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IN VITRO STUDY OF THE ANTI-INFLAMMATORY ACTIVITY OF SOME MEDICINAL AND EDIBLE PLANTS GROWING IN RUSSIA*

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The effects of the ethanolic extracts of 105 plants used in Russian traditional medicine, 26 vegetables and fruits and 2 mushrooms on the release of tumor necrosis factor α (TNF-α) and prostaglandin E_2 (PGE_2) in lipopolysaccharide (LPS)-stimulated differentiated human acute monocytic leukemia THP-1 cells were studied using TNF-α and PGE_2 assays, respectively. We found that 16 plant extracts inhibited TNF-α production and 15 extracts decreased PGE_2 release in the cells in a concentration-dependent manner. Guilder rose, tansy, shrubby cinquefoil, wintergreen and prince's pine, the last two of which belong to the Pyrolaceae family, notably inhibited the levels of both of the inflammatory mediators.

Keywords: anti-inflammatory, plant extracts, TNF-α assay, PGE_2 assay.

Introduction

Inflammation, a multi-step process, is mediated by activated inflammatory or immune cells. If not controlled, inflammation can lead to the development of such diseases as chronic asthma, rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease [1]. Macrophages play a central role in this process by mediating the immunopathological changes, including the overproduction of pro-inflammatory cytokines and mediators induced by activated tumor necrosis factor α (TNF-α) and cyclooxygenase 2 (COX-2) [2, 3]. The activation of macrophages is mediated by the activation of pattern recognition receptors (e.g., Toll-like receptor 4; TLR 4) by their ligands (e.g., lipopolysaccharide; LPS), which are released from bacteria or viruses [4]. TNF-α is a toxic cytokine involved in inflammation and other pathological processes, such as rheumatoid arthritis and microbial infections [5, 6]. COX-2 is an inducible immediate-early gene product in inflammatory cells and immune cells; it is mostly involved in chronic inflammatory processes and is markedly stimulated by LPS, cytokines, growth factors and tumor promoters [7]. COX-2 produces prostaglandins (PGs) that contribute to the pain and swelling of inflammation [8, 9]. Both TNF-α and COX-2 are essential to the inflammatory response to pathogenic germs or toxicants. Thus, the suppression of these mediators may be an effective therapeutic strategy for preventing inflammatory reactions and diseases [10]. However, the adverse effects of some nonsteroidal anti-inflammatory drugs have stimulated an interest in identifying natural products for the prevention and treatment of inflammatory disorders [11]. Herbs and spices are extensively used in traditional medicine to relieve the symptoms of inflammatory disorders. Indeed, *in vitro* and *in vivo* studies have shown that diverse nonnutritive dietary compounds, such as curcumin, diallyl sulfide, capsaicin, eugenol and gingerol, that are present in herbs and spices suppress the expression of pro-inflammatory gene products, including cytokines, chemokines, adhesion factors and enzymes [10, 12, 13]. Several biologically

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active food additives have been developed (e.g., Antiartrol, Bambuflex, Dokholodan and Joint Flex) by Russian firms (see the Federal Register of Biologically Active Food Additives, Moscow, 2000, Chapter 10). To date, very few anti-inflammatory drugs of herbal origin have been identified; however, a number of plants from ethno-medicinal databases are under laboratory investigation worldwide [14].

Here, we describe the screening of the anti-inflammatory activities of the extracts of 133 plants and mushrooms available from Russian suppliers (105 dried plants and 2 mushrooms) and supermarkets (26 vegetables and fruits).

LPS-stimulated human differentiated acute monocytic leukemia THP-1 cells have been used as a cell model to study the anti-inflammatory potencies of plant ethanolic extracts. The results demonstrate that non-cytotoxic quantities of several extracts attenuated the LPS-mediated TNF-α and prostaglandin E_2 (PGE_2) production in THP-1 cells.

Experimental conditions

Plant materials. Whole plants and plant parts were purchased from Moscow drugstores or supermarkets or were obtained from official suppliers as ready-to-use dried herbal preparations in 2009 (see Supplementary materials, Table 1). The materials were collected within the territory of the Russian Federation from various regions, depending on the supplier. Dr. V. Karandashov identified the plant materials. Voucher specimens have been deposited in our laboratory at the Ajinomoto-Genetika Research Institute (ZAO AGRI). The herbal materials were stored in a dry, dark location under standard laboratory conditions.

Preparation of plant extracts. The preparation of the plant extracts was carried out by Dr. I. Malfanov and Dr. S. Ivanov (Ajinomoto-Genetika Research Institute), and the dried plant material was prepared as previously described [15]. Briefly, approximately 10 g of each plant was dried and crushed in a mill (if necessary) and soaked in 70% aqueous ethanol (1 : 40, w/v) with stirring (200 rpm) overnight at room temperature. The suspensions were filtered through filter paper (Whatman №4), and the ethanol was removed using a rotary evaporator. After freeze-drying, the crude extracts that were obtained had a mean yield of approximately 31%, ranging from 3,7% for *Salsola collina* to 94,1% for *Beta vulgaris*. The extracts were stored at -20 °C in the dark until further analysis. Prior to the analysis, the dried extracts were solubilized in dimethyl sulfoxide (DMSO)/complete RPMI-1640 medium (1 : 100 v/v) at concentrations of 10,0, 1,0 and 0,1 mg/ml before the final dilution (1 : 9) with culture medium.

Chemicals and equipment. The nutrient medium (RPMI-1640), glutamine, penicillin and streptomycin were obtained from NPP PanEko (Russia). The reactions were performed in 96-well flat-bottom clear polystyrene microplates (Corning, USA) with the aid of a Thermostatic Shaker ST-3 (Elmi, Latvia). The optical density (at 450 nm for the TNF-α and PGE_2 assays and 540 nm for the MTT assay) was measured using a microtiter plate reader, Multiskan Ascent (Thermo Electron, USA).

Growth and activation of cells. Human THP-1 monocytic leukemia cells [16] were routinely maintained in RPMI-1640 medium containing with 10% FBS (HyClone, USA), 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine. To reduce the risk of contamination of the nutrient medium with endotoxins, the FBS was heated at 56 °C for 30 min and filter-sterilized before application. Freshly thawed THP-1 cells were cultured for each set of experiments and were not used for more than a few (3–5) passages. The cells were seeded at $2x10^5$ cell/ml ($4x10^4$ cell/well) and $8x10^5$ cell/ml ($16x10^4$ cell/well) for the TNF-α and PGE_2 assays, respectively, and were grown in 96-well plates sealed with a gas-permeable Breathe-Easy membrane BEM-1 (Diversified Biotech, USA,) at 37 °C under 5% CO_2.

The differentiation of the THP-1 monocytes into macrophage-like cells was carried out with phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich, USA) applied at 10 ng/ml (16 nM), a concentration sufficient to induce stable cell differentiation without undesirable gene upregulation [17]. PMA was prepared as a stock solution (10 mg/ml) in DMSO and then diluted with complete RPMI-1640 medium to obtain the final concentrations. The cells were incubated in the presence of PMA for 24 h.

TNF-α assay. LPS from *E. coli* O127B:B8 (Sigma-Aldrich, USA) was used to induce an inflammatory response in the differentiated THP-1 macrophages. The cells were grown in the presence of 12,5 ng/ml LPS in complete RPMI-1640 medium plus either 0,01, 0,1, or 1,0 mg/ml of the plant extracts for 4 h. The medium was then collected for the determination of the TNF-α level, and the cells were washed and incubated for 40 min with fresh medium containing 0,1 mg/ml MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Alfa

Aesar, USA) to evaluate the cytotoxic effects of the plant extracts. The absorbance was measured at 540 nm, and the cell viability (percent of the control) was calculated relative to the untreated control. A TNF-α synthesis inhibitor, dexamethasone (Sigma-Aldrich, USA), was applied at 10 μM, and these samples served as positive controls. The amount of TNF-α was determined with a Human TNF-α ELISA Ready-SET-Go! kit (eBioscience, USA) according to the manufacturer's instructions.

PGE_2 assay. Differentiated THP-1 cells were grown in the presence of 1250 ng/ml LPS in complete RPMI-1640 medium plus 0,01, 0,1, or 1 mg/ml of the plant extracts for 24 h. The medium was then collected for the PGE_2 analysis, and the cells were washed and incubated for 40 min with fresh medium containing 0.1 mg/ml MTT to evaluate the cytotoxic effects of the plant extracts. The COX-2-specific inhibitor nimesulide (Sigma-Aldrich, USA) was used as a positive control to suppress the COX-2 activity and, therefore, to inhibit PGE_2 synthesis and release by the differentiated THP-1 macrophages. The nimesulide was dissolved in DMSO and applied at 5–10 μM with a final DMSO concentration ≤0,1%. The PGE_2 production was measured using a R&D Systems Parameter PGE_2 kit (R&D Systems, USA) (1 : 2 sample dilution, the high-sensitivity assay option) according to the manufacturer's recommendations.

Results and discussion

The anti-inflammatory activities of the extracts of 105 plants used in Russian traditional medicine, 26 vegetables and fruits and 2 mushrooms were determined using LPS-stimulated differentiated human acute monocytic leukemia THP-1 cells as a cell model. The effects of these extracts were compared with the effects of two synthetic reference anti-inflammatory drugs: dexamethasone, an inhibitor of IL-1β, TNF-α, NO and PGE_2 production [18], and nimesulide, a COX-2-specific inhibitor [19].

In the TNF-α assay, LPS-stimulated THP-1 cells were incubated with one of three concentrations of plant extract (0,01, 0,1 and 1,0 mg/ml) for 4 h, after which the TNF-α level in the medium was evaluated. Seventy-five ethanolic plant extracts, applied at 1,0 mg/ml, strongly inhibited (>50%) the TNF-α production of the THP-1 cells (see Supplementary materials, Table 1); however, twelve of these extracts showed significant levels of cytotoxicity (data not shown). In contrast, only sixteen of the extracts applied at a 0,1 mg/ml concentration caused significant reductions in the TNF-α release (>30%) by THP-1 cells (Supplementary materials, Table 1, and Fig. 1), a result that was similar to that of 3,9 μg/ml (10 μM) dexamethasone. Twelve of the extracts did not exhibit any cytotoxicity (Fig. 1). The extracts of lentil (seeds), parsnip, horseradish (both roots), guilder rose (bark), meadowsweet, prince's pine, shrubby cinquefoil, wintergreen, Gmelin's wormwood, silverweed cinquefoil, crowberry (all of the aerial parts), tansy (flowers), strawberry and blackcurrant (both leaves), kohlrabi and golden chanterelle demonstrated notable concentration-dependent effects on the inhibition of TNF-α release; however, the extracts of prince's pine, wintergreen, Gmelin's wormwood and tansy significantly (>35%) reduced the THP-1 cell survival when applied at 1,0 mg/ml (Fig. 1).

In the PGE_2 assay, a higher extract concentration and a longer incubation time (24 h) than required in the TNF-α assay were needed to achieve obvious levels of inhibition of PGE_2 release by the THP1 cells. Fifteen plant extracts applied at 1,0 mg/ml notably decreased (>33%) the PGE_2 release in the cells (Supplementary materials, Table 1, and Fig. 2); however, only six of them, basil (green), bistort, water pepper, redshank (all of the aerial parts), feijoa (fruits) and dwarf everlasting (flowers), did not exhibit cytotoxicity. The levels of cell survival were lower than 80% in the presence of the following extracts: wintergreen, shrubby cinquefoil, prince's pine, sidebells wintergreen, wormwood sage, (both aerial parts), marsh cinquefoil (aerial parts and roots), guilder rose, tansy and madder (rhizomes). Only the wintergreen extract demonstrated a significant level (approximately 30%) of PGE_2 release inhibition when applied at 0.1 mg/ml without causing any cytotoxic effect. In the PGE_2 assay, the application of 10 μM nimesulide, a COX-2 inhibitor, decreased the PGE_2 release level by 83%. There were also some extracts (alpine bistort, barberry, common aspen, field pansy, Jacob's ladder, kelp, long-leaved speedwell, redcurrant and rhubarb) that exhibited stimulatory effects on the PGE_2 release by the THP-1 cells (Supplementary materials, Table 1).

The lentil seed and golden chanterelle extracts demonstrated the highest potency in the TNF-α assay yet did not cause significant inhibitory effects on the PGE_2 release by the THP-1 cells. Conversely, the water pepper, redshank, sidebells wintergreen and basil (green) extracts, the most active PGE_2 release inhibitors, exhibited low activities in the TNF-α assay when applied at 0,1 mg/ml.

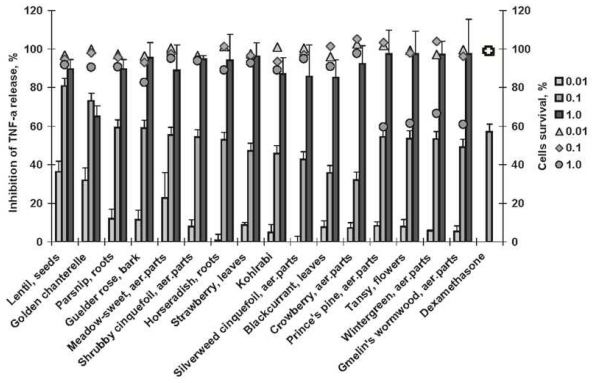

Fig. 1. Effect of plant extracts (0,01, 0,1 or 1,0 mg/ml) and dexamethasone (10 μM) on the inhibition of TNF-α release (%, in bars) and the viability (%, symbols) of LPS-stimulated macrophage THP-1 cells. The results represent the mean±SD for two independent assays performed in triplicate

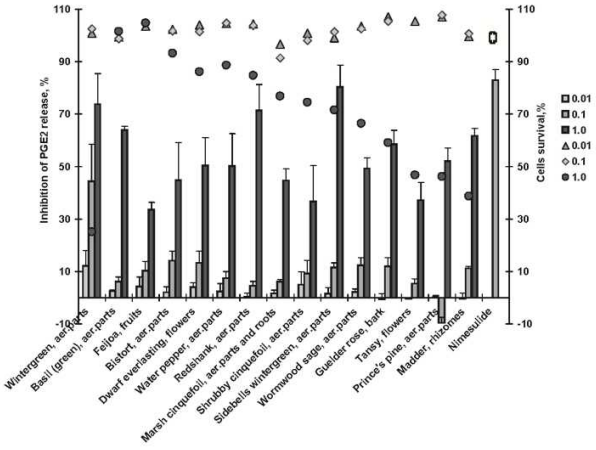

Fig. 2. Effect of plant extracts (0,01, 0,1 or 1,0 mg/ml) and nimesulide (10 μM) on the inhibition of PGE2 release (%, in bars) and the viability (%, symbols) of LPS-stimulated macrophage THP-1 cells. The results represent the mean±SD for two independent assays performed in triplicate

Only a few of the plant extracts, guilder rose (Adoxaceae), tansy (Asteraceae), shrubby cinquefoil (Rosaceae), wintergreen and prince's pine (both Pyrolaceae), showed significant inhibitory effects on the release of both TNF-α and PGE_2, inflammatory mediators in LPS-stimulated THP-1 cells. However, the last two extracts were cytotoxic when applied at 1,0 mg/ml. Another member of the Pyrolaceae family, sidebells wintergreen, was more effective in the PGE_2 assay. It is possible that the different mechanisms of TNF-α and PGE_2 release inhibition are affected by different plant constituents or different concentrations.

Wintergreen (*Pyrola rotundifolia*), used as a whole herb, has long been indicated as a remedy, mostly for arthritic diseases in traditional Chinese medicine [20]. The dried material of another plant of the Pyrolaceae family, *P. decorata*, a perennial herbaceous plant growing throughout China («Lu xian cao» in Chinese) is officially listed in the *Chinese Pharmacopoeia*. Lu xian cao has been used as a tonic, sedative, hemostatic agent, anti-inflammatory agent, and analgesic remedy for the treatment of rheumatoid arthritis in China since ancient times [20, 21]. Two wintergreen compounds shown to be active in anti-inflammatory tests (carrageenan-induced hind paw edema in rats) and analgesic tests (acetic acid-induced writhing in mice) were identified as chimaphilin and ursolic acid [22]. The inotropic and platelet aggregation inhibitory activity of *Pyrola* plants has also been attributed to chimaphilin [23]. Clearly, chimaphilin plays an important role in the bioactivity of *P. decorata* and, possibly, in other plants of the Pyrolaceae family.

Although the role of phenolic compounds and sesquiterpene lactones, particularly parthenolide, in mediating the anti-inflammatory effects of *Tanacetum* species (Asteraceae, *T. parthenium*, *T. artemisioides*, and *T. microphyllum*) has attracted much attention in recent years [24], the closely related cosmopolitan species, tansy (*T. vulgare*), has remained little investigated in this field. However, the anti-inflammatory effect of extract against mouse-ear edema and its antioxidant activity [25] have been demonstrated.

There are few scientific data on the medicinal properties of guilder rose. The bark and berries of this plant are widely used in Russian traditional and folk medicine. It has been previously shown that phenolic compounds are responsible for the high antioxidant activity of guilder rose berries [26], whereas the bark phenolic acid complex is rich in caffeic, protocatechuic, ellagic, 3,4-dihydroxyphenylacetic and homogentisic acids and their esters [27]. In the present study, we showed novel effects of guilder rose extracts on the inhibition of the release of TNF-α and PGE_2 by THP-1 cells.

The lentil (*Lens culinaris*) is a popular food in many countries. However, little is known about the phenolic composition of these seeds. In lentil seed coat extracts, the major monomeric flavan-3-ols, (+)-catechin-3-glucose, (+)-catechin and (-)-epicatechin; oligomeric proanthocyanidins composed of catechin, gallocatechin, catechin

gallate units; and several procyanidins and prodelphinidins (from pentamers to nonamers) were identified [28]. There have been no previous data reported on the anti-inflammatory activity of lentil seeds.

The anti-inflammatory properties of basil extracts are well established; its constituents block both the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism [29].

Mushrooms have a number of components that are potentially immuno-modulatory. *In vitro*, the extracts of edible mushrooms, such as white button and, to a lesser extent, shiitake, crimini and oyster mushrooms, reduced the IL-10 production and increased the IL-1β and TNF-α production by macrophages in a way that is predicted to be beneficial for boosting anti-tumor immunity [30]. Our data showed that the golden chanterelle extract inhibited the release of TNF-α by macrophages and possibly possessed an anti-inflammatory activity, which is a novel finding.

Medicinal plants, including members of the Polygonaceae family, are widely distributed in Asia and North America. For example, Japanese knotweed (*Polygonum cuspidatum*) is used as an analgesic, antipyretic, diuretic and expectorant remedy in traditional Chinese medicine; anti-inflammatory properties, such as the inhibition of NF-κB, have also been reported for this plant [31]. Indeed, interest in Japanese knotweed has increased due to the high concentration of resveratrol and its glycosides in the roots. In addition to resveratrol, Japanese knotweed extracts contain such compounds as quercetin and emodin, which have anti-inflammatory activities [32]. The anti-inflammatory properties of certain other polygonaceous species have also been explored: *P. stagninum* and *P. multiflorum* have been investigated using animal models [33] and their cyclooxygenase-2 activity has been investigated in LPS-induced mouse RAW264.7 macrophage cells [34]. The water pepper and redshank plants of the Polygonaceae family tested in the present work are, as yet, less well studied.

Conclusion

We found that the LPS-stimulated differentiated human acute monocytic leukemia THP-1 cell model provides a simple and cost-effective approach for the initial screening of the anti-inflammatory activities of extracts from numerous medicinal herbs and food items. In this study, we found that, when applied at 0,1 mg/ml, the ethanolic extracts of 16 plants efficiently inhibited TNF-α production and that 15 extracts applied at 1.0 mg/ml decreased the PGE_2 release by THP-1 cells. All of these extracts displayed their activities in a concentration-dependent manner. The inhibition of the PGE_2 synthesis occurred at higher concentrations of the plant extracts and required longer incubation times than the inhibition of TNF-α release. Some of the plant extracts exhibited significant cytotoxic effects in the PGE_2 assay. Further investigation is needed to identify the specific active compounds in the plant extracts that are responsible for these biological activities and to evaluate their bioavailability and efficacy *in vivo*.

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References

1. Medzhitov R. Nature, 2008, vol. 454, pp. 428–435.
2. Allam R., Anders H.J. *Current Opinion in Rheumatology*, 2008, vol. 20, pp. 538–544.
3. Uchida K. *Molecules and Cells*, 2008, vol. 25, pp. 347–351.
4. Lin W.J., Yeh W.C. *Shock*, 2005, vol. 24, pp. 206–209.
5. Makarov S.S. *Molecular Medicine Today*, 2000, vol. 6, pp. 441–448.
6. Goldberg R.B. *The Journal of Clinical Endocrinology & Metabolism*, 2009, vol. 94, pp. 3171–3182.
7. Martel-Pelletier J., Pelletier J.P., Fahmi H. *Seminars in Arthritis and Rheumatism*, 2003, vol. 33, pp. 155–167.
8. Hla T., Neilson K. *Proceedings of the National Academy of Sciences USA*, 1992, vol. 89, no. 16, pp. 7384–7388.
9. Yamada M., Niki H., Yamashita M., Mue S., Ohuchi K. *The Journal of Pharmacology and Experimental Therapeutics*, 1997, vol. 281, pp. 1005–1012.
10. Iwalewa E.O., McGaw L.J., Naidoo V., Eloff J.N. *African Journal of Biotechnology*, 2007, vol. 6, pp. 2868–2885.
11. Garret A., FitzGerald M.D. *The New England Journal of Medicine*, 2004, vol. 351, pp. 1709–1711.
12. Chainani-Wu N. *The Journal of Alternative and Complementary Medicine*, 2003, vol. 9, pp. 161–168.
13. Aggarwal B.B., Shishodia S. *Biochemical Pharmacology*, 2006, vol. 71, pp. 1397–1421.
14. Kong D.X., Li X.J., Zhang H.Y. *Drug Discovery Today*, 2008, vol. 14, pp. 115–119.
15. Ivanov S.A., Nomura K., Malfanov I.L., Sklyar I.V., Ptitsyn L.R. *Fitoterapia*, 2011, vol. 82, pp. 212–218.
16. Tsuchiya S., Yamabe M., Yamaguchi Y., Kobayashi Y., Konno T., Tada K. *International Journal of Cancer*, 1980, vol. 26, pp. 171–176.
17. Park E.K., Jung H.S., Yang H.I., Yoo M.C., Kim C., Kim K.S. *Inflammation Research*, 2007, vol. 56, pp. 45–50.

18. Li G., Liu Y., Tzeng N.S., Cui G., Block M.L., Wilson B., Qin L., Wang T., Liu B., Liu J., Hong J.S. *Biochemical Pharmacology*, 2005, vol. 69, pp. 233–240.
19. FitzGerald G.A., Patrono C. *The New England Journal of Medicine*, 2001, vol. 345, pp. 433–442.
20. *Dictionary of Chinese Material Medicine*. Ed. Jiangsu New Medical College, Shanghai Scientific and Technological Publisher, Shanghai, 1977, 2243 p.
21. Li X., Zhou Y., Huang X., He G., Zhou S., Huang H. *Zhong Yao Cai*., 1997, vol. 20, pp. 402–406.
22. Kosuge T., Yokota M., Sugiyama K., Mure T., Yamazawa H., Yamamoto T. *Chemical & Pharmaceutical Bulletin*, 1985, vol. 33, pp. 5355–5357.
23. Kagawa K., Tokura K., Uchida K., Kakushi H., Shike T., Nakai H. *Chemical & Pharmaceutical Bulletin*, 1992, vol. 40, pp. 2083–2087.
24. Sur R., Martin K., Liebel F., Lyte P., Shapiro S., Southall M. *Inflammopharmacology*, 2009, vol. 17, pp. 42–49.
25. Schinella G.R., Giner R.M., Recio M.C., Mordujovich de Buschiazzo P., Ríos J.L., Máñez S. *Journal of Pharmacy and Pharmacology*, 1998, vol. 50, pp. 1069–1074.
26. Cam M., Hisil Y., Kuscu A. *Chemistry of Natural Compounds*, 2007, vol. 43, pp. 460–461.
27. Turek S., Cisowski W. Free and chemically bonded phenolic acids in barks of *Viburnum opulus* L. and *Sambucus nigra* L. // Acta Poloniae Pharmaceutica – Drug Research, 2007, vol. 4, pp. 377–383.
28. Dueñas M., Sun B., Hernández T., Estrella I., Spranger M.I. *Journal of agricultural and food chemistry*, 2003, vol. 51, pp. 7999–8004.
29. Benedec D., Pârvu A.E., Oniga I., Toiu A., Tiperciuc B. *Revista medicochirurgicala a Societatii de Medici si Naturalisti din Iasi*, 2007, vol. 111, pp.1065–1069.
30. Yu S., Weaver V., Martin K.I., Cantorna M.T. *BMC Immunology*, 2009, vol. 10, no. 12, pp. 1–13.
31. Bralley E.E., Greenspan P., Hargrove J.L., Wicker L., Hartle D.K. *Journal of Inflammation*, 2008, vol. 5, no. 1, pp. 1–7.
32. Li H.L., Chen H.L., Li H., Zhang K.L., Chen X.Y., Wang X.W., Kong Q.Y., Liu J. *International Journal of Molecular Medicine*, 2005, vol. 16, pp. 41–47.
33. Mazid M.A., Datta B.K., Bachar S.C., Bashar S.A., Nahar L., Sarker S.D. *Pharmaceutical Biology*, 2010, vol. 48, pp. 770–774.
34. Zhang Y.Z., Shen J.F., Xu J.Y., Xiao J.H., Wang J.L. *Journal of Asian natural products research*, 2007, vol. 9, pp. 355–363.

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