Radical scavenging activity of *Caesalpinia spinosa*

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Abstract

OBJECTIVES: To assess protective effects of the tara (Caesalpinia spinosa) extract against hyaluronan (HA) degradation evoked by cupric ions and ascorbate.
METHODS: Uninhibited/inhibited HA degradation was assayed by a decrease in dynamic viscosity of the HA solutions, whereas as a method rotational viscometry was used. To determine radical scavenging capacity of the tara extract, the ABTS and DPPH assays were performed.
RESULTS: The results of rotational viscometry showed remarkable protective effects of the tara extract against the degradation of HA. In the ABTS and DPPH assays the IC_{50} values of the tara extract 1.59 and 30.8 μg/mL indicated quite high radical scavenging properties.
CONCLUSION: The tara extract is an efficient antioxidant as demonstrated by the methods used.

INTRODUCTION

Tara (*Caesalpinia spinosa*) is a native leguminous tree from South America consisting of red or pale pods of 8–10 cm length. Tara widely grows in the Peruvian coast and Andean region at altitude from 1,000 to 2,900 m above sea level. Tara infusions have been traditionally and extensively used by the Peruvian folk medicine to treat inflamed tonsils, fever and cold. Ground tara pods concentrate a high tannin content (~40–60 weight %). Tara pods are a good source to produce tannic, galltannic and gallic acid. Tara tannins are used in the leather industry to obtain very bright and light-colored leathers. Tara powder is used in the fabric printing process, as a mordant, and to make dyes using ferric salts. Moreover, it can serve as adhesives, wine clarifier, malt substitute, a source to obtain the antioxidant gallic acid, which is used in the oil industry. Tara tannins are also employed as a component of gastroenterological medicaments to treat ulcers and help cicatrization. Astrigent, antiinflammatory, antifungal, antibacterial, antiseptic, antidiarheal and antiviral properties have been also attributed to tara tannins (Chambi *et al.* 2013; Bellotti *et al.* 2012; Aguilar-Galvez *et al.* 2014).

From tara seeds a gum is extracted. Because of a high viscosity of the gum, it can be used in food processing as a thickener and stabilizer. Since 1995, Tara gum (E417) is an internationally recognised additive that is used in the production of ice cream, jelly, sauce, yoghurt, and bakery and meat

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products. It also has huge potential as a replacement of fats in light products (Trade for Development Centre 2013).

In general, most tannins can chelate metallic ions such as iron and copper due to their o-diphenyl groups; this feature allows the formation of metal-tannin complexes and decreases metallo-enzyme activities (Aguilar-Galvez *et al.* 2014).

The aim of the study was to assess effects of the tara extract as a potential inhibitor of hyaluronan (HA) oxidative degradation. Moreover, the ABTS and DPPH assays were used to assess radical scavenging capacity of the tara extract.

MATERIALS AND METHODS

Chemicals

A HA sample (sodium salt) of molar mass 970.4 kDa coded P0207-1A was purchased from Lifecore Biomedical Inc., Chaska, MN, U.S.A. Analytical purity grade NaCl and $CuCl_2 \times 2H_2O$ were purchased from Slavus Ltd., Bratislava, Slovakia. Tara powder SILVA made of roots was obtained from Silvateam S.p.A., Italy. L-Ascorbic acid and potassium persulfate ($K_2S_2O_8$ p.a. purity, max. 0.001% nitrogen) were the products of Merck KGaA, Darmstadt, Germany. 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS; purum, >99%) was from Fluka, Germany. 2,2-Diphenyl-1-picrylhydrazyl (DPPH; 95%) was from Aldrich, Germany. Methanol was the product of MikroChem, Pezinok, Slovakia. Redistilled deionized high quality grade water, with conductivity of <0.055 μS/cm, was produced using the TKA water purification system from Water Purification Systems GmbH, Niederelbert, Germany.

Preparation of stock and working solutions

The samples of HA, solutions of ascorbate and cupric chloride were prepared as published by Baňasová *et al.* (2012). The tara extract (1 mg/mL) was prepared as follows:

The tara powder (50 mg) was leached for 15 min in boiled distilled water (40 mL). The tara extract was colded down, filtered and completed with 5 mmol/L phosphate buffer (pH 7.4) to the volume 50 mL.

Uninhibited/inhibited hyaluronan degradation

Uninhibited and inhibited HA degradation was performed as published by Valachová *et al.* (2009).

Rotational viscometry

Dynamic viscosity of the reaction mixture (8 mL; 0.15 mol/L aqueous NaCl) composed of HA (2.5 mg/mL), ascorbate (100 μmol/L) plus Cu(II) ions (1 μmol/L) in the absence and presence of the extract of tara (6.25, 25 and 625 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 (Valachová *et al.* 2008; 2011; Dráfi *et al.* 2010). The herbal extract as a potential protective agent was introduced into the HA reaction system before or 1 h after evoking HA degradation.

ABTS assay

Standard experimental conditions were used as published by Valachová *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 the *Caesalpinia spinosa* extract. UV/VIS spectra were recorded in 1, 5, 10, 15 and 20-min intervals using a UV-VIS 1800 spectrophotometer (Shimadzu, Japan).

ABTS and DPPH assays – determination of the IC_{50} values

The $ABTS^{\bullet+}$ solution (250 μL), prepared from 7 mmol/L ABTS and 2.45 mmol/L $K_2S_2O_8$, 1:1 v/v ratio of aqueous solutions, was added to 2.5 μL of the tara extract and the absorbance of the sample mixture was measured at 734 nm after 6 min, followed by determination of the radical scavenging capacity of the tara extract.

The DPPH•, prepared from 1.1 mg DPPH dissolved in distilled methanol (50 mL), was added to 25 μL of the tara extract and the absorbance of the sample mixture was measured at 517 nm after 30 min, followed by determination of the radical scavenging capacity of the tara extract.

The light absorbance was measured quadruplicately in 96-well Greiner UV-Star microplates (Greiner-Bio-One GmbH, Germany) with Tecan Infinite M 200 reader (Tecan AG, Austria). The IC_{50} value was calculated with CompuSyn 1.0.1 software (ComboSyn, Inc., Paramus, USA).

RESULTS AND DISCUSSION

Figure 1 illustrates the results of examining the extract of tara (curves 1–3) on HA degradation mediated by the oxidative system composed of Cu(II) and ascorbate (curve 0). Since the tara extract is a mixture of various components, its molar mass is unknown. For this reason we prepared 1.0 mg/mL stock solution as mentioned above, whereas below are mentioned its concentrations in the HA oxidative mixture. The tara extract showed concentration-dependent decreased rate of HA degradation. This extract at the concentration 625 μg/mL was demonstrated to be very effective in scavenging •OH radicals (left panel) reaching only a slight decrease in dynamic viscosity (η) values of the HA solution (by 0.42 mPa.s; curve 3). Even at the concentration 6.25 μg/mL the extract was partially effective (curve 1).

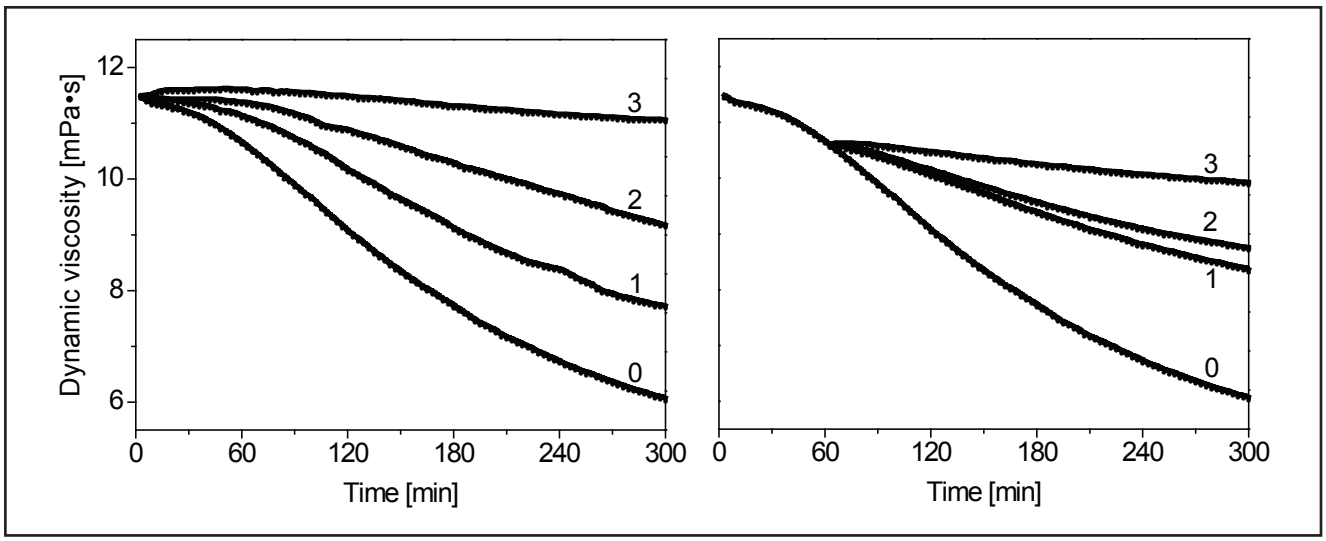

Fig. 1. Time-dependent changes of dynamic viscosity of HA solution in the presence of tara extract added to the reaction mixture before initiating HA degradation (left panel) or after 1 h (right panel) in μg/mL: 0 (0), 6.25 (1), 25 (2), 625 (3).

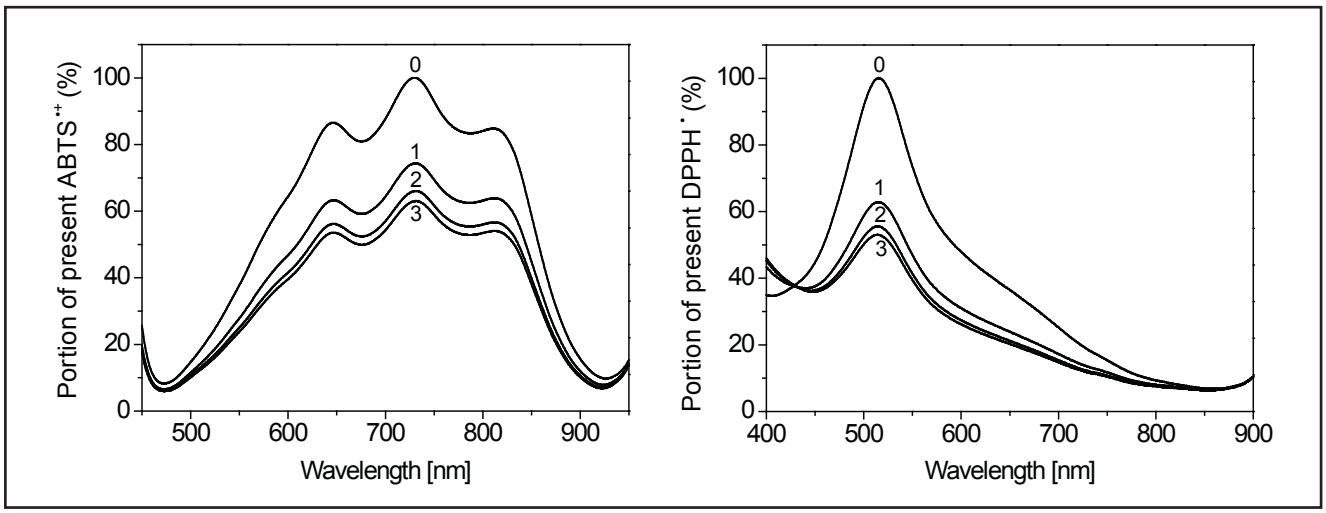

Fig. 2. Portion of ABTS•+ in % (0, left panel) and DPPH• (0, right panel) present in the medium after addition of the tara extract. In the ABTS and DPPH assay the tara extract (1 mg/mL) was examined in time intervals: 1 min (1), 10 min (2), 20 min (3).

The extract of tara was demonstrated to be effective also in the medium producing peroxy-type radicals (right panel). The value of η of the HA solution dropped only by 0.68 mPa.s at the highest concentration of the extract after 5 h of the measurement (curve 3).

A high effectiveness of tara is supposed to be due to a high content of polyphenols especially tannins, which are known chelators of cupric ions. The method of rotational viscometry showed the ability of this extract to donate H• to both hydroxyl (Figure 1, left panel) and peroxy-type radicals (Figure 1, right panel).

Figure 2 shows the results of the portion of ABTS•+ and DPPH• (in %) not scavenged after the addition of the tara extract representing the values 63 and 53%, respectively after 20 min of the measurement. On the other hand, Figure 3 displays the portion of scavenging ABTS•+ and DPPH• by the extract: the values were 37 and 47%, respectively after 20 min of the measurement.

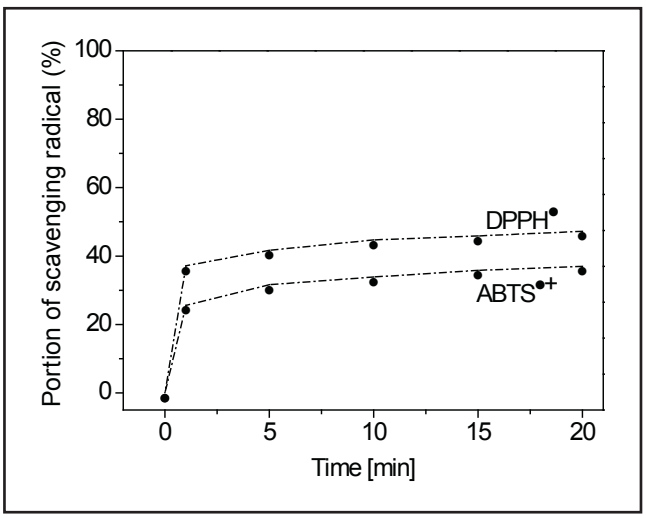

Fig. 3. Portion of scavenging ABTS•+ and DPPH• at 730 and 517 nm (in %), respectively by the tara extract (1 mg/mL).

A high reducing power of the tara extract was observed in the ABTS and DPPH assays by determing the IC_{50} values 1.59 and 30.8 μg/mL, respectively.

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