity.

Objective: The applicative purpose of the study was to estimate the actual solubility of phytochemicals – $S_{|real|}$ [mol/dm³] in water and in water-ethanol solutions of diversified polarity (ϵ_M) for their selective extraction and optimal formulation of oral solid dosage form.

Methods: The basic physico-chemical and structural quantities of phytochemicals and corresponding mathematical equations of general Hildebrand-Scatchard-Fedors theory of solubility were used to calculate the actual solubility – $S_{|real|}$ and the level of hydrophilic-lipophilic balance (HLB).

Results: The calculated actual solubility values – $S_{|real|}$ [mol/dm³] collated with correlation equations enabled the assessment of phytochemical capability for the process of mass exchange on phase boundary. Correlation equations for the dependence $\log P = f(-\log S_{|real|})$ point to the structural preferences of phytochemicals in the kinetics of the mass exchange (diffusion) through the natural phase boundary.

Conclusions: Calculations and correlations between the values characterizing the actual solubility – $S_{|real|}$, media polarity (water, ethanol and their solutions) and the partition coefficient (log P) including the level of hydrophilic-lipophilic balance (HLB) show that basing on thermodynamic components of the general Hildebrand-Scatchard-Fedors theory of solubility, the diffusion profile of phytochemicals contained in the green tea extract (*Ext. Camellia sinensis L. aqu. siccum*) through the biological phase boundary as well as optimal choice of the extraction medium for selective extraction of the class of phytochemicals can be estimated.

Key words: *Camellia sinensis*, actual solubility ($S_{|real|}$), phytochemicals, partition coefficient (log P), Hildebrand-Scatchard-Fedors theory of solubility

INTRODUCTION

Studies (documented in publications) on the phytochemical composition of e.g. green tea infusions (*Folium Camellia sinensis L.*) allowed to explain pharmacologically differentiated – within infusions of different types of tea available in the market – profile of activity as well as their antioxidant properties [1-5]. The technology used in the green tea production process of hot water steam inactivation of polyphenol oxidase results in obtaining the infusion composition similar to the composition of phytochemicals contained in fresh leaves. The above process prevents enzymatic oxidation of catechins and, above all, the condensation of simple polyphenols, which are responsible for the characteristic color and taste of black tea infusion [6-9].

The green tea infusion contains, among others: catechins 30-32% (at principally EGCG – 11%, EC – 10%, ECG – 2% and EGC – 2%), hydrocarbons 15%, methylxanthines 7-9%, including caffeine 3.5%, tannin 4-6%, theogallin 2-3%, quinic acid 2%, mineral compounds 10% which supplemented with procyanidins and depsides (esters of two or more phenolic acids), determine its therapeutic properties [1].

Phytochemicals: phenolic acids and their derivatives (46.5586 mg/g), catechins (131.0343 mg/g), purine alkaloids (26.4800 mg/g), proanthocyanidins (0.8660 mg/g), O-glycosylated flavonols (11.7543 mg/g), flavonols and flavone (0.2865 mg/g), acylated glycosylated flavonols (0.3633 mg/g) were identified and determined by UPLC/DAD/MS method in different types of tea (green, pu-erh and white) [10, 11].

The published results of clinical studies on phytochemicals contained in green tea leaves (*Folium Camellia sinensis L.*) indicate that they can be used as dry extract (*Ext. Camellia sinensis L. siccum*) in the treatment of e.g. hypertension [12], inhibition of breast proliferation, skin and gastrointestinal tract cancer [13, 14], and the decrease of lipid profile parameters including total cholesterol [15-17]. The beneficial effect of epigallocatechin gallate on glycemia profile [18], form of menopause [19] and induction of apoptosis [20] were also confirmed.

The isolation and identification of basic physico-chemical parameters of phytochemicals – chemical individuals – that determine the profile of pharmacological activity of the green tea leaf extract (*Ext. Camellia sinensis L. aqu. siccum*) allow to calculate their actual

solubility in water and extraction media of diversified polarity (ϵ_M) on the basis of general Hildebrand-Scatchard-Fedors theory of solubility [21-26].

This enables to estimate the content of phytochemicals in the therapeutically suggested dose which can be significantly increased by micellar and hydrotropic solubilization in the produced extract of green tea leaves (*Ext. Camellia sinensis L. aqu. siccum*) [27].

Calculation – on the basis of selected thermodynamic parameters (ΔH_f^{app} , $\delta^{1/2}$, T_m °K) and on the chemical structure of phytochemicals – of the structural and solubility level of hydrophilic-lipophilic balance (HLB_D , HLB_C , $HLB_{Requ.}$) and, above all, the estimation of the actual solubility – $S_{|real.|}$ [mol/dm³] allow, by means of the correlation with partition coefficient ($\log P$), the determination of the predicted mechanism of phytochemical penetration through lipid layer of the physiological phase boundary, *i.e.* the process of mass exchange on phase boundary.

The calculated with QSAR methods [28-34] numerical values of the partition coefficient of phytochemicals in the form of a logarithm ($\log P$), supplemented by the estimated actual solubility $S_{|real.|}$ are an application indication for the inclusion into the prescription of the designed formulations containing dry green tea extract (*Camellia sinensis L. aqu. siccum*) and the appropriate types of solubilizers (micellar or hydrotropic) that will significantly improve the bioavailability of phytochemicals.

Moreover, the obtained results allow for optimal selection of the extraction system of compatible polarity (ϵ_M) for the class of phytochemicals which will enable selective isolation of chemical individual (structural) or a class of phytochemicals from plant raw material [35-37].

MATERIAL AND METHODS

Dry aqueous extract from green tea leaves – *Ext. Camellia sinensis L. folium aqu. siccum* (100% native) was obtained from Europlant Group – Phytopharm Klęka S.A., Poland.

Solubility of phytochemicals in the extraction medium of diversified polarity – ϵ_M

Currently, due to the applicative aspect, the general Hildebrand-Scatchard-Fedors theory of solubility deserves special attention [26]. It allows to calculate the predicted actual solubility in the form of molar fraction logarithm ($-\log x_2$) on the basis of determined melting temperature of the phytochemical

($T_m = 273.15 + t^\circ\text{C}$).

The fundamental Hildebrand-Scatchard equation has the following form:

$$-\log x_2 = \frac{\Delta H_f^{app}}{2.303 \cdot R \cdot T} \cdot \frac{T_m - T}{T_m} + \frac{V_2 \cdot \varphi^2}{2.303 \cdot R \cdot T} \cdot (\delta_1 - \delta_2)^2,$$

where: ΔH_f^{app} – apparent enthalpy of melting, T – solution temperature in absolute scale (in °K), R – gas constant, V_2 – phytochemical molar volume, φ^2 – solvent volume fraction, δ_1 , δ_2 – phytochemical parameter (2) and solvent parameter (1).

Values were calculated. Procedure of the above equation requires calculation of ΔH_f^{app} , that is apparent enthalpy of melting, from the dependence:

$$\Delta H_f^{app} = \frac{0.01(T_m - T) \cdot R}{T_m/T} \cdot T_m$$

and of the parameter of solubility $\delta^{1/2} = \sqrt{\Sigma \Delta E_i / \Sigma \Delta V_i}$ from the equation given by Fedors [38].

The solubility parameter allows to calculate from the equation:

$$HLB_{Requ.} = [(\delta^{1/2} + 7)/8]^4$$

the so-called required solubility level of hydrophilic-lipophilic balance $HLB_{Requ.}$.

Calculated (using a molar fraction (x_2)) numerical value of actual solubility in water or in extraction medium of defined polarity, considering the dielectric constant (ϵ_M), can be expressed in mol/dm³ with the equation:

$$S_{|real.|} = \frac{1000}{M_{molec.} \cdot (1/x_2 - 1)}$$

where: $M_{molec.}$ – molecular mass of the solvent.

The calculated values were $\Sigma \Delta E_i$, $\Sigma \Delta V_i$, $\delta^{1/2}$, ΔH_f^{app} , $-\log x_2$ and $S_{|real.|}$.

Structural level of hydrophilic-lipophilic balance (HLB) of the phytochemicals

Numerical value of the structural level of hydrophilic-lipophilic balance (HLB) of phytochemicals is a measure of the ratio between the hydrophilic part of the molecule and its lipophilic part. Griffin

suggested calculating the level from the dependence:

$$HLB_G = 20 \cdot \frac{M_p}{M}$$

where: M_p – molar ratio of the polar part in the phytochemical molecule, M – its molecular weight (in g/mol).

In the alternative method proposed by Davies [39], the HLB of the organic structure is calculated from the dependence:

$$HLB_D = 7 + \sum_1^i W_{h(+)} + \sum_1^i W_{l(-)}$$

where: $W_{h(+)}$ and $W_{l(-)}$ – mean proportion of hydrophilic $h_{(+)}$ and lipophilic $l_{(-)}$ groups in the structure of the phytochemical.

Numerical values of the level of hydrophilic-lipophilic balance (HLB) were calculated in the notifications of Griffin method, Davies method and HLB_{Requ} .

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS AND DISCUSSION

The structure of phytochemicals contained in *Ext. Camellia sinensis L. aqu. siccum* [40], supplemented with selected physicochemical values, was the basis for calculating by Fedors' method [38] the thermodynamic values: $\Sigma\Delta E_i$ (cal/mol) and $\Sigma\Delta V_i$ (cm³/mol) that allow to calculate the solubility parameter – $\delta^{1/2}$ and HLB_{Requ} . This part of the research was supplemented with the structural level of hydrophilic-lipophilic balance calculated by Davies' method (HLB_D) and Griffin's method (HLB_G).

The thermodynamic (ΔH_f^{app} , $\Sigma\Delta E_i$, $\Sigma\Delta V_i$, $\delta^{1/2}$, HLB_{Requ}) and physicochemical values (t , melting °C, HLB_D , HLB_G) are presented in table 1.

The estimated polarity of the extraction medium – (ϵ_M) of the water/ethanol system and its solubility parameter – $\delta^{1/2}$ allowed to calculate the predicted solubility of phytochemicals contained in *Ext. Camellia sinensis L. aqu. siccum* by Hildebrand-Scatchard method in the form of molar fraction ($-\log x_2$). The calculated values ($-\log x_2$) for the investigated structures of the derivatives of: xanthine, catechins, polyphenols and flavonoids are demonstrated in table 2.

Table 1.

Calculated thermodynamic values and the level of hydrophilic-lipophilic balance HLB_{Requ} , HLB_D and HLB_G of phytochemicals contained in *Ext. Camellia sinensis L. folium aqu. siccum*

Phytochemicals	Melting t.°C	ΔH_f^{app} cal·mol ⁻¹	$\Sigma\Delta E_i$ cal·mol ⁻¹	$\Sigma\Delta V_i$ cm ³ ·mol ⁻¹	$\delta^{1/2}$	HLB_{Requ}	HLB_D	HLB_G
1. Caffeine	238.0	9241.53	20565	102.6	14.157	48.92	43.40	9.07
2. Theobromine	357.0	12791.69	20440	82.8	15.712	64.96	44.35	9.88
3. Theophylline	273.0	10238.88	20440	82.8	15.713	65.17	43.80	9.77
4. (-) Catechin [2S,3R]	175-176	7560.59	51580	96.9	23.072	199.64	9.90	13.51
5. (+) Catechin [2R,3S]	214.0	6689.04	51580	96.9	23.072	199.64	9.90	13.51
6. (+) Epicatechin [2S,3S]	235-237	9186.83	51580	96.9	23.072	199.64		
7. (-) Epicatechin [2R,3R]	240.0	9297.43	51580	96.9	23.072	199.64		
8. Epicatechin gallate (-)	257-258	9792.29	75425	130.3	24.059	227.20	14.02	84.09
9. Epigallocatechin gallate (-)	140-142	6689.04	83420	130.9	25.244	263.91	12.13	7.81
10. Procyanidin B-1	231-232	9060.68	10097	175.9	23.958	224.26	19.10	6.98
11. (-) Epigallocatechin	219-221	8744.12	66330	150.3	27.28	336.97	14.95	7.78
12. Astragalín	178.0	7625.29	70650	173.5	20.179	133.22	15.52	8.70
13. iso-Quercetin	238-242	9297.45	75710	156.5	21.994	172.55	18.37	9.13
14. Myricetin	357.0	12795.76	70520	79.4	29.802	447.84	14.35	9.17
15. Kaempferol	316-318	11548.42	64910	102.1	25.214	262.91	13.40	8.54
16. Quercetin	231-232	9060.68	10097	175.9	23.958	224.26	19.10	6.98

Table 2.

Calculated predicted solubility – $\log x_2$ of phytochemicals depending on solubility parameter $\delta^{1/2}$ of extraction medium at ethanol concentration progression

Extraction medium	$\delta^{1/2}$	ϵ_M	– $\log x_2$							
			Caffeine	Theobromine	Theophylline	(–) Catechin	(+) Catechin	(+) Epi-catechin	(–) Epi-catechin	(–) Epi-catechin gallate
1. H ₂ O	24.520	78.50	10.908	7.742	8.083	2.008	2.589	3.004	2.939	1.423
2. 50% aqu. solution of C ₂ H ₅ OH	18.572	49.92	4.292	5.235	3.896	4.371	4.952	5.367	5.302	5.643
3. 70% aqu. solution of C ₂ H ₅ OH	16.192	30.87	3.135	4.947	3.420	6.784	7.365	7.780	7.715	9.233
4. 90% aqu. solution of C ₂ H ₅ OH	13.813	28.10	2.935	5.069	3.630	9.999	10.581	10.996	10.932	13.908
5. C ₂ H ₅ OH	12.629	24.30	2.996	5.283	w3.989	11.900	12.481	12.896	12.832	16.639

Extraction medium	$\delta^{1/2}$	ϵ_M	– $\log x_2$							
			Epigallo-catechin gallate (–)	Procyanidin B-1	(–) Epigallo-catechin	Astragalín	iso-Quercetin	Myricetin	Kaempferol	Quercetin
1. H ₂ O	24.520	78.50	1.423	2.759	2.971	4.301	3.584	6.558	4.216	4.419
2. 50% aqu. solution of C ₂ H ₅ OH	18.572	49.92	5.643	14.587	6.905	2.223	4.194	12.251	4.704	7.488
3. 70% aqu. solution of C ₂ H ₅ OH	16.192	30.87	9.233	27.396	9.638	3.916	6.702	15.678	6.711	10.277
4. 90% aqu. solution of C ₂ H ₅ OH	13.813	28.10	13.908	44.831	13.015	7.048	10.511	19.761	9.754	13.913
5. C ₂ H ₅ OH	12.629	24.30	16.639	55.230	14.944	7.595	12.888	22.037	11.654	16.037

They allowed to trace the correlation between the predicted solubility ($-\log x^2$) of chemical structures of phytochemicals and the polarity of extraction system (water-ethanol mixture) – ϵ_M : $(-\log x_2) = f(\epsilon_M)$. The course of this correlation is presented in figures 1-4.

It results from the course of this correlation for xanthine derivatives (caffeine, theobromine, theoflavin) – figure 1 – that with the increase of ethanol concentration in the extraction medium the numerical value ϵ_M decreases, whereas their solubility increases significantly.

However, in the case of catechins – figure 2, catechins and polyphenols – figure 3 and flavonoids – figure 4, the increase of ethanol concentration in the extraction system by more than 50% ($\epsilon_M = 49.92$) results in dramatic drop in solubility which is reflected in the numerical value ($-\log x_2$) – table 2.

The course of the correlations between ($-\log x_2$) in the function of dielectric constant of the extraction system (ϵ_M) at $p=0.05$ is described by correlation

equations presented in table 3. They reflect the solubility preferences of phytostructures contained in *Ext. Camellia sinensis L. aqu. siccum*, the concentration of which in pharmaceutical and cosmetic formulations produced on the basis of dry extract determines the therapeutic and cosmetic efficacy.

The partition coefficient ($\log P$), predicted actual solubility ($-\log S_{[real]}$) and the level of hydrophilic-lipophilic balance (HLB) of phytochemicals, secondary metabolites contained in *Ext. Camellia sinensis L. aqu. siccum* collected from databases [40, 41] and demonstrated in tab. 4 partition coefficients of the phytochemicals expressed in the form of $\log P$:

$$\log P = \frac{[\text{non-ionized form of the compound}]_{\text{n-octan}}}{[\text{non-ionized form of the compound}]_{\text{water (buffer)}}$$

point to their preferences in the process of mass exchange on phase boundary. It results from the available literature that at the values $0 < \log P < 3$ the process of mass exchange – penetration can be optimal, but with increasing lipophilic properties $\log P > 3$ (weak

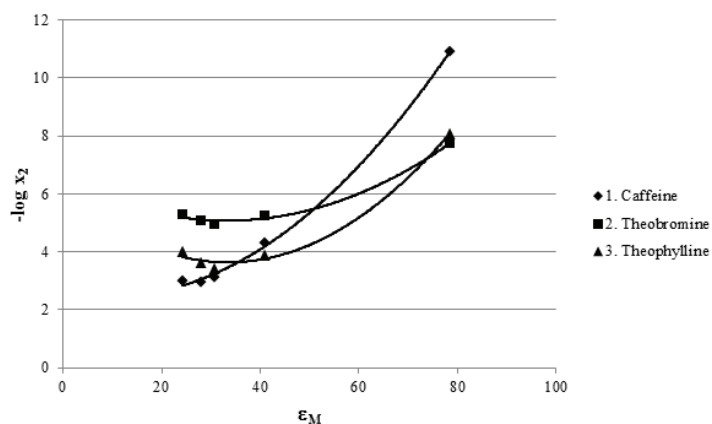


Figure 1.

The course of the correlation between $-\log x_2$ of xanthine derivatives (caffeine, theobromine, theophylline) and the dielectric constant ϵ_M of the system of solvents: water-ethanol

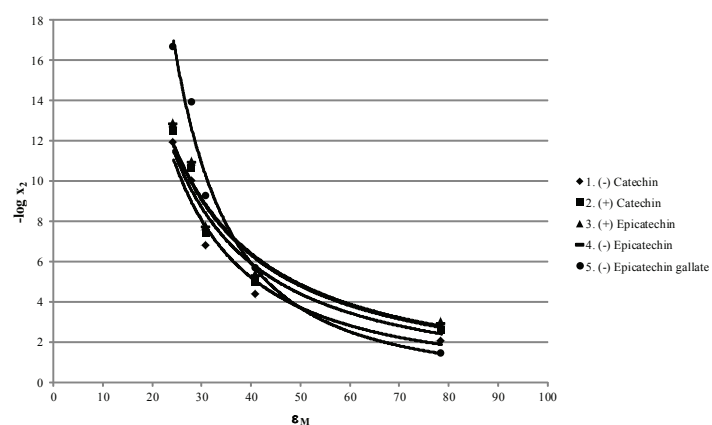


Figure 2.

The course of the correlation between $-\log x_2$ of catechins and the dielectric constant ϵ_M of the system of solvents: water-ethanol

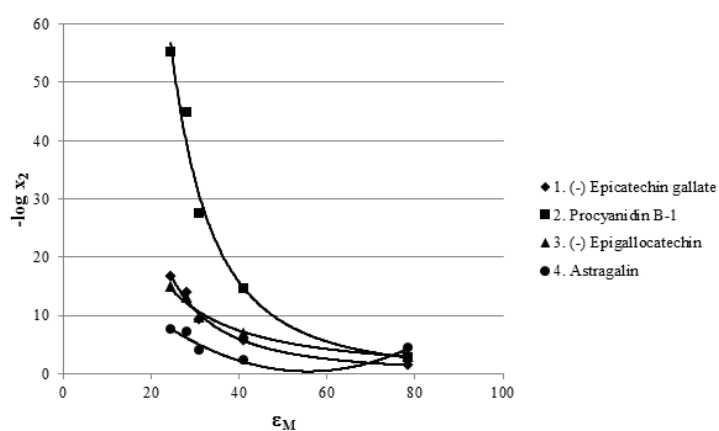


Figure 3.

The course of the correlation between $-\log x_2$ of the phytochemicals of the type of catechins and polyphenols and the dielectric constant ϵ_M of the system of solvents: water-ethanol

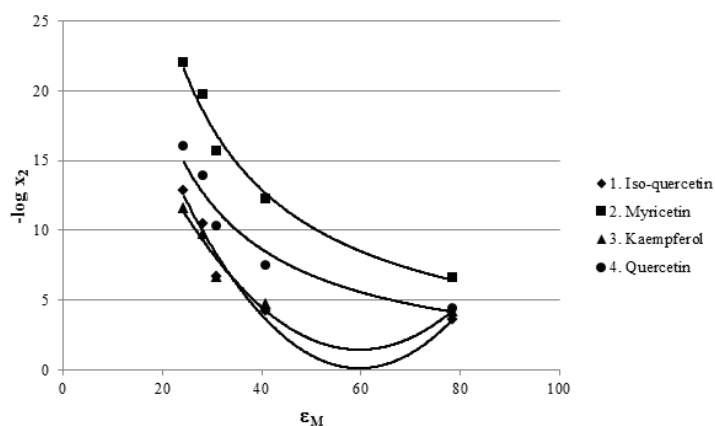


Figure 4.

The course of the correlation between $-\log x_2$ of flavonoids (1 – iso-quercetin, 2 – myricetin, 3 – kaempferol, 4 – quercetin) and the dielectric constant ϵ_M of the system of solvents: water-ethanol

Table 3.

Correlation equations describing the dependence at $p = 0.05$ between calculated predicted solubility – $\log x_2$ and medium polarity – ϵ_M ; – $\log x_2 = f(\epsilon_M)$

Phytochemicals	Type of approximation equation	Correlation coefficient	Slope coefficients	
			a±da	b±db
1. Caffeine	1. $1/y = a+b \cdot x$	0.9964	0.4610±0.0341	-0.0046±0.0073
	2. $\log y = a+b \cdot x$	0.9854	0.1735±0.1571	0.0106±0.0034
	3. $y = a + b \cdot x$	0.9612	-1.3442±3.6176	0.1470±0.0775
2. Theobromine	1. $1/y = a+b \cdot x$	0.9077	0.2327±0.0479	-0.0012±0.0102
	2. $\log y = a+b \cdot x$	0.9092	0.6081±0.1284	0.0032±0.0027
	3. $y = a + b \cdot x$	0.9099	3.4666±1.8622	0.0476±0.00398
3. Theophylline	1. $1/y = a+b \cdot x$	0.8983	0.3528±0.1124	-0.0027±0.0024
	2. $\log y = a+b \cdot x$	0.9062	0.3785±0.2469	0.0061±0.0034
	3. $y = a + b \cdot x$	0.9072	1.2583±3.1223	0.0793±0.0668
4. (-) Catechin	1. $1/y = a+b \cdot x$	0.9919	-0.1047±0.0823	0.0075±0.0073
	2. $\log y = a+b \cdot \log x$	0.9896	3.0956±0.6232	-1.4704±0.3912
	3. $y = a + b \cdot x$	0.9816	0.0237±0.0284	26.2285±9.3732
	4. $y = a+b \cdot 1/x$	0.9748	-2.6091±4.3007	338.1191±141.9176
5. (+) Catechin	1. $1/y = a+b \cdot x$	0.9945	-0.0535±0.0496	0.0055±0.0011
	2. $\log y = a+b \cdot x$	0.9843	2.8745±0.5853	-1.2994±0.3674
	3. $y = a + b \cdot x$	0.9843	0.1568±0.2320	23.2763±7.6594
	4. $y = a+b \cdot x$	0.9748	-2.0283±4.3015	338.1322±142.0014
6. (+) Epicatechin	1. $1/y = a+b \cdot x$	0.9948	-0.0338±0.0413	0.0047±0.0008
	2. $\log y = a+b \cdot \log x$	0.9874	2.7707±0.5711	-1.2172±0.3586
	3. $\log y = a + b \cdot 1/x$	0.9853	0.2237±0.2104	21.8472±6.9489
	4. $y = a+b \cdot 1/x$	0.9748	-1.6789±4.3031	338.1689±142.0530
7. (-) Epicatechin	1. $1/y = a+b \cdot x$	0.9948	-0.0308±0.0402	0.0046±0.0008
	2. $\log y = a+b \cdot \log x$	0.9872	2.7531±0.5686	-1.2031±0.3570
	3. $\log y = a + b \cdot 1/x$	0.9854	0.2353±0.2070	21.6023±6.8344
	4. $y = a+b \cdot x$	0.9748	-1.6133±4.3015	338.1323±142.0014
8. Epicatechin gallate (-)	1. $1/y = a+b \cdot x$	0.9927	-0.0547±0.0048	0.0046±0.0011
	2. $\log y = a+b \cdot \log x$	0.9891	3.1025±0.5970	-1.3701±0.3748
	3. $\log y = a + b \cdot 1/x$	0.9815	0.2394±0.2653	24.4544±8.7595
	4. $y = a+b \cdot 1/x$	0.9759	-3.0592±5.7234	460.6401±188.9396
9. Epigallocatechin gallate (-)	1. $\log y = a+b \cdot x$	0.9914	1.6442±0.2137	-0.0189±0.0457
	2. $\log y = a+b \cdot \log x$	0.9828	4.0317±1.1051	-2.0134±0.6937
	3. $y = a + b \cdot x$	0.9793	-5.3512±5.9496	517.3046±196.4071
10. Procyanidin B-1	1. $\log y = a+b \cdot x$	0.9923	2.2627±0.2477	-0.0231±0.0053
	2. $\log y = a+b \cdot \log x$	0.9842	5.1854±1.2988	-2.4644±0.8154
	3. $y = a + b \cdot 1/x$	0.9723	-22.1122±23.9789	1794.7970±791.5887
	4. $1/y = a+b \cdot x$	0.9541	-0.1623±0.1686	0.0062±0.0036
11. Epigallocatechin	1. $\log y = a+b \cdot x$	0.9883	1.4367±0.1627	-0.0123±0.0034
	2. $\log y = a+b \cdot \log x$	0.9860	3.0061±0.6529	-1.3207±0.4099
	3. $1/y = a + b \cdot x$	0.9835	-0.0591±0.0764	0.0048±0.0016
	4. $y = a+b \cdot x$	0.9830	-2.0193±4.2019	404.6184±138.7133
12. iso-Quercetin	1. $\log y = a+b \cdot 1/x$	0.9707	0.2513±0.2783	20.2290±9.1881
	2. $\log y = a+b \cdot \log x$	0.9345	2.5394±1.2068	-1.0826±0.7576
	3. $y = a + b \cdot 1/x$	0.9343	-1.6514±6.8872	343.2620±227.3610
	4. $1/y = a+b \cdot x$	0.9311	0.0134±0.00112	0.0036±0.0026
13. Myricetin	1. $1/y = a+b \cdot x$	0.9888	-0.0021±0.0002	0.0019±0.0005
	2. $\log y = a+b \cdot \log x$	0.9859	2.7117±0.4907	-0.9886±0.3081
	3. $y = a + b \cdot x$	0.9834	0.4435±5.3467	520.5738±176.5871
	4. $\log y = a+b \cdot x$	0.9871	1.5364±0.1284	-0.0092±0.0027
14. Kaempferol	1. $\log y = a+b \cdot 1/x$	0.9645	0.3813±0.2435	15.9751±8.0405
	2. $y = a+b \cdot x$	0.9343	0.0307±0.0550	259.2439±181.6204
	3. $\log y = a+b \cdot \log x$	0.9246	2.1828±1.0264	-0.8514±0.6444
	4. $1/y = a+b \cdot x$	0.9107	0.0437±0.1048	0.0027±0.0022
15. Quercetin	1. $1/y = a+b \cdot x$	0.9939	-0.0057±0.0027	0.0029±0.0005
	2. $\log y = a+b \cdot 1/x$	0.9841	0.4347±0.1908	19.0216±6.2991
	3. $\log y = a+b \cdot \log x$	0.9871	2.6339±0.5029	-1.0607±0.3157
	4. $y = a+b \cdot 1/x$	0.9782	-0.8413±4.6764	395.9855±154.3769

Table 4.

Molar fraction (x_2), logarithm from partition coefficient ($-\log P$) and calculated actual solubility $-S_{|real|}$ (mol/dm³) of phytochemicals contained in dry extract from green tea (*Ext. Camellia sinensis L. aqu. siccum*)

Phytochemicals	Mol. mass [g·mol ⁻¹]	Solubility molar fraction x_2	Calculated solubilities $S_{ sol }$ [mol/dm ³]	$-\log S_{ sol }$	$\log P$
					* **
1. Caffeine	194.19	1.2359·10 ⁻¹¹	6.3645·10 ⁻¹¹	10.1962	-0.24;-0.55
2. Theobromine	180.16	1.8000·10 ⁻⁸	9.9908·10 ⁻⁸	7.0004	-0.78;-0.77
3. Theophylline	180.16	8.0000·10 ⁻⁹	4.4403·10 ⁻⁹	8.3525	-0.26;-0.77
4. Catechin (-)	290.26	9.8174·10 ⁻³	3.3822·10 ⁻²	1.4709	1.02;1.80
5. Catechnin (+)	290.26	2.5763·10 ⁻⁴	8.8995·10 ⁻³	2.0506	1.02;1.80
6. Epicatechin (+)	290.26	9.9085·10 ⁻⁴	3.4168·10 ⁻³	2.4663	1.02;1.80
7. Epicatechin (-)	290.26	1.1508·10 ⁻³	3.9691·10 ⁻³	2.4013	1.02;1.80
8. Epicatechin gallate (-)	442.37	3.7757·10 ⁻²	8.5252·10 ⁻²	1.0687	2.55;3.88
9. Epigallocatechin gallate (-)	458.37	3.7757·10 ⁻²	8.5604·10 ⁻²	1.0675	3.38;3.08
10. Procyanidin B-1	578.52	1.74180·10 ⁻³	3.0160·10 ⁻³	2.5205	2.29;3.12
11. Epigallocatechin	306.26	1.0691·10 ⁻³	3.4943·10 ⁻³	2.4566	0.71;1.49
12. Astragalin	448.37	5.0003·10 ⁻⁵	1.1115·10 ⁻⁴	3.9541	0.52; 0.16
13. iso-Quercetin	464.37	2.6061·10 ⁻⁴	5.6135·10 ⁻⁵	4.2507	0.47;-0.14
14. Myricetin	318.23	2.7600·10 ⁻⁷	8.6700·10 ⁻⁷	6.0619	1.66;1.85
15. Kaempferol	286.23	6.0813·10 ⁻⁵	2.1247·10 ⁻⁴	3.6726	1.99;2.46
16. Quercetin	302.23	3.8106·10 ⁻⁵	1.2608·10 ⁻⁴	3.8993	1.81;2.16

*ALOGPS
**Chem Axon

solubility in water) the above mentioned process can be made difficult if through the micellar solubilization there does not come to the increase of physiological solubility in the balance system.

Table 4 also includes the calculated predicted solubility of phytochemicals $S_{|real|}$ (mol/dm³, SI) as well as its logarithmic form $-\log S_{|real|}$.

It allows to trace within classes of phytochemicals contained in the *Ext. Camellia sinensis L. aqu. siccum* the correlation between the calculated predicted actual solubility ($-\log S_{|real|}$) and the partition coefficient $\log P$; $\log P = f(-\log S_{|real|})$ specified in two independent notification systems ALOGPS and Chem Axon, fig. 5 and 6.

The course of the dependence between solubility HLB_{Requ} , structural HLB_D and partition coefficient ($\log P$) was investigated. The course of the correlation ($\log P$) = ($-\log S_{|real|}$) – figure 7 and $\log P = f(HLB_{Requ}, HLB_D)$ at $p=0.05$ is described by correlation equations, which are presented in table 5.

CONCLUSIONS

1. The general Hildebrand-Scatchard theory of solubility supplemented by Fedors' solubility parameter $-\delta^{1/2}$ was used for calculating the predicted solubility by $-\log x_2$ (logarithm of the molar fraction) of phytochemicals, secondary metabolites contained in *Ext. Camellia sinensis L. aqu. siccum*. The relation between ($-\log x_2$) and polarity of the extraction medium ϵ_M ; ($-\log x_2$) = $f(\epsilon_M)$ described by correlation equations allows applicative optimal choice of water/ethanol mixture for selective separation of proper class of phytochemicals from raw material.
2. It results from the course of the dependence ($-\log x_2$) = $f(\epsilon_M)$, figures 1–4 and correlation equations from table 3 that the process of solubility of phytochemicals in the class of derivatives of xanthine, catechins, polyphenols and flavonoids is most fully and reliably described – at high values of correlation coefficient (r) – by

Table 5.

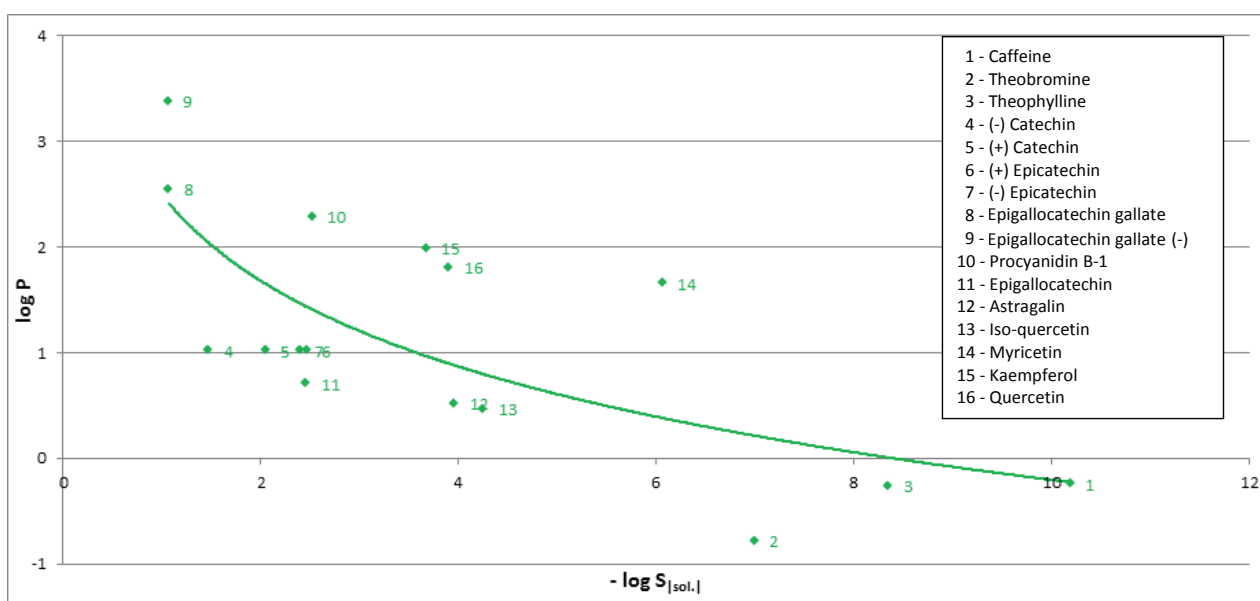
Correlation equations describing dependence between $\log P$ and calculated solubility of phytochemicals ($-\log S_{|\text{real}|}$) and their level of hydrophilic-lipophilic balance $\text{HLB}_{\text{Requ.}}$ and HLB_{D}

Phytochemical	Type of approximation equation	Correlation coefficient	Slope coefficients	
			a±da	b±db
1. $\log P = f(-\log S_{ \text{real} })$	1. $\log y = a+b \cdot x$	0.7195	0.7208±0.1661	-0.1921±0.1063
	2. $1/y = a+b \cdot x$	0.7057	0.1977±0.1505	0.1674±0.0963
	Notification ALOGPS	3. $y = a + b \cdot 1/x$	0.7881	4.1408±0.9197
2. $\log P = f(-\log S_{ \text{real} })$	1. $\log y = a+b \cdot x$	0.8002	0.7387±0.1413	-0.1631±0.0701
	2. $1/y = a+b \cdot x$	0.7398	0.1939±0.1409	0.1340±0.0698
	Notification Chem. Axon	3. $y = a + b \cdot 1/x$	0.7845	6.0281±1.3230
3. $\log P = f(\text{HLB}_{\text{Requ.}})$	1. $\log y = a+b \cdot 1/x$	0.7683	1.1070·10 ² ±0.2783·10 ⁻²	-2.7782·10 ⁻³ ±1.4554·10 ⁻³
	2. $\log y = a+b \cdot \log x$	0.7687	2.0435±0.1503	0.1403±0.0734
	Notification Chem. Axon	3. $y = a + b \cdot 1/x$	0.6512	141.1778±68.5819
4. $\log P = f(\text{HLB}_{\text{Requ.}})$	1. $1/y = a+b \cdot x$	0.7424	0.0112±0.3231·10 ⁻²	-3.5040·10 ⁻³ ±1.9886·10 ⁻³
	2. $\log y = a+b \cdot \log x$	0.7428	2.0356±0.1630	0.1778±0.1003
	Notification ALOGPS	3. $y = a + b \cdot x$	0.6225	138.2749±72.5470
5. $\log P = f(\text{HLB}_{\text{D}})$	1. $\log y = a+b \cdot 1/x$	0.8601	21.2515±3.9540	-5.7670±2.1515
	2. $y = a+b \cdot x$	0.7105	29.6851±7.7334	-7.6395±4.7587
	Notification ALOGPS	3. $\log y = a+b \cdot \log x$	0.6707	1.4056±0.1439
6. $\log P = f(\text{HLB}_{\text{D}})$	1. $1/y = a+b \cdot x$	0.7386	29.3823±7.1485	-6.0839±3.4926
	2. $\log y = a+b \cdot 1/x$	0.7148	1.4042±0.1309	-0.1040±0.0639
	Notification Chem. Axon	3. $\log y = a+b \cdot \log x$	0.64841	4.6292·10 ⁻² ±1.6286·10 ⁻²

equations of the type: $1/y=a+b \cdot x$, $\log y=a+b \cdot \log x$ (logarithmic form of the exponential equation of the type $y=a \cdot x^b$) and $\log y=a+b \cdot 1/x$ with significant differentiation in the case of the change of numerical value of the hydrophilic-lipophilic balance ($\text{HLB}_{\text{Requ.}}$, HLB_{D} , HLB_{G}).

3. The calculated numerical values of the predicted actual solubility of secondary classes of

metabolites contained in the *Ext. Camellia sinensis L. aqu. sicum* – $S_{|\text{real}|}$ (mol/dm³, SI) allowed to assess their capacity of the process of mass exchange on phase boundary (diffusion) by tracing the correlation between the partition coefficient ($\log P$), tab. 4, and their applicative parameter ($-\log S_{|\text{real}|}$) and $\log P = f(-\log S_{|\text{real}|})$. Taking into account alternative numerical values of $\log P$ calculated using ALOGPS and

**Figure 5.**

Correlation between $\log P$ (ALOGPS) and the value $-\log S_{|\text{sol}|}$

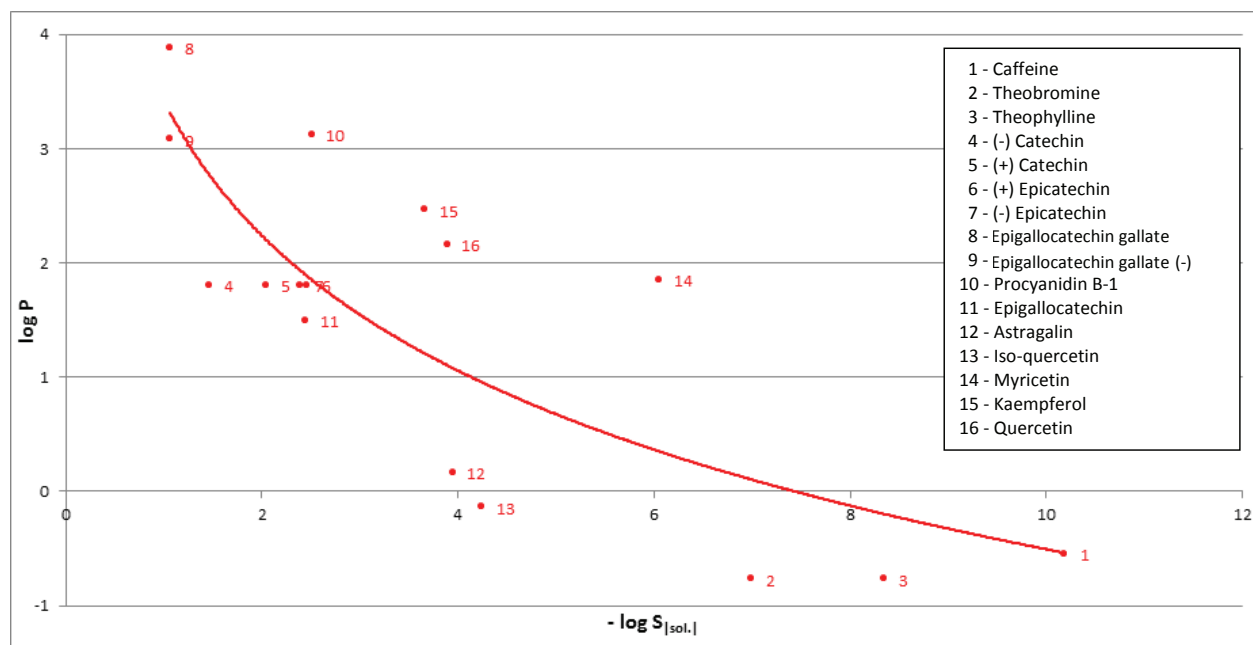


Figure 6.

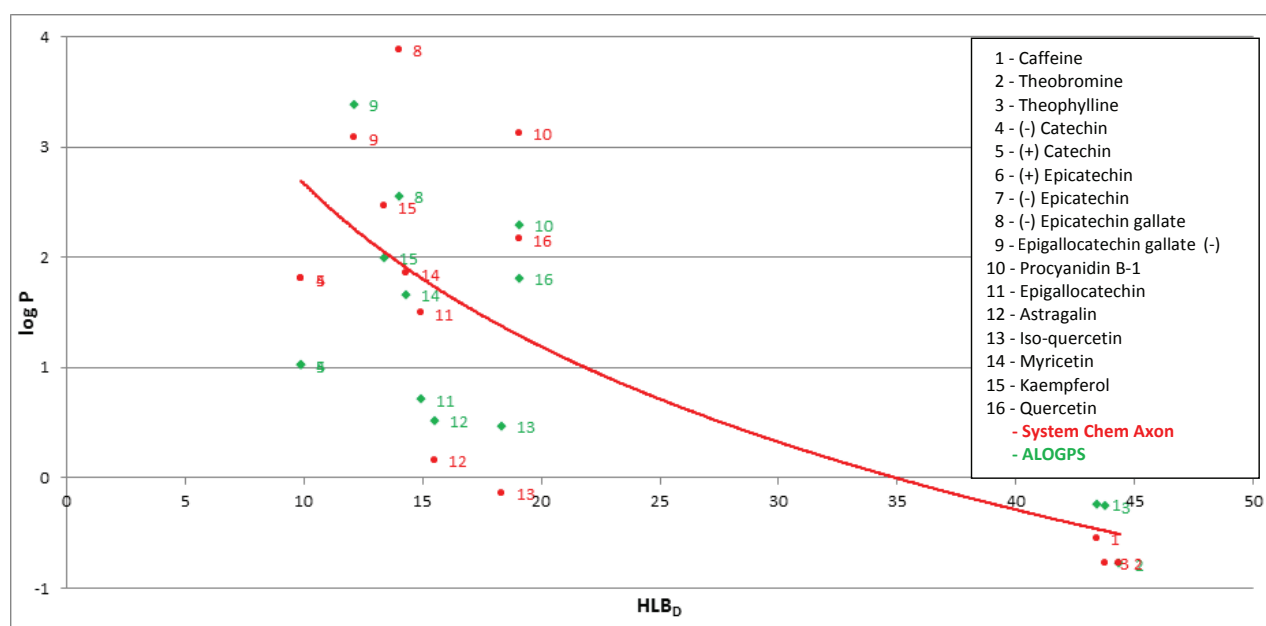
Correlation between $\log P$ (Chem Axon System) and the value $-\log S_{\text{sol.}}$ 

Figure 7.

Correlation between $\log P$ (Chem Axon System) and the structural value HLB_D in the notification of Davies method

Chem. Axon software, the above correlation was described by correlation equations, which with appropriate for them correlation coefficient (r) allow for their use for the real estimation of the partition coefficient (P , $\log P$) – *in vitro* (*in vivo*).

- The calculated values of hydrophilic-lipophilic balance HLB_{Requ} and its structural alternative HLB_D were also correlated with the partition coefficient ($\log P$); $\log P = f(HLB_{\text{Requ}}, HLB_D)$ – to

estimate prospectively – within the preformulation studies, the capability of secondary metabolites for cascade kinetics of mass exchange on phase boundary. It results from the correlation equations presented in table 5 that chemical structure of phytochemicals contained in *Ext. Camellia sinensis L. aqu. siccum* may be the sufficient basis for initial estimation of the partition coefficient ($\log P$) and may also point to the preferences of the selected structures in the

process of mass exchange on phase boundary *in vivo*.

Conflict of interest: Authors declare no conflict of interest.

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Rozpuszczalność rzeczywista ($S_{|rz.|}$), poziom równowagi hydrofilo-lipofilowej ($HLB_{Req.}$, HLB_D , HLB_G) oraz współczynnik podziału ($\log P$) fitozwiązków wchodzących w skład *Ext. Camellia sinensis L. aqu. siccum* w świetle ogólnej teorii rozpuszczalności Hildebranda-Scatcharda-Fedorsa

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Streszczenie

Wstęp: Wykorzystując ogólną teorię rozpuszczalności Hildebranda-Scatcharda-Fedorsa wyliczono ułamek molowy (x_2) rozpuszczalności fitozwiązków zawartych w suchym ekstrakcie z liści zielonej herbaty, które decydują o profilu działania farmakologicznego.

Cel: Aplikacyjnym celem prowadzonych badań było oszacowanie rzeczywistej rozpuszczalności fitozwiązków – $S_{|rz.|}$ (mol/dm^3) w wodzie i roztworach wodno-etanolowych o zmiennej polarności (ϵ_M) w celu selektywnej ich ekstrakcji i optymalnej formulacji stałej doustnej postaci leku.

Metody: Do wyliczenia rozpuszczalności rzeczywistej – $S_{|rz.|}$ i poziomu równowagi hydrofilo-lipofilowej (HLB) wykorzystano podstawowe wielkości fizyko-chemiczne i strukturalne fitozwiązków oraz stosowne równania matematyczne z ogólnej teorii rozpuszczalności Hildebranda-Scatcharda-Fedorsa.

Wyniki: Wyliczone wielkości rozpuszczalności rzeczywistej – $S_{|rz.|}$ (mol/dm^3) zestawione w równaniach korelacyjnych umożliwiły ocenę zdolności fitozwiązków do procesu wymiany masy na granicy faz. Równania korelacyjne dla zależności $\log P = f(-\log S_{|rz.|})$ wskazują preferencje strukturalne fitozwiązków w kinetyce procesu wymiany masy (dyfuzji) przez naturalną granicę faz.

Wnioski: Z przeprowadzonych obliczeń i korelacji między wielkościami, które charakteryzują rozpuszczalność rzeczywistą – $S_{|rz.|}$, polarność mediów (woda, etanol i ich roztwory) oraz współczynnik podziału ($\log P$), a w tym również poziom równowagi hydrofilo-lipofilowej (HLB) wynika, że w oparciu o składowe termodynamiczne ogólnej teorii rozpuszczalności Hildebranda-Scatcharda-Fedorsa można oszacować profil dyfuzji fitozwiązków zawartych w ekstrakcie z zielonej herbaty (*Ext. Camellia sinensis L. aqu. Siccum*) przez biologiczną granicę faz, a także optymalnie dobrać medium ekstrakcyjne w celu selektywnego pozyskiwania klasy fitozwiązków.

Słowa kluczowe.: *Camellia sinensis*, rozpuszczalność rzeczywista ($S_{|rz.|}$), fitozwiązki, współczynnik podziału ($\log P$), teoria rozpuszczalności Hildebranda-Scatcharda-Fedorsa.