EXPERIMENTAL PAPER

Maidenhair tree (*Ginkgo bilobae*) leaf extraction products in the light of Biopharmaceutics Classification System (BCS) requirements and medium of diversified polarity (ε_M)

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S u m m a r y

Model maidenhair tree (*Ginkgo bilobae*) leaf extracts were created basing on medium of diversified polarity (ε_M). Chromatographic analysis was performed with the HPLC method, with the so-called dry residue remaining after evaporating the dissolving agent from saturated aqueous solutions and from 0.1 mol HCl. Viscosity measure and surface activity estimations were conducted on phase boundary. Then, basic values of viscosity ($[\eta]$, M_η) and hydrodynamic values (R_o, $R_{obs.}$, Ω) were calculated. Moreover, reference quercetin and rutin (rutoside) were used to mark the conversion contents of flavonoids in produced extracts with the UV method.

Key words: Ginkgo bilobae, dry extract, polarity, viscosity

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INTRODUCTION

The fact that amidon [1-3] incorporated the assumptions underlying Biopharmaceutics Classification System (BCS) into drug formation process indicated the necessity to consider the fundamental relation between real solubility characteristic for the maximal dosage of a medicinal product in model volume of the acceptor fluid and the mass exchange process observed on phase boundary.

Modern phytopharmacy uses properly prepared quantified plant extracts, as their granulometric, physical and chemical properties are decisive as far as quality and form of the preparation introduced into population therapy is concerned [4-8].

Qualitative and quantitative content within the quantified dry extract is associated with polarity of the extraction system, and the practical emanation of the abovementioned lies in dielectric constant (ε_M) characteristic for the dissolving agent or a mixture of the abovementioned [9].

Hence, the solubility of medicinal products (and/or their mixtures) included in pharmacopoeial plant material stands as derivative of compatibility of the required hydrophilic-lipophilic balance (HLB_R) observed in the phytocompound and polarity of the extraction medium (ε_M).

Basing on the technology underlying the production of solid oral drug form (tablet, dragée, capsule), suspension or cosmetic forms, when selecting excipients it is crucial to consider ballast substances present in extracts, as they decide on the pharmaceutical availability profile in model acceptor fluids [10].

This was the reason to conduct comparative studies on dry extracts obtained from maidenhair tree (*Ginkgo bilobae*) leaves. Extracts were obtained by etching the leaves wit medium of diversified polarity, namely water (ε_{H20}=78.50) and aqueous ethanol solutions: 45% (ε_M=55.0–49.0) and 70% (ε_M=38.0–43.40).

The study testing solubility of extracts in model acceptor fluids, mainly focused on the estimation of viscosity parameters, next to surface activity [11-13].

The abovementioned segment of the study was additionally expanded with HPLC chromatographic analysis of obtained dry extracts and the so-called "dry residue" after the exposure in water and 0.1 mol HCl (200 mOsmol/dm^3) [14].

Furthermore, a spectrophotometric (UV) method was used to mark conversion contents of flavonoids in dry extracts, basing on reference quercetin and rutin (rutoside).

Obtained results constitute the basis to consider the relation between proposed BCS classification of extracts and their properties concerning application in drug form or cosmetic form technology.

MATERIAL AND METHODS

Extracts

1. Extracts: Extractum *Ginkgo bilobae* aq. siccum
   Extractum *Ginkgo bilobae* spir. siccum – 45%(*v/v*)

Extractum *Ginkgo bilobae* spir. siccum – 70%(*v/v*)
Created with standard industrial technology in Phytopharm Klęka S.A.
From *Ginkgo bilobae* leaves of the following quality: Ph. Eur. 01/2008:1828
1. Reference extract:
Extractum *Ginkgo bilobae* spir. siccum – 80% (*V/V*)
24% maltodextrin participation: producer – China

Methods and equipment

Marking average molecular masses in model *Ginkgo bilobae* leaf extracts with HPLC method.

Marking the molecular mass layout in produced extracts lies in the division depending on hydrodynamic volume in each molecule. This method is practically relative (GPC method) due to calibration of the system and maintaining stable conditions of analysis. As far as calibration of the system is concerned, the research was based on polystyrene structural models characterized by linear structure and familiar physical and chemical parameters, such as an average number of molecular mass (M_{CZ}).

Research equipment

Chromatographic set: L7100 pump (Merck Hitachi) with degasser (Knauer) and manual dosing valve (Knauer) with 20 μl loop volume, used column PL gel mini-mix E3 μm, 250x4,6 mm, refractometric detector (Varian) and „GRAMS-386 for Chromatography" computer programme (Galactics) to collect and process experimental data.

Flow of the eluent (THF – tetrahydrofuran) reached 0.3 cm^3/min with temperature of the column equalling 30±0.1°C, and retention time deviation not exceeding 0.2%. In all chromatograms the retention time was corrected by changing the final time of analysis in order to obtain constant retention time of the system peak –22.350±0.001 min [14].

Concentration of solutions prepared for calibration reached 10-16 μg PS (polystyrene)/ 10 cm^3 of the eluent. Efficiency related with division of column marked for PS 9200 and PS 93 300 standards equalled 4.32.

The test was performed in ICSO "Blachownia" in Kędzierzyn Koźle.

Surface activity of aqueous extracts obtained from *Ginkgo bilobae* leaves

According to recommendations of the Polish Standard (PN/ISO), stalagmometric method was used to determine the number value of surface tension coefficient

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$-\gamma^{25}$ which constituted the basis to estimate the critical micelle concentration (CMC) for examined extracts from the course of dependence $\gamma^{25} = f(c, \log c; g \times 100 \, cm^{-3})$. Line equations with $p = 0.05$ and $r^2 \geq 0.9975$ were used to describe dependence between $-\gamma^{25}$ surface tension coefficient and $\log c$ within the scope of small concentrations ($y_1 = a_1 \times \log c + b_1$) and large concentrations ($y_2 = a_2 \times \log c + b_2$). Both lines cross at the point where the scope of concentrations reach equality, and this point reflects critical micelle concentration (CMC), and they are calculated basing on the following equation

$$\log cmc = b_2 - b_1/a_1 - a_2$$

Simultaneously, in case of such a complex structure as aqueous plant extract solution, the number of CMC values (mol/dm^3) enabled to calculate

$$-\Delta G^0_M = 2.303 \, RT \times \log CMC,$$

namely the thermodynamic potential of micelle formation (ΔG^0_M).

Viscosity of extractum *Ginkgo bilobae* siccum aqueous solutions

LVN (limiting viscosity number, η) aqueous solutions of extracts of the so-called dry residue left after the evaporation of the saturated solution was determined according to the Polish Standard with Ubbelohde type viscosimeter [16].

It constituted the basis, analogically as in publications [17], to calculate selected viscosity values: M_η, R_o, $R_{obs.}$, Ω.

Results obtained from conducted pre-formulation tests are presented in tables 3 and 4.

Marking conversion contents of flavonoids in extractum *Ginkgo bilobae* siccum

Spectroscopic (UV) method was used to mark conversion contents of three flavonoids in the created so-called dry residue remaining after dissolving *Ginkgo bilobae* extracts in water and in 0.1 mol HCl, and then evaporating the saturated solutions to a solid mass.

Approximating equation describing the relation between concentration (c_w) and the measured absorbance value ($-A$) with $p = 0.05$ and $r \geq 0.9997$ for quercetin (methanol solution – w in $g/100 \, cm^3$):

1. $A = 3.8704 \times 10^{-2} + 744.9264 \times c$,
2. $\log A = 2.6121 + 0.9012 \times \log c$
   and for rutin (rutoside, methanol solution – w in $g/100 \, cm^3$)

1. A= 0.1061 + 31.4565 x c,
2. log A = 0.7389 + 0.5621 x log c

After conversion to the form –c = A; (log A) – a/b made calculation of the amount of flavonoids in each extract possible.

Obtained results are presented in tables 3 and 4.

DISCUSSION OF RESULTS

Determining the structure of flavonoid components observed in extracts from maidenhair tree (*Ginkgo bilobae*) leaves (fig. 1), next to diterpenes and sesquiterpenes (f. ex. bilobalide) constituted the basis to conduct HPLC analysis of the extracts obtained with the medium of diversified polarity ($\mathcal{E}_M$). The division covered the so-called dry residue that was obtained after dissolving created extracts in water (mOsmol/dm^3=0) and 0.1 mol HCl (nOsmol/dm^3=200), and then evaporating them in the temperature of 37°C from saturated dissolvent solution, finally reaching the remaining material in a form of a solid mass.

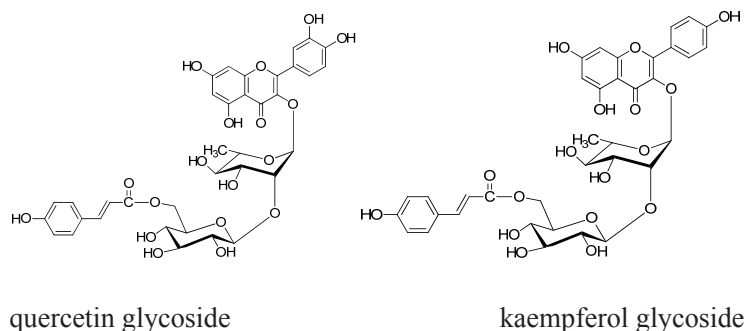quercetin glycoside   kaempferol glycoside

Figure 1.

Results of chromatographic analysis are presented in table 1.

Table 1.

Results of chromatographic analysis performed with the so-called HPLC method, the so-called dry residue obtained after evaporating the saturated solution to a dry mass; medium – model acceptor fluids according to Polish Pharmacopoeia VIII – 0.1 mol HCl and water

MODELS Type of extract	Mn	Mw	Mw/Mn	t.p.	M.p.	Mp for particular peaks	Percentage (%) share in the whole extract
1. Quercetin	261	266	1.02	18.22	271	271	100
2. Rutin (1)	789	1058	1.34	10.72	1308	1308	100
3. Rutin (2)	727	995	1.37	10.83	1201	1201	100
4. Rutin (3)	734	1001	1.36	10.82	1206	1206	100

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MODELS Type of extract	Mn	Mw	Mw/Mn	t.p.	M.p.	Mp for particular peaks	Percentage (%) share in the whole extract
5. Extractum Ginkgo aq. siccum (H_2O)	1111	6350	5.72	12.09	540	23619	17.38
						2362	36.39
						1230	6.89
						856	11.77
						540	27.57
6. Extractum Ginkgo spir. siccum 45% (*v/v*)	1340	8061	6.01	8.15	27623	27623	18.87
						2626	43.28
						1295	5.97
						923	12.43
						552	19.45
7. Extractum Ginkgo spir. siccum 70% (*v/v*)	1175	6107	5.20	10.02	2389	25054	15.69
						2389	43.75
						1223	7.90
						859	15.07
						533	17.59
8. Extractum Ginkgo spir. siccum 80% (*v/v*):24% mixt on maltodextrin	1177	4073	3.46	10.03	2372	21897	7.39
						2372	49.81
						1154	12.97
						867	13.01
						646	16.82
9. Extractum Ginkgo aq. siccum (0.1 mol HCl)	1435	3544	2.47	9.69	3301	23192	3.37
						3301	84.03
						555	12.60
10. Extractum Ginkgo spir. siccum 45%(*v/v*) (0.1 mol HCl)	1415	5437	3.84	9.54	3851	21241	9.21
						3851	64.59
						955	11.75
						546	14.45
11. Extractum Ginkgo spir. siccum 70% (*v/v*) (0.1 mol HCl)	1698	5748	3.38	9.35	4675	20295	9.19
						4675	66.75
						966	12.99
						548	11.07
12. Extractum Ginkgo spir. siccum 80% (*v/v*):24% mixt on maltodextrin	1527	7009	4.59	9.54	4219	19605	14.92
						4219	63.99
						921	8.51
						548	12.58

Mn – molecular mass
Mw – the weight-average molecular mass
M.p – molecular mass calculation maximum peak

Taking into consideration the value of rutin (rutoside) molecular mass (tab. 1) as well as kaempferol glycoside (molecular mass = 743.74) and quercetin glycoside (molecular mass = 759.74) it is possible to indicate fractions in the analysed extracts with molecular mass similar to stated reference structures. Unexpectedly it turned out that the contents of the tested extracts includes – with considerable quantitative participation, biopolymer structures with molecular mass $\geq$20 000.

Due to exposure of extracts in 0.1 mol HCl, their overall participation drops by about 50%. Quantitative participation of biopolymers with molecular mass $\geq$20 000 still remains on the level ranging between 3 and 9%, which may pose significant influence on the manner of formulating the solid oral form of the drug.

Critical micelle concentration (CMC) was indicated, and – what is more – a number value concerning the lowering of the surface tension within the critical area $-\gamma^{25}_{cmc}$ enabled to calculate the micelle creating a potential for such a thermodynamic complex $-\Delta G^0_m$ and elevating lipophilic glycoside segment above phase boundary $-A_m$.

Calculated physical and chemical values ΔG^0_m, Am and HLB $_{1HNMR}$ [18] are presented in table 2.

As it can be seen from $-\gamma^{25}_{cmc}$, numerical values of the surface activity of di- and sesquiterpene contents in extracts does not lead to considerable lowering of surface tension coefficient in model acceptor fluids, and this is of noteworthy significance not only in marking the effective process relating pharmaceutical availability of phytocompounds, but also on reducing the adverse reactions observed on mucosa (phase boundary during the mass exchange process) after the disintegration of the tablet containing the extract in gastric juice.

In practice – basing on results obtained in *in vitro* conditions, it is possible to state that the so-called post-drug gastroesophageal reflux shall not occur.

Relatively low ΔG^0_m (kJ/mol) content for water reaching 13.74-17.08 KJ/mol and in case of 0.1 mol HCl ranging between 13.84 and 16.27 KJ/mol indicate rather not too high (physiological) thermodynamic stability of micelle adduct with phytocompound. In the light of the above – this state indicates a relatively high bioavailability of the phytocompound.

As far as it can be seen from basic viscosity values presented in tables III and IV, lowered polarity within the extracting system is associated with considerable viscosity drop in average molecular mass – M_η and symmetrically hydrodynamic values (R_o, R_{abs}, Ω).

Calculated markings of flavonoids (reference substances: quercetin and rutin-rutoside) lead to a conclusion that 45% (*v/v*) water ethanol solution stands as an optimal system supporting obtaining flavonoids in the extract.

Nonetheless, after exposure of extracts in 0.1 mol HCl we can obtain – with smaller differentiation in basic viscosity values (tab. 4; M_η, R_o, R_{abs}, Ω), considerable growth in the marked conversion flavonoids content for Extractum *Ginkgo bilobae* aq. siccum and extractum *Ginkgo bilobae* spir. siccum – 70%(*v/v*).

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Basic physical and chemical values characterising surface activity of extracts from *Ginkgo bilobae* and their structural HLB_{1HNMR} level

Extract – type	Test portion of the medium	Dry residue	% of soluble contents	cmc g/100cm³	cmc * mol/dm³	DG^0_m kJ/mol	g^{25} mJ/m²	Am*10^{-18} M²	HLB ¹HNMR
				H_2O					
1. Extractum Ginkgo aq. siccum	2.1702	2.1510	99.12	0.2746	1.0162*10⁻³	-17.0870	71.39	6.9762	19.17
2. Extractum Ginkgo spir. siccum 45% (*v/v*)	3.3726	3.2525	96.44	0.6025	3.9082*10⁻³	-13.7474	71.01	4.2432	19.13
3. Extractum Ginkgo spir. siccum 70% (*v/v*)	1.6700	1.5600	93.41	0.2020	1.8109*10⁻³	-15.6747	71.33	6.3322	18.80
4. Extractum Ginkgo spir. siccum 80% (*v/v*):24% mixt on maltodextrin	2.7006	1.3525	50.08	0.3012	2.4680*10⁻³	-14.8871	61.91	0.4087	-
				0.1 mol HCl					
5. Extractum Ginkgo aq. siccum	2.1655	2.1374	98.70	0.4005	1.7623*10⁻³	-15.7221	71.47	7.2210	18.47
6. Extractum Ginkgo spir. siccum 45% (*v/v*)	2.1330	2.0546	96.32	0.2884	1.4109*10⁻³	-16.2735	70.62	3.0264	18.73
7. Extractum Ginkgo spir. siccum 70% (*v/v*)	3.1628	2.9970	94.75	0.2892	1.9471*10⁻³	-15.4749	71.39	6.9762	18.77
8. Extractum Ginkgo spir. siccum 80% (*v/v*):24% mixt on maltodextrin	3.4449	1.6970	49.26	0.3050	3.7568*10⁻³	-13.8454	63.50	0.4853	-

Cmc – critical micelle concentration
DG^0_m – thermodynamic potential for micelle formation
g^{25} – surface tension calculationin the cmc area
Am – the surface occupied by the lipophilic structure at the phase boundary
HLB – hydrophilic-lipophilic balance

Basic viscosity values of Extr. *Ginkgo bilobae* siccum aqueous solutions (saturated solutions)

Type of extract	Medium C_{exp} G*100cm³	GLL, [h]	M_h	R_o*10⁻⁷ cm	R_{obs}*10⁻⁸ cm	Ω * 10⁻²⁰ cm³	$c_{/k/}^*$ g/100cm³	$c_{/R/}^{**}$ g/100cm³
1. Extractum Ginkgo aq. siccum	1.1003	0.106350	2702.01	4.3743	3.5715	1.9084	4.1520*10⁻²	0.7381
2. Extractum Ginkgo spir. siccum 45%(*V/V*)	1.2694	0.075776	1541.61	3.2404	2.6457	0.7781	7.4275*10⁻²	1.5234
3. Extractum Ginkgo spir. siccum 70%(*V/V*)	1.2279	0.063104	1115.45	2.7369	2.2346	0.4674	4.8098*10⁻²	0.9313
4. Extractum Ginkgo spir. siccum 80%(*V/V*):24% mixt on maltodextrin	1.3525	0.065804	1220.42	2.8599	2.3351	0.5333	14.9987*10⁻²	3.3566

Basic viscosity values of Extr. *Ginkgo bilobae* siccum solutions in 0.1 mol HCl (saturated solutions)

Type of extract	Medium C_{exp} G*100cm^3	GLL, [h]	M_h	R_o*10^{-7} cm	R_{obs}*10^{-8} cm	Ω * 10^{-20} cm^3	$c_{/k/}^*$ g/100cm^3	$c_{/R/}^{**}$ g/100cm^3
1. Extractum Ginkgo aq. Siccum	1.3415	0.095791	2272.47	3.9876	3.2557	1.4456	6.5683*10^{-2}	1.2859
2. Extractum Ginkgo spir. siccum 45% (*v/v*)	1.2563	0.089852	2043.98	3.7679	3.0764	1.2196	5.5347*10^{-2}	1.0769
3. Extractum Ginkgo spir. siccum 70% (*v/v*)	1.7054	0.074091	1485.24	3.1765	2.5036	0.7308	6.4072*10^{-2}	1.3081
4. Extractum Ginkgo spir. siccum 80% (*v/v*):24% mixt on maltodextrin	1.6970	0.055901	931.63	2.4754	2.0211	0.3458	15.7639*10^{-2}	3.5186

$c_{/k/}^*$ – marking flavonoids per quercetin
$c_{/R/}^{**}$ – marking flavonoids per rutoside
GLL, [h] – the limiting viscosity number
M_h – viscosity average
Ω – effective volume
R_o, R_{obs} – hydrodynamic parameter of the micelle

CONCLUSIONS

1. Performed tests reveal that created Extractum *Ginkgo bilobae* aq. siccum, spir. siccum 45%(*v/v*) and spir. siccum 70%(*v/v*) are characterized by high content of high molecular weight biopolymers, which maintain their structural stability in 0.1 mol HCl (model acceptor fluid, PP VIII).
2. Indicated basic viscosity values indicate that decrease in the polarity of the extracting system is reflected by considerable viscosity differentiation in average molecular mass – M_h and basic hydrodynamic values (R_o, R_{obs}., Ω). Observed regularity was confirmed with viscosity measures in 0.1 mol HCl.
3. It turned out – unexpectedly, that we do not observe a significant participation of structures extracts from *Ginkgo bilobae* leaves, which would decrease the surface tension on phase boundary in a natural manner. Calculated thermodynamic micelle creating potential – DG^0_m indicates a physiologically satisfying endurance of complex micelle, which is a carrier for the phytocompound.
4. Despite conducting symmetrical comparative studies with Extractum *Ginkgo bilobae* spir. siccum – 80%(*V/V*); 24% mixt on maltodextrin (producer: China), yet the lack of significant data related with its creation does not provide reliable grounds to perceive it as a reference system.

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PRODUKTY EKSTRAKCJI LIŚCI Z MIŁORZĘBU JAPOŃSKIEGO (*GINKGO BILOBAE*) W ŚWIETLE WYMAGAŃ BIOFARMACEUTYCZNEGO SYSTEMU KLASYFIKACJI (BCS) I MEDIUM O ZRÓŻNICOWANEJ POLARNOŚCI ($\mathcal{E}_M$)

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S t r e s z c z e n i e

Wytworzono modelowe ekstrakty z liści miłorzębu japońskiego (*Ginkgo bilobae*), korzystając z medium o zróżnicowanej polarności (ε_M). Przeprowadzono analizę chromatograficzną metodą HPLC tzw. suchej pozostałości uzyskanej po odparowaniu rozpuszczalnika z nasyconych wodnych roztworów i z 0,1 mol HCl. Przeprowadzono pomiary lepkościowe i aktywności powierzchniowej na granicy faz. Wyliczono podstawowe wielkości lepkościowe ($[\eta]$, $M\eta$) i hydrodynamiczne (Ro, Robs., Ω). Korzystając z referencyjnej kwercetyny i rutyny (rutozydu) przeliczeniową metodą UV oznaczono również zawartość flawonoidów w wytworzonych ekstraktach.

Słowa kluczowe: Ginkgo bilobae, suchy ekstrakt, lepkość, polarność