Journal of Herbal Medicine 8 (2017) 17–23

Contents lists available at ScienceDirect

Journal of Herbal Medicine

journal homepage: www.elsevier.com/locate/hermed

Research paper

Arbutin and its metabolite hydroquinone as the main factors in the antimicrobial effect of strawberry tree (*Arbutus unedo* L.) leaves

Karlo Jurica[a,*], Ivana Gobin[b], Dario Kremer[c], Dubravka Vitali Čepo[c], Renata Jurišić Grubešić[c], Irena Brčić Karačonji[d], Ivan Kosalec[c]

[a] Special Security Operations Directorate, Ministry of the Interior, Ulica grada Vukovara 33, Zagreb, Croatia
[b] Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[c] Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia
[d] Institute for Medical Research and Occupational Health, Zagreb, Croatia

ARTICLE INFO

Article history:
Received 12 September 2016
Received in revised form 11 March 2017
Accepted 15 March 2017
Available online 18 March 2017

Keywords:
Arbutus unedo leaves
Antioxidative properties
Antimicrobial activity
Arbutin
Hydroquinone
Urinary pathogens

ABSTRACT

Strawberry tree (*Arbutus unedo* L.) leaves are used in folk medicine for treating inflammation, hypertension, and diabetes. The aims of this study were to evaluate the antimicrobial effects of *A. unedo* leaf extracts on 15 uropathogens and test the extent to which arbutin, as the most abundant bioactive compound in the leaves, is responsible for their antimicrobial activity. Antimicrobial activity of leaf extracts against urinary pathogenic microorganisms was tested by agar well diffusion assay and twofolded microdilution method. Since the polyphenolic content responsible for *A. unedo* leaves' antimicrobial activity depends on climate and geolocation, the authors determined the total phenolic, tannin, flavonoid and phenolic acid content using spectrophotometric methods, arbutin and hydroquinone mass fraction using high performance liquid chromatography coupled with a diode array detector (HPLC–DAD) and antioxidative activities by FRAP, ABTS, and DPPH assay. Although methanol was shown to be a more effective solvent for the extraction of polyphenols from leaves, the traditionally used aqueous extract exhibited similar antimicrobial properties. While arbutin did not show direct antimicrobial activity, its metabolite hydroquinone showed strong antimicrobial activity against the tested uropathogens. The strongest antimicrobial activity of leaf extracts was detected for uropathogenic strains of *Enterococcus faecalis*, which was probably associated with the ability of bacterial β-glucosidase, exerting strong activity in *E. faecalis*, to convert arbutin to hydroquinone. Our study suggested that the aqueous extract of strawberry tree leaves has the potential for use as a phytotherapeutic in clinical application and should be further investigated.

1. Introduction

Plants produce secondary metabolites like polyphenols that play a vital role in the antioxidant and antimicrobial activity of strawberry tree leaves. The significance of phenolic compounds in their antimicrobial activity has been well-documented and there is a growing interest in plant extracts that could be used as an alternative to current antimicrobial agents with increasing antimicrobial resistance (Cushnie et al., 2003; Ferreira et al., 2012).

The strawberry tree (*Arbutus unedo* L., Ericaceae) is an evergreen shrub that grows in the Mediterranean region, Asia Minor, and Western Europe (Amel, 2013; Malheiro et al., 2012; Mariotto et al., 2008). Its leaves have uroantiseptic, diuretic, and astringent properties and they have been used in folk medicine for treating inflammation, hypertension, and diabetes (Oliveira et al., 2009). A number of constituents such as tannins, flavonoids, phenolic, and iridoid glucosides have been found in the phenolic fraction of strawberry tree leaves exerting potent antioxidative and antimicrobial activity (Carcache-Blanco et al., 2006; Males et al., 2006; Sanjust et al., 2008).

The phenolic glycoside arbutin was identified as the main bioactive compound in *A. unedo* leaves (Fiorentino et al., 2007; Oliveira et al., 2011) High arbutin content was also present in the *Ericaceae* species *Arctostaphylos uva-ursi* (L.) (bearberry), well known in herbal medicine as an ancient urinary antiseptic and astringent (European Medicines Agency, 2012; Pavlović et al.,

* Corresponding author.
E-mail address: juricakarlo@gmail.com (K. Jurica).

http://dx.doi.org/10.1016/j.hermed.2017.03.006
2210-8033/

2009). Arbutin is absorbed from the gastrointestinal tract where it begins to split up into aglycone hydroquinone and glucose by intestinal microflora under the influence of enzyme β-glucosidase (Blaut et al., 2006). The antimicrobial effect of arbutin is directly dependent on β-glucosidase activity (European Medicines Agency, 2012). Hydroquinone is recognized as an active substance at the site of action (urinary tract) and it might be important for the therapeutic activity of a herbal preparation (Blaut et al., 2006; Schindler et al., 2002). Since polyphenolic content (including arbutin content) in *A. unedo* leaves depends on climate and geolocation, we investigated the Croatian population of this plant, abundant in the country's coastal area.

To the best of the authors knowledge, there is no published data on the leaf phenolic acid content or the leaf extract antimicrobial activity for *Klebsiella pneumoniae* ESBL and the clinically isolated microorganisms' strains used in this research. Furthermore, there is no data characterizing the antioxidant and antimicrobial properties of the Croatian population of *A. unedo*. The aims of this study were therefore to determine the content of the major antioxidant compound groups: total phenols, tannins, flavonoids, and phenolic acids and *in vitro* antioxidant and antimicrobial activity of aqueous and methanolic extracts of *A. unedo* leaves. For comparative purposes, the aqueous extract as traditionally used was tested along with methanol, described in the literature as the most effective solvent regarding phenolic extraction from different herbal extracts (Khoddami et al., 2013). In addition, the antimicrobial properties of arbutin as the main compound and hydroquinone as the active metabolite were determined and compared with the antimicrobial properties of *A. unedo* leaf extracts so as to test the extent to which arbutin is responsible for antimicrobial activity.

2. Materials and methods

2.1. Leaf samples

A. unedo leaves were randomly collected in May 2013 on the island of Mali Lošinj (GPS coordinates: 44°31′50″ N; 14°28′06″ E; 14 m a.s.l.). The collected leaves were dried in a dark place at room temperature. Herbal material was identified by Dr. Dario Kremer and deposited in the Fran Kušan Pharmaceutical BotanicalGarden at the Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia. The dried leaves were ground in a laboratory mill and stored in plastic containers at room temperature until analysis.

2.2. Extraction

Powdered dried leaves of *A. unedo* were extracted with water and methanol. The sample (3 g of powdered leaves mixed with 80 mL of water or methanol) was placed in an ultrasound bath (Bandelin, Sonorex) at 50 °C for 60 min with vortexing every 15 min. Extracts were cooled to room temperature, centrifuged at 4000*g* for 15 min and filtered through a thick filter paper. Aliquots of water and methanolic extracts were used for determination of total phenolic and tannin content and antioxidant activity. For the determination of antimicrobial properties, water extracts were lyophilized while methanolic extracts were evaporated under reduced pressure at 40 °C (Heidolph Laborota 4000 efficient, HB digital) before lyophilisation. Both *A. unedo* lyophilized extracts were stored in a desiccator due to the plant's hygroscopic characteristics. Yields for water and methanolic extracts were 28.6 and 32.4%, respectively. To standardize the extract, we determined the total phenolic, tannin, flavonoid and phenolic acid content and the content of arbutin, the most abundant bioactive compound.

2.3. Phytochemical investigations

2.3.1. Total phenolic content

Total phenolic content was determined in water and methanol extracts using the Folin-Ciocalteu spectrophotometric assay (Gao et al., 2002) with slight modifications. Two hundred microliter of diluted extract (1:50, v/v) was mixed with 1.35 mL of water and 150 μL of Folin-Ciocalteu reagent. The reaction mixture was incubated at room temperature for 5 min and mixed with 1.5 mL of Na_2CO_3 solution (6% w/w). Absorbance was measured at 725 nm after 30 min at 50 °C and the results were expressed as mg of gallic acid equivalents (GAE) per g of dry leaf weight.

2.3.2. Total tannin content

Total tannin content was determined by the method of Makkar et al. (1993) using the polyvinyl-polypyrrolidone (PVPP) to precipitate tannins. Briefly, 30 mg of PVPP was mixed with 2 mL of water, shaken to yield a suspension, and then mixed with 2 mL of diluted leaf extract (1:50, v/v). The solution was well shaken and allowed to stand at 4 °C for 30 min with occasional vigorous shaking. Then, the suspension was centrifuged for 10 min at 3000 rpm and clear supernatants were used for the determination of non-tannin phenolics by the Folin-Ciocalteu method described in Subsection 2.3.1. Tannin content was calculated as the difference between total phenolic and non-tannin phenolic content in the extract.

2.3.3. Total phenolic acids content

The amount of total phenolic acids was determined by spectrophotometric method according to the Rosmarini folium monograph from European Pharmacopoeia (2005). Briefly, 0.200 g of powdered dried leaves was mixed with 80 mL of 50% ethanol and then extracted in a boiling water bath with a reflux condenser for 30 min. After cooling, the extract was filtered into a volumetric flask and diluted with 50% ethanol up to 100 mL. One milliliter of the resulting extract was mixed with 2.0 mL of 0.5 M HCl, 2.0 mL of nitrite-molybdate reagent (10 g of sodium nitrite and 10 g of sodium molybdate was dissolved in 100 mL of distilled water), 2.0 mL of 8.5% sodium hydroxide solution and with distilled water up to 10 mL. A compensatory solution was made by diluting 1.0 mL of extract with distilled water up to 10 mL. Mass fraction (%) of total hydroxycinnamic acid derivatives, expressed as rosmarinic acid, was calculated according to the formula: $A \times 2.5/m$, where A was absorbance of the test solution at 505 nm, and m was the substance mass in grams.

2.3.4. Total flavonoids content

The amount of total flavonoids was determined by a spectrophotometric method according to Christ and Müller (1960). Powdered dried leaves (0.2 g) were separately extracted for 30 min with 20 mL of acetone, 2 mL of 25% HCl and 1 mL of 0.5% solution of hexamethylenetetramine by heating in a water bath with a reflux condenser until water was brought to the boil. The hydrolysate was passed through cotton wool, and drug residues were extracted again with 20 mL of acetone, heated to boiling for 10 min. This solution was passed through cotton wool again, and the previously described extraction with acetone was repeated three times. The combined filtrates were diluted with acetone to 100 mL. Then, 20 mL of the hydrolysate was mixed with 20 mL of water, extracted with 15 mL of ethyl acetate and after that three times with 10 mL of ethyl acetate. Combined ethyl acetate phases were washed twice with 40 mL of water, then passed through cotton wool and diluted with ethyl acetate to 50 mL. Ten milliliter of this solution was transferred to two 25 mL flasks. A total of 0.5 mL of 0.5% aqueous sodium citrate was added to each flask. In one flask, 2 mL of a solution of aluminium chloride (2 g of aluminium chloride hexahydrate dissolved in 100 mL of 5%