Research Article

The role of solvent dielectric constant in modeling of the extraction process of phenolic compounds from *silybum marianum* l. Fruits

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ABSTRACT

Objective: The objective of this work was to study the influence of solvent physicochemical properties on qualitative and quantitative content of extracts by high-performance liquid chromatography (HPLC) for substantiation of a rational technology of silibinin extraction from *Silybum marianum* l. fruits. **Methods:** For experiments, we used ground plant raw material with 0.1–0.5 mm particle size, distilled water, ethanol, n-hexane, methanol, 1-propanol, 2-propanol, acetone, and ethyl acetate. Qualitative analyses of substances were carried out by reversed-phase HPLC on a chromatograph of Agilent technologies 1200 infinity type. **Results:** Flavonolignans dominated among the phenolic compounds detected in the extracts. In this study, it was found that a ketone functional group in the solvent's molecule has a stronger influence on the extraction of flavonolignans from the plant raw material matrix into the solvent than a hydroxyl group, and a linear structure of hydrophobic part of molecules was preferred to a non-linear one. An optimal range of the dielectric constant of the solvent for maximum extraction of silibinin from the plant raw material was determined, and it equals $\varepsilon = 40 \pm 4$ units. **Conclusion:** It has been shown that dependence between the natural logarithm of silibinin concentration and the reverse dielectric constant value of the ethanol–water solution approximates well by a quadric equation. The following sequence of preferred solvents as for their extraction properties for silibinin was arranged: Acetone $\geq$ ethanol 70% v/v $\geq$ methanol 86% v/v $\geq$ methanol $\geq$ 2-propanol 68% v/v $\geq$ acetone 68% v/v $\geq$ 1-propanol $\geq$ ethyl acetate $\geq$2-propanol.

KEY WORDS: Dielectric constant, Silibinin, *Silybum marianum* l. fruits, Solvent

INTRODUCTION

Drugs from *Silybum marianum* l. fruits are important in the treatment of toxic damages and chronic liver diseases.[1] At the same time, according to scientific data, the main active substances that have a positive effect on hepatic metabolism are phenolic compounds, such as flavonolignans and taxifolin.[2]

These substances have multiple pharmacologic effects: Antioxidant, detoxicant, hepatoprotective, capillary reinforcing, antiviral, antibacterial, as well as a number of other useful properties.[3-5] A wide range of scientific works are dedicated to phytochemical and pharmacological studies of *S. marianum* l. fruits and drugs on their basis.[6]

One particular work[7] is worth noting, as it presents data on solvents used for extraction of flavonolignans by manufacturers in the European Union (EU), as well as discussion of scientifically based pharmacological properties, side effects, dosage, etc. As described in the work indicated above, all manufacturers in the EU use four main types of solvents: Ethanol, methanol, acetone, and ethyl acetate.

Thus, according to scientific literature, the following solvents are most often used for flavonolignans' extraction: Ethanol of various concentrations in ethanol-water solution, methanol, acetone, and ethyl acetate. At the same time, the authors could not find any systematic works dedicated to the influence of solvent physicochemical properties on the qualitative and quantitative content of extracts from *S. marianum* l. fruits for substantiation of a rational solvent choice.

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The aim of this work was to study the influence of solvent physicochemical properties on the qualitative and quantitative content of extracts by high-performance liquid chromatography (HPLC) analysis for substantiation of a rational technology of flavonolignans extraction from *S. marianum* l. fruits.

Theoretical Part

For theoretical substantiation of experimental dependencies, we used energetic principle related to changes of Gibbs energy in the system and Van der Waals forces between molecules.[8]

For example, on the one hand, Gibbs energy is related to the equilibrium constant of the distribution process of biologically active substances between phases by the following Equation (1):

$$\Delta G = RT \ln K \tag{1}$$

Where ΔG is Gibbs energy, J/mol; R is gas constant, J/(K·mol); T is absolute temperature, K; K is the equilibrium constant that at a first approximation can be related to the equilibrium concentration of biologically active substances in the extract (C) by the following Equation (2):

$$\ln K = \ln C \tag{2}$$

On the other hand, the energy of molecular interaction between biologically active substances, plant raw material, and the solvent at a first approximation can be represented by the combination of energies:

$$\Delta G = \Delta G_{solid} + \Delta G_{solv} + \Delta G_{unpred} \tag{3}$$

Where ΔG_{solid} is cohesive energy of biologically active substances and plant raw material matrix molecules, J/mol; ΔG_{solv} is interaction energy of biologically active substances and solvent molecules, J/mol; ΔG_{unpred} is unpredicted energy processes, J/mol.

These types of energy are related to the dielectric constant of the medium by means of equations describing Van der Waals forces.[8]

To create a simplified mathematical model, we used the following assumptions: All molecules are polar, Debye forces can be neglected, plant raw material matrix is represented by cellulose, and the dielectric constant of the plant raw material matrix impregnated by the solvent (ε_y) is equal to the sum of product of components' volume ratio by their dielectric constant, i.e., cellulose (ε_1) and solvent (ε_x).

Thus, using these assumptions and formulas from work,[8] we were able to compose the following sequence of Equations (4-6):

$$\Delta G_{solid} = NA \cdot \left(\frac{\mu1 \cdot \mu2}{(4 \cdot \pi \cdot \varepsilon_0 \cdot \varepsilon_y) \cdot r^3} + \frac{3 \cdot \alpha1 \cdot \alpha2}{(4 \cdot \pi \cdot \varepsilon_0 \cdot \varepsilon_y)^2 \cdot 2 \cdot r^6} \cdot \left(\frac{I_1 \cdot I_2}{I_1 + I_2} \right) \right) \tag{4}$$

$$\Delta G_{solv} = -NA \cdot \left(\frac{\mu_3 \cdot \mu_2}{(4 \cdot \pi \cdot \varepsilon_0 \cdot \varepsilon_x) \cdot r^3} + \frac{2 \cdot \mu_3^2 \cdot \mu_2^2}{(4 \cdot \pi \cdot \varepsilon_0 \cdot \varepsilon_x)^2 \cdot 3 \cdot k \cdot T \cdot r^6} + \frac{3 \cdot \alpha_3 \cdot \alpha_2}{(4 \cdot \pi \cdot \varepsilon_0 \cdot \varepsilon_x)^2 \cdot 2 \cdot r^6} \left(\frac{I_3 \cdot I_2}{I_3 + I_2} \right) \right) \tag{5}$$

$$\varepsilon_y = \varphi_1 \varepsilon_1 + (1 - \varphi_1) \varepsilon_x \tag{6}$$

Where NA is Avogadro number, $6.02 \cdot 10^{23}$/mol; π is mathematical constant, 3.14; ε_0 is electrical constant, $8.85 \cdot 10^{-12}$ F/m; ε_1 and ε_x are relative dielectric constants of the plant raw material matrix and the solvent, respectively; μ_1, μ_2, and μ_3 are dipole moment of molecules for plant raw material matrix, biologically active substances, and the solvent, respectively, cm; α_1, α_2, and α_3 are molecular polarization for plant raw material matrix, biologically active substances, and the solvent, respectively, m³; I_1, I_2, and I_3 are ionization energy of molecules for plant raw material matrix, biologically active substances, and the solvent, respectively, J; r is the distance between the molecules, m; and φ_1 is volume ratio of plant raw material matrix.

After rearrangement of these equations and extraction of a solvent's dielectric constant from them, we can write finite Equation (7) that relates the equilibrium concentration of substances in the extract and the dielectric constant of the solvent:

$$\frac{R \cdot T}{N_A} \cdot \ln C = \frac{1}{\varepsilon_x^2} \left((D + E) - \frac{\varepsilon_x^2}{\varepsilon_y^2} B \right) + \frac{1}{\varepsilon_x} \left(G - \frac{\varepsilon_x}{\varepsilon_y} A \right) + \Delta G_{unpred} \tag{7}$$

Where

$$D = \frac{2 \cdot \mu_3^2 \cdot \mu_2^2}{(4 \cdot \pi \cdot \varepsilon_0)^2 \cdot 3 \cdot k \cdot T \cdot r^6}, E = \frac{3 \cdot \alpha_3 \cdot \alpha_2}{(4 \cdot \pi \cdot \varepsilon_0)^2 \cdot 2 \cdot r^6} \cdot \left(\frac{I_3 \cdot I_2}{I_3 + I_2} \right), B = \frac{3 \cdot \alpha_1 \cdot \alpha_2}{(4 \cdot \pi \cdot \varepsilon_0)^2 \cdot 2 \cdot r^6} \cdot \left(\frac{I_1 \cdot I_2}{I_1 + I_2} \right), G = \frac{\mu_3 \cdot \mu_2}{(4 \cdot \pi \cdot \varepsilon_0)^2 \cdot r^3}, A = \frac{\mu_1 \cdot \mu_2}{(4 \cdot \pi \cdot \varepsilon_0)^2 \cdot r^3}$$

This equation under certain conditions predicts the extremum of ln C within a certain range of dielectric

constant values. Furthermore, the properties of Equation (7) depend on the balance of energies (D+E)-B and (G-A).

MATERIALS AND METHODS

Plant Raw Material

Commercial product "*S. marianum* l. fruits" by "Biocor" LLC, Penza, Russia, manufacturing date and batch number: 19.01.15, shelf life: 24 months, was used for the studies.

Commercial product "Milled *S. marianum* l. fruits SE" by "Farmacom" LLC, Kharkov, Ukraine, manufacturing date and batch number: 01.03.17, shelf life: 24 months, was used for the studies.

Chemicals and Reagents

For experiments, we used ground plant raw material with 0.1–0.5-mm particle fraction, distilled water, ethanol (pharmaceutical grade, Russia), n-hexane, methanol, 1-propanol, 2-propanol, acetone, and ethyl acetate (high grade, Russia).

Qualitative analyses of substances were carried out by UV-spectra and retention time of compounds, as well as with reference substance of silibinin (Sigma-Aldrich, Germany, CAS Number 22888-70-6).

Preparation of Extracts

Extracts for analysis were obtained under the following conditions: Before flavonolignans' extraction, the plant raw material was defatted with n-hexane to depletion degree of ≥98% as for lipophilic substances. For this purpose, some portions of plant raw material, 2 g each (exact weight [wt.]), were extracted for 30 min with 50 ml of n-hexane at the boiling point. Then, the plant raw material was dried at room temperature for 24 h. Extraction of hydrophilic compounds was carried out in a hermetic glass vial under the following conditions: 1 g (exact weight) of plant raw material was extracted with 10 ml (exact weight) of the solvent (plant raw material/solvent ratio: 1:10 (wt./v)), at temperature 25 ± 1°C, and maceration time of 24 h. The following substances were used as solvents: Ethanol–water solution 23, 43, 60, 72, 81, and 96 ± 1% v/v, methanol, methanol–water solution 86 ± 1% v/v (80% wt.), 1-propanol, 2-propanol, 2-propanol-water solution 68 ± 1% v/v (60% wt.), acetone, acetone-water solution 68 ± 1% v/v (60% wt.), and ethyl acetate.

The extracts were decanted and centrifuged at 13,000 rpm for 5 min, and then, reversed-phase HPLC was carried out.

HPLC Analysis

A reversed-phase HPLC analysis was carried out on a chromatograph of "Agilent technologies 1200 infinity" type, the USA.

The process of reversed-phase HPLC was carried out under the following conditions: Mobile phase (a) 1% water solution of formic acid and (b) ethanol 96% v/v in linear gradient elution; chromatographic column: *Supelco Ascentis express* C$_{18}$, sized 2.7 μm × 100 mm × 4.6 mm; mobile phase velocity: 0.5 ml/min; chromatographic column temperature: +35°C; and sample volume: 1 μl.

RESULTS AND DISCUSSION

In the first series of experiments, we studied and modeled empirical dependences between the content of phenolic compounds in the extract and ethanol concentration in the solvent for the substantiation of its optimal concentration.

Table 1 shows tabulated values of peak area for compounds identified by reversed-phase HPLC in extracts based on ethanol–water solutions from plant raw material "Biocor" LLC, Penza, Russia.

As it can be seen from the data of Table 1, the largest values of peak area for all phenolic compounds fall within ethanol concentration of 72% v/v.

To construct a dependency in coordinates ln C = f(ε), according to Equation (7), the authors used experimental data presented in Table 1, reference data for the dielectric constant of ethanol mixtures with water,[9] and a linear equation of silibinin concentration/peak area dependency C = (3.61 ± 0.19)·10^{-5}·S; the dependency obtained is shown in Figure 1.

As it can be seen from Figure 1, the dependency between the natural logarithm of silibinin concentration and the reverse dielectric constant value of ethanol–water solutions approximates well by a quadric equation, which under certain conditions is equal to Equation (7) predicted by the theory with determination coefficient (R² = 0.981) within the range of dielectric constant values between 27 and 60 units. Maximum silibinin content in the extract according to this equation falls within the range of solvent dielectric constant 36 ± 4, which is equivalent to ethanol concentration of 80 ± 10% v/v and consistent with other works.[10]

The second series of experiments was dedicated to study the relationship between extracts' content and the structure and dielectric constant of the solvent.

Table 2 shows tabulated peak area values for compounds identified by reversed-phase HPLC in extracts obtained from plant raw material by "Farmacom" LLC, Kharkov, Ukraine.

As it can be seen from the data in Table 1, maximum peak areas for silibinin are observed in extracts based on acetone, ethanol 70% v/v, methanol, and 1-propanol.

Ethyl acetate and 2-propanol have much worse extraction properties than acetone, approximately 2 and 5 times, respectively.

Basing on the data of Table 1, we ranged the solvents by their increasing extracted properties for silibinin: Acetone, ethanol 70% v/v, methanol, 1-propanol, ethyl acetate, and 2-propanol.

It is interesting to note an abnormal difference of extraction properties of 2-propanol versus acetone, 2-propanol versusmethanol, and 2-propanol versus 1-propanol.

This fact may be explained by better ability of the ketone functional group in acetone to form hydrogen bonds with flavonolignans and/or by the fact that an irregular hydrocarbon radical in 2-propanol makes it difficult to form hydrogen bonds with flavonolignans and with each other.

To check this suggestion, we decided to dilute 2-propanol, acetone, and methanol with water [Table 1] to achieve the optimal value of the dielectric constant for the solvent. From denominator data of Table 1, we can see that dilution of 2-propanol and methanol with water leads to a significant increase of
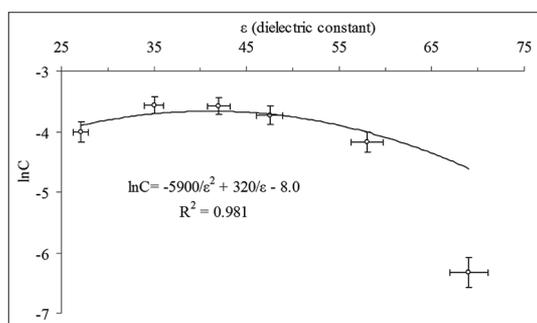
silibinin content in the extract when compared to the pure solvent, but for acetone solution, we can see an opposite tendency: Silibinin content in acetone-water solution is 12% less than that in pure acetone.

Thus, the structure of a solvent molecule has a great influence on extraction properties of silibinin, and the presence of a donor–acceptor group in the hydrophilic part of the molecule (for example, a ketone functional group or a hydroxyl group) is important. In addition, the presence of water molecules in the solvent and the structure of its hydrophobic part (linear or non-linear) have a great influence on extraction properties of silibinin.

Basing on the data from Table 1 and reference data for the dielectric constant of solvents and their mixtures with water,[9] we constructed a dependency between silibinin content in extracts and the dielectric constant of the solvent. Figure 2 presents this dependency.

As it can be seen from the graph presented in Figure 2, the dependency of silibinin content in extracts approximates satisfactorily by a simple hyperbolic equation with a determination coefficient ($R^2 = 0.953$) and predicts maximum silibinin content in the extract within the range of solvent dielectric constant 43 ± 4 units. The value of silibinin content in 2-propanol and acetone is abnormal and falls out of the dependency.

Thus, we can generally conclude that for maximum extraction of silibinin from *S. marianum* l. fruits, we need to use a solvent with a dielectric constant within $\varepsilon = 40 \pm 4$ units. Moreover, the solvent should contain a donor–acceptor group in the hydrophilic part of the molecule (a ketone functional group is in preference to a hydroxyl group). Finally, a linear structure of carbonic skeleton in the hydrophobic part of the molecule is in preference to a non-linear one.

Figure 1: Dependency between silibinin content in extracts and the dielectric constant of the solvent

Table 1: Peak area values for phenolic compounds identified in extracts from *S. marianum* l. fruits based on ethanol–water solutions

Compound (λ, nm)	Retention time, min*	Compound peak area, mAU·s*					
		Ethanol 23% v/v	Ethanol 43% v/v	Ethanol 60% v/v	Ethanol 72% v/v	Ethanol 81% v/v	Ethanol 96% v/v
1. Hydroxycinnamic acid derivative I (325.4)	6.60±0.38	62±3	142±4	200±6	205±6	169±5	13.42±0.43
2. Taxifolin II (290.4)	12.50±0.14	285±8	460±14	552±15	524±14	451±13	161±6
3. Coniferyl alcohol derivative III (310.4)	16.33±0.14	16.34±0.49	66±2	92±3	95±3	86±2	45.60±1.24
4. Flavonolignan IV (290.4)	18.47±0.14	190±6	232±7	332±10	363±11	349±10	187±6
5. Flavonolignan V (Silibinin) (290.4)	24.80±0.25	21.32±0.64	185±5	295±8	343±11	349±10	231±7
6. Flavonolignan VI (Silibinin) (290.4)	25.50±0.25	28.40±0.83	241±7	370±12	430±12	434±10	273±8
7. Flavonolignan VII (290.4)	27.03±0.52	28.04±0.60	205±6	326±9	355±11	339±9	198±6
8. Flavonolignan VIII (290.4)	27.57±0.38	14.86±0.21	113±3	181±5	198±5	185±5	102±3

Note: *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 mean, *S. marianum: Silybum marianum*

Nikolay N. Boyko, *et al.*

Table 2: Peak area values for phenolic compounds identified in extracts from *S. marianum* l. fruits based on different solvents

Compound (λ, nm)	Retention time, min*	Compound peak area, mAU·s*					
		Ethanol 70% v/v	Methanol**	Acetone**	1-propanol	Ethyl acetate	2-propanol**
1. Hydroxycinnamic acid derivative I (325.4)	6.57±0.14	182±5	128±5 251±9	0 210±5	0	0	0 157±4
2. Taxifolin II (290.4)	12.47±0.14	592±16	501±15 312±11	413±14 500±16	233±7	247±7	41.2±1.0 607±15
3. Coniferyl alcohol derivative III (310.4)	16.13±0.14	261±7	278±8 218±5	48.1±1.3 -	0	1963±0.63	0 276±7
4. Flavonolignan IV (290.4)	18.55±0.33	2660±80	2381±80 2394±70	2650±85 2430±60	1796±55	1370±43	357±12 2546±67
5. Flavonolignan V (Silibinin) (290.4)	24.87±0.14	1871±57	1757±64 1848±53	1906±51 1725±28	1416±50	956±28	354±13 1725±45
6. Flavonolignan VI (Silibinin) (290.4)	25.57±0.29	2139±65	1976±51 2136±59	2142±61 1873±37	1632±42	995±21	385±11 1989±52
7. Flavonolignan VII (290.4)	27.10±0.25	813±24	755±25 819±24	819±28 733±25	564±23	367±11	119±4 766±31
8. Flavonolignan VIII (290.4)	27.27±0.29	268±8	251±8 280±9	267±8 233±5	166±6	103±4	31.5±0.9 257±7

*The mean value and its confidence interval (mean±SEM) are calculated with repeat counts n=3 and significance level P=0.95. **The numerator presents the value for 100% solvent, and the denominator presents the value for water solution of the 2-propanol and acetone with concentration of 60% wt. (68% v/v) and methanol 80% wt. (86% v/v). SEM: Standard error mean, *S. marianum: Silybum marianum*
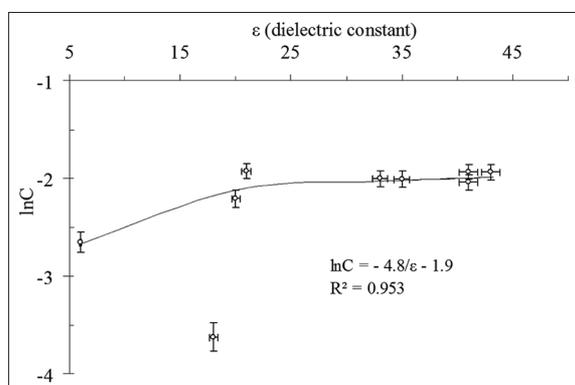
Figure 2: Dependency between silibinin relative content in extracts and the dielectric constant of the solvent

For neutralizing a negative influence of the hydrophobic part of the solvent's molecule on flavonolignans' extraction from raw plant material, water can be added into the solvent to reach the optimal range of the dielectric constant.

CONCLUSION

The influence of solvent physicochemical properties on qualitative and quantitative content of extracts has been studied by a HPLC analysis for substantiation of a rational technology of flavonolignans' extraction from *S. marianum* l. fruits.

The influence of the hydrophilic and hydrophobic part of the solvent's molecule on the extraction of phenolic compounds from the plant raw material has been determined.

It has been discovered that a ketone functional group unlike a hydroxyl group in the hydrophilic part of the solvent's molecule has a better influence on the extraction of phenolic compounds from the plant raw material.

It has also been discovered that the presence of a linear structure in the hydrophobic part of the solvent's molecule is in preference to a non-linear one and it increases extraction of phenolic compounds from the plant raw material.

It has been shown that the dependence between the natural logarithm of silibinin concentration and the reverse dielectric constant value of ethanol–water solutions approximates well by a quadric equation. Thus, an optimal range of the dielectric constant for maximum extraction of silibinin from the raw plant material has been determined; it is ε = 40 ± 4 units.

A sequence of preferred solvents in terms of their extraction properties for silibinin has been arranged: Acetone ≥ ethanol 70% v/v ≥ methanol 86% v/v ≥ methanol ≥ 2-propanol 68% v/v ≥ acetone 68% v/v ≥ 1-propanol ≥ ethyl acetate ≥ 2-propanol.

ACKNOWLEDGMENT

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