ESTIMATION OF THE THERAPEUTICAL VALUE OF DRIED POLYPHENOLIC EXTRACTS FROM TWO PLANTS OF DOBROGEA SPONTANEOUS FLORA

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Reviewed by: Assoc. Prof. T. Yankova, MD, PhD

ABSTRACT

Our study involves *Sophora japonica* L. and *Holoschoenus vulgaris* L. both plants from Dobrogea' spontaneous flora. In order to establish the therapeutic value we measured the total polyphenol content (Folin – Ciocalteu method) and the total antioxidant activity (by ABTS radical decolorization assay) of the dried extracts obtained under different working conditions. All the dried extracts' features presented are according to the general monograph Extracta quality conditions, <0,04% iron and heavy metals content, and dried substance <5%. The higher total antioxidant capacity and total polyphenol content were observed in *Sophora* extract no.1, correlated with a certain raw material/solvent ratio (1/5), time of extraction (8 hours). These preliminary data obtained for TEAC and TP content lead us to conclude that the extracts could have an important therapeutical value possibly confirmed by future studies involving animal testing.

Keywords: antioxidant capacity, polyphenol content

INTRODUCTION

Holoschoenus vulgaris L. (Cyperaceae), synonym *Scirpus holoschoenus* L. (Figure 1) is a rare herbaceous perennial plant, one meter high, with a round-headed club-rush, flowering in April - July.

European distribution: France, Greece, Italy, Portugal, Spain. Widespread in the entire Mediterranean basin, extending along the coasts of the Black Sea, in particular in dunal systems, north to the Dobrogea and the Danube Delta, in valleys of the Balkan peninsula north to the Banat (15).

From the phytochemical point of view, *Holoschoenus vulgaris* L. has not been previously investigated.

From a medicinal point of view, the crude extract of *Scirpus americanus* (11) and *Scirpus maritimus* (12) exhibited significant activity against lymphocytic leukemia, rhizomes extract from *Scirpus lacustris* showed bacterial activity against *Escherichia coli* (8) and stilbene trimers isolated from the tubers of *S. fluvialis* behave as antiallergic and anti-inflammatory agents (1). Traditionally, people from Dobrogea region use the boiled extract from the rhizomes of *Holoschoenus vulgaris* L. for its hepatic-protective activity.

Figure 1. *Holoschoeni rhizoma*

Sophora buds come from the *Sophora japonica* L. (Fabaceae) tree (Figure 2), (also known as the Japanese pagoda), a deciduous tree native to east Asia and cultivated in China, Japan and Korea and in Bulgaria and Romania. The flowers of the *sophora* tree are hermaphrodite and flower in late August and early September. The flower buds represent raw material for rutin (flavonoid) extraction over 15-20%, and quercetin (obtained by hydrolysis) and pectines (2).

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Flavonoids (mainly rutin and quercetin) are able to modulate the activity of some enzymes and to modify the behaviour of many cell systems suggesting that they could exert a multitude of biological activities, particularly significant antioxidant, vasculoprotective, antihepatotoxic, antiallergic, anti-inflammatory, antiulcerogenic and even antitumoral activities and are capable to exhibit metal ion chelating effect. (3,4,7).

Figure 2. *Sophorae* flower buds

MATERIAL AND METHODS

Plant material

Sophora japonica flower buds were collected from Constanta area in July - August 2005, they were dried in a dark place, at room temperature. Botanical identification was done according to the morphologic features (macro and microscopic examinations).

Table 1. Extraction procedure for *Sophora* flower buds and *Holoschoeni* rhizoma

Extract No.	Raw material/ 40% methanol	Time (hours)	Hydrolysis (HCl 10%, 1:1), (hours)
1. <i>Sophora</i> flower buds	1:5	-	8
2. <i>Sophora</i> flower buds	1:10	-	16
3. <i>Sophora</i> flower buds	1:15	-	24
4. <i>Holoschoeni</i> rhizoma	1:7.5	6	-

The plant rhizomes from *Holoschoenus vulgaris* (*Cyperaceae*) were collected from Tulcea County during September - October 2005, dried on air, grounded and extracted with different solvents, in order to separate the components. Botanical identification was done according to the morphologic features (macro and microscopic examinations). Dried extracts were obtained under different working conditions from flower buds of *Sophora japonica* (Table 1) and roots of *Holoschoenus vulgaris* (Table 1) and were analysed according to Extracta monograph (European

Pharmacopoeia and Romanian Pharmacopoeia Xth edition) (3,4,7).

The chemical composition of all extracts was established by a general chemical analysis of aetheric, alcoholic and water extracts (5,6).

Determination of the total polyphenol content

Total polyphenol content was measured using the Folin-Ciocalteu reagent as described by Singleton and Rossi (1965) (14) with slight modifications. Samples (150 μ L) were introduced into test tubes; 750 μ L of Folin-Ciocalteu's reagent and 600 μ L of sodium carbonate (7.5%) were added. The tubes were mixed and incubated at 50° C for 10 min. Absorption at 760 nm was measured on spectrophotometer Spekol 11 (Carl Zeiss Jena, Germany). Quercetin was used as a standard. The total phenolic content was expressed as mM Quercetin Equivalents (QE). Results are presented as means $\pm$ S.D. Each measurement was performed at least in triplicate. Quercetin, sodium carbonate and Folin-Ciocalteu's reagent were obtained from Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany.

Measurement of antioxidant activity of the working solution

0.5 g of *Sophora japonica* and *Holoschoenus vulgaris* extracts were diluted in 100 ml 40% ethanol and stirred continuously at RT to the full dilution of the extract. After 10 min of centrifugation ($\times$ 4000 g) the supernatant was used for biochemical measurements. The total antioxidant capacity of aqueous-alcoholic plant extracts was determined by the ABTS (2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) radical decolorization assay (13). The method is based on the consumption of preformed in the presence of potassium persulfate ABTS radical (ABTS^{•+}) whose absorption maximum is at 734 nm. Addition of antioxidants to ABTS^{•+} reduces it to ABTS. 10 μ L plant extract was added to 1000 μ L ABTS^{•+} in 5 μ M phosphate buffered saline, pH 7.4, (PBS), with absorption adjusted to 0.700 $\pm$ 0.02. The decrease of the absorption was measured after six minutes.

Alpha-tocopherol in methanol and Trolox (registered trade-mark of Hoffman-LaRoche, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were used as a standard. The antioxidant activity is presented as mM Trolox equivalent antioxidant capacity, (TEAC). The results are presented as means $\pm$ S.D. Each measurement was performed at least in triplicate. Analysis was carried out on spectrophotometer Spekol 11 (Carl Zeiss Jena, Germany).

RESULTS AND DISCUSSION

Botanical identification of the plant material was carried out by macro and macroscopic examinations of the flower buds from *Sophora japonica* L. (*Fabaceae*) and of rhizome from *Holoschoenus vulgaris* L. (*Cyperaceae*).

The 3 dried extracts obtained in different working conditions from *Sophora japonica* flower buds L. (Table 1) presented as brown crystals, soluble in methanol and ethanol had an iron and heavy metal content <0,04% and dried substance 3,89% (extract 1), 3,94% (extract 2), 4,56% (extract

3). The dry extract from *Holoschoenus vulgaris* roots presented as brown crystals, soluble in methanol, ethanol, contained mainly polyphenols (Arnow test), and had an iron and heavy metal content $<0,04\%$ and dried substance $2,30\%$. These data were in accordance to the general monograph Extracta quality conditions for dried extracts (less than $<0,04\%$ iron and heavy metals content, and $<5\%$ dried substance).

Chemical analysis of the dried extracts showed the presence of flavonoids (rutin), polyphenols (Arnow test), mucilages and pectines for *Sophora flower buds* extracts and the presence of sterols, triterpenes, coumarines, reducing compounds, oses and poliozes, tannins, contain mainly polyphenols (Arnow test) for *Holoschoenus vulgaris* roots extract (Table 2).

Aqueous ethanolic extracts corresponding to the 3 dried ex-

Table 2. Chemical analysis of dried extracts from *Sophora flower buds* and *Holoschoeni rhizoma*

Nr.	Solution	Reaction	Intensity	Active principles identified
1. <i>Sophora</i> flower buds extract	Aetheric	Shibata	+++	flavonols
2. <i>Holoschoeni</i> rhizome extract	Aetheric	Shibata	+++	flavonols
		Lieberman - Burchard	++	sterols
		U.V. ($\lambda = 365$ nm)	++	coumarins
3. <i>Sophora</i> flower buds extract	Alcoholic	Fehling	++	reduced compounds
4. <i>Holoschoeni</i> rhizome extract	Alcoholic	Fehling	++	reduced compounds
5. <i>Sophora</i> flower buds extract	Alcoholic hydrolised	Shibata	++	flavonols
		Arnow	++	polyphenols
6. <i>Holoschoeni</i> rhizome extract	Alcoholic hydrolised	Lieberman - Burchard	++	sterols
		Arnow	++	polyphenols
7. <i>Sophora</i> flower buds extract	Aquaous	Fehling	++	reduced compounds
		H ₂ SO ₄ conc. + timol	++	oze, polioze-
8. <i>Holoschoeni</i> rhizome extract	Aquaous	Fehling	++	reduced compounds
		H ₂ SO ₄ conc. + timol	++	oze, polioze
		FeCl ₃	++	taninuri
		Styasny	+	tanin catehic

Table 3. Total polyphenol and total antioxidant capacity of *Sophora flower buds* extracts and *Holoschoeni rhizoma* extract.

Extract No	Total polyphenol content (TP) $\mu\text{M} \pm \text{SD}$	Total antioxidant capacity (TEAC) $\text{mM} \pm \text{SD}$
1. <i>Sophora</i> flower buds	3732.16 7.23	16.94 0.15
2. <i>Sophora</i> flower buds	3585.08 4.55	15.53 0.71
3. <i>Sophora</i> flower buds	3118.42 23.73	14.81 0.23
4. <i>Holoschoeni</i> rhizoma	2060.04 29.75	8.21 0.27

tracts from *Sophora japonica* flower buds and that from *Holoschoenus vulgaris* roots were studied for TEAC and TP content (Table 3) in order to select the extract with the greatest content in TP and a higher antioxidant potential, possibly explaining their therapeutical uses and select appropriate conditions for extraction.

Aqueous ethanolic extracts from *Sophora japonica* buds exhibited high TEAC values that could be measured only at 8 times dilution. The results indicated a good antioxidants content, especially in extract 1 (16.94 ± 0.15 mM), and were related to the results of the chemical analysis (rutin, polyphenols). Higher dilutions (20 times) produced similar results for the TEAC, especially for extracts 1 and 2 (data not shown). According to our previous results the method produces slightly higher values when measurements are performed at higher dilutions (9).

TP content was measured at 20 times dilution and extract 1 was found to be not only the one with the highest TEAC value but also with highest TP content ($3732.16 \pm 7.23 \mu\text{M}$).

This allows us to conclude that TEAC and TP values are also related to the extraction procedure, and the results suggest that a raw material/solvent ratio (1:5), 8 h hydrolysis with HCl 10%, resulted in the highest TP content. High polyphenol content might be the explanation for the high TEAC values, according to a previously established correlation (10).

TEAC and TP values for aqueous ethanolic extracts from *Holoschoenus vulgaris* roots were determined at 8 times dilution (Table 3). These values also show a high polyphenol content, correlated with the results of chemical analysis, and a good value for the antioxidant potential.

CONCLUSION

Our preliminary data about TEAC and TP content lead us to conclude that the extracts could have an important therapeutic value, which will possibly be confirmed by future studies involving animal testing. We assume that these are preliminary results and that we will improve the extraction procedure (raw material / solvent ratio, vary in solvents, and hydrolysis time).

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