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Antioxidant activity of different extracts of dried and frozen fruits of *Schisandra chinensis* (Turcz.) Baill

A. V. Terzieva[1], R. Z. Vrancheva[2*], N. D. Delchev[2]

[1]*Student of specialty "Analysis and Control of Food Products",*
Department of Analytical Chemistry, University of Food Technologies-Plovdiv,
26 Maritza Str., 4000 Plovdiv, Bulgaria

[2] *Department of Analytical Chemistry, University of Food Technologies-Plovdiv,*
26 Maritza Str., 4000 Plovdiv, Bulgaria

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Schisandra chinensis has been used in traditional Chinese medicine for hundreds of years. In the last decades, the pharmacological and chemical studies of this drug revealed various valuable biological and pharmacological activities, such as antihepatotoxic, antioxidant, detoxificant, anticarcinogenic, tonic and anti-inflammatory effects. The purpose of current study was to assess the antioxidant activity and phenolic profiles of different extracts (water by maceration at ambient temperature; water, 70% and 96% ethanol extracts by heat-reflux extraction at 70°C) of dried and frozen fruits of *Schisandra chinensis* (Turcz.) Baill. Antioxidant potential of investigated extracts was determined by four most applied spectrophotometric methods, namely DPPH, ABTS, FRAP and CUPRAC. The 70% ethanol extracts of dried fruits showed the highest antioxidant activity by all of the tested methods. The highest antioxidant potential of frozen fruits was defined in water extract obtained by heat-reflux extraction at 70°C. Besides, that were the extracts with highest total phenol contents, defined by Folin-Ciocalteu`s assay. HPLC analysis of phenol profiles of the investigated extracts revealed the presence of chlorogenic, p-cumaric and sinapic acids.

The established strong antioxidant activity of analyzed extracts of dried and frozen fruits of *S. chinensis* is a prerequisite for their future application as natural preservatives in different food systems.

Key words: *Schisandra chinensis, antioxidants, phenol acids, biological activity, HPLC*

INTRODUCTION

Currently, about 50 000-70 000 plant species are used in food and cosmetic industries, as well as in folk and traditional medicine all over the world. Apart from their better organoleptic impact, the plants also amplify the chemical composition of food with biologically active substances that exhibit high antioxidant, antimicrobial, antiviral, antitumor, immunostimulatory and other valuable activities for human health [1].

Antioxidants are important compounds used against oxidative damages in food systems and free radical induced oxidative stress-associated diseases in humans, such as cancer, atherosclerosis, neurodegenerative diseases and inflammation [2].

In the recent years, there was increased interest in the natural antioxidants with plant origin because of the proved toxic, mutagenic and carcinogenic effects of commercial synthetic ones [3]. Usually, the antioxidant potential of the plant extracts was related to their polyphenolic compounds with strong redox properties (absorbed and neutralized free radicals, quenched singlet and triplet oxygen or decomposed peroxides) [4]. Besides, they exhibit other valuable biological properties, such as, cardioprotective, antimutagenic, antibacterial, anti-viral and anti-inflammatory activities [5, 6]. Considering the important role of phenolic compounds in human health and nutrition, it is mandatory to provide new data on their amounts or variety in medicinal plants and natural foods.

Schisandra chinensis is a traditional Chinese medicine and has been used for hundreds of years [7].

The dried fruits of *Schisandra chinensis* are extensively used in traditional medicine to treat asthma, gonorrhea, enuresis, dysentery, diarrhea, diabetes, atopic dermatitis and others [8]. Fruits of this species also exhibited anti-inflammatory, antiviral, anticancer and neuroprotective effects [9, 10].

Chemical composition of *S. chinensis* consists of amino acid, polysaccharides, sesquiterpenes, vitamins, organic acids, volatile oil, lignans and triterpenoids. Most of the biological activities of this plants were associated with the presence of lignans and triterpenoids that exhibit hepatoprotective, anti-inflamotory, antioxidant, anti-HIV and anticancer activities [11].

Although the fruits of *S. chinensis* have been

* To whom all correspondence should be sent.
E-mail: radka_vrancheva@yahoo.com

used in traditional medicine for many years, their health-promoting properties are still being investigated, and new extraction methods were searched in order to use this fruits in a better way in the food and pharmaceutical industries [12]. The differences in the composition and antioxidant activity of plant extracts were due to the genetic factors, the degree of maturity of plants, cultivation techniques, post-harvest handling, storage conditions and solvents and conditions applied for extractions [13]. Therefore the purpose of current study was comparative analysis of antioxidant activity and phenol profile of different extracts from dried and frozen fruits of *S. chinensis.*

MATERIALS AND METHODS

Plant material

The samples of *Schisandra chinensis* were cultivated in the area of Pleven city (Bulgaria). Part of the fruits was stored in freezer at -18ºC. Fresh fruits were dried in shade at ambient temperature and grounded before analysis.

Extraction procedure

Each fresh and frozen sample (1.0 g) was extracted three times with 10 mL of the relevant extraction solvent (96% ethanol, 70% ethanol (v/v) and distilled water) under reflux-heat at 70ºC for 20 min. Water extracts obtained by maceration were extracted three times for 24 h with 10 mL distilled water at ambient temperature. The residue of plant material was removed through filter paper filtration and the combined extracts were evaporated to dryness under vacuum. The dried extracts were stored in refrigerator at 4ºC in dark and dissolved in appropriate solvent before analyses.

Total phenolics

The total phenolic contents were measured using a Folin-Ciocalteu assay according to the procedure described by Stintzing *et al.* [14] with some modifications. Folin - Ciocalteu reagent (1mL) (Sigma) diluted five times was mixed with 0.2 mL of sample and 0.8 mL 7.5% Na_2CO_3 (Sigma). The reaction was 20 min at room temperature in darkness. After reaction time, the absorption of sample was recorded at 765 nm against blank sample, developed by the same way but without extract. The results were expressed in mg equivalent of gallic acid (GAE) per g dry weight (DW), according to calibration curve, build in range of 0.02 - 0.10 mg.mL^{-1} gallic acid (Sigma) used as a standard.

HPLC analysis

Qualitative and quantitative determination of phenolic acids was performed by using Elite LaChrome (Hitachi) HPLC system equipped with DAD and ELITE LaCHrome (Hitachi) software. Separation of the phenolic acids was performed by Supelco Discovery HS C_{18} column (5 µm, 25 cm × 4.6 mm), operated at 30ºC under gradient conditions with mobile phase consist of 2% (v/v) acetic acid (solvent A) and acetonitrile (solvent B). The gradient program used was: 0-1 min – 95% A and 5% B; 1-40 min: 50% A and 50% B; 40-45 min: 100% B; 46-50 min: 95% A and 5% B. The detection of phenolic acids was carried out at 280 and 320 nm and the flow rate was 0.8 mL.min^{-1}.

Antioxidant activity

Antioxidant activity of the obtained extract was defined by four spectrophotometric methods: DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), FRAP (ferric reducing antioxidant power) and CUPRAC (cupric reducing antioxidant capacity), described previously by Ivanov *et al.* [15].

Statistical analysis

Three independent extracts with the relevant solvent (96% ethanol, 70% ethanol (v/v) and distilled water) were prepared from the analyzed samples of fresh and frozen fruits and each extract was analyzed for total phenols, phenolic acid content and antioxidant activitiy in triple replication. The presented values are means (n = 3) with standard deviations (± SD). Figures were made by Microsoft Office Excel ® 2010.

RESULTS AND DISCUSSIONS

Total phenols

The highest total phenol content was defined in the 70% ethanol extract of dried fruits, as the water extract, obtained by maceration was with the lowest amount of phenols. Water extract obtained under reflux-heat at 70°C showed the highest total phenol content, among the investigated extracts of frozen fruits of *S. chinensis* (Table 1).

Cai *et al.* [16] also reported that alcohol extract (80% methanol) of dried fruits of *S. schinensis* contained about five times higher amount of total phenols (1.05 g for 100 g DW) compared to that in the water extract obtained in the same conditions (in a water bath shaker at 80°C). Pliszka *et al.* [12] found that 80% methanol extract of frozen fruits contain 128.3 ± 5.5 mg GAE for 100 g FW total phenols. This value is close to the total phenol content determined in the water extract under heat-reflux in our study (Table 1).

Table 1. Total phenolic content of extracts of dried and frozen fruits of *S. schinensis*

Extracts	Total phenols in dried fruits, mg GAE.g^{-1} DW	Total phenols in frozen fruits, mg GAE.g^{-1} FW
96% ethanol	1.08 ± 0.02	0.84 ± 0.03
70% ethanol	2.15 ± 0.06	0.55 ± 0.01
water	0.91 ± 0.01	0.68 ± 0.02
water,70°C	1.76 ± 0.26	1.23 ± 0.02

Considering that water is non-toxic and eco-friendly solvent it is more appropriate solvent for obtaining the extracts with high phenols content compared to the 80% methanol.

HPLC analysis

Provided HPLC analysis of obtained extracts revealed the presence of chlorogenic, *p*-coumaric and sinapic acids (Table 2). The dominant phenolic acid in all of the investigated extracts is chlorogenic acid. That is of great importance because this phenolic acid has been shown to possess multiple beneficial properties, including analgesic, anti-carcinogenic, antioxidant, anti-diabetic, anti-inflammatory, anti-microbial, cardioprotective, hypotensive and neuroprotective effects [17]. Sinapic and *p*-coumaric acids were in highest concentration in the 70% ethanol extract of dried fruits and water extract of frozen fruits, obtained under heat-reflux at 70°C. These phenolic acids also possessed valuable biological activities. It was found that sinapic acid has antiinflammatory effect by suppressing production of some proinflammatory mediators [18]. *p*-coumaric acid could be used against oxidative stress by protecting DNA from oxidative damages [19].

HPLC-UV-MS analysis of extracts of dried fruits of *S. schinensis* revealed the presence of chlorogenic (3.26 ± 0.25 μg.g^{-1} plant material), *p*-coumaric (<0.02 μg.g^{-1} plant material) and gentisic acids (<0.02 μg.g^{-1} plant material) [20].

Comparing with the results obtained by Mocan *et al.* [20] our samples were with higher concentration of phenolic acids. The differences could be explained with the different extraction method used, as well as genetic factors, geographical and climate conditions, type of soil, season of harvesting, drying and storage of herbs.

Analysis of antioxidant activity

The highest ability to scavenge DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ radicals possess 70% ethanol extract of dried fruits of *S. schinensis.* This extract also showed the highest reduction ability defined by FRAP and CUPRAC methods (Fig. 1). Besides, this was the extract with highest total phenol content (Table 1) and highest amount of chlorogenic, *p*-coumaric and sinapic acids (Table 2).

Among the investigated extracts of frozen fruits the highest antioxidant activity defined by all of the tested methods possessed the compounds extracted with water under reflux-heat at 70°C (Fig. 2). The highest antioxidant potential of this extract could be explained with the highest amount of total phenols and phenolic acids compared to the other extracts. The other three extracts (96% ethanol, 70% ethanol, water extracts, obtained at ambient temperature) of frozen fruits showed similar antioxidant potential.

Hot water extracts of dried and frozen fruits of *S. schinensis* were with higher total phenol content and antioxidant activity than the water extracts, obtained by maceration at ambient temperature. Heating the water extracts at 70°C resulted in obtaining the extracts with higher contents of biologically active compounds.

The observed differences in the total phenol content, phenolic acid concentration and antioxidant activity of the investigated extracts could be explained with the different solvent and methods for extraction used, as well as different type of used fruits (dried and frozen).

Table 2. HPLC analysis of phenolic acids of obtained extracts of dried and frozen fruits of *S. schinensis.*

Extract	Chlorogenic acid, μg.g^{-1} DW	*p*-coumaric acid, μg.g^{-1} DW	Sinapic acid, μg.g^{-1} DW	Chlorogenic acid, μg.g^{-1} FW	*p*-cumaric acid, μg.g^{-1} FW	Sinapic acid, μg. g^{-1} FW
96% ethanol	297.9 ± 5.2	104.5 ±1.6	32.6 ± 0.9	32.4 ± 2.1	18.8 ± 0.5	10.4 ± 1.1
70% ethanol	556.2 ± 6.4	157.3 ± 1.4	62.2 ± 2.7	19.2 ± 1.1	14.2 ± 1.4	11.8 ± 1.1
Water	133.5 ± 1.8	77.3 ± 1.1	26.2 ± 0.8	21.4 ± 1.4	16.3 ± 1.4	12.1 ± 1.4
Water, 70°C	265.1 ± 3.1	89.6 ±1.5	28.9 ± 0.8	55.6 ± 3.5	28.3 ± 2.1	16.5 ± 1.4

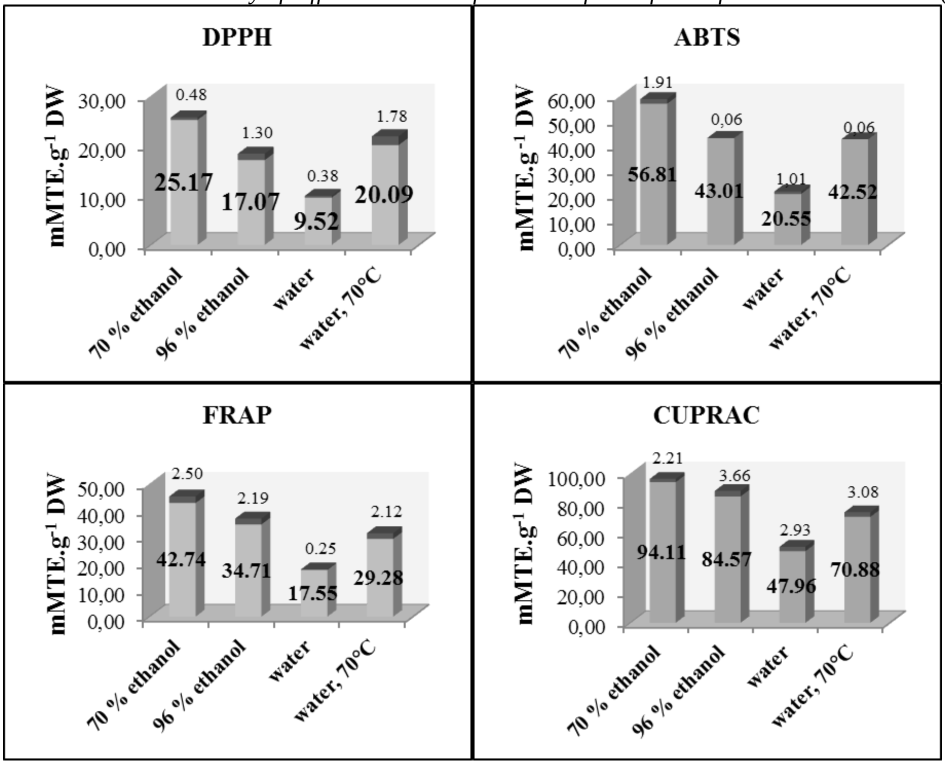

Figure 1. Antioxidant activity of extracts of dried fruits of *S. schinensis* defined by DPPH, ABTS, FRAP and CUPRAC methods.

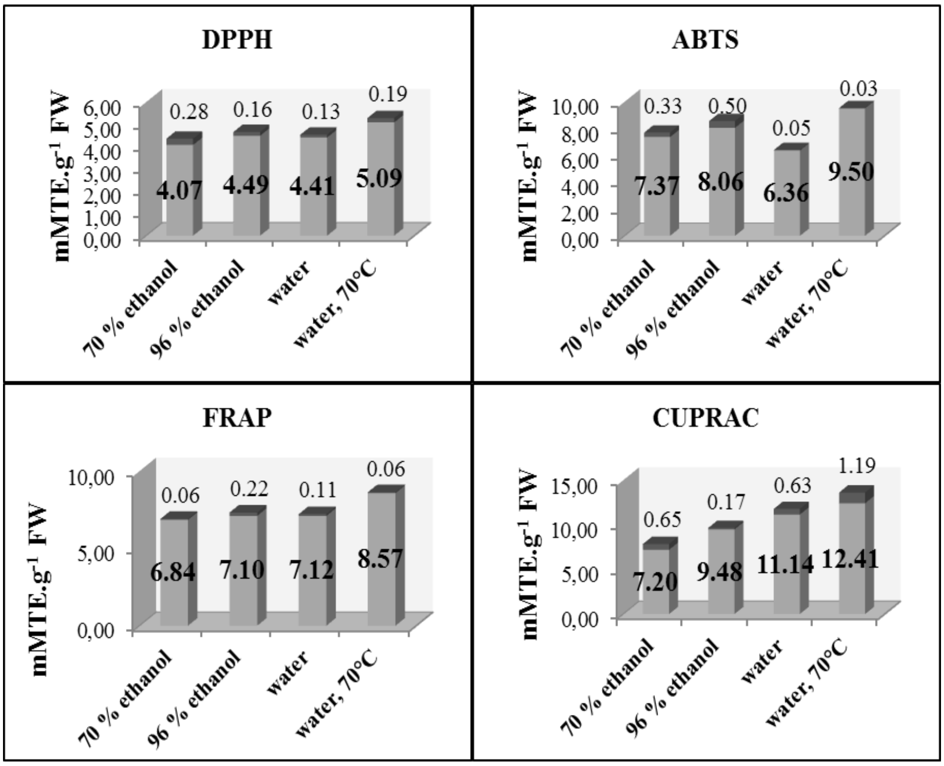

Figure 2. Antioxidant activity of extracts of frozen fruits of *S. schinensis* defined by DPPH, ABTS, FRAP and CUPRAC methods.

CONCLUSION

The present study indicates that the selected solvents are able to obtain extracts from *S. chinensis* fruits with a high antioxidant activity and high phenolic acids content. This is a base for potentially future application of *S. chinensis* fruits as source of natural antioxidants in different food systems.

REFERENCES

1. N. J. Temple, *Nutrition Research*, **20,** 449 (2000).
2. S. C. Jeong, R. Tulasi, S. R. Koyyalamudi, *Cancers*, **8,** 1 (2016).
3. B. Kaurinovic, M. Popovic, S. Vlaisavljevic, S. Trivic, *Molecules*, **16**, 7401 (2011).
4. D. Benedec, L. Vlase, D. Hanganu, I. Oniga, *Dig. J. Nanomater Biostruct.*, **7,** 1263 (2012).
5. J. Dai, R. J. Mumper, *Molecules*, **15**, 7313 (2010)
6. M. Serafini, R. Bellocco, A. Wolk, A. M. Ekstrom, *Gastroenterology*, **123**, 985 (2002).
7. Y. Lu, D.-F. Chen, *J. Chromatogr. A.*, **1216** 1980 (2009).
8. K.-P. Lee, S. Kang, S.-J. Park, J.-M. Kim, J.-M. Lee, A.-Y. Lee, H.-Y. Chung, Y.-W. Choi, Y.-G. Lee, D.-S. Im, *J. Ethnopharmacol.*, **173** 361 (2015).
9. L. Y. Guo, T. M. Hung, K. H . Bae, E. M. Shin, H. Y. Zhou, Y. N. Hong, S. S. Kang, H. P. Kim, Y. S. Kim, *Eur. J. Pharm.*, **591**, 293 (2008).
10. S. J. Kim, H. Y. Min, E. J. Lee, Y. S. Kim, K. Bae, S. S. Kang, S. K. Lee, *Phytother. Res.,* **24**, 193 (2010).
11. L. Zhang, H. Chen, J. Tian, S. Chen, *Ind. Crops Prod.*, **50**, 690 (2013).
12. B. Pliszka, G. Huszcza-Ciołkowska, E. Wierzbicka, *Acta Sci. Pol. Technol. Aliment*., **15**, 57 (2016).
13. A. Dabija, C. G. C. Pop, A. Buculei, I. Rebenciuc, Annals and Proceedings of DAAAM International 2011, **22,** 1003 (2011).
14. F. C. Stintzing, K. M. Nerbach, M. Mosshammer, R. Carle, W. Yi, S. Sellappan, C. C. Acoh, R. Bunch, P. Felker, *J. Agric. Food Chem.*, **53**, 442 (2005).
15. I. Ivanov, R. Vrancheva, A. Marchev, N. Petkova, I. Aneva, P. Denev, V. Georgiev, A. Pavlov, *Int. J. Curr. Microbiol. App. Sci.*, **3**, 296 (2014).
16. Y. Cai, Q. Luo, M. Sun, H. Corke, *Life Science*s. **74,** 2157, (2004).
17. M. Plazas, J. Prohens, A. Cuñat, S. Vilanova, P. Gramazio, F. Herraiz, I. Andújar, *Int. J. Mol. Sci.*, **15,** 17221 (2014).
18. K. Yun, D. Koh, S. Kim, S. J. Park, J. H. Ryu, D. Kim, J. Lee, K. Lee, *J. Agric. Food Chem.*, **56**, 10265 (2008).
19. F. Guglielmi, C. Luceri, L. Giovannelli, P. Dolara, M. Lodovici, *Br. J. Nutr*., **89**, 581 (2003).
20. A. Mocan, G. Crişan, L. Vlase, O. Crişan, D. C. Vodnar, O. Raita, A.-M. Gheldiu, A. Toiu, R. Oprean and I. Tilea, *Molecules*, **19**, 15162 (2014).

АНТИОКСИДАНТНА АКТИВНОСТ НА РАЗЛИЧНИ ЕКСТРАКТИ ОТ ИЗСУШЕНИ И ЗАМРАЗЕНИ ПЛОДОВЕ ОТ *Schisandra chinensis* (Turcz.) Baill.

А. В. Терзиева[1], Р. З. Вранчева[2]*, Н. Д. Делчев[2]

[1]Студент от специалност „Анализ и контрол на хранителните продукти", катедра „Аналитична химия", Университет по хранителни технологии-Пловдив, бул. „Марица" №26, 4000 Пловдив

[2] Катедра „Аналитична химия", Университет по хранителни технологии-Пловдив, бул. „Марица" №26, 4000 Пловдив

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(Резюме)

Китайският лимонник *(Schisandra chinensis)* е използван в традиционната китайска медицина в продължение на стотици години. През последните десетилетия, фармакологичните и химични изследвания на това растение разкриха различни ценни биологични и фармакологични активности, като антихепатотоксична, антиоксидантна, детоксикираща, антитуморна, тонизираща и противовъзпалителна активност. Целта на настоящото изследване е да се определи антиоксидантна активност и фенолния профили на различни екстракти (воден при стайна температура, воден, 70% и 96% етанолен екстракт чрез екстракция на водна баня при 70 °C и обратен хладник) на изсушени и замразени плодове от *Schisandra chinensis* (Turcz.) Baill. Антиоксидантния потенциал на изследваните екстракти е определен чрез четири най-често използвани спектрофотометрични методи, а именно DPPH, ABTS, FRAP и CUPRAC. 70% етанолен екстракт от сушените плодове притежава най-висока антиоксидантна активност, определена чрез всички тествани методи. Най-висок антиоксидантен потенциал на замразените плодове е определен във водния екстракт, получен чрез екстракция на обратен хладник при 70° С. Освен това, тези екстракти са с най-високо съдържание на общо феноли, определени по метода на Folin-Ciocalteu. Чрез HPLC анализ на фенолният профил на изследваните екстракти се установи наличието на хлорогенова, *p*-кумарова и синапова киселина.

Установената висока антиоксидантна активност на изследваните екстракти от изсушени и замразени плодове от *S. chinensis* е предпоставка за бъдещото им прилагане като естествени консерванти в различни хранителни системи.

Ключови думи: *Schisandra chinensis, антиоксиданти, фенолни киселини, биологична активност, HPLC*