ORIGINAL ARTICLE

CORRELATION BETWEEN TECHNOLOGICAL PROCESS AND ANTIOXIDANT CAPACITY FOR *SOPHORAE FLORES* FLAVONES SELECTIVE DRY EXTRACTS

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Abstract

Because flavones act as antioxidants and oxidative stress characterizes lead poisoning we determined the antioxidant capacity and TPC (total polyphenol content) for *Sophorae flores* flavones selective dry extracts that could be used in lead poisoning treatment. The antioxidant capacity was performed by FRAP (Ferric Reducing Antioxidant Power), TEAC (Trolox Equivalent Antioxidant Capacity) and chemiluminescence (CL) methods and TPC (total polyphenol content) assay by Folin – Ciocalteu method. Because the greatest antioxidant capacity correlated with TPC (total polyphenol content) assay was noticed for B flavones selective dry extract, we suggest as a technological process correlated with highest antioxidant capacity the following scheme: vegetal product – solvent ratio (1:10); 40% CH_3OH under reflux extraction, 2N HCl hydrolysis, hydrolysis time = 15 h, ethyl acetate aglycon extraction.

Rezumat

Datorită acţiunii antioxidante a flavonelor, stresul oxidativ prezent în cazul intoxicaţiilor cu plumb, am considerat importantă cercetarea activităţii antioxidante şi a conţinutului în polifenoli totali a extractului flavonozidic selectiv obţinut din *Sophorae flores*, care ar putea fi folosit în intoxicaţiile cu plumb. Capacitatea antioxidantă a extractelor a fost evaluată prin următoarele metode: TEAC (*2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt*), FRAP (*the Ferric reducing antioxidant power*), CL (chemiluminiscenţă), iar polifenolii totali au fost determinaţi prin metoda Folin-Ciocâlteu. Cea mai mare capacitate antioxidantă şi cel mai crescut conţinut în polifenoli totali a prezentat extractul selectiv B, de aceea propunem ca pentru obţinerea lui să se folosească următorul procedeu: raportul optim produs vegetal / solvent = 1/10, extracţie prin refluxare cu metanol 40%, hidroliza acidă (HCl 2N) a soluţiei extractive metanolice, timp de hidroliză optim = 15 ore şi extracţia agliconilor cu acetat de etil.

Keywords: *Sophorae flores* flavones selective dry extracts, technological process, antioxidant capacity

Introduction

From literature data it results that quercetol and its heteroside (rutoside) have an important therapeutic potential. From this point of view, flavones, especially quercetol, are evaluated for their antioxidant capacity correlated to their use in the treatment of different diseases induced by ROS (Reactive Oxygen Species). These substances act at cellular membrane level, (reducing lipid per oxidation and increasing membrane resistance) and endoplasmatic reticulum [3, 5, 6, 13, 14].

The antioxidant capacity of flavones from selective dry extracts can be determined in aqueous as well as in different concentrations of alcoholic solutions.

The methods used for antioxidant capacity assessment are based upon antioxidants possibility to annihilate free radicals action by hydrogen atom transfer (HAT method) or an electron transfer (SET = single electron transfer). Using these methods they can reduce different oxidant substrates. HAT methods are solvent type and pH range independent and more rapid compared to SET methods [14]. The most cited methods for antioxidant capacity determination are FRAP (Ferric reducing antioxidant power) (from SET methods) and TEAC (Trolox Equivalent Antioxidant Capacity) (from SET and HAT methods).

Previously conducted researches on mice poisoned with lead acetate and treated with quercetol revealed the quercetol role in lowering enzymatic activity of superoxide dismutase (SOD), catalase, demonstrating the indirect action as possible antidote (ratio of lead acetate bioinactivation / quercetol = 2.18) [13, 14].

The aim of the present study was to develop technologies for flavones selective dry extracts obtaining, from *Sophorae flores*, correlated to the

highest TPC (total polyphenol content) and to assay their antioxidant capacity using different methods: FRAP (Ferric Reducing Antioxidant Power), TEAC (Trolox equivalent antioxidant capacity) and chemiluminescence (CL).

Materials and Methods

For this purpose we used 3 technological processes for flavones selective dry extracts obtaining. We used the extraction under reflux with 40% methanol (heterozides extraction) and ethylacetate for the resulted aglycons by 2N HCl hydrolysis for 10 hours (A), 15 hours for (B) and 24 hours for (C). The combined extractive solutions, filtered, dry on anhydrous sodium sulfate were concentrated at the Rotavapor at 30°C and 0.6 barr pressure, then dry on vacuum oven at the same temperature [10, 16, 19].

TPC (Total polyphenol content)

TPC *(Total polyphenol content)* assay is based upon the oxidation-reduction reaction between polyphenols and Folin – Ciocalteu reagent (as reducing agent). The reaction product between Folin – Ciocalteu reagent (a molybdenum heteropolyphosphotungstate) is a mix of tungsten and molybdenum oxides of different valences, blue coloured, with a maximum absorbance at 760 nm [2, 7, 15, 17, 18].

Working solution obtaining

Each 0.5 g of flavones selective dry extracts obtained by the 3 processes, noted A, B and C were dissolved in 100 mL 40% ethanol, and the ethanol solutions were centrifuged 10 minutes (x 4000g). The supernatants obtained from each flavones selective dry extract were noted as SA, SB and SC and were used to determine TPC and the antioxidant capacity.

Procedure for experimental conditions

150 µL of each SA, SB and SC were mixed with 750 µL of Folin – Ciocalteu reagent and 600 µL of a 7.5% sodium carbonate solution, shaked and incubated for 10 minutes at 50^0C. The absorbance was determined at λ=760 nm using a Spekol 11, Carl Zeiss Jena spectrophotometer. Total polyphenols content was expressed as mg quercetol equivalents (QE) /g selective dry extract, as mean ± SD (standard deviation) of three replicates. Because the obtained absorbance values did not fit the calibration curve, we diluted the samples in 1: 8 or 1:20 ratios.

TEAC assay

The method was first described by Miller and Rice Evans (1993) and is based on $ABTS^{\bullet+}$ oxidation by peroxyl radicals or other oxidants (potassium persulfate aqueous solution) and obtained cation radicals ($ABTS^{\bullet+}$ blue - green coloured) captation. ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt solution reacts with potassium persulfate in a 2:1 molar ratio and the obtained radical has a half-life of 24 hours. The radical is soluble in hydrophobic and hydrophilic medium in a large pH scale. The decreasing radical $ABTS^{\bullet+}$ absorbance value in the presence of the working solution of (Trolox = (S) (2)-6-hydroxi-2,5,7,8 tetramethylchroman-2 carboxylic acid, vitamin E) is measured in a 6 minutes period of time [8, 9, 12].

The calibration curve was linear for the range of Trolox concentrations between 0,25 – 2 mM and the results were expressed in mmols as Trolox equivalents/g extract [16, 17].

Procedure for experimental conditions

Each 10 µL of SA, SB and SC solutions we mixed with 1000 µL of $ABTS^{\bullet+}$ in a 5 mM saline phosphate buffer solution (pH = 7.4 and absorbance value is 0.700 ± 0.02). As standards we used α-tocopherol and Trolox 40% methanol solutions. Absorbance decrease was determined at λ = 734 nm, in three replicates after 6 minutes using a Spekol 11, Carl Zeiss Jena, spectrophotometer. The results are expressed as mM Trolox equivalents / g of dry flavones extract (mean ± SD).

Because the solutions obtained as described above had absorbance values that did not fit the calibration curve, we diluted them in 1:8 or 1:20 ratios.

The TEAC value was obtained using the following equation based on the calibration curve:

$$TEAC\ mM = \Delta A \times F.D. / 0.274$$

where ΔA is the absorbance difference, F.D. the dilution factor and 0.274 the regression coefficient.

FRAP (Ferric Reducing Antioxidant Power) assay

This method measures the capacity of a substance (antioxidant) to reduce at low pH values (3.6) ferric tripyridyltriazine (Fe^{3+}-TPTZ) (2, 4, 6 tripiridiltriazil) complex to the ferrous tripyridyltriazine (Fe^{2+}-TPTZ blue coloured complex), with a maximum absorbance at 593 nm. The reaction emphasizes compounds with a redox potential < 0.7 V. The reducing capacity is correlated to the molecule hydroxylation degree and the conjugated double bonds presence from polyphenols structure [1, 8, 11, 17, 18].

Working solution obtaining

0.1 g of A, B and C extracts were dissolved in 30 mL 40% methanol solution, centrifuged at 4000g, for 10 minutes. 120 µL of each supernatant were mixed with 3 mL of FRAP reagent and incubated for 10 minutes in a water bath at 37°C. After cooling we determined the absorbance at λ = 593 nm using a Cecil Bio 2000 UV – VIS spectrophotometer in three replicates, compared to a blank that contained only the reagents (200 mL TPTZ solution, 20 mL $FeCl_3$x 6 H_20 and 24 mL of distilled water).

The calibration curve was linear for the range of 0.5 - 3 mM $FeSO_4$ x 7 H_20 solutions. The results were expressed as mmol $Fe^{2/}$mL (mean ± SD).

The antioxidant capacity determined by chemiluminiscence (CL) assay

Chemiluminiscence (CL) is a physical mechanism that consists in electrons excitation from atoms and molecules of substances and the release of the absorbed energy as light.

Working solutions obtaining

We obtained 10^{-5} mol/L DMSO (dimethylsulfoxide) solutions from A, B, C flavones dry extracts and rutoside and quercetol, used as reference substances. The chemiluminiscence generator system used contained luminol 10^{-5} mol/L – H_2O_2 10^{-5} mol/L in Tris – HCl buffer (pH = 8.4) as standard, in a final reaction volume of 1 mL. All the measurements were performed on the Turner Designs TD 20/20 – USA chemiluminometer. The signal was recorded every 5 seconds, and the chemiluminescent signal was used to determine the time evolution curve type CL = f (t). The luminol signal intensity was I_0 = 3770. The measurements were done in five replicates and the results are expressed as mean [4, 6].

Results and Discussion

The best results for flavones dry extracts were obtained by refluxing the vegetal product with 40%methanol, followed by 2N HCl hydrolysis for 15 hours and ethylacetate extraction (procedure B).

Using different extraction conditions we obtained flavones selectives dry extracts noted as A, B, C, yellow – brown coloured, expressed in grams as 1g% (A), 7.01 g% (B) and 0.66 g% (C).

Increasing hydrolysis time from 10 hours (procedure A) to 15 hours (procedure B) assures a better aglycon obtaining, confirmed by the highest content of flavones selective dry extract. The total polyphenols content results are presented in Table I.

Table I

Total polyphenols content assay for *Sophorae flores* flavones selective dry extracts

Extract	Absorbance (λ = 760 nm), dilution 1/20	TPC (mg QE ± SD) / g dry selective extract
A	0.769	68 ± 3.23
	0.784	
	0.776	
	0.765	
B	0.734	64 ± 4.55
	0.745	
	0.745	
	0.743	
C	0.643	55.5 ± 3.73
	0.608	
	0.666	
	-	

The antioxidant capacity values determined by FRAP **(**Ferric Reducing Antioxidant Power**)** method are presented in Table II. From the obtained data we noticed that the greatest antioxidant capacity was registered for extract B.

Table II

Antioxidant capacity of dry flavonoids extracts from *Sophorae flores* by FRAP **(**Ferric Reducing Antioxidant Power**)** assay

Extract	Absorbance (λ = 593 nm)	$\delta a_1 = a_1 - a_{standard}$	Fe^{2+} (mmoles / mL)
A	0.605	0.563	3.35
B	0.879	0.837	5.07
C	0.745	0.703	4.23

Table III

Antioxidant capacity of dry flavonoids extracts from *Sophorae flores* by TEAC (Trolox Equivalent Antioxidant Capacity) assay

Extract	Antioxidant capacity mmoles Trolox / g dry selective extract, 1/8 dilution	Antioxidant capacity mmoles Trolox / g dry selective extract, 1/20 dilution
A	16.94 ± 0.15	20.10 ± 0.15
B	15.53 ± 0.71	20.36 ± 0.91
C	14.81 ± 0.23	17.06 ± 0.19

From Table III data analysis (1/20 dilution) we can conclude that both A and B extracts have an important antioxidant capacity, having very closed values (20.10 ± 0.15 – A, and 20.36 ± 0.91 – for B). We assume these data to appropriate total polyphenol content and a different time for acid hydrolysis (A procedure – 8 hours, B procedure – 15 hours).

From Table IV data analysis one can see that compared to the used standards (rutoside and quercetol) it results that extract B has the greatest antioxidant capacity and a high content of aglycons (quercetol).

Table IV

Antioxidant capacity of dry selective flavonoids extracts from *Sophorae flores* by chemiluminiscence assay

Sample	Antioxidant Capacity (AC %)
Rutoside	77.4%
Quercetol	92.8%
Extract A	45.5%
Extract B	69.6%
Extract C	68.2%

Conclusions

From the obtained results we suggest as a technological process for obtaining *Sophorae flores* flavones selective dry extract, correlated to highest antioxidant capacity and polyphenol content the following scheme: vegetal product – solvent : ratio (1:10), 40% CH_3OH extraction under reflux, 2N HCl acid hydrolysis, 15 hours, ethylacetate aglycon extraction.

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