Food Chemistry 127 (2011) 1540–1548

Contents lists available at ScienceDirect

Food Chemistry

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

Achillea millefolium L. s.l. herb extract: Antioxidant activity and effect on the rat heart mitochondrial functions

Sonata Trumbeckaite [a,b,*], Raimondas Benetis [c], Lina Bumblauskiene [a], Deividas Burdulis [a], Valdimaras Janulis [a], Adolfas Toleikis [b], Pranas Viškelis [d], Valdas Jakštas [a]

[a] Department of Pharmacognosy of Lithuanian University of Health Sciences, A. Mickeviciaus Str. 9, LT-44307 Kaunas, Lithuania
[b] Institute for Biomedical Research of Lithuanian University of Health Sciences, Eiveniu Str. 4, LT-50009 Kaunas, Lithuania
[c] Department of Drug Chemistry of Lithuanian University of Health Sciences, A. Mickeviciaus Str. 9, LT-44307 Kaunas, Lithuania
[d] Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Kauno Str. 30, LT-54333 Babtai, Kaunas Reg., Lithuania

ARTICLE INFO

Article history:
Received 2 November 2010
Received in revised form 1 February 2011
Accepted 3 February 2011
Available online 2 March 2011

Keywords:
Achillea millefolium
Yarrow
Mitochondria
Antioxidant
Phenolic compounds
Flavonoids

ABSTRACT

An extract of *Achillea millefolium* herb (YE) was investigated for antioxidant activity using chemical and biological assays. Qualitative and quantitative analysis of some major phenolics was carried out by HPLC. An on-line HPLC-DPPH assay showed that YE possesses significant antiradical activity which is due to the presence of active components amongst phenolic compounds. Furthermore, direct effects of YE and a mixture of its identified phenolic compounds (MPC) on isolated rat heart mitochondrial function were investigated. We found that YE in concentration-dependent manner induce a decrease in State 3 respiration rate without any changes in the integrity of inner mitochondrial membrane. Thus, pyruvate oxidation was affected only by the highest used YE concentrations; meanwhile succinate oxidation was reduced even at lower YE concentrations. MPC had no effect on mitochondrial State 3 respiration rate. Fluorimetric measurements demonstrated that YE at concentrations that had no effect on the State 3 respiration rate significantly decreased H_2O_2 production in mitochondria.

1. Introduction

The genus *Achillea* L. (yarrow) comprises over 100 perennial herb species indigenous to the Northern Hemisphere (Si, Zhang, Shi, & Kiyota, 2006). In Lithuania *Achillea millefolium* L. s.l., the best-known and most widespread species of yarrow, is listed amongst the most commonly used plant species in both folk and conventional medicine (Radušienė & Gudaitytė, 2005). The diversity and complexity of the phytochemical composition of yarrow species may explain their polyvalent pharmacological activity. The raw material of *A. millefolium* L. s.l. contains terpenes, alkaloids and bases, tannins, coumarins, saponins, sterols, vitamins, amino and fatty acids (Blumenthal, Goldberg, & Brinckmann, 2000; Si et al., 2006). Phenolic compounds, such as flavonoids and phenolcarbonic acids, constitute one of the most important groups of pharmacologically active principles in yarrow. It is suggested that anti-inflammatory (Blumenthal et al., 2000), antimicrobial (Aljancic et al., 1999), choleretic (Benedek, Geisz, Jager, Thalhammer, & Kopp, 2006) and cytotoxic (Trifunović et al., 2006) activities of *Achillea* plants are mainly attributed to the flavonoid and phenolcarbonic acid complex. However, further scientific investigations are still required to clarify its phytochemicals as well as the spectrum of their potential utilisation.

Oxidation reactions and the decomposition of oxidation products are major causes of deterioration of various food products. To prevent these processes, antioxidants are widely used as additives in some foods. Owing to increased safety concerns about synthetic antioxidants and their possible involvement in chronic diseases, research efforts have been directed toward natural antioxidants (Shahidi, 2000). Medicinal plants and their extracts constitute one of the most important targets to search for new sources of natural antioxidants for consideration as components for functional ingredients and nutraceuticals as well as feasible and natural alternatives to synthetic antioxidants in the food industry. Since plant-derived antioxidants are generally considered to be multifunctional and their activity depends on various parameters, any herb or its extract should be thoroughly tested involving several methods of assessing antioxidant activity.

It has been shown that the anti-diabetic (Yazdanparast, Ardestani, & Jamshidi, 2007) and gastroprotective (Potrich et al., 2010) properties of extracts from *Achillea* plants may be

* Corresponding author at: Department of Pharmacognosy of Lithuanian University of Health Sciences, A. Mickeviciaus Str. 9, LT-44307 Kaunas, Lithuania. Tel.: +370 37 302968; fax: +370 37 302959.
E-mail addresses: sonatai@centras.lt (S. Trumbeckaite), raimondas.benetis@med.kmu.lt (R. Benetis), lina3b@yahoo.com (L. Bumblauskiene), dvdsburdulis@yahoo.com (D. Burdulis), farmakog@kmu.lt (V. Janulis), adolfas.toleikis@kmu.lt (A. Toleikis), viskelis@parabole.lt (P. Viškelis), valdasjakstas@itc.kmu.lt (V. Jakštas).

0308-8146/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2011.02.014

linked to their antioxidant potential, therefore, it is of high importance to investigate their antioxidant effectiveness. Recent reports indicate that the *Achillea* genus displays a relevant antioxidant activity that is associated or correlated well with its flavonoid and total phenolic contents (Giorgi, Mingozzi, Madeo, Speranza, & Cocucci, 2009; Konyalioglu & Karamenderes, 2005). However, the observed similarities or close correlation between the profiles of the antioxidant capacity and of the total phenolic and flavonoid contents must be interpreted with care, since the latter parameter is usually measured using traditional spectrophotometric assays, which are based on non-specific reaction of phenolic compounds with Folin–Ciocalteu's reagent and complexation of flavonoids with Al(III) (Naczk & Shahidi, 2004).

The relevance of botanicals as antioxidants in biological systems cannot be reliably predicted according to the results shown solely using the assays based on chemical reactions. Thereby they should be tested using *in vitro* biological techniques (MacDonald-Wicks, Wood, & Garg, 2006). Thus, for a more thorough evaluation of the antioxidant potential of the *A. millefolium* herb extract (YE), it is important to investigate its behaviour in both chemical (free radical-scavenging activity) and biological systems (for instance, in isolated mitochondria). Mitochondria are an important intracellular source of reactive oxygen/nitrogen species (ROS/RNS) and, on the other hand, are also a critical target of their damaging effects (Cadenas, 2004). Moreover, oxidative stress-mediated impairments in mitochondrial function are considered to be implicated in a large variety of diseases and patho-physiological processes, such as cardiovascular, neurodegenerative diseases, ageing and carcinogenesis (Gellerich et al., 2004). Assessment of influence of YE on cellular energy metabolism would gain more insights into the mechanism of their action by counteracting detrimental effects of ROS. As mitochondria produce most of the cell ATP required for cell function and play an important role in health and diseases, the characterisation of functional status of mitochondria exposed to various biologically active compounds is of great importance.

Nowadays, international attention has been directed toward bioactive food components such as polyphenols that could have the potential to modulate mitochondrial function. Our previous study showed that flavonoids (quercetin and its derivatives as rutin, hyperoside, quercitrin) even at low concentrations cause concentration-dependent heart mitochondrial uncoupling, while at higher concentrations they reduce mitochondrial State 3 respiration rate (Trumbeckaite et al., 2006). Moreover, we revealed a powerful uncoupling effect of *Ginkgo biloba* leaves extract (Baliutyte, Baniene, Trumbeckaite, Borutaite, & Toleikis, 2010; Trumbeckaite et al., 2007) and slight uncoupling effect of *Crataegus monogyna* fruit extract (Bernatoniene et al., 2009), both rich in flavonoids. Recently, it was shown that *A. millefolium* exhibits hypotensive, cardiosuppressant, and vasodilatory activities, thereby confirming the traditional use of yarrow in cardiovascular disorders (Khan & Gilani, 2010). However, despite the fact that many of cardiovascular diseases are associated with disturbances of the mitochondria, to our knowledge, no study has been carried out on the effects of *A. millefolium*, which also contains phenolic compounds such as flavonoids and phenolcarbonic acids, on heart mitochondrial oxidative phosphorylation.

Therefore, the aims of this study were (1) to evaluate antioxidant activity of *A. millefolium* herb extract (YE) assessed by chemical (on-line HPLC-DPPH) and biological (in relevance to ROS production in rat heart mitochondria) systems and (2) to investigate *in vitro* the direct influence of YE on the respiration of isolated rat heart mitochondria.

2. Materials and methods

2.1. Chemicals

Authentic standards of reference compounds apigenin, apigenin 7-*O*-glucoside, luteolin, luteolin 7-*O*-glucoside, luteolin 3′,7-di-*O*-glucoside, rutin, vicenin-2, and chlorogenic acid were purchased from Fluka (Buchs, Switzerland), Roth (Karlsruhe, Germany), ChromaDex (Santa Ana, CA) and Quality Phytochemicals LLC. Trifluoroacetic acid was obtained from Sigma–Aldrich (Seelze, Germany). Acetonitrile, labelled as HPLC grade, was supplied by Sigma–Aldrich (Buchs, Switzerland). Ethanol 96.3% (v/v) was provided by Stumbras (Kaunas, Lithuania). (R)-6-Methoxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox®) was supplied by Acros Organics (Geel, Belgium), while DPPH (1,1-diphenyl-2-picrylhydrazyl) radical, glutamic acid, malic acid, succinic acid, cytochrome *c* from bovine heart, adenosine 5′-diphosphate (ADP) sodium salt , ethylene glycol-bis-(β-aminoethylether)-*N*,*N*,*N*′*N*′-tetraacetic acid (EGTA), Tris–HCl, KH_2PO_4, antimycin A, atractyloside were obtained by Sigma–Aldrich Chemie (Steinheim, Germany). Sodium citrate, citric acid, KCl and magnesium chloride were purchased from Roth (Karlsruhe, Germany). Amplex® Red was purchased from Invitrogen (Carlsbad, CA). All other chemicals used were analytical grade and obtained from Sigma–Aldrich Chemie (Steinheim, Germany) and Fluka (Buchs, Switzerland). Ultrapure water was used throughout and was prepared using a Millipore water purification system (Bedford, MA).

2.2. Preparation of YE

The plant material represents tops of *A. millefolium* L. s.l. plants (~10 cm in length) collected at full flowering stage in 2007 from the field collection of the Institute of Botany, Lithuania. The raw material was air dried at room temperature (20–25 °C), in a ventilated lodge, avoiding direct sunlight for two weeks. Dry material was packed into multilayer paper bags and stored in a dark room at ambient temperature. The air-dried aerial parts of *A. millefolium* were milled at room temperature and sieved using a sieve with 355 μm mesh. The hydroalcoholic extract of powdered plant material was prepared by maceration with 40% ethanol at room temperature (1:10, v/v), initially for 48 h and thereafter until exhaustion. The hydroalcoholic extract obtained was filtered and concentrated under vacuum (at 50 °C) and then subjected to freeze drying. Extract was frozen at −30 °C in a glass jar then freeze dried for 48 h using a laboratory freeze dryer with shell freezer FD8512S (Ilshin Lab. Co., Ltd., Gyeonggi-do Korea). Sublimation was carried out at −80 °C condenser temperature and a pressure of 0.007 mbar. A thermocouple PT100 was used to measure the temperature in the centre of the sample during freeze drying. Freeze-dried yarrow powder was packed into a glass jar.

2.3. Quantitative analysis of phenolic compounds by HPLC

HPLC analysis of YE was performed using a liquid chromatographic Waters 2690 Alliance HPLC system (Waters Corporation, Milford, MA) equipped with Waters 2487 dual λ absorbance detector (UV/Vis) and a Waters 996 photodiode array (PDA) detector. Separations were carried out using a 5-μm Ascentis™ RP-Amide analytical column (150 × 4.6 mm) with guard column 5-μm Supelguard™ Ascentis™ RP-Amide (20 × 4.00 mm) (Supelco, Bellefonte, PA). The chromatographic separation was carried out using 0.1% trifluoroacetic acid solution in water as solvent **A** and 0.1% trifluoroacetic acid solution in acetonitrile as solvent **B** with a previously published gradient elution programme (Benetis, Radušienė, Jakštas, Janulis, & Malinauskas, 2008). The elution was monitored