ORIGINAL ARTICLE

Determination of antioxidative properties of herbal extracts: *Agrimonia herba*, *Cynare folium*, and *Ligustri folium*

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Abstract

OBJECTIVES: Hyaluronan (HA) molecules were exposed to free radical-mediated degradation performed by the reaction mixture Cu(II) and ascorbate, the so-called Weissberger biogenic oxidative system, which mimics the situation of acute inflammation. To achieve protection of HA from degradation, herbal extracts such as *Agrimonia herba, Cynare folium,* and *Ligustri folium* were selected.
METHODS: Time- and dose- dependent changes of dynamic viscosity of the HA solutions in the presence and absence of the herbal extracts were recorded by rotational viscometry (RV). Radical scavenging capacity of the extracts was investigated by the spectrocolorimetric ABTS and DPPH assays.
RESULTS: The results of RV revealed that the extracts of *Agrimonia herba* and *Cynare folium* were effective in inhibiting the degradation of HA. On the other hand, the extract of *Ligustri folium* increased the rate of HA degradation. The highest radical scavenging capacity of $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ was observed in *Agrimonia herba* extract followed by the extracts of *Ligustri folium* and *Cynare folium*.

INTRODUCTION

Medicinal plants have been identified and used throughout human history. At least 12,000 compounds synthesized by plants have been isolated so far; a number estimated to be less than 10% of the total. Compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects (Tapsell *et al.* 2006). Many of the pharmaceuticals currently available have a long history of use as herbal remedies (e.g. aspirin, digitalis, quinine, opium). The use of herbs to treat diseases is almost universal, and is often more affordable than purchasing expensive modern pharmaceuticals (Fabricant & Farnsworth 2001). Pharmaceuticals are expensive for the most of the world population. In comparison, herbal medicines can be grown from seed or

gathered from nature for little or no cost. In 2002, the U.S. National Center for Complementary and Alternative Medicine of the National Institutes of Health began funding clinical trials into the effectiveness of herbal medicine. In a 2010 research of 1,000 plants was carried, of which 356 had clinical trials published evaluating their "pharmacological activities and therapeutic applications" while 12% of the plants, although available in the Western market, had "no substantial studies" of their properties (Cravotto *et al.* 2010). Herbal remedies are very common in Europe. Prescription drugs are sold alongside essential oils, herbal extracts, tinctures. Herbal remedies are seen by some as a treatment to be preferred to pure medical compounds, which have been industrially produced (Duke 2000).

Agrimonia herba (commonly known as agrimony) is used as a herbal medicine with little understanding of the underlying mechanism for its therapeutic effects. *Agrimonia* has been listed as one of the 38 plants that are used to prepare Bach flower remedies, a kind of alternative medicine promoted for its effect on health. However according to Cancer Research UK, "there is no scientific evidence to prove that flower remedies can control, cure or prevent any type of disease, including cancer" (Vohra 2005). Previous studies have shown that *agrimonia* possesses anti-bacterial, anti-tumor, and hepatoprotective activities. The pharmacological activities of *agrimonia* may be primarily due to phenolic compounds such as agrimonin, catechin, quercetin, etc. (Jung *et al.* 2010).

Cynare sp. (artichoke) shows interesting tendency of protection against degenerative diseases such as cancer. In folk medicine, many parts of artichoke have been widely used as astringent, blood cleanser, cardiotonic, detoxifier, digestive stimulant, diuretic, hypoglycemic and hypocholesterolemic. Extracts *Cynare folium*, have been used against liver complaints and the extracts or its constituents have been claimed to exert a beneficial action against hepato-biliary diseases and to improve liver regeneration after partial hepatectomy (Elsayem *et al.* 2012). Moreover, *cynare* leaf extracts was proved to have antioxidative and anti-inflammatory properties, antibacterial, anti-HIV and urinative activities as well as the ability to inhibit cholesterol biosynthesis and LDL oxidation. These variable therapeutic functions cannot be attributed to a single active compound, however it could be due to the presence of several bioactive components which generate synergistic pharmacologic effects. The most active components found in *cynare* species consists of flavones, their glycosides, coumarins, sterols caffeoylquinic acids and triterpenoid saponins (Alghazeer *et al.* 2012)

Ligustri folium (privet) is useful in making a tonic that is commonly used as an immune booster (it is often given to patients of cancer or HIV as it helps towards improving their immunity). It is also very good to improve vision. This herb is a part of the traditional remedy for treating vertigo and other related problems. In some cases, it has been shown that regular ingestion can even help in the prevention of cancer. It is also known to have antibacterial and antiviral effects. The herb in its different forms can help many types of various diseases, which result from chronic infection and inflammation. In traditional Chinese medicine, it is commonly used for treatment of chronic bronchitis, hepatitis, hyperlipidemia and inflammatory disorders (Wu *et al.* 2011).

MATERIALS AND METHODS

Chemicals

High-molar-mass HA sample P0207-1 (M_w=970.4 kDa) was purchased from Lifecore Biomedical Inc., Chaska, MN, U.S.A Lyophilized aqueous extract of *Agrimonia* (aerial parts), Cynara (leafs) were obtained from FYTOPHARMA, a.s., Slovakia and lyophilized aqueous extract of Ligustri was produced from leafs collected in arboretum Mlyňany, Slovakia. Further chemicals used were identical to those reported previously (Banasova *et. al.*, 2012, Valachova *et. al.*, 2008).

Preparation of stock and working solutions

The hyaluronan samples (20 mg) were dissolved in 0.15 mol/L of aqueous NaCl solution for 24 h in the dark. HA sample solutions were prepared in two steps: first, 4.0 mL and after 6 h, 3.85 mL of 0.15 mol/L NaCl were added. Solutions of ascorbate (16 mM) and cupric chloride (16 mM diluted to a 160 M solution) were prepared also in 0.15 mol/L aqueous NaCl.

Uninhibited/inhibited hyaluronan degradation

Uninhibited/inhibited hyaluronan degradation was performed as published by Valachova *et al.* (2011).

Rotational viscometry

Dynamic viscosity of the reaction mixture (8 mL; 0.15 mol/L aqueous NaCl) containing HA (2.5 mg/mL), ascorbate (100 μmol/L) plus Cu(II) ions (1 μmol/L) in the absence and presence of herbal extracts (307 μg/mL) was monitored by a Brookfield LVDV-II+PRO digital rotational viscometer (Brookfield Engineering Labs., Inc., Middleboro, MA, U.S.A.) at 25.0±0.1 °C and at a shear rate of 237.6 s^{-1} for 5 h in the Teflon® cup reservoir. The herbal extracts as potential protective agents were introduced into the HA reaction system before or 1 h after evoking HA degradation (Drafi *et al.* 2010, Soltes *et al.* 2006).

ABTS assay

Radical-scavenging activity was measured as published by Valachova *et al.* (2011).

DPPH assay

Radical-scavenging activity was measured by the DPPH assay as follows: DPPH (1.1 mg) was dissolved in methanol (50 mL) reaching the final 55 μmol/L concentration.

The investigated samples comprised of 2 mL of DPPH• solution and 50 µL of herbal extracts. UV/VIS spectra were recorded in defined time intervals using a UV-VIS 1800 spectrophotometer (Shimadzu, Japan) (Valachová *et al.* 2010).

RESULTS AND DISCUSSION

At first, HA was exposed to degradation by reactive oxygen species, whereas as a source of their formation cupric ions and ascorbate were selected. The results of HA degradation are presented in Figures 1–3, curve 0, which show the decrease of dynamic viscosity (η) of the HA solutions by 3.88 mPa.s after a 5-h treatment. To protect HA from degradation, herbal extracts such as *Agrimonia herba*, *Cynare folium*, and *Ligustri folium* were added to the HA reaction mixture. Since the herbal extracts are a mixture of various components, their molar mass is unknown. For this reason the concentration of their stock solutions was 4.92 mg/mL, which corresponds to the concentration of glutathione used in previous experiments (Valachova *et al.* 2010).

Figure 1, panel A displays the results of protective effects of the herbal extracts in an environment producing •OH radicals, whereas the extract of *Agrimonia herba* (curve 1) was supposed to be the most effective scavenger of •OH radicals, followed by *Cynare folium* (curve 2). On the other hand, the extract of *Ligustri folium* (curve 3) functioned as a promoter of the HA oxidative degradation.

After applying the extracts to the HA reaction mixture 1 h later, i.e. during prevailing production of peroxy-type radicals, the results are somewhat different (Figure 1, panel B). The extract of *Cynare folium* (curve 2) inhibited the degradation of HA more markedly than the extract of *Agrimonia herba* (curve 1). The extract of *Ligustri folium* (curve 3) again pronounced HA oxidative degradation, thereby it differs from other two extracts denoted as H• donating substances. The possible explanation for this phenomenon can be that likewise the Weissberger biogenic oxidative system, the extract of *Ligustri folium* might contain similar components.

Figure 2 displays comparison of the amount of $ABTS^{\bullet+}$ and DPPH• remaining in the medium after addition of the extracts. In the 5th min of the measurement the amount of not scavenged $ABTS^{\bullet+}$ was 42.4%, when using *Agrimonia herba* extract, while in the extracts of *Ligustri folium* and *Cynare folium* the values were 67.8 and 77.3%. The same order of the extracts was observed in the DPPH assay. In the 5th min of the measurement the amount of non scavenged DPPH• was 38.3% in *Agrimonia herba* extract, while in the extracts of *Ligustri folium* and *Cynare folium* the values were 52.0 and 60.9%.

The results in Figure 3 express the portion of scavenging $ABTS^{\bullet+}$ and DPPH• (in %) by the herbal extracts at 730 and 517 nm, respectively. The highest radical scavenging capacity was observed in the extract of *Agrimonia herba* observing the reduction of $ABTS^{\bullet+}$ and DPPH• by 64 and 70% after a 20- and 15-min measurement, respectively. The order of the extracts efficacy was identical in both methods.

Unlike rotational viscometry, the ABTS and DPPH assays determine electron (e^-) donor properties, thus the extract of *Agrimonia herba* was the most efficient electron donator, the mechanisms are as follow:

$$ABTS^{\bullet+} + e^- \rightarrow ABTS$$
$$DPPH^{\bullet} + e^- \rightarrow DPPH^-$$

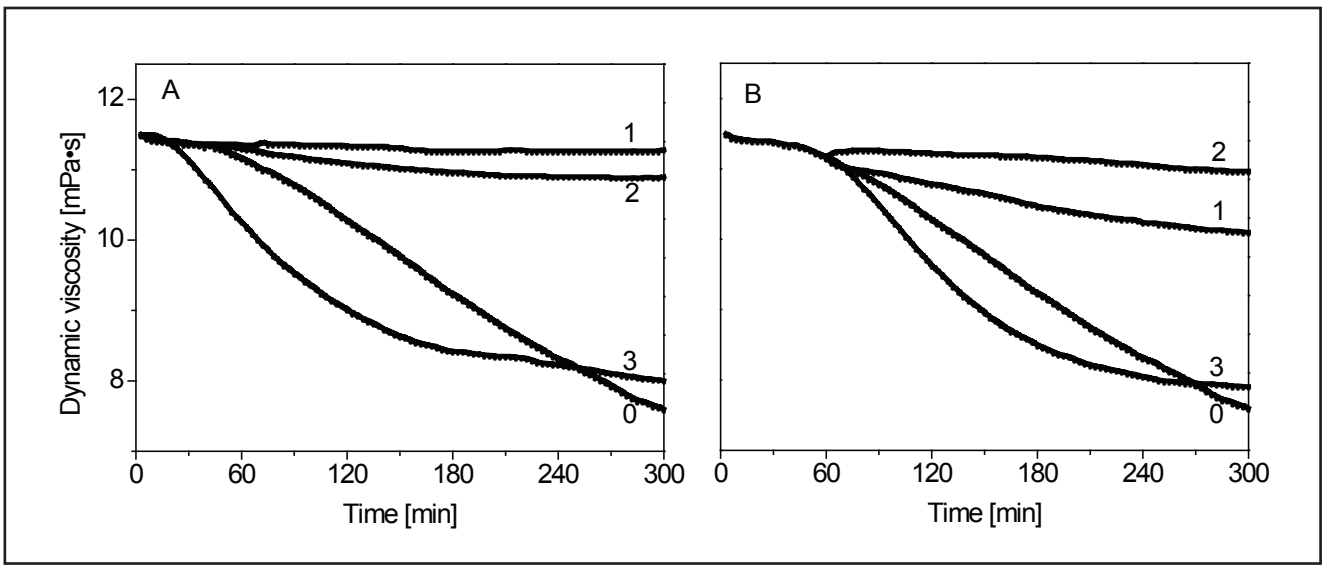

Fig. 1. Effect of *Agrimonia herba* (1), *Cynare folium* (2), *Ligustrum folium* (3) extracts against HA degradation initiated by the oxidative system containing 1.0 µmol/L $CuCl_2$ and 100 µmol/L ascorbic acid. Concentration of the extracts in the system before the start of HA degradation (A) or after 1 h (B) was 307 µg/mL.

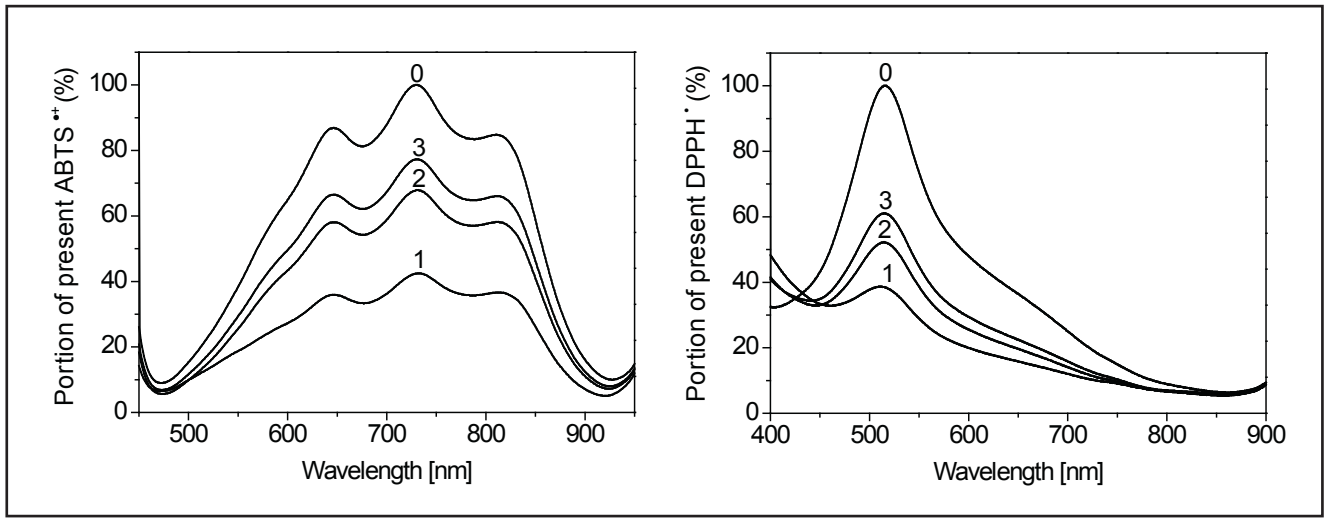

Fig. 2. Left panel: Portion of ABTS•+ (0) present in the medium (in %) after addition of the extracts of *Agrimonia herba* (1), *Ligustri folium* (2) *Cynare folium* (3). The data were reported in the 5th min, the concentrations of the extracts was 307 μg/mL. Right panel: Portion of DPPH• (0) present in the medium (in %) after addition of the extracts of *Agrimonia herba* (1), *Ligustri folium* (2) *Cynare folium* (3). The data were reported in the 5th min, the concentrations of the extracts was 0.063 mg/mL.

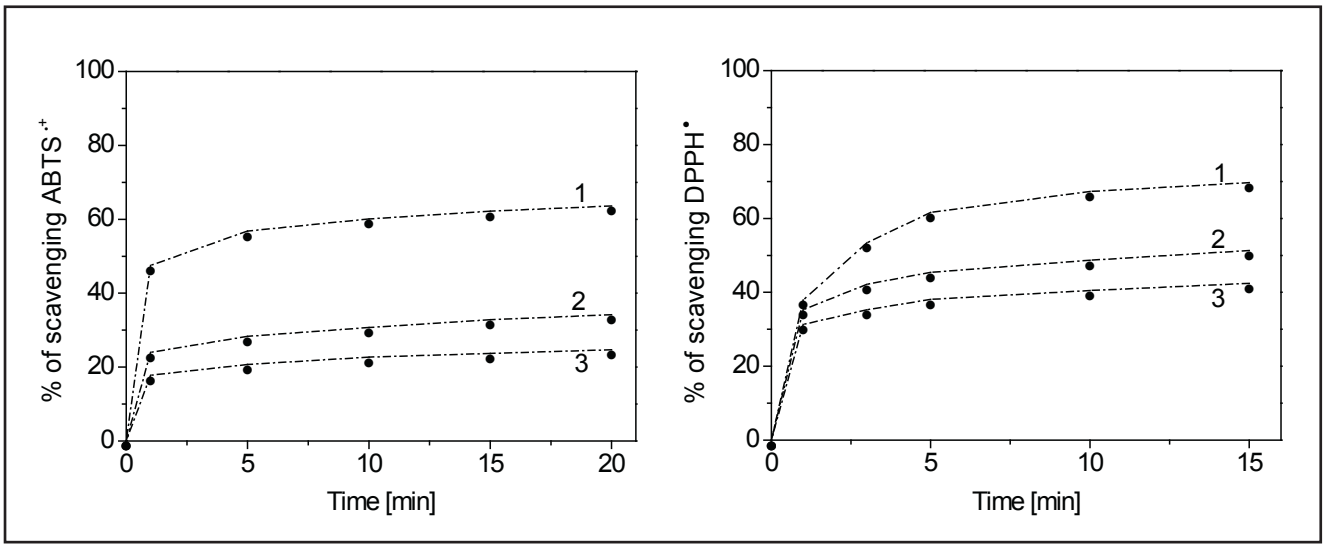

Fig. 3. Left panel: Kinetics of scavenging ABTS•+ by the extracts of *Agrimonia herba* (1), *Ligustri folium* (2), *Cynare folium* (3). The concentration of the extracts was 307 μg/mL. Right panel: Kinetics of scavenging DPPH• by the extracts of *Agrimonia herba* (1), *Ligustri folium* (2), *Cynare folium* (3). The concentration of the extracts was 0.063 mg/mL.

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