Food Chemistry 271 (2019) 216–223

Contents lists available at ScienceDirect

Food Chemistry

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

Anticholinergic effects of *Actinidia arguta* fruits and their polyphenol content determined by liquid chromatography-photodiode array detector-quadrupole/time of flight-mass spectrometry (LC-MS-PDA-Q/TOF)

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ARTICLE INFO

Keywords:
LC-MS-PDA-Q/TOF
Flavonols
Flavanols
Phenolic acid
Anthocyanin
AChE
BuChE
Inhibition

ABSTRACT

This study discusses polyphenolic compounds identified and quantified in *Actinidia arguta* fruits by LC-MS-PDA-Q/TOF method and *in vitro* anticholinergic activity. Notably, of 31 compounds, including 16 flavonols, 7 flavanols, 7 phenolic acids, and 1 anthocyanin were identified or tentatively identified on the basis of their retention times, accurate mass measurements and subsequent mass fragmentation data, or by comparison with reference substances and literature. Among the detected compounds, 27 were reported for the first time in *A. arguta* fruits. The content of total polyphenols equal 845.54 mg/100 g dry weight (dw), and flavanols predominat (92% of total phenolic compounds). Flavonol derivatives, mainly glycosylated and acetylated forms of quercetin (22.64 mg/100 g dw) and kaempferol (18.40 mg/100 g dw) were quantified. The total content of phenolic acids was 29.63 mg/100 g dw, and neochlorogenic acid predominant. This anticholinergic activity effect of *A. arguta* fruits can be explained by the Pearson's correlation found between flavonols (r = 0.709 and 0.678), phenolic acids (r = 0.513 and 0.487), flavan-3-ols (r = 0.466 and 0.443) and anthocyanins (r = 0.312 and 0.301) for acetylcholinesterase (AChE) or butylcholinoesterase (BuChE), respectively. The data compiled from the quantitative polyphenol indicate that *A. arguta* fruits could be regarded as a promising source of bioactive functional food.

1. Introduction

Human diet is important in protection against oxidative stress and degenerative diseases, such as Alzheimer's or Parkinson's disease (Hasbal, Yilmaz-Ozden, Can, 2015; Morris et al., 2002). Its health-protecting role has been partly attributed to compounds with antioxidant capacity, e.g. phenolic, with fruits and vegetables serving as major sources of dietary antioxidants (Schijlen, Ric de Vos, van Tunen, & Bovy, 2004). Therefore, one of the current research trends is to demonstrate a relationship between fruit consumption and reduced risk of diseases or prevention of different health conditions.

Kiwi belongs to the genus of Actinidia, which is extremely diverse and comprises about 65 species (Bursal & Gülcin, 2011). One of the most popular is *Actinidia deliciosa,* introduced to the world market from New Zealand in 1950s, and now most popularly grown in Chile, Korea, China, and Japan. Nowadays, one of the most important species is *Actinidia arguta* ((Sieboldet Zucc.) Planch. ex Miq., called 'grape kiwi', 'northern kiwi', 'baby kiwi', 'kiwi berry', 'arctic kiwi', 'mini kiwi', 'hardy kiwifruit' or 'Bower Actinidia'). In recent years, it has gained popularity in the USA, and some European countries, e.g. Poland, Switzerland, and France (Latocha & Jankowski, 2011).

Current trends and worldwide development on new fruits with multiple functionalities aim at demonstrating significant bioactivity of tropical or exotic fruits. Actinidia species bear fruits that offer health benefits and are attractive to consumers (Park et al., 2011). Previous studies demonstrated that biologically active compounds from kiwi fruit exhibited strong antioxidant and protective activity against cardiovascular diseases, both *in vitro* (Jung et al., 2005) and *in vivo* (Duttaroy & Jørgensen, 2004). According to Collins Horskà, Hotten, Riddoch, and Collins (2001), eating kiwi fruit might provide substantial protection against DNA damage that triggers cancer and, more significantly, might greatly speed up DNA repair. Basic chemical composition and antioxidant activity of kiwi fruits have been described (Wojdyło, Nowicka, Oszmiański, & Golis, 2017) but there are no reports on their anticholinergic effects and inhibition of acetylcholinesterase (AChE; EC 2.3.1.6) and butyrylcholinesterase (BuChE; EC 3.1.1.8).

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https://doi.org/10.1016/j.foodchem.2018.07.084
Received 23 April 2018; Received in revised form 10 July 2018; Accepted 12 July 2018

Human brain contains two major forms of cholinesterases: AChE and BuChE. Inhibition of AChE is a treatment strategy in neurodegenerative diseases such as Alzheimer's disease (Hasbal, et al., 2015; Kösea, et al., 2015). A loss of acetylcholine plays a crucial role in learning and memory deterioration in Alzheimer's patients. The neurotransmitter disturbances and insufficient cholinergic functions are identified among pathological features in the central nervous system disorders. Some studies suggest that polyphenol compounds may slow down mild cognitive impairment in Alzheimer's disease (Morris et al., 2002).

Additionally, the available information on phenolic compounds identified in *A. arguta* fruits is still limited. Research publications from the last decade have provided only general data on the content of total phenolic compounds in *A. arguta* (Latocha, Krupa, Wołosiak, Worobiej, & Wilczak, 2010; Latocha, Wołosiak, Worobiej, & Krupa, 2013; Park et al., 2011) or on the content of such flavonoids as quercetin derivatives and some phenolic acids in *A. deliciosa* (Bursal & Gülcin, 2011). Since then, these fruits have been studied only to a minor extent. Until now, little is known about the leaf flavonols in Actinidia genus species (Webby, Wilson, & Ferguson, 1994).

To improve the situation, the aim of this study was to identify and quantify the phenolic compounds, such as flavanols (monomers and polymers), phenolic acids, flavonol glycosides, and anthocyanins in kiwi fruits. Furthermore, accurate mass measurement technique of LC-MS-PDA-Q/TOF was successfully applied for the first time in *A. arguta* species to elucidate the elemental composition of the investigated polyphenols. Secondary aims of this work were to check potential positive effects by *in vitro* study of anticholinergic activity as cholinesterase inhibitors (acetyl- and butyryl-cholinoesterase, AChE and BuChE). The study involved the fruits of cultivar 'Genewa', one of the most commonly grown cultivar of *A. arguta* species.

2. Material and methods

2.1. Reagents and standards

(−)-Epicatechin, (+)-catechin, procyanidin B_1, B_3, B_4, C_1, caffeic acid, quercetin and kaempferol of: -3-*O*-glucoside, -3-*O*-galactoside, and -3-*O*-rutinoside, and cyanidin-3-*O*-sambubioside were purchased from Extrasynthese (Genay, France). Chlorogenic, neochlorogenic, and cryptochlorogenic acids were purchased from TRANS MIT GmbH (Giessen, Germany). All solvents for LC/MS grade purchased from Sigma-Aldrich (Steinheim, Germany). UPLC grade water prepared by using an HLP SMART 1000s system (Hydrolab, Gdańsk, Poland), was additionally filtered through a 0.22 μm membrane filter immediately before use.

2.2. Plant material

A. arguta fruits 'Genewa' cv. was collected in Sepember 2017 from the Medical Garden of Wrocław Medical Academy (Wrocław, Poland). Fully mature fruits were manually harvested and transported directly to the lab. Fruits (≈500 g; two replications of 20 randomly chosen fruits from three bushes) were directly frozen in liquid nitrogen and after wards were lyophilized 24 h (Christ Alpha 1–4 LSC Freeze dryer; Martin Christ GmbH, Osterode am Harz, Germany), which was pre-cooled to −50 °C for 1 h at 1 mbar. Fruits after liophilization were crushing by laboratory mill (IKA 11A; Staufen, Germany). The collected material was kept at −80 °C until further analyzed, no longer than 5 days.

2.3. Extraction of phenolic compounds

The extraction procedure of polyphenolic compounds was the same as that used by Wojdyło, et al. (2017). Briefly, the freeze-dried powder of fruits (~1 g) was vortexed for 1 min with 5 mL methanol/water/acetic acid/ascorbic acid (30:68:1:1, v/v) and sonicated for 20 min (Sonic 6D, Polsonic, Warsaw, Poland). Two additional extractions were performer for sample with addition 5 mL of the same solvent, as described above, all supernatant was collected after centrifuged at 19.000 ×*g* for 10 min at 4 °C. Finally, the extract was filtred by 0.20 μm hydrophilic PTFE membrane (Millex Simplicity Filter; Merck, Germany) and used for phenolic compounds identification by LC-MS-PDA–Q/TOF and quantification by UPLC-PDA.

2.4. Identification and quantification of phenolic compounds by the LC-MS-PDA-Q/TOF and UPLC-PDA method

Methanolic extracts of *A. arguta* fruits powder were analyzed using an ACQUITY Ultra Performance LC system consisting of an autosampler, binary solvent manager and photodiode array detector (PDA; Waters Corporation, Milford, MA, USA), coupled to mass detector G2 Q/TOF mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization (ESI) as a source operating in negative and positive ion modes with spectra acquired over a mass range from *m/z* 100 to 1800 as described previously by Wojdyło, Nowicka, Carbonell-Barrachina, and Hernández (2016) and Kolniak-Ostek (2016).

2.5. Anticholinergic activity analysis as AChE and BuChE inhibition activity

The inhibition of AChE and BuChE activity was determined based on Ellman's method, as reported previously Gozde, Yilmaz-Ozden, and Can (2015). The results were expressed as % of inhibition.

2.6. Statistical

The correlation Persona were calculated using STATISTICA 12.0 program (StatSoft, Poland) with the mean values of five (n = 5) independent experiments in triplicate.

3. Results and discussion

3.1. Phenolic compounds of A. arguta characterized by LC-MS-PDA-Q/TOF

Fruit extracts of *A. arguta* 'Geneva' cv. was assessed and found to contain a highly complex mixture of flavonoids and phenolic acids.

The molecules that were certainly or putatively identified in negative ion mode belonged to phenolic acids, catechins and procyanidins, and flavonols. Positive ionization was used for identification of compound belonging to anthocyanins. About 200 mass spectrum outputs were studied for each analytical replicate. This procedure allowed for tentative identification of up to 31 compounds – the broadest characterization of the phenolic fingerprint of *A. arguta* fruit to date.

In the present work, sixteen compounds were identified by comparison with reference standards, while the remaining 15 compounds were confirmed by the extract mass data (MS), and the MS/MS-Q/TOF fragmentation pattern, absorption UV of PDA spectrum profiles at 200–600 nm, retention times (R_t), mass measurement errors (Δm), and a comparison with standard reference compounds, when available, and the literature focusing on some fruits and species. Table 1 and Fig. 1 shows the list of 31 compounds identified in *A. arguta* fruits. Little is known on the phenolics in Actinidia species, because only a few of these compounds had been previously detected in Actinidia genus (Webby et al., 1994).

The occurrence of tannic acid, 2,5-dihydroxybenzoic acid and chlorogenic acid in *A. arguta* was reported by Latocha et al. (2010). (+)-Catechin, chlorogenic acid and (−)-epicatechin were reported in *A. arguta* by Kim, Beppu, and Kataoka (2009). However, high-resolution MS analyses revealed the presence of other 27 polyphenols that have been not previously reported in Actinidia genus, especially in *A. arguta* and they were tentatively identified for the first time, as far as we know, in *A. arguta* fruits. The newly characterised phenolic compounds were classified according to the phenol family to which they belong.