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EXPERIMENTAL PAPER

Predicted real solubility ($-\log x_2$) and the level of hydrophilic-lipophilic balance ($HLB_{\text{Requ.}}$) of phytochemicals contained in extracts isolated from linden inflorescence (*Tiliae flos*) with solvents of diversified polarity (ϵ_M)

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Summary

Introduction: The broad spectrum of pharmacological properties of linden inflorescence extracts results from polarity and the level of hydrophilic-lipophilic balance of solvents (medium) used to separate compatible phytochemical structures with the expected pharmacotherapeutic profile.

Objective: The use of the general Hildebrand-Scatchard-Fedors theory of solubility to calculate the predicted solubility of classes of phytochemicals contained in linden inflorescences (*Tiliae flos*) and the indication of those structures which, due to their high solubility in the medium, are responsible for the profile of pharmacological activity.

Material and methods: The Hildebrand, Scatchard equation, supported with computational technique proposed by Fedors, allows calculation of the solubility parameters of the extraction medium. Despite application reservations, it is a fundamental tool for estimating the predictable solubility of phytochemicals in real solution.

Results: The structure of phytochemicals isolated from linden inflorescences (*Tiliae flos*) owing to the use of solvents of significantly diversified polarity ($-\epsilon_M$) was the basis for calculating the molar evaporation energy $-\Sigma\Delta E_i$ (cal/mol) and molar volume $-\Sigma\Delta V_i$ (cm³/mol) by Fedors method, which are fundamental quantities necessary to estimate the solubility parameter $-\delta^{1/2}$ and required solubility level of hydrophilic-lipophilic balance $-\text{HLB}_{\text{Requ.}}$.

Conclusions: Results of the presented research indicate that basing on the parameters characterizing the structure of phytochemicals and the calculated ideal ($-\log x_1$) and predicted real ($-\log x_2$) solubility, it is possible – using the general Hildebrand-Scatchard-Fedors theory of solubility – to choose selectively the cascade of extraction media in order to distinguish in the plant material chemical and structural individuals of different polarity.

Key words: *real solubility, phytochemicals, Tiliae flos, level of hydrophilic-lipophilic balance ($\text{HLB}_{\text{Requ.}}$)*

Słowa kluczowe: *rozpuszczalność rzeczywista, fitozwiązki, Tiliae flos, poziom równowagi hydrofilo-lipofilowej ($\text{HLB}_{\text{Requ.}}$)*

INTRODUCTION

Dried leaves and inflorescences of both types of linden: small-leaved (*Tilia cordata* Mill.) and large-leaved (*Tilia platyphyllos* Scop.) are rich in mucus and phenolic compounds (chlorogenic acid, caffeic acid, vanillic acid, *p*-coumaric acid) and above all in flavonoids (about 1%) among which the fundamental structures are tiliroside, astragalin, rutin, kaempferol and quercetin derivatives as well as galatechin tannins and proanthocyanidins [1-4].

In bracts and inflorescences (*Tiliae flos*) there are small amounts of essential oil the main components of which are mono- and sesquiterpenes (linalool, germacrene, farnesene) [5-7].

Galenic preparations obtained from linden inflorescence have been used in folk medicine as sedative for anxiety [1] and they also lower the threshold of stimulation of thermoregulatory centers [8] whereas the presence of mucous polysaccharides relieves throat irritation and dry cough [9].

Terpene compounds are responsible for diuretic effect [8, 9] and high content of flavonols (quercetin and kaempferol) in water-alcoholic extracts promotes inhibition of lipid peroxidation and free radical scavenging [10, 11].

Extracts of linden inflorescences (*Tiliae flos*) with lipophilic solvents (ethanol, dichloromethane) inhibit lymphoma cell proliferation by inducing apoptosis, while having significant antiproliferative properties [12]. The methanol extracts, especially those made with ethyl acetate, were characterized by high

antimicrobial activity [13, 14]. Water extracts do not demonstrate these properties [12]. High presence of tiliroside in the flavonoid fraction promotes anti-inflammatory activity of extracts [12-16].

The broad spectrum of pharmacological properties of linden inflorescence extracts results from polarity and the level of hydrophilic-lipophilic balance of solvents (medium) used to separate compatible phytochemical structures with the expected pharmacotherapeutic profile.

The above was the reason for using the general Hildebrand-Scatchard-Fedors theory of solubility [17-27] to calculate the predicted solubility of classes of phytochemicals contained in linden inflorescences (*Tiliae flos*) and the indication of those structures which, due to their high solubility in the medium, are responsible for the profile of pharmacological activity.

The calculated solubility not only indicates the quantitative composition of phytochemicals, but also the proper composition of ballast substances, granulometric properties of which decide on the optimal selection of excipients and lubricants in the technology of model solid drug form manufacturing [28, 29].

MATERIAL AND METHODS

Material

1. Dry titrated ethanol extract of linden inflorescence (*Tilia cordata* Mill./*Tilia platyphyllos*

Scop.): yellow-brown powder with characteristic sweetish scent.

Extraction agent: ethanol

Extract composition:

85% pure extract (DER 6-7:1)

15% low-sugar maltodextrin

Standardized extract for flavonoid content estimated in terms of rutin equivalent; limit 3.0% (m/m), content in the extract – 3.2% (m/m).

2. Extraction media:

water (H_2O), methanol (CH_3OH), ethanol (C_2H_5OH), dichloromethane ($ClCH_2Cl$), ethyl acetate ($CH_3COOC_2H_5$)

Measurements characterizing the polarity of solvents and their solubility level of hydrophilic-lipophilic balance ($HLB_{\text{Requ.}}$).

Solubility of phytochemicals in the extraction medium of diversified polarity – ϵ_{M+}

The Hildebrand-Scatchard equation, supplemented with the computational technique proposed by Fedors [30], allows calculation of the solubility parameters of the extraction medium and phytochemical – despite application reservations, it is a fundamental tool for estimating the predicted solubility of phytochemicals in the real solution.

The fundamental equation is the following:

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

where: ΔH_f^{app} – apparent molar enthalpy of melting, R – gas constant, T – temperature in K° ($273.15 + t^\circ C$) V_2 – phytochemical molar volume of defined structure and melting temp. ($^\circ C$),

φ – solvent volume fraction, δ_1 , δ_2 – solubility parameter of medium (1) and phytochemicals (2), allows the calculation of the solubility in the form of molar fraction – $\log x_2$.

A thermodynamic supplement to the above equation requires the calculation of an apparent enthalpy of melting $-\Delta H_f^{\text{app}}$ – from the dependence –

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

where: T_m – melting temperature of the dissolved substance (phytochemical),

T – temp. (in K°) in which – in the application option solubility is calculated.

The numerical value – ΔH_f^{app} prompts the estimation

of the logarithm of the ideal molar fraction solubility ($-\log x_2^i$) of the therapeutic agent or phytochemical from the dependence:

$$-\log x_2^i = \frac{\Delta H_f^{\text{app}}}{2.303 \cdot R} \cdot \frac{T_m - T}{T \cdot T_m}$$

Yalkowski and Misha [25] simplified the Hildebrand-Scatchard equation by calculating the logarithm of the ideal molar fraction solubility ($-\log x_2^i$) from the dependence –

$$-\log x_2^i = 0.01 \cdot (T_m - T)$$

However, the solubility parameter – $\delta^{1/2}$ was calculated from the dependence given by Fedors [30] –

$$\delta^{1/2} = \sqrt{\frac{\sum \Delta E_i}{\sum \Delta V_i}}$$

Its numerical value allows to estimate the solubility level of hydrophilic-lipophilic balance ($HLB_{\text{Requ.}}$) from the equation –

$$HLB_{\text{Requ.}} = \left[\frac{7 + \delta^{1/2}}{8} \right]^4$$

Calculated numerical value of solubility using a molar fraction (x_2 , x_2^i) can be expressed in mol/dm^3 with the equation:

$$S_{(\text{real.})} = \frac{1000}{M_{\text{molec.}}} \cdot \left(\frac{1}{x} - 1 \right)$$

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

RESULTS AND DISCUSSION

The structure of phytochemicals isolated from linden inflorescences (*Tiliae flos*), owing to the use of solvents of significantly diversified polarity (– dielectric constant – ϵ_M) [28], was the basis for calculating by Fedors method [30] the molar evaporation energy – $\sum \Delta E_i$ (cal/mol) and molar volume – $\sum \Delta V_i$ (cm^3/mol), which are fundamental quantities necessary to estimate the solubility parameter – $\delta^{1/2}$ and the required solubility hydrophilic-lipophilic level – $HLB_{\text{Requ.}}$.

The values for media are demonstrated in table 1 and for classes of isolated phytochemicals in tables 2 and 3.

It results from the values presented in tab. 1 – $\delta^{1/2}$, $HLB_{\text{Requ.}}$ and the dielectric constant of the extraction media – ϵ_M that their numerical values decrease radically with the regression of polarity.

Table 1Calculated thermodynamic values and level of hydrophilic-lipophilic balance $HLB_{\text{Requ.}}$ of the extraction media [2, 30]

Extraction medium ¹⁾	Dielectric constant ϵ_M	$\Sigma\Delta E_i$ cal/mol	$\Sigma\Delta V_i$ cm ³ /mol	$\delta^{1/2}$	$HLB_{\text{Requ.}}$
1. Water H ₂ O	78.38	–	–	24.52	240.98
2. Methanol CH ₃ OH	32.62	8245	43.5	13.76	45.41
3. Ethanol C ₂ H ₅ OH	24.55	9425	59.6	12.57	35.84
4. Dichloromethane Cl-CH ₂ -Cl	8.93	5780	68.1	9.21	16.86
5. Ethyl acetate CH ₃ COOC ₂ H ₅	6.02	7730	101.1	8.74	15.00

1) Poradnik Fizykochemiczny, Wydawnictwo Naukowo-Techniczne. Warszawa 1974; Dział B, str. 114

Table 2Calculated thermodynamic and biopharmaceutical values and the level of hydrophilic – lipophilic balance $HLB_{\text{Requ.}}$ of phytochemicals contained in *Tiliae extractum aqu. siccum* [2, 17, 18, 30]

Phytochemicals M _w ; [g/mol]	MeltingT [°C]	log P	pK _a	Bioavailability	$\Sigma\Delta E_i$ [cal/mol]	$\Sigma\Delta V_i$ [cm ³ /mol]	$\delta^{1/2}$	$HLB_{\text{Requ.}}$
1. Chlorogenic acid C ₁₆ H ₁₈ O ₉ ; 354.31	205–209	0.17 ¹⁾ –0.27 ²⁾	3.33	1	60090	118.8	22.4902	184.65
2. Caffeic acid C ₉ H ₈ O ₄ ; 180.15	225	1.67 ¹⁾ 1.53 ²⁾	3.14	1	33750	91.8	19.1741	114.58
3. Vanillic acid C ₈ H ₈ O ₄ ; 168.15	211.5	1.7 ¹⁾ 1.17 ²⁾	4.16	1	21825	99.8	14.7880	55.02
4. p-Coumaric acid C ₉ H ₈ O ₃ ; 164.15	211.5	1.74 ¹⁾ 1.84 ²⁾	4.00	1	26630	100.8	16.2538	84.48
5. Tiliroside C ₃₀ H ₂₅ O ₁₃ ; 594	269–271	2.5	3.33	–	88100	224.3	19.8186	126.29
6. Rutin Quercetin 3–O–β–D– rutinoside C ₂₇ H ₃₀ O ₁₆ ; 610.51	125	0.15 ¹⁾ –0.87 ²⁾	3.14	0	91065	130.5	26.4162	304.41
7. Quercetin C ₁₅ H ₁₀ O ₇ ; 302.23	316–318	1.81 ¹⁾ 2.16 ²⁾	4.16	1	54970	82.6	25.7972	282.48
8. Isoquercetin 3–O–β–D–glucoside C ₂₁ H ₁₀ O ₆ ; 464.37	238–242	0.47 ¹⁾ –0.14 ²⁾	4.00	0	83210	169.3	22.1696	176.75
9. Kaempferol C ₁₅ H ₁₀ O ₆ ; 286.24	285–287	1.99 ¹⁾ 2.46 ²⁾	4.00	1	47850	102.6	21.5957	163.24
10. Astragalin; Kaempferol– 3–O–β–D–glucoside C ₂₁ H ₂₀ O ₁₁ ; 448.10	178.0	0.52 ¹⁾ 0.16 ²⁾	6.43	0	72190	107.4	25.9261	286.94
11. Kaempferol 3-glucosyl –(1→2)–rhammoside C ₂₇ H ₃₀ O ₁₅ ; 594.52	–	0.08 ¹⁾ –0.57 ²⁾	6.43	0	94390	164.9	23.9250	223.29
12. Catechin C ₁₅ H ₁₄ O ₆ ; 290.26	214.0	1.02 ¹⁾ 1.8 ²⁾	9.0	1	47880	90.9	22.9506	224.04
13. Procyanidin B2 C ₃₀ H ₂₆ O ₁₂ ; 578.53	–	2.29 ¹⁾ 3.12 ²⁾	8.69	0	98680	136.2	27.3211	338.75
14. Scopoletin C ₁₀ H ₈ O ₄ ; 192.17	204.0	1.65 ¹⁾ 1.32 ²⁾	8.26	1	22235	93.9	15.3881	61.33

¹⁾Calculated ALOGPS program²⁾Calculated Chem Axon program

Approximation equations at $p=0.05$ describe correlations between the solubility parameter $\delta^{1/2}$ in the function ϵ_M and $\delta^{1/2}=f(\epsilon_M)$;

$$\delta^{1/2}=7.1721 + 0.2188 \cdot \epsilon_M \text{ with } r^2=0.9989$$

$$\log \delta^{1/2}=0.9225 + 6.1155 \cdot 10^{-3} \cdot \epsilon_M \text{ with } r^2=0.9936$$

and between $\delta^{1/2}$ a $HLB_{\text{Requ.}}$; $\delta^{1/2}=f(HLB_{\text{Requ.}})$

$$\delta^{1/2}=(-7.2515) + 13.1380 \log \text{ with } r^2=0.9951$$

$$\log \delta^{1/2}=0.5144 + 0.3703 \log \text{ with } r^2=0.9989$$

The above equations, after the estimation of $\delta^{1/2}$ and $HLB_{\text{Requ.}}$ of the class of phytochemicals allow to optimal selection of the medium for their effective quantitative extraction from plant material.

The determined melting temperatures of the phytochemicals – T_m w K° ($273.15 + t^\circ C$) [5] prompted to calculate the apparent enthalpy of melting – ΔH_f^{app} , which was a fundamental value to estimate the ideal solubility ($-\log x_2^i$) and – from the Hildebrand-Scatchard equation – the predicted real solubility using ($-\log x_2$).

The numerical value of the ideal solubility ($-\log x_2^i$) and the predicted real solubility ($-\log x_2$) of phytochemicals isolated from linden inflorescences (*Tiliae flos*) are demonstrated in table 4.

These values (tab. 4) were the basis for tracing the correlation between the predicted real solubility of phytochemicals using $\log x_2$ ($-\log x_2$, logarithm of the molar fraction of the dissolved substance – x_2) and the extraction medium polarity – ϵ_M (dielectric constant); ($-\log x_2$)= $f(\epsilon_M)$. The course of this correlation is presented in fig. 1, 2, 3, 4 and described by correlation equations listed in table 5.

It results from the course of this correlation that the solubility of the discussed class of phytochemicals is a derivative of the hydrophilic-lipophilic balance $HLB_{\text{Requ.}}$ related to the polarity level of the extraction medium. Actual solubility of flavonoids hydrophilized by glycoside sugar structures, e.g. rutin and astragalin in relation to their aglycones – quercetin is noteworthy.

The dominant type of equations, which with high correlation coefficients describe the solubility of phytochemicals from the linden inflorescence (*Tiliae flos*) are $y=a+b \cdot \log x$ (tiliroside, isoquercetin, kaempferol, catechin) and $\log x=a+b \cdot x$ (rutin, quercetin, astragalin).

Unexpectedly, the solubility profile of tiliroside and kaempferol in solvents of the regressive value of dielectric constant (ϵ_M) is described by the logarithmic form of the exponential equation $y=a \cdot x^b$; $\log y=a+b \cdot \log x$.

In case of structures of phytochemicals included in the composition of essential oils, thermodynamic parameters and the calculated expected solubility of

which are presented in tables 3 and 4, far-reaching compatibility should be noted between the polarity of the extraction medium (ϵ_M , $\delta^{1/2}$, $HLB_{\text{Requ.}}$) and the progression of their solubility ($-\log x_2$).

The above has been reliably documented by the course of the correlation between ($-\log x_2$) and polarity of the extraction medium ϵ_M ; ($-\log x_2$)= $f(\epsilon_M)$, fig. 4. The course of this correlation has been described at $p=0.05$ by approximation equations $y=a+b \cdot x$ and by logarithmic form of exponential equation $y=a \cdot x^b$ ($\log y=a+b \cdot \log x$), the directional coefficients (slopes) are listed in table 5.

It results from the table 5 data that a significant progression of solubility is directly related to the increase in lipophilicity – the regression of the numerical value of $\delta^{1/2}$ and $HLB_{\text{Requ.}}$ of the extraction medium.

In terms of thermodynamics, the melting temperature T_m ($273.15 + t^\circ C$) is a fundamental value which allows to calculate ΔH_f^{app} (apparent enthalpy of melting) and above all, ideal ($-\log x_2^i$, $S_{(i)}$ – mol/dm³) and predicted real solubility ($-\log x_2$, $S_{(n)}$ – mol/dm³).

This results from the Clausius-Clapeyron equation, which concerns – in the application record – the average velocity – V – of particles during the transition of the state of matter – (where $V=\sqrt{3RT/M \text{ mol}}$) and, above all, from the kinetic definition $T_m(K^\circ) - T_m = \frac{1}{3} E_{k(\text{aver.})}$. From this dependence (after transformations), the average velocity of the particle during the transition of the state of matter – V – can be calculated from the equation $V=\sqrt{6T/m}$ where $m=Mg/\text{mol}/6.023 \cdot 10^{23}$.

It results from the calculated in tab. 6 ideal solubility – $S_{(i)}$, that the dissolution profiles of phytochemicals with the real introduction of thermodynamic parameters of water as the pharmacopoeial medium remain in significant numerical convergence.

The use of thermodynamic values: ΔH_f^{app} , $\Sigma \Delta V_i$, $T_m(K^\circ)$ and extraction medium solubility parameters – $\delta^{1/2}$ as well as extracted phytochemical – $\delta^{1/2}$ to calculate the real solubility by ($-\log x_2$) determines the application reliability of the calculated values listed in tab. 4 and 6 also when the medium polarity (ϵ_M) is changed.

CONCLUSIONS

1. Modern studies in pharmacognosy largely focus on the isolation of phytochemical individuals from plant material with the application of commonly used extractive techniques and after determination of their structure, characterizing them, among others, by the melting temperature ($T_{\text{mel}} K^\circ = 273.15 + t_{\text{mel}}^\circ C$) which is the basis for calculat-

Figure 1

The course of the correlation between logarithm of the real molar fraction solubility ($-\log x_2$) and dielectric constant (ϵ_M), polarity of the extraction media for phenolic acids; ($-\log x_2 = f(\epsilon_M)$)

Figure 2

The course of the correlation between logarithm of the real molar fraction solubility ($-\log x_2$) and dielectric constant (ϵ_M), polarity of the extraction media for flavonoids; ($-\log x_2 = f(\epsilon_M)$)

Figure 3

The course of the correlation between logarithm of the real molar fraction solubility ($-\log x_2$) and the dielectric constant (ϵ_M), polarity of the extraction media for phytochemicals contained in *Tiliae extr.*; ($-\log x_2 = f(\epsilon_M)$)

Figure 4

The course of the correlation between logarithm of the real molar fraction solubility ($-\log x_2$) and the dielectric constant (ϵ_M), polarity of the extraction media for the components of the essential oil from *Tiliae flos*; ($-\log x_2 = f(\epsilon_M)$)

Table 3

Calculated thermodynamic and biopharmaceutical values and the level of hydrophilic – lipophilic balance HLB_{Requ} of components of the oil isolated from *Tiliae flos*

Phytochemical M_w ; [g/mol]	Melting T [°C]	log P	pK_a	Bioavailability	$\Sigma\Delta E_i$ [cal/mol]	$\Sigma\Delta V_i$ [cm ³ /mol]	$\delta^{1/2}$	HLB_{Requ}
1. Linalool 3,7-Dimethyl-1,6-octadien-3-ol $C_{10}H_{18}O$; 154.25	<25.0	2.68 ¹⁾ 2.65 ²⁾	18.46	1	17325	173.5	9.9227	20.35
2. Anethol $C_{10}H_{12}O$; 148.20	22.5	3.40 ¹⁾ 2.94 ²⁾	-4.8	1	11290	230.8	6.9941	9.36
3. β -Caryophyllene $C_{15}H_{24}$; 204.35	< 25.0	5.35 ¹⁾ 4.52 ²⁾	–	1	16105	225.0	8.4604	13.94
4. Geranyl acetate $C_{13}H_{22}O$; 194.31	–	4.59 ¹⁾ 3.68 ²⁾	19.6	1	17490	225.2	8.8127	15.26
5. Ent-16-Kaurene $C_{20}H_{32}$; 272.47	50.0	5.14 ¹⁾ 5.62 ²⁾	–	1	18560	195.6	9.7410	19.17
6. Nonanal $C_9H_{18}O$; 142.24	–	3.81 ¹⁾ 2.99 ²⁾	15.56	1	14485	168.5	9.2716	17.11
7. Linoleic acid $C_{18}H_{32}O_2$; 280.44	-8.5	7.06 ¹⁾ 6.42 ²⁾	4.99	0	26005	309.2	9.1708	16.69
8. Heneicosane $C_{21}H_{44}$; 296.57	–	9.98 ¹⁾ 9.80 ²⁾	–	0	24920	372.9	8.1748	12.94
9. Tricosane (alkane) $C_{23}H_{48}$; 324.62	–	10.30 ¹⁾ 10.69 ²⁾	–	0	27280	405.1	8.2062	13.05

¹⁾Calculated ALOGPS program

²⁾Calculated Chem Axon program

Table 4

Calculated ideal ($-\log x_2^i$) and predicted real ($-\log x_2$) solubility of phytochemicals depending on solubility parameter $\delta^{1/2}$ of the extraction medium

Phytochemicals	Molar enthalpy of melting Δ	Calculated ideal solubility -log x_2^i	Predicted real solubility (– log x_2)				
			Water $\epsilon_M=78.38$	Methanol $\epsilon_M=32.62$	Ethanol $\epsilon_M=24.55$	Dichloromethane $\epsilon_M=8.93$	Ethyl acetate $\epsilon_M=6.02$
<u>Phenolic acids</u>							
1. Chlorogenic acid	35109.98	2.3311	2.4179	3.9037	4.3630	5.9752	6.2369
2. Caffeic acid	37154.68	2.6129	3.0758	3.0829	3.3131	4.2083	4.3620
3. Vanillic acid	35617.96	2.4008	4.8537	3.2474	3.3147	3.7725	3.8677
4. <i>p</i> -coumaric acid	35617.96	2.4008	3.6131	2.5099	2.6397	3.2761	3.3965
<u>Flavonoids and coumarin</u>							
1. Tiliroside	42475.08	2.8630	3.7388	4.2816	4.9042	7.2923	7.6814
2. Rutin	26454.97	1.1725	1.2530	14.0462	17.3853	24.9864	26.3014
3. Quercetin	48318.23	4.1876	4.2105	6.2814	6.7169	8.1669	8.3950
4. Isoquercitrin	38900.25	2.8548	3.0215	4.9284	5.5846	7.8333	8.1999
5. Kaempferol	44431.23	3.6328	3.7885	4.7241	5.0951	6.3885	6.6009
6. Astragalin	31904.06	1.8952	1.9313	4.6763	5.2434	7.1502	7.4489
7. Catechin	35901.55	2.4397	2.5414	3.7825	4.1537	5.4397	5.6532
8. Scopoletin	34772.87	2.2849	3.6928	3.3281	2.4151	2.9122	3.0109
<u>Essential oil compounds</u>							
1. Linalool	15516.72	–0.0463	6.4469	0.4028	0.1674	–0.0310	0.0042
2. β -caryophyllene	15516.72	–0.0463	6.4469	0.4028	0.1674	–0.0310	0.0042
3. Anethol	16804.75	–0.0248	12.4209	1.8297	1.2343	0.1245	0.0682
4. Ent-16-kaurene	18953.43	0.2602	7.7634	0.8238	0.5353	0.2792	0.2942
5. Linoleic acid	15672.42	–0.0002	12.6328	0.9839	0.4673	–0.1603	–0.1505

Table 5

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

Phytochemicals	Type of correlation equation	Correlation coefficient	Directional (slope) coefficients of correlation equations	
			a±da	b±db
<u>Phenolic acids</u>				
1. Chlorogenic acid	1. $\log y = a + b \cdot x$	0.9862	0.8067±0.0691	-5.6317·10 ³ ±1.7364·10 ³
	2. $y = a + b \cdot \log x$	0.9961	9.1496±0.7771	-3.5004±0.5691
	3. $1/y = a + b \cdot x$	0.9963	0.1395±0.0069	0.0035±0.0002
2. Caffeic acid	1. $\log y = a + b \cdot \log x$	0.9446	0.7566±0.1365	-0.1566±0.0999
	2. $\log y = a + b \cdot 1/x$	0.9686	0.4715±0.0485	1.1127±0.5242
	3. $y = a + b \cdot 1/x$	0.9721	2.9234±0.3881	9.4539±4.1894
3. Vanillic acid	1. $y = a + b \cdot \log x$	0.9952	4.5894±0.3199	-0.8967±0.2667
	2. $\log y = a + b \cdot \log x$	0.9949	0.6762±0.0405	-0.1097±0.0338
	3. $1/y = a + b \cdot x$	0.9971	0.2481±0.0204	0.0019±0.0009
4. p-coumaric acid	1. $y = a + b \cdot \log x$	0.9954	4.4260±0.4403	-1.2699±0.3677
	2. $\log y = a + b \cdot \log x$	0.9945	0.6842±0.0719	-0.1876±0.0601
	3. $1/y = a + b \cdot x$	0.9940	0.2706±0.0297	0.0040±0.0013
<u>Flavonoids and coumarin</u>				
1. Tiliroside	1. $y = a + b \cdot \log x$	0.9862	10.6738±2.2941	-3.9032±1.6801
	2. $\log y = a + b \cdot \log x$	0.9961	1.1287±0.1402	-0.3065±0.1027
2. Rutin	1. $y = a + b \cdot x$	0.9892	27.1012±3.7092	-0.3424±0.0932
	2. $\log x = a + b \cdot x$	0.9837	1.6194±0.2477	-0.0185±0.0062
3. Quercetin	1. $\log y = a + b \cdot x$	0.9972	0.9369±0.0219	-4,0486·10 ⁻³ ±0,5506·10 ⁻³
	2. $1/x = a + b \cdot x$	0.9988	0.1091±5.6569·10 ⁻³	1,6232·10 ⁻³ ±1,4208·10 ⁻⁴
4. Isoquercetin	1. $y = a + b \cdot \log x$	0.9968	12.1510±0.9476	-4.7773±0.6939
	2. $1/y = a + b \cdot x$	0.9994	0.1051±7.0243·10 ⁻³	2.9030·10 ⁻³ ±1.7642·10 ⁻⁴
5. Kaempferol	1. $y = a + b \cdot \log x$	0.9972	8.7422±0.4987	-2.6210±0.3651
	2. $\log y = a + b \cdot \log x$	0.9943	1.0052±0.0588	-0.2207±0.0431
6. Astragalin	1. $y = a + b \cdot x$	0.9832	-7.5496±10179	-7.5038·10 ⁻² ±2.5566·10 ⁻²
	2. $\log y = a + b \cdot x$	0.9996	0.9249±0.0155	-8.1248·10 ⁻³ ±3.9116·10 ⁻⁴
7. Catechin	1. $y = a + b \cdot \log x$	0.9955	10.6738±2.2941	-3.9032±1.6801
	2. $1/y = a + b \cdot x$	0.9984	1.1287±0.1402	-0.3065±0.1027
8. Scopoletin	1. $y = a + b \cdot \log x$	0.9955	3.8046±0.3401	-0.9823±0.2840
	2. $\log y = a + b \cdot \log x$	0.9948	0.6092±0.0594	-0.1605±0.0496
	3. $1/y = a + b \cdot x$	0.9911	0.3105±0.0332	0.0038±0.0016
<u>Essential oil compounds</u>				
1. Linolool	1. $y = a + b \cdot x$	0.9464	-1.3663±2.2929	9.1839·10 ⁻¹ ±5.7590·10 ⁻²
2. β-caryophyllene	1. $y = a + b \cdot x$	0.9464	-1.3663±2.2929	9.1839·10 ⁻¹ ±5.7590·10 ⁻²
3. Anethol	1. $y = a + b \cdot x$	0.9704	-2.1217±3.1785	0.1746±0.0798
	2. $\log y = a + b \cdot \log x$	0.9988	-2.8046±0.2457	0.2457±0.1799
4. Ent-16-kaurene	1. $y = a + b \cdot x$	0.9487	-1.2597±2.5848	0.1062±0.0649
	2. $\log y = a + b \cdot x$	0.9970	-0.7198±0.1134	0.0203±0.0028
5. Linoleic acid	1. $y = a + b \cdot x$	0.9548	-2.7122±4.1357	0.1816±0.1038

ing basic thermodynamic quantities, i.e. apparent melting enthalpy $-\Delta H_f^{\text{app}}$, solubility parameter $\delta^{1/2}$ and the required solubility level of hydrophilic-lipophilic balance HLB_{Requ} . The above values allow – using the general Hildebrand-Scatchard-Fedors theory of solubility [28] – to calculate by

the logarithm of the molar fraction ($-\log x_2$) the predicted real solubility (S_{real}) in extraction media with designed, progressive HLB_{Requ} values.

- In the case of the extraction of plant material with water, the calculated ideal solubility ($-\log x_2^i$) is of

Table 6Calculated ideal and predicted real solubility of phytochemicals contained in linden inflorescence *Tiliae flos*

Phytochemicals ¹⁾ Molecular Weight; [g/mol]	Ideal solubility		Real solubility calculated in:			
	$x_2^{i\ 2)}$	$S_{(i)}^{3)}$ [mol·dm ⁻³]	– water		– ethanol	
			x_2	$S_{(real)}$ [mol·dm ⁻³]	x_2	$S_{(real)}$ [mol·dm ⁻³]
<u>Phenolic acids</u>						
1. Chlorogenic acid MWT 354.31	$4.6655 \cdot 10^3$	$1.3229 \cdot 10^{-2}$	$3.8203 \cdot 10^{-3}$	$1.0782 \cdot 10^{-2}$	$4.3350 \cdot 10^{-5}$	$1.2235 \cdot 10^{-4}$
2. Caffeic acid MWT 180.15	$2.4383 \cdot 10^3$	$1.3209 \cdot 10^{-2}$	$8.3984 \cdot 10^{-4}$	$4.6619 \cdot 10^{-3}$	$4.8629 \cdot 10^{-4}$	$2.6994 \cdot 10^{-3}$
3. Vanillic acid MWT 168.15	$3.9737 \cdot 10^3$	$1.4005 \cdot 10^{-5}$	$1.4005 \cdot 10^{-5}$	$8.3288 \cdot 10^{-5}$	$4.8629 \cdot 10^{-4}$	$2.8920 \cdot 10^{-5}$
4. <i>p</i> -Coumaric acid MWT 164.15	$3.9737 \cdot 10^3$	$2.4305 \cdot 10^{-2}$	$2.4372 \cdot 10^{-4}$	$1.4843 \cdot 10^{-3}$	$2.2924 \cdot 10^{-3}$	$1.3965 \cdot 10^{-3}$
<u>Flavonoids and Coumarin</u>						
1. Tiliroside MWT 594.52	$1.3708 \cdot 10^{-3}$	$2.3090 \cdot 10^{-3}$	$1.8247 \cdot 10^{-4}$	$3.0692 \cdot 10^{-4}$	$1.2468 \cdot 10^{-5}$	$2.0971 \cdot 10^{-5}$
2. Rutin MWT 610.51	$6.7220 \cdot 10^{-2}$	$1.1010 \cdot 10^{-1}$	$5.5840 \cdot 10^{-2}$	$9.1475 \cdot 10^{-2}$	$1.1181 \cdot 10^{-18}$	$1.8314 \cdot 10^{-18}$
3. Quercetin MWT 302.23	$6.4923 \cdot 10^{-5}$	$2.1483 \cdot 10^{-4}$	$6.1588 \cdot 10^{-5}$	$2.0377 \cdot 10^{-4}$	$1.9100 \cdot 10^{-7}$	$6.3196 \cdot 10^{-7}$
4. Isoquercitrin MWT 464.37	$1.3970 \cdot 10^{-3}$	$3.0084 \cdot 10^{-3}$	$9.5169 \cdot 10^{-4}$	$2.0494 \cdot 10^{-3}$	$2.6020 \cdot 10^{-6}$	$5.6033 \cdot 10^{-6}$
5. Kaempferol MWT 286.24	$1.6274 \cdot 10^{-4}$	$5.6855 \cdot 10^{-4}$	$1.6274 \cdot 10^{-4}$	$5.6855 \cdot 10^{-4}$	$8.0330 \cdot 10^{-6}$	$2.8064 \cdot 10^{-5}$
6. Astragalin MWT 448.10	$1.2729 \cdot 10^{-2}$	$2.8407 \cdot 10^{-2}$	$1.1713 \cdot 10^{-2}$	$2.6141 \cdot 10^{-2}$	$5.6440 \cdot 10^{-6}$	$1.2594 \cdot 10^{-5}$
7. Catechin MWT 290.9	$1.6332 \cdot 10^{-3}$	$1.2517 \cdot 10^{-2}$	$1.8747 \cdot 10^{-3}$	$9.9040 \cdot 10^{-3}$	$7.0194 \cdot 10^{-5}$	$2.4183 \cdot 10^{-4}$
8. Scopoletin MWT 192.12	$5.1891 \cdot 10^{-3}$	$2.7003 \cdot 10^{-2}$	$2.0286 \cdot 10^{-4}$	$1.0556 \cdot 10^{-3}$	$3.8450 \cdot 10^{-3}$	$2.008 \cdot 10^{-2}$
<u>Essential oil compounds</u>						
1. Linolool MWT 154.25	1.1124	64.1095	$3.5700 \cdot 10^{-7}$	$2.3144 \cdot 10^{-6}$	0.6799	13.2287
2. β -Caryophyllene MWT 204.35	61.1125	48.3936	$3.5700 \cdot 10^{-7}$	$1.755 \cdot 10^{-6}$	0.6799	10.4493
3. Anethol MWT 148.20	1.0587	121.5703	$7.7940 \cdot 10^{-13}$	$5.2591 \cdot 10^{-12}$	5.8304	0.4177
4. Ent-16-Kaurene MWT 272.47	1.0587	121.5703	$1.7000 \cdot 10^{-8}$	$6.2392 \cdot 10^{-7}$	0.2915	1.5103
5. Linolenic acid MWT 280.44	1.0005	7768.80	$3.3291 \cdot 10^{-13}$	$1.07108 \cdot 10^{-13}$	0.3409	1.8448

¹⁾ Molecular Weight (MWT), [g/mol]; ²⁾ x_2^i - molar fraction ideal solubility; $S_{(i)}$ – ideal solubility

significant applicative importance because it allows to estimate the quantitative and qualitative composition of the aqueous extract in a significant way. However, as the polarity of the extraction system decreases at a low value of $\delta^{1/2}$ and HLB_{Requ} , a significant lack of compatibility between the ideal ($-\log x_2^i$) and predicted real solubility ($-\log x_2$) is observed (tab. 6).

- Results of the presented research indicate that basing on the parameters characterizing the structure of phytochemicals and the calculated ideal ($-\log x_2^i$) and predicted real ($-\log x_2$) solubility, it is possible – using the general Hildebrand-Scatchard-Fedors theory of solubility [28] – to choose selectively the cascade of extraction media in order to distinguish in the plant material chemical and structural individuals of different polarity. The solubility parameter $\delta^{1/2}$ and HLB_{Requ} of the extraction medium should be taken into account in the selective extraction optimization process as well as the direction of technological process i.e. from lipophilic to hydrophilic structures, so that during extraction with water and aqueous ethanol solutions, adverse effects on the quality of the hydrotropic and micellar solubilization extract are eliminated [28, 29].

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