Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.4.31

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (4): 849–852

ORIGINAL PAPER

Evaluation of the antioxidant activity of *Berberis jaeschkeana* C. K. Schneid. fruits using the ABTS assay

Anna Och [1], Agata Ćwikła [1], Karolina Orzechowska [1], Anna Cwener [2]

[1] Department of Pharmaceutical Botany, Medical University of Lublin, Lublin, Poland
[2] Botanical Garden of Maria Curie-Skłodowska University in Lublin, Lublin, Poland

ABSTRACT

Introduction and aim. The assessment of the antioxidant activity of plant extracts is an important research direction in the search for new chemopreventive substances. The aim of this study was to demonstrate the antioxidant potential of the methanol extract of *Berberis jaeschkeana* C. K. Schneid fruit.

Material and methods. Spectrophotometric tests were carried out: testing of the total content of polyphenols (TPC) in extracts, testing of the total content of flavonoids (TFC) in extracts. Antiradical activity was determined using the improved ABTS+• decolorization test with modifications.

Results. TPC extracts was determined at the level of 43.52±2.37 mg/g, while TFC extracts was determined at the level 6.08±0.48 mg/g. The ABTS activity test showed 31.15 mg/g expressed as mg Trolox per g of dry extract.

Conclusion. It was assessed that the methanolic extract of the fruit of Berberis jaeschkeana C. K. Schneid. has an antioxidant properties, what makes it a potential chemopreventive agent in civilizational diseases.

Keywords. antioxidants, *Berberis jaeschkeana* C. K. Schneid., chemoprevention, civilization disease, fruit extract, traditional medicine

Introduction

Plants have been used for medical purposes for several thousand years. In ancient times, they were the only source of obtaining substances with medicinal properties. Botanical ingredients include chemical compounds that inhibit or delay the oxidation process and play a key role in chemoprevention. The search for new plant species as sources of antioxidants is one of the most important tasks in modern science.[1]

Free radicals are molecules or atoms that contain unpaired electrons. They arise through endogenous processes necessary for life, but also as a result of the influence of external factors, for example, radiation, air pollution, and pose a threat to the body and its proper functioning. Currently, there is a great demand for compounds with antioxidant properties that prevent the harmful effects of free radicals, both in the field of cosmetology and in the prevention of lifestyle diseases. Most antioxidants are produced synthetically, but natural antioxidants are more effective, including secondary plant metabolites.[2]

Berberis jaeschkeana C. K. Schneid. is a shrub that grows up to 1 m high. Leaves – oblong elliptical, serrated, 1-2 cm wide. Yellow flowers, gathered in clusters of 3-5. Fruit – red oblong-oval berries. Thick angular branches with spines 1-1.5 cm long. It is native to Assam, the East Himalaya, Nepal, Pakistan, Tibet, the West Himalaya[3,4] (Fig. 1, 2 and 3). All parts of the plant contain the alkaloid berberine most concentrated in the roots, stems and inner bark. In the fruits there is the

Corresponding author: Anna Och, e-mail: annaoch@umlub.pl

Received: 1.09.2023 / Revised: 15.09.2023 / Accepted: 20.09.2023 / Published: 30.12.2023

Och A, Ćwikła A, Orzechowska K, Cwener A. Evaluation of the antioxidant activity of Berberis jaeschkeana C. K. Schneid. fruits using the ABTS assay. *Eur J Clin Exp Med.* 2023;21(4):849–852. doi: 10.15584/ejcem.2023.4.31.

lowest concentration of this alkaloid. The fruit of this genus *Berberis* L. are edible.[5,6]

Fig. 1. *B. jaeschkeana* C. K. Schneid. fruits

Fig. 2. *B. jaeschkeana* C. K. Schneid. shrub

Fig. 3. *B. jaeschkeana* C. K. Schneid. dried stems with fruits

Material and methods

Plant material and reagents

The plant material of *Berberis jaeschkeana* C. K. Schneid. fruits were obtained from the Maria Curie-Skłodowska University Botanical Garden in Lublin in October 2022. The raw material was separated and dried at room temperature in the shade with ventilation. The raw material was weighed and ground in an electric mill and portioned, vacuum packed, and stored in a closed package at –30°C until the start of the tests. Trolox, gallic acid, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+), Folin-Ciocalteu reagent was purchased from Sigma-Aldrich (Stenheim, Germany); methanol and aluminium chloride hexahydrate of analytical grade were purchased from POCH (Gliwice, Poland).

Sample extraction and process

A 2 g amount of powdered *B. jaeschkeana* C. K. Schneid. fruits were extracted by accelerated solvent extraction (ASE). Accelerated solvent extractions with an 80% methanol concentration (3 cycles for 10 min each at 80°C) were performed on an ASE 150 system from Dionex Corporation (Sunnyvale, CA, USA). The extract was prepared in triplicate. The extract obtained was evaporated to dryness under reduced pressure and lyophilised in a Free Zone 1 apparatus (Labconco, Kansas City, KS, USA). Samples for testing were prepared immediately prior to analysis by dissolving them in an ultrasonic bath. A weighed amount of the extract after lyophilization was dissolved in a measuring volume of 80% methanol to obtain starting solutions with a concentration of 40 mg/mL. As required for the determinations, it was diluted with the same solvent to a specific concentration.

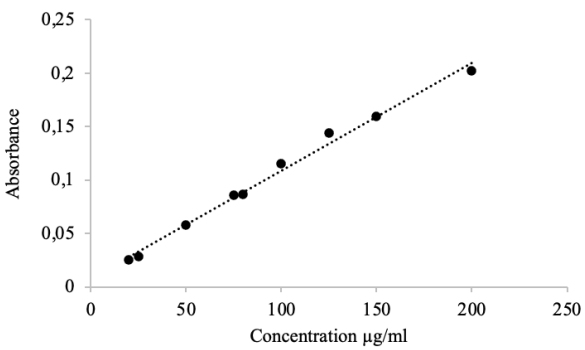

Fig. 4. Standard curve for gallic acid for TPC testing – a graph of absorbance versus gallic acid concentration

Determination of total phenolic (TPC) and total flavonoid contents (TFC)

The analysis of the total phenolic content was carried out using the modified Folin–Ciocalteu method.[8] The TPC was determined using a standard curve prepared for gallic acid (Fig. 4). The absorbance was read at 680 nm after a 20-min incubation using the Tecan microplate reader Infinite 200 Pro-Elisa with I-control Tecan system (Mannedorf, Switzerland). The results were expressed in mg of gallic acid per 1 g of dry weight of dry extract – gallic acid equivalent. The total flavonoid

content was determined according to the method proposed by Lamaison and Carret with modifications. The TFC was determined using a standard curve prepared for quercitin (Fig. 5). The absorbance was measured at 430 nm after a 30 min incubation against a blank containing methanol instead of the test sample. Results were expressed in mg of quercetin per 1 g of dry extract.

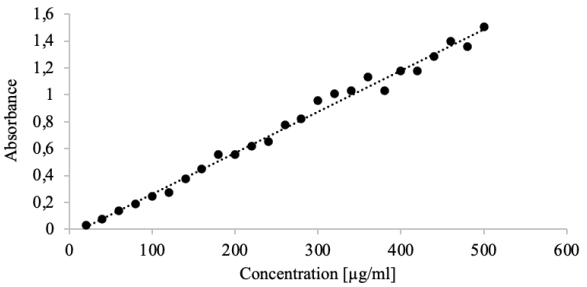

Fig. 5. Standard curve for quercetin for TFC testing – a graph of absorbance versus quercetin concentration

Antiradical activity analysis

Antiradical activity was determined with the ABTS+• discolouration test, with modifications.[9,10] The ability of the extract to quench ABTS+• free radicals was determined using equation:

$$\text{Capture \%} = [(AC - AA)/AC] \times 100,$$

where: AC is the absorbance of the control and AA is the absorbance of the sample. The absorbance was measured at 734 nm after a 6-min incubation. The results were obtained from measurements made for each sample and expressed as milligrammes of Trolox of dry extract (Trolox equivalents).

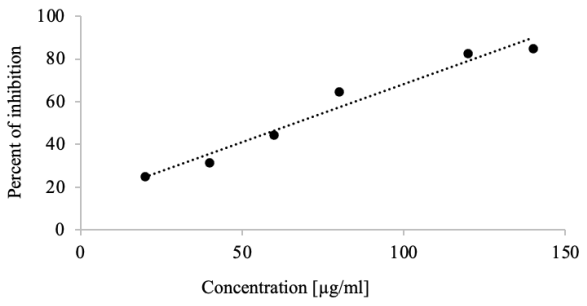

Fig. 6. Standard curve for Trolox for testing anti-radical activity – a graph of the dependence of the percentage of inhibition on the concentration of Trolox.

Results

The tests carried out allowed for the determination of the total polyphenol content in the TPC extracts (total phenolic content), expressed as the equivalent of gallic acid in plant extracts (mg/g) at the level of 43.52±2.37 mg/g. The analysis performed allowed the determination of the flavonoids in the TFC extracts, expressed as the equivalent quercetin in the tested extracts at the level of 6.08±0.48 mg/g. The ABTS activity test showed significant antioxidant potential of the *B. jaeschkeana* C.K. Schneid. fruit extract. Antiradical capacity (ABTS+•) expressed as mg Trolox per g of dry extract was 31.15 mg/g.

Discussion

Despite the long tradition of medicinal use of barberry species, relatively few of them have been tested in terms of chemical composition, chemopreventive potential and nutraceutical use. The content of polyphenols and flavonoids as well as antioxidant properties were determined only for selected species of *Berberis* L. For example, in fresh fruits of *Berberis heteropoda*, the TPC value was 68.55 mg GAE/g, expressed in milligrams of gallic acid equivalent per gram of fresh fruit weight, and the TFC value was 108.42 mg QE/g, expressed in milligrams of rutin equivalent per gram of fresh fruit pulp.[11] For *Berberis cretica*, the TPC value was determined to be 190 mg GAE/g, and for *B. sibirica*, the TPC value was determined to be 159 mg GAE/g.[12,13] Traditional eastern medicine knows genus from the antimicrobial activity of its roots rich in alkaloids. It is known that fruits of *Berberis* L. contain lower level of alkaloids so they are safe and eadible.[5,6] Until now antioxidant potential of this fruits has been very poorly examined. Bewal et al. has assessed the influence of extraction method on antioxidant metabolites in *B. jaeschkeana* C.K. Schneid. fruits and they obtained higher level of TPC and TFC using microwave extraction.[14,15] The fruits of *Berberis jaeschkeana* C.K. Schneid. examined in this study seem to be worth further research because it is as valuable in this respect as other species of the genus *Berberis* L. Thea are source of antioxidants which can be used as a chemoprevention in nutraceutics or can be potential as antioxidants in cosmetology.

Conclusion

B. jaeschkeana C.K. Schneid. are characterised by a high content of polyphenols and high antioxidant activity. Therefore, they can be considered as chemoprevetive agent. It can be also considered for potential use in cosmetology as natural antioxidants or for naturally extending the shelf life of food products.

Declaration

Funding

This research received no specific grant from any funding agency.

Author contributions

Conceptualization, A.O.; Methodology, A.O.; Software, A.O; Validation, A.O. and A.C.; Formal Analysis, A.O.; Investigation, K.O. and A.Ć.; Resources, A.O.; Data Cu-

ration, A.C.; Writing – Original Draft Preparation, A.O.; Writing – Review & Editing, A.O. and K.O.; Visualization, A.O.; Supervision, A.O.; Project Administration, A.O.; Funding Acquisition, A.O.

Conflicts of interest

The author declare no competing interests.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

Not applicable.

References

1. Bober Z, Stępień A, Aebisher D, Ożóg Ł, Bartusik-Aebisher D. Fundamentals of the use of Berberis as a medicinal plant. *Eur J Clin Exp Med*. 2018;16(1):41–46. doi: 10.15584/ejcem.2018.1.7
2. Seung-Kwon M. Efficacy of vitamin and antioxidant supplements in prevention of cardiovascular disease: systematic review and meta-analysis of randomised controlled trials. *BMJ*. 2013;346:f10 doi: 10.1136/bmj.f10
3. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines*. 2018;5(3):93. doi: 10.3390/medicines5030093
4. eFloras. http://www.efloras.org. Accessed May 9, 2023.
5. Temperate Plants Database. http://www.temperate.theferns.info. Acessed August 1, 2023.
6. Medicinal Herbs. http://www.naturalmedicinalherbs.net/ Acessed August 1, 2023.
7. PlantNet. https://identify.plantnet.org. Accessed August 28, 2023.
8. Singleton VL, Rossi JA. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am J Enol Vitic.* 1965;16:144-158. doi 10.5344/ajev.1965.16.3.144
9. Pieczykolan A, Pietrzak W, Nowak R, Pielczyk J, Łamacz K. Optimization of Extraction Conditions for Determination of Tiliroside in *Tilia* L. Flowers Using an LC-ESI-MS/MS Method. *J Anal Methods Chem.* 2019;9052425. doi: 10.1155/2019/9052425
10. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radic Biol Med.* 1999;26:1231-1237. doi: 10.1016/S0891-5849(98)00315-3
11. Sun J, Li Q, Li J, Liu J, Xu F. Nutritional Composition and Antioxidant Properties of the Fruit of Berberis Heteropoda Schrenk. *PLoS ONE.* 2022;17:e0262622. doi: 10.1371/journal.pone.0262622
12. Kukula-Koch W, Aligiannis N, Halabalaki M, Skaltsounis AL, Glowniak K, Kalpoutzakis E. Influence of Extraction Procedures on Phenolic Content and Antioxidant Activity of Cretan Barberry Herb. *Food Chem.* 2013;138:406-413. doi: 10.1016/j.foodchem.2012.10.045
13. Kukula-Koch W, Koch W, Angelis A, Halabalaki M, Aligiannis N. Application of PH-Zone Refining Hydrostatic Countercurrent Chromatography (HCCC) for the Recovery of Antioxidant Phenolics and the Isolation of Alkaloids from Siberian Barberry Herb. *Food Chem.* 2016;203: 394-401. doi: 10.1016/j.foodchem.2016.02.096
14. Belwal T, Giri L, Bhatt ID, Rawal RS, Pande V. An improved method for extraction of nutraceutically important polyphenolics from *Berberis jaeschkeana* C.K. Schneid. Fruits. *Food Chemistry*. 2017;15(230):657-666. doi: 10.1016/j.foodchem.2017.03.086
15. Belwal T, Bhatt ID, Cravotto G. Effect of ultrasound on extraction and stability of polyphenols from *Berberis jaeschkeana* C.K. Schneid fruits: A comparative study. *Sustainable Chemistry and Pharmacy*. 2022;27:100649. doi: 10.1016/j.scp.2022.100649