SHORT COMMUNICATION

Investigation of phenolic compounds at the leaves and shoots Arctostaphylos spp. and their antioxidant and antityrosinase activities

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ABSTRACT
Comparative phytochemical and pharmacological analysis of leaves and shoots of Arctostaphylos uva-ursi (L.) Spreng. and endemic of Transcaucasia and North Caucasia Arctostaphylos caucasica Lipsch. was carried out. Phenolic compounds in methanol extracts were investigated by HPLC-DAD-ESI-MS. Pharmacological investigation was carried out at the models of generation of superoxide, DPPH, hydroxyl and nitrosyl radicals. The antityrosinase properties were evaluated in the reaction of tyrosinase inhibition using L-tyrosine as a substrate. Five new compounds were identified in the shoots and leaves of A. uva-ursi: 4,6-di-\text{C142}-galloylglucose; 2,3-di-\text{C142}-galloylglucose; 1,4,6-tri-\text{C142}-galloylglucose; 1,2,4,6-tetra-\text{C142}-galloylglucose; tetra-\text{C142}-galloylhexose. Thirty-five phenolic compounds and two saponins were identified for the first time in A. caucasica. Water-alcohol extract (70% ethanol) from A. caucasica had the highest antiradical activity. Water extracts from both species had antityrosinase activity and possessed comparable efficiency (IC\textsubscript{50} = 36.4 ± 1.2 \mu g/ml and IC\textsubscript{50} = 35.5 ± 2 \mu g/ml, respectively).

ARTICLE HISTORY
Received 12 October 2021
Accepted 25 December 2021

KEYWORDS
Arctostaphylos uva-ursi (L.) Spreng.; Arctostaphylos caucasica Lipsch.; HPLC-DAD-ESI-MS; antioxidant activity; antityrosinase activity

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2021.2025370.

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1. Introduction

Genus *Arctostaphylos* according the data base The Plant List in 2021 includes 75 species (The Plant List 2021). Two species of genus *Arctostaphylos* grow at the area of Russian Federation: *Arctostaphylos uva-ursi* (L.) Spreng. and relict endemic of Transcaucasia and North Caucasia *Arctostaphylos caucasica* Lipsch. (Czerepanov 1995; Takhtadjan 2009), for which there are not any data about chemical content and pharmacological activity. *Arctostaphylos uva-ursi* (L.) Spreng. is included in national pharmacopoeias of different countries as diuretic and antiseptic agent due to presence total amount phenologlycosides, the dominant among which is arbutin (Linnenbrink and Kraus 1986; Kubo et al. 1990). Analysis of literature data showed that *Arctostaphylos uva-ursi* (L.) Spreng. is a rich source for its chemical content including not only phenologlycosides (Sticher et al. 1979; Matsuda et al. 1992; Alam et al. 2011), but flavonoids, phenylpropanoids, galloylglycosides and catechins (Olennikov and Chekhirova 2013), polysaccharides (Olennikov and Nazarova 2009), iridoids (Jahodar et al. 1978), saponins (Takada et al. 2010; Caligiani et al. 2013), essential oil (Radulović et al. 2010), macro- and microelements (Afanasyeva and Ayushina 2018). It is known that in the folk medicine bearberry leaves are used for their antihelmintic, astringent, sedative, haemostatic, adaptogenic properties, and normalize metabolism (Budancev and Lesiovskaya 2001). Several scientists detected antibacterial (Larsson et al. 1993), nephrolytic (Grases et al. 1994), diuretic (Beaux et al. 1999), depigmenting (Matsuda et al. 1992), anti-inflammatory (Kubo et al. 1990), antidiabetic (Swanson-Flatt et al. 1989), neuroprotective (Chandler et al. 2010), antioxidant (*in vitro*) (Mohd Azman et al. 2016) and antiproliferative activities (Amarowicz and Pegg 2013). Also it is important to control quality of *A. uva-ursi* leaves and products from its. For this purpose HPLC-PDA-ESI-MS can be used. It is a modern analytical method using to get fingerprint identification or to get away from column and preparative chromatography and investigated crude extract without additional purification (Gallo et al. 2013).

Due to high rule of tyrosinase in the melanogenesis inhibitors of this enzyme often are used in cosmetic purposes as depigmentation agents or for therapy myeloleucosis (Furue and Tsuji 2019). Also the prevention of oxidative reactions is a potential target of pathogenic therapy of wide range of pathological conditions (Zhang et al. 2015).

So the aim of this work was investigation of phenolic complex in the leaves and shoots of both species of genus *Arctostaphylos* and evaluation of direct antioxidant
2. Results and discussion

Thirty-four phenolic compounds were identified at the leaves and sprout of *Arctostaphylos uva-ursi*, five of them for the first time: 4,6-di-O-galloylglucose; 2,3-di-O-galloylglucose; 1,4,6-tri-O-galloylglucose; 1,2,4,6-tetra-O-galloylglucose; isomers of tetra-O-galloylhexose (Figure S1). These results were compared with work of Olennikov (Olennikov and Chekhirova 2013). All of thirty-seven compounds were identified at the first time for *A. caucasica* (Figure S2, Table S1) due to this species is not investigated previously. The main phenolic compounds for both species are: arbutin; 4,6-di-O-galloylglucose; 2,3-di-O-galloylglucose; 1,6-di-O-galloylglucose; 6-O-galloylarbutin; 1,4,6-tri-O-galloylglucose; 1,2,4,6-tetra-O-galloylglucose; tetra-O-galloylhexose; quercetin-3-O-galactoside; 1,2,3,4,6-penta-O-galloylglucose. Mass spectra of several compounds are presented in the Figure S3. Results of quantitative analysis of compounds are showed in the Table S2 and validation parameters are presented in the Table S3.

The complex of pharmacological tests of the analysed dry residues allowed us to establish that the most pronounced antiradical activity has the extract from *A. caucasica* obtained by extraction with 70% alcohol. *IC*\(_{50}\) in all cases for this compound was comparable with the reference trolox solution. Less indirect antioxidant activity was characterized by extracts that were obtained by extracting raw materials with ethyl alcohol 40% and 95% concentration, and the level of activity did not depend on the type of raw materials that was used (Table S4). Dry residues of aqueous extracts from *A. caucasica* and *A. uva-ursi* did not show significant antiradical activity.

The antiradical activity of the test extracts may be a background for their use in the correction of oxidative stress. It has been established that oxidative stress is part of the pathogenesis of numerous diseases, such as atherosclerosis, hypertension, diabetes mellitus, chronic heart failure, Alzheimer’s disease and some types of cancer. It is known that the main trigger of oxidative stress is the hyperproduction of free radicals (ROS), represented by superoxide and hydroxide radicals, as well as reactive forms of nitrogen (nitrosyl radical, peroxonitrite). Having a non-specific effect on the cell, ROS are extremely cytotoxic agents (Poprac et al. 2017). At the same time, as a number of numerous studies show, the suppression of ROS production or their effects is the leading method of treating oxidative stress. The group of direct scavengers of ROS stands out, which combines both synthetic and natural objects; moreover, plant extracts occupy a leading position (Parham et al. 2020). The obtained results suggest the presence of ROS scavengers in ethanol extracts, as evidenced by the value of *IC*\(_{50}\) in the assessment of superoxide, hydroxyl, and nitrosyl antiradical activity. Also, in addition to direct antioxidant activity, the ethanol extracts under study may be characterized by the presence of reductive properties, which is confirmed by the data of the DPPH test (Sirivibulkovit et al. 2018). The high antioxidant properties of the studied extracts are probably associated with the presence of a large number of polyphenolic compounds.
An assessment of the anti-tyrosinase properties was shown that the extracts from *A. caucasica* and *A. uva-ursi* obtained by water extraction demonstrate the most pronounced tyrosinase-inhibiting properties similar to kojic acid (Table S4). At the same time, ethanol extracts did not have anti-tyrosinase activity. The obtained data open up certain prospects for the use of aqueous extracts from the therapy of a number of diseases, the basis of which is abnormal melanogenesis due to the high content of arbutin.

Melanogenesis is a complex process represented by a complex cascade of enzymatic reactions, in which the determining role is assigned to proteins related to tyrosinase TYRP1 and TYRP2-multifunctional copper-containing metalloenzymes that catalyze the reaction that limits the rate of melanin synthesis. Therefore, it can be assumed that tyrosinase inhibition may be one of the approaches to correcting skin hyperpigmentation. Among tyrosinase inhibitors, hydroquinone and kojic acid are the most well-known, the use of which in clinical practice is limited by a significant number of side effects. Thus, hydroquinone is recommended to be used only in combination with isotretinoin (in the gel form), which significantly increases the drug load on the patient (Chandra et al. 2012). Kojic acid is a natural metabolite produced by fungi that inhibits tyrosinase. Kojic acid is characterized by high biocompatibility, which, however, does not increase the effectiveness and does not contribute to reducing the toxicity of the compound (Saeedi et al. 2019). Thus, medicines obtained on the basis of water extracts of *A. uva-ursi* and *A. caucasica* can be a safer alternative to existing depigimenting agents.

3. Conclusions

The comparative study of chromatographic profiles both species of bearberry, *A. uva-ursi* and *A. caucasica* were carried out also as chromatographic profile of extracts from leaves and shoots *A. caucasica* were obtained at the first time. Thus, medicines obtained on the basis of water extracts of *A. uva-ursi* and *A. caucasica* can be a safer alternative to existing depigimenting agents. At the same time, water-alcohol extraction based on raw materials *A. caucasica* and *A. uva-ursi* can have a wide range of therapeutic applications due to the presence of anti-radical activity.

Acknowledgments

Authors acknowledge Pyatigorsk Medical-Pharmaceutical Institute (PMPI), Branch of Volgograd State Medical University; Institute of General and Experimental Biology, Siberian Branch, Russian Academy of Science; Kursk State Medical University (KSMU).

Disclosure statement

The authors declare that they don’t have any conflict of interest.

Funding

The author(s) reported there is no funding associated with the work featured in this article.
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