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Antioxidant Activity and Total Phenols in Different Extracts of Four *Staphylea* L. Species

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Abstract: *Staphylea* L. is a deciduous ornamental shrub that possesses significant cytotoxic and antibacterial activity, although the chemical composition of its extracts and the identity of the structures responsible for these biological activities are not yet known. In this study we have determined the total phenolic content in chloroform and ethyl acetate extracts of four *Staphylea* species: *Staphylea colchica* Stev., *S. elegans* Zab., *S. holocarpa* Hemsl. and *S. pinnata* L.. The antioxidant potential (DPPH radical and peroxynitrite scavenging activity) of these extracts was also determined and a correlation between the phenolic content and antioxidant activities of the ethyl acetate extracts has been found. Ethyl acetate extracts were more active and one of them, obtained from *S. colchica* Stev., possessed the highest activity.

Keywords: *Staphylea*, antioxidant activity, radicals, total phenols.

Introduction

Staphylea L., (bladdernut, Staphyleaceae), belongs to a well-known group of deciduous ornamental shrubs that are widely distributed throughout the Northern Hemisphere. In Slovakia they are cultivated for their nice bright green foliage and small white and whitish flowers. *S. pinnata* L. grows naturally in

this territory and is designated a protected species. A decoction prepared from the fruit of *S. bumalda* DC. has been used in Traditional Chinese Medicine as a cough remedy. The fresh roots are supposed to possess a blood refreshing effect after delivery. The dried fruit is also used as a folk anti-diarrhoeal medicine. Indians used the infusion from *S. trifolia* L. for its antirheumatic, dermatological, sedative and gynecological activities. The seeds were considered sacred and were used in gourd rattles for dream and medicine dances [1].

In 2000 Jantová *et al*. found that the plants possesses significant cytotoxic and antibacterial activity [2, 3], but the chemical composition of the extracts was not elucidated. Only a limited number of papers have described the various flavonoid, amino-containing, triterpene, steroid, megastigmane, saccharide, organic and fatty acid compounds found therein [4]. For this reason we have focused on establishing a relationship between the total phenolic content of the non-polar (chloroform) and polar (ethyl acetate) extracts of four *Staphylea* species (*Staphylea colchica* Stev., *S. elegans* Zab., *S. holocarpa* Hemsl. and *S. pinnata* L.) and their biological activity, measured via a determination of their antioxidant activities (DPPH and peroxynitrite scavenging activity).

Results and Discussion

Total phenolic content

These measurements were performed using a modified Folin-Ciocalteu colorimetric method according the procedure described in the Experimental section. The results, presented in Table 1, show that the total phenolic content varies among different *Staphylea* L. species and in the different extracts. As might be expected, the ethyl acetate extracts are generally richer in phenols than the chloroform ones. Thus, the phenolic content in ethyl acetate extracts from the various species tested varied from 10.6 to 2.3 gallic acid equivalents (GAE) in the following order: *S. colchica* (SCE) > *S. holocarpa* (SHE) > *S. elegans* (SEE) > *S. pinnata* (SPE). Chloroform extracts contain less phenolic compounds, from 4.2 to 2.9 GAE, but in the same order: *S. colchica* (SCCH) > *S. holocarpa* (SHCH) > *S. elegans* (SECH) > *S. pinnata* (SPCH).

DPPH radical scavenging activity

The antioxidant potential of the ethyl acetate extracts correlates with the phenolic content. The most active one was the extract prepared from *S. colchica* (SCE). The determination of this activity in the chloroform extracts was not possible, probably due to some complex interaction occurring after the addition of the reagent. Thus, it would appear that peroxynitrite scavenging activity measurements are a better technique to evaluate the antioxidant potential of *Staphylea* L. extracts.

Peroxynitrite scavenging activity

To obtain an extended picture of the antioxidant activity in the mentioned species and their extracts, the protective activity against peroxynitrite-induced tyrosine nitration has also been investigated. A correlation between the phenolic content and the intensity of this biological activity was again observed in the ethyl acetate extracts, which were more active than the chloroform ones in the range from 26.8 CE (SCE) to 2.1 CE (SPCH).

Table 1. Total phenolic content and antioxidant activities of *Staphylea* L. extracts.

Sample*	Total phenolic content [GAE]	SD	DPPH radical scavenging activity [CE]	SD	Peroxynitrite scavenging activity [CE]	SD
SCE	10.6	0.1	22.0	0.2	26.8	0.4
SEE	6.1	0.2	10.9	0.2	13.3	0.3
SHE	7.2	0.2	15.5	0.4	17.2	0.4
SPE	2.3	0.1	2.2	0.2	4.6	0.1
SCCH	4.2	0.5	−	−	6.1	1.7
SECH	3.5	0.1	−	−	8.0	1.8
SHCH	3.8	0.1	−	−	4.0	1.0
SPCH	2.9	0.1	−	−	2.1	0.7

* XXE = ethyl acetate extract; XXCH = chloroform extract.

Conclusions

This study reports for the first time the antioxidant activity and total phenolic content of several *Staphylea* L. extracts. A relationship between the biological activities and the total phenolic content was found [5]. Chemical elucidation of contents of the ethyl acetate extract from *S. colchica* Stev., as the most promising species based on the findings of this study, should be the main objective of further investigations. This should lead to the structural identification of the active metabolites and the determination of their antioxidant profile in pure form.

Experimental Section

Plant material

The leaves of *Staphylea colchica* Stev. (SC), *S. elegans* Zab. (SE), *S. holocarpa* Hemsl. (SH) and *S. pinnata* L. (SP) were collected at the Dendrobiology Institute, Slovak Academy of Sciences – Arboretum Mlyňany, in June 2005. The fresh leaves were dried at room temperature (22 °C) for 3 weeks and then processed in a laboratory mill (Fritsch, Germany). Voucher specimens (No. SC - IM/015, SE - IM/016, SH - IM/012, SP - IM/014) were deposited at Faculty of Pharmacy, Comenius University, Bratislava.

Preparation of extracts

The dried, minced leaves (100 g) of four *Staphylea* L. species were macerated in sequence at one-week intervals with petroleum ether, chloroform, ethyl acetate and water (300 mL). Extracts were filtered and evaporated under vacuum. The residues were dried, weighed and the ethyl acetate (E) and chloroform (CH) extracts were analyzed. The presence of selected secondary metabolites was determined using specific reagents on silica gel chromatographic plates.

Quantitative determination of total phenols

The total content of phenols in the crude chloroform and ethyl acetate extracts was determined using a modified Folin-Ciocalteu colorimetric method with gallic acid as a standard. The extract solution in DMSO (700 µL) was transferred to a 10 mL volumetric flask, the Folin-Ciocalteu reagent (400 µL) was added and after 3 min, each flask was made up to the mark with sodium carbonate (Na_2CO_3) solution (75 g/L). After 2 hours, the suspension was centrifuged (5000 r.p.m., 5 min) and the absorbance of the solution was measured at 760 nm. The total phenolic content was expressed as a gallic acid equivalent (GAE) in g/100 g of dry extract. Data are reported as mean ± SD for at least three replicates [6].

Evaluation of antioxidant activity by scavenging of DPPH radical

The free radical scavenging activity of the extracts was determined with the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay. The extract solution (30 µL) in DMSO (1 mg/mL) was made up to 2.0 mL with 0.1 mM DPPH in methanol. After 5 min, the absorbance was measured at 517 nm. The reduction of DPPH was calculated relative to the measured absorbance of the control. The obtained data were compared to the calibration curve of catechin. The results were expressed as an antioxidant activity equivalent of catechin (CE) in mmol/100 g of dry extract (mean ± SD of at least three measurements) [7].

Evaluation of antioxidant activity by scavenging of peroxynitrite

Peroxynitrite solutions (10.0 mM, 8 µL) in 0.1 mM NaOH were drawn and mixed rapidly in the injector of the HPLC autosampler with 1.0 mM tyrosine solution in 0.05 mM KH_2PO_4-Na_2HPO_4 buffer (42 µL, pH = 6.0) containing the tested extract (0.1 mg/mL) and DMSO (in a 1:1 ratio with water). The reaction mixture was injected directly into a HP 1100 HPLC system (Agilent Technologies, Germany) equipped with an autosampler, quaternary pump and DA detector. The separation was carried out on a Supelcosil ABZ + Plus column (250 × 4.6 mm, 5 µm particle size, column temperature 25 °C); the mobile phase consisted of 90% 40 mM HCOOH and 10% CH_3CN (v/v) at a flow rate of 1 mL/min. The chromatograms were detected at 276 nm ± 20 nm. The obtained results were compared to the calibration curve of catechin. Data were expressed as an antioxidant activity equivalent of catechin (CE) in mmol/100 g of dry extract (mean ± SD of at least three measurements) [8].

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Sample Availability: Available from the authors.