SHORT COMMUNICATION

Anticholinesterase, antioxidant activity and phytochemical investigation into aqueous extracts from five species of Agrimonia genus

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Aqueous extracts of aerial flowering parts of five Agrimonia species (Rosaceae): Agrimonia coreana Nakai, Agrimonia japonica (Miq.) Koidz, Agrimonia procera Wallr., Agrimonia eupatoria L. and Agrimonia leucantha Kunze were investigated on their antioxidant activity, measured using five different methods; the best was the extract from A. procera with IC50 values from 6 to 29 μg/mL. All the extracts displayed inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) at the tested concentration of 100 μg/mL. We found the highest inhibition of cholinesterase in the extract of A. japonica with inhibition 70.4% for AChE and 79.8% for BuChE. These findings are statistically significant in comparison with those of other extracts (p < 0.001). The phytochemical analyses showed that the antioxidant activity of Agrimonia extracts can be affected especially by hexahydroxydiphenoyl (HHDP)-glucose and quercetin glycosides, and inhibition of cholinesterases by apigenin, luteolin and quercetin glycosides.

Keywords: Agrimonia species; flavonoids; polyphenols; antioxidant activity; cholinesterase inhibition

1. Introduction

The Agrimonia species have been reported to possess several biological activities, such as antioxidant, anti-inflammatory, antiviral, antibacterial, anti-diabetic and diuretic (Copland et al. 2003; Kwon et al. 2005). Decoction can be used in traditional medicine for the treatment of diabetes mellitus or coronary diseases (Gray & Flatt 1998; Correia et al. 2006). Antioxidants from Agrimonia herbs can prevent oxidative stress associated with diabetes mellitus and could exert a beneficial effect in the diabetic environment (Gray & Flatt 1998). Earlier reports have
revealed that oxidative injury plays a main role also in the pathogenesis of neurodegenerative disorder Alzheimer’s disease (AD), and a high dietary consumption of antioxidants in people may reduce the risk of AD. On the other hand, the inhibition of acetylcholinesterase (AChE) along with butyrylcholinesterase (BuChE), the key enzymes which hydrolysed mediators at the cholinergic synapse, is currently the most established approach to treat AD (Şenol et al. 2010). Thus, antioxidant activity has been also evaluated in our study together with investigation of cholinesterases inhibition abilities. Previous scientific investigations focused on research of the most common *Agrimonia eupatoria* (Gray & Flatt 1998; Kwon et al. 2005). In the methanolic extract of *A. eupatoria* were found flavonoids with a neuroprotective effect (Lee et al. 2010). There is not much information about *Agrimonia japonica* and *Agrimonia coreana* growing in eastern Asia. Therefore, we aimed to investigate them together with *A. eupatoria*, *Agrimonia procera* and *Agrimonia leucantha*.

2. Results and discussion

The HPLC profile of the *A. eupatoria* aqueous extracts was recorded at 254 nm (Figure S1). This extract is the richest of its kind among polyphenolic compounds of all tested *Agrimonia* plants. It contains quercetin, apigenin, luteolin and kaempferol glycosides, compounds with high antioxidant activity. Aglycones were confirmed by the hydrolysis of the extracts. Compounds of the *Agrimonia* extracts identified by HPLC/DAD/ESI-MS/MS (Table S1) as hyperoside, isoquercitrin, rutin, luteolin 7-O-glucoside, luteolin 7-O-glucuronide, apigenin 7-O-glucoside and apigenin 7-O-glucuronide. Isovitexin and vitexin with the fragmentation of mono-C-hexosides were present only in the *A. eupatoria* extract.

Fragment at *m/z* 285 [M − H]− corresponding to kaempferol aglycone and loss sugar moiety in MS spectrum were obtained for kaempferol 3-O-glucoside, kaempferol O-rutinoside and kaempferol O-(coumaroyl)-glucoside. Kaempferol 3-O-(6"O-p-coumaroyl)-glucoside and others kaempferol, quercetin and apigenin glycosides were isolated previously from *A. eupatoria* (Correia et al. 2006). Luteolin glucuronide and apigenin glucuronide were also previously isolated from *Agrimonia pilosa*. They were the major components of the aqueous extract and can improve estrogen deficiency-related menopausal symptoms or can treat diseases in postmenopausal women (Young et al. 2012). Our data indicate that apigenin 7-O-glucuronide is also a dominant flavonoid in *A. japonica* and *A. coreana* and, with regard to the fact that *A. pilosa* Ledeb. is distributed primarily over Korea and Japan, is content of glucuronides chemotaxonomic significance in *Agrimonia* species growing native especially in Eastern Asia. The species of *Agrimonia* have great numbers of synonyms and *Agrimonia japonica* (Miq.) Koidz. has also been referred to as *A. pilosa* subsp. *japonica* (Miq.) H. Hara. or *A. pilosa* var. *japonica* (Miq.) Nakai (Czerepanov 1981).

With a molecular ion at *m/z* 289, [M − H]− was identified as catechin. Dimeric and trimeric procyanidins were also identified by HPLC/DAD/MS in all extracts of *Agrimonia*. These compounds were founded early and support antioxidant activity of extracts (Correia et al. 2006). The antioxidant activity of *Agrimonia* extracts especially *A. procera* and *A. leucantha* can also be affected by hexahydroxydiphenoyl (HHDP)-glucose.

The aqueous extracts from *Agrimonia* herbs exhibited high antioxidant activity (Table S2). The aerial parts of *A. eupatoria* are used as infusion in folk medicine for haemostatic, diuretic and anti-inflammatory properties. *A. procera* is less used than *A. eupatoria* in the Czech Republic, but its polyphenol content is higher than in the aqueous extract of *A. eupatoria*. In a previous study (Venskutonis et al. 2007), the acetone–hexane extract of *A. procera* had demonstrated better DPPH scavenging activity than the acetone–hexane extract of *A. eupatoria*. The aqueous extracts of *A. japonica* also demonstrated strong antioxidant activity.
Table 1 summarises the inhibitory potency of tested extracts against AChE and BuChE. All of the extracts displayed inhibition of enzymes at the tested concentration of 100 μg/mL. According to the data obtained in the study on Scutellaria species (Şenol et al. 2010), our results imply that the extracts showed remarkable activity. We found the best inhibition of cholinesterase activity in the extract of A. japonica. As was reported previously, flavonoid glycosides were shown to be inhibitors of cholinesterases (Jung & Park 2007). From aglycones included in the glycosides of Agrimonia species, the best AChE inhibition potency was reported for luteolin and quercetin and BuChE inhibition potency for apigenin (Katalinic et al. 2010). The dominant flavonoids of A. japonica are apigenin 7-O-glucuronide and rutin.

3. Conclusion
On the basis of our results, the highest antioxidant activity caused by the highest polyphenols content, possess the aqueous extract of A. procera. The best inhibition of cholinesterase activity we found in the aqueous extract of A. japonica which is rich in content of polyphenols and also has high content of flavonoid glycosides. These findings are statistically significant in comparison with other extracts (p < 0.001) A. japonica represents the best dietary adjunct for treatment of AD with high antioxidant activity.

Supplementary material
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