

**EFFECT OF WATER EXTRACTS OF TANNINS-CONTAINING BULGARIAN PLANTS
ON RAT PLATELET AGGREGTION**

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ABSTRACT

The search of a new chemical substances influencing platelet aggregation is a very important endeavor for medical practice. The *in vitro* effect of water extract from 20 tannins-containing plants from Bulgarian flora on rat platelet aggregation was investigated. The water extracts of 9 medicinal plants: *Agrimonia eupatoria* L., *Arctostaphylos uva-ursi* L. Spreng, *Corylus avellana* L., *Cydonia oblonga* Mill, *Dryopteris filix-mas* (L.) Schott, *Ephedra distachya* L., *Geum urbanum* L., *Primula officinalis* (L.) Hill and *Punica granatum* L. inhibited platelet aggregation, initiated by adenosine diphosphate. The analysis of literature data indicated these plants contained between 10% and 30% tannins, except of *Primula officinalis* (L.) Hill.

Key words: medicinal plant, tannin, platelet aggregation, rat.

INTRODUCTION

Tannins are from 500 to 20 000 D high-molecular polyphenolic compounds with a bitter taste. Accordingly to their chemical structure K. Freudenberg divided tannins into hydrolysable and condensed. A number of Bulgarian medicinal plants are rich in tannins, which are regarded as the active principle. The water-soluble nature of tannins allows easy extraction and is useful in various applications in the chemical and pharmaceutical industry. Plants containing tannins have predominantly astringent, hemostatic, antiseptic and toning properties. The platelet functions play a central role in the processes of blood clotting and many cardiovascular diseases associated with change of platelet activity (Andrioli *et al.*, 1996; Fabre and Gurney, 2010). For correction of the platelet activity can be used substances isolated from foods (green tea, garlic and tomato) and medicinal plants (Atanasov, 1994; Mekfi *et al.*, 2006; Rahman and Billington, 2000; Dutta-Roy *et al.*, 2001; Sagesaka-Mitane *et al.*, 1990). Thus, the alternative medicine appears an additional source for searching of healing remedies. In this direction, the recent investigations are directed to isolation of tannins-containing substances, including medicinal plants (Haouari. *et al.*, 2006; Mekfi *et al.*, 2006; Mosa *et al.*, 2011). Wide spectrum of plants from Bulgarian flora contain from 10 to 20% tannins (Asenov *et al.*, 1989; Petkov, 1982). The main tannins containing plant accordingly to Bulgarian pharmacopeia are: *Agrimonia eupatoria* L. (herbal material) – up to 5% catechins, and up to 8% gallotannins; *Achillea millefolium* L. (herbal material) – up to 2.8%; *Alchemilla vulgaris* L. (herbal material, rhizome) – about 10% tannins with high content of gallic and ellagic acid; *Arctostaphylos uva-ursi* L. (Spreng) (folia) – about 20% gallotannins; *Cotinus cogicria Scop.* (folia) – 15-20% gallotannins; *Corylus avellana* L. (cortex) – about 10% tannins; *Cydonia oblonga* Mill. (folia); *Dryopteris filix-mas* (L.) Schott (rhizome) – up to 10% fillixic acid; *Ephedra distachya* L. (herbal material) – up to 10% pyrocatechins; *Geum urbanum* L. (radix, rhizome) – up to 30% tannins; *Juglans regia* L. (folia) – up to 5% tannins; *Hypericum perforatum* L. (herbal material) – up to 10% catechins; *Lavandula angustifolia* Mill. (flores) – up to 12% tannins; *Ocimum basilicum* L. (herbal material) – up to 5% tannins; *Primula officinalis* (L.) Hill. (folia); *Punica granatum* L. (cortex) – up to 25% tannins; *Rosmarinus officinalis* L. (folia) – up to 8% tannins; *Rubus sp. diversa* (folia) – from 5% to 14% tannins; *Symphytum officinale* L. (radix) – up to 6.5% tannins; *Vaccinium myrtillus* L. (folia, juice) – up to 20% tannins (Asenov *et al.*, 1989; Petkov, 1982). The aim of the study is to test the effect of water extracts of these Bulgarian tannin-containing medicinal plants on rat platelet aggregation.

MATERIALS AND METHODS

Plant material. High tannins-containing Bulgarian medicinal plants were used: *Agrimonia eupatoria* L., *Achillea millefolium* L., *Alchemilla vulgaris* L., *Arctostaphylos uva-ursi* L. (Spreng), *Cotinus cogigria* Scop., *Corylus avellana* L., *Cydonia oblonga* Mill., *Dryopteris filix-mas* (L.) Schott, *Ephedra distachya* L., *Geum urbanum* L., *Juglans regia* L., *Hypericum perforatum* L., *Lavandula angustifolia* Mill., *Ocimum basilicum* L., *Primula officinalis* (L.) Hill, *Punica granatum* L., *Rosmarinus officinalis* L., *Rubus sp. diversa*, *Symphytum officinale* L., *Vaccinium myrtillus* L.

Extract preparation. The water extracts of these plants was prepared by maceration of 2g dry material in 20 ml distilled water for 20-24h at 18-20°C. The fresh extracts were filtered and the effects of extracts on platelet aggregation were studied.

Platelet aggregation study. The platelet aggregation was investigated by method of Born (Born, 1962). The platelet-rich plasma (PRP) from Wistar rats was prepared. The PRP was standardized to 250 000-300 000 platelets in 1 microliters with Hank's solution without Ca^{2+} containing: NaCl-8g; KCl-4g; $MgSO_4 \cdot 7H_2O$ - 0.1g; $MgCl \cdot 6H_2O$ -0.1g; $Na_2HPO_4 \cdot 12H_2O$ -0.15g; glucose -1g and distilled water up to 1000 ml. The effect of water extracts on platelet aggregation was investigated by mixing of 350 μ l PRP and 17-23 μ l water extract from given medicinal plant. As aggregating agent 20 μ l ADP 1×10^{-3} M (Reanal, Hungary) was used. The percentage of platelet inhibition (I, %) was calculated using the equation: $I (\%) = (A-B) \times 100 / A$, where A is the maximum platelet aggregation of the control PRP and B is the maximum aggregation of the mix between PRP and given plant extract.

Statistics. Data were expressed as Mean \pm SEM for n=3 experiments for given plant extract.

RESULTS

It was found that the water extracts of 9 tannins-containing medicinal plants inhibited platelet aggregation. These plants are: *Agrimonia eupatoria* L. with 22.4 \pm 3.6 mg/ml dry material in extract, *Arctostaphylos uva-ursi* L. (Spreng) with 56.3 \pm 4.8 mg/ml dry material in extract, *Cydonia oblonga* Mill. with 16.1 \pm 2.8 mg/ml dry material in extract, *Corylus avellana* L. with 24.8 \pm 3.6 mg/ml dry material in extract, *Dryopteris filix-mas* (L.) Schott with 52.1 \pm 5.2 mg/ml dry material in extract, *Ephedra distachya* L. with 20.2 \pm 1.8 mg/ml dry material in extract, *Geum urbanum* L. with 42.5 \pm 4.2 mg/ml dry material in extract, *Primula officinalis* L. (Hill) with 26.4 \pm 3.5 mg/ml dry material in extract, *Punica granatum* L. with 18.6 \pm 2.6 mg/ml dry material in extract. The percentage of platelet inhibition (I, %) from these plant extracts varied between 25 and 75% (SEM falls in the interval of \pm 4.3% -10.6% for n=3). Only the extract of *Punica granatum* L. inhibited platelet aggregation up to 80-90%. All plants that inhibited platelet aggregation contained about 10% tannins, except of *Cydonia oblonga* Mill and *Primula officinalis* L. For these two plants the literature data showed relatively low tannins content. Provocative is the fact, that the other high tannins-containing plants given in 'Materials and Method' do not inhibited platelet aggregation, independently to high tannin concentration in them. It exist the small possibility that inhibition of platelet aggregation from the established nine plants to be connected with chemical substances different from tannins.

DISCUSSION

The Bulgarian Pharmacopoeia contains a wide range of data on the biologically active action of medicinal plants with a high content of tanning substances. Especially for the nine medicinal plants that suppress the platelet aggregation, the pharmacopoeia data show similar biological effects and medical applications of these plants. Concretely, *Agrimonia eupatoria* L. has a hemostatic and anti-inflammatory action, *Arctostaphylos uva-ursi* L. (Spreng) has antiseptic action and lowers blood sugar, *Cydonia oblonga* Mill has a bloodthirsty action, *Corylus avellana* L. has a haemostatic

and anti-inflammatory action, *Geum urbanum* L has anti - inflammatory action, *Ephedra dystachia* L. has a temperature-limiting effect. The data in the scientific literature showed that from these medicinal plants are isolated substances that inhibited platelet aggregation. For example: the proanthocyanidins and tannins-like compounds are isolated from *Agrimonia eupatoria* L. (Santos-Buelga and Scalbert, 2000), flavonoids (their glycosides) and anthocyanins are isolated from *Primula veris* L. (Veitch and Grayer, 2008), some components of *Punica granatum* inhibits *ex vivo* platelet aggregation (Jurenka, 2008). From full spectrum of tannins compounds the main substances inhibiting platelet aggregation are catechins and elagotannins (Won-Seek Kang *et al.*, 1999). These substances inhibited platelet aggregation in combination with flavonoids too (Pignatelli *et al.*, 2000). The find nine anti-aggregating plants from Bulgarian flora are do not analyzed for chemical components of the tannins compounds. However, this problem may be a task for future research.

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