ANTIOXIDATIVE POTENTIAL OF *AGRIMONIA EUPATORIA* L.

Ivanova D., Tasinov O*., Vankova D., Kiselova-Kaneva Y.
Department of Biochemistry, Molecular Medicine and Nutrigenomics,
Medical University "Prof. Dr. P. Stoyanov", 9002 Varna, Bulgaria;
**Corresponding author: oskan@mail.bg*

ABSTRACT

In folk medicine Agrimonia eupatoria (Agrimony) is well known with its beneficial effects in various diseases. Based on its use, studies on aqueous and aqueous-alcoholic extracts are directed on elucidating of the antioxidant, antidiabetic and anti-inflammatory activity. We have shown that antioxidant activity of Agrimony is associated with high polyphenolic content, and the extracts modified the expression of pro-inflammatory factors and enzymes from glutathione metabolism in cell culture and animal models. Administration of extracts prevented body mass gain and fat accumulation and normalized serum lipid profile in high fructose fed rats. The herb is shown to be a perspective therapeutic especially for treatment of social significant diseases such as diabetes and obesity, accompanied by low-grade inflammation.

***Keywords**: Agrimonia eupatoria, antioxidant, antidiabetic, anti-inflammatory, glutathione*

Introduction. *Agrimonia eupatoria* L. is traditionally used in Bulgarian folk medicine to treat various inflammatory diseases. Studies in the recent years suggest that its therapeutic potential is also related to social significant diseases such as type 2 diabetes. However, the mechanisms of its effects are not well understood.

Botanical characteristics. The genus *Agrimonia* are perennial herbaceous flowering plants classified in Division Magnoliophyta, Class: Magnoliopsida, Family: Rosaceae, Genus: *Agrimonia*. Two species of the plant, *Agrimonia eupatoria* and *Agrimonia procera,* are naturally spread in Bulgaria (16). Agrimony (*A. eupatoria*) is flowering with 5 parts yellow blossoms. It has erect pubescent stems (50-150 cm high) covered with soft hairs. The leaves are pinnate, toothed with velvety undersides. Bright yellow flowers with five small petals occur on long slender spikes from June to September. Small cone-shaped fruits are enclosed in a characteristic bristled calyx-tube. The hooked bristles enable widespread dispersal of seeds on animal fur. The plant spreads vegetatively by stout woody deep-lying rhizomes (15).

Habitat. *A. eupatoria* is found abundantly in meadows, pastures, and lowlands as well in mountains up to 1500 m altitude. Agrimony likes sun lights and grows on moderate humid and dry soils.

Uses in folk medicine. Agrimony is well known with its beneficial effects in various diseases such as liver complaints, gall-bladder stones; diarrhea, edemas and kidney diseases. Thanks to its diuretic properties the herb is widely used against atony of the bladder and disuria. Many other uses of Agrimony in Bulgarian folk medicine are known such as rheumatism; hemorrhoids, bleeding gums, varicose ulcers; laryngitis; pulmonary and cutaneous tuberculosis. The extracts could be used externally as compress or gargle as well as internally as infusion. (15).

Phytochemical composition. As presented in Table 1, many *A. eupatoria* phytochemical composition assays have focused on alcoholic and aqueous-alcoholic extracts.

Shabana et al. have found that the major compounds in aqueous-alcoholic extract are flavonoids, great amounts of tannins, as well as phenolic acids. Five of the flavonoids identified in this study were not reported earlier for *A. eupatoria* extract. Polyphenolic profile of the herb extract is characterizated mainly by HPLC technique (Table 1). The main presumption of the cited studies was to identify the most potent compounds related to the antioxidative activity of *A. eupatoria* (4, 14, 22, 23).

Table 1. *A. euaptoria* phytochemical composition assays

References	Extract	Phytochemical composition	Methods
Shabana et al. (2003)	aqueous-alcoholic	tannins (10.08%), flavonoids (0.33%) and phenolic acids (2.26%) (luteolin 7-O-sophoroside, luteolin 7-O (6"- acetylglucoside), acacetin 7-O-glucoside, luteolin 7-O-glucoside and apigenin 7-O-glucosidep, protocatechuic, vanillic acids, p-hydroxybenzoic acid)	chemical and spectral methods
Correıa et al., (2006)	ethyl acetate fraction from aqueous-alcoholic	flavan-3-ols (catechin and procyanidins B1, B2, B3, B6, B7, C1, C2 and epicatechin-epicatechin-catechin), quercetin 3-O-glucoside, quercetin 3-O-galactoside, kaempferol 3-O-glucoside, kaempferol 3-O-(6"-O-p-coumaroyl)-glucoside, apigenin 6-C-glucoside;	HPLC
Venskutonis et. al., (2008)	methanolic	luteolin-7-*O*-glucoside, hyperoside, and apigenin, glycoside	HPLC
Zhang et al., (2009)	NA	apigenin-7-O-3-D-glucopyranoside, catechin, quercetin, rutin, kaempferol-3-O-alpha-L-rhamnoside, Kampferol-3-O-beta-D-glucopyranoside, lutcolin-7-O-beta-D-glucopyranoside, 19alpha, 24-dihydroxy ursolic acid, 3,3'-di-O-mcthyl ellagic acid4-O-beta-D-glucopyranoside	HPLC
Lee et al., (2010)	methanolic	kaempferol 3-O-β-D-(2"-O-acetyl-6"-(E) *p*-coumaroyl)-glucopyranoside (2"-acetyl tiliroside), kaempferol 3-O-β-D-(2"-O-acetyl-6"-(E) *p*-coumaroyl)-glucopyranoside, tiliroside, astragalin, apigenin 7-O-β-D-glucuronide, rutin, quercitrin, isoquercitrin, luteolin 7-O-β-D-glucuronide, luteolin 7-O-β-D-glucopyranoside;	HPLC

Biological activities of *A. eupatoria* extracts. Based on its wide use in folk medicine Agrimony provokes a great scientific interest. Table 2 summarizes results from various investigations aimed to assess some important pharmacological properties of the *A. eupatoria* extracts. (1, 3-7, 9-11, 13, 14, 19, 21, 22).

Table 2. *A. eupatoria* extracts activity assays

References	Extract	Object	Activities	Methods
Kiselova et al., (2011)	aqueous-alcoholic	rats	antioxidant activity (AOA), anti-inflammatory	gene expression
Ivanova et al., (2011)	aqueous-alcoholic	mice 3T3-L1 preadipocytes cells	AOA, anti-inflammatory	gene expression
Kiselova et al., (2004)	aqueous-alcoholic	extract	AOA	ABTS assay
Ivanova et al., (2005)	aqueous	extract	AOA	ABTS assay
Correıa et al., (2006)	aqueous-alcoholic	extract	AOA	DPPH assay, ABTS assay
Correıa et al., (2007)	aqueous-alcoholic, polyphenol-enriched fraction	extract	AOA	DPPH assay
Venskutonis et. al., (2007)	acetone, methanol, aqueous, acetone-hexane, acetone-t-butylmethylether, acetone-n-BuOH, acetone-aqueous	extract	AOA	DPPH assay, ABTS assay
Venskutonis et. al., (2008)	supercritical (SC)-CO_2 extracts	extract	AOA	DPPH assay, ABTS assay
Gião et al., (2010).	aqueous	mice	AOA	enzyme activity, hepatic glutathione level
Lee et al., (2010)	ethanolic	mice HT22 hippocampal cells	neuroprotective,	glutamate-induced oxidative neurotoxicity evaluated by MTT assay
Copland et al., (2003)	*n*-hexane, and methanol	*B. cereus* *B. subtilis*	antibacterial, AOA	96-well microplate-based broth dilution assay, DPPH assay

Cwikla et al., (2010)	aqueous-ethanolic	*H. pylori*	antibacterial	micro-dilution assay
Kwon et al., 2005	aqueous	hepatitis B virus	antiviral	microbiolological assays
Swanston-Flatt et al., (1990)	dried leaves	mice	antidiabetic	clinical biochemistry
Gray et al., (1998)	aqueous	mice, BRIN-BD11 pancreatic B-cell line	antidiabetic (antihyperglycaemic, insulin-releasing and insulin-like activity)	clinical biochemistry
Bae et al., (2010)	NA	mice BV2 microglial cells	anti-inflammatory	ELISA and western blotting for measurement of cytokines levels : LPS stimulation
Bratoeva et al., (2010)	aqueous-ethanolic	rats	anti-obesity	Biochemical methods for measurement of cholesterol and triglycerides
Vankova et al., (2011)	aqueous	healthy volunteers	AOA	ABTS assay, FRAP assay

High correlation between polyphenolic content and total AOA have been demonstrated in aqueous-alcoholic Agrimony extracts (11). These results have been confirmed by other authors applying various methods (4, 5, 7, 21, 22). Antioxidant activity of agrimony aqueous extracts have been demonstrated *in vivo* and *in vitro* by measurements of changes in hepatic catalase and superoxide dismutase activities in mice (9). Due to its antioxidant properties the herb has shown to exert neuroprotective effects (14).

Extracts of *A. eupatoria* possess powerful antibacterial activity (3). The inhibitory effect of the herb on *Helicobacter pylori* reveals the mechanism of its antiulcer potential (6). Antiviral effect of *A. eupatoria* has been investigated in aqueous extract prepared from the aerial parts which inhibited HBsAg release against hepatitis B virus (13). The anti-inflammatory properties of agrimony have been demonstrated by its inhibitory effects on lipopolysaccharide-induced nitric oxide and proinflammatory cytokines production (1).

Special attention should be paid on investigations of antidiabetic properties of *A. eupatoria.* In experimental model of type 1 diabetes antihyperglycaemic, insulin-releasing and insulin-like activities of agrimony's aqueous extract and dried leaves have been demonstrated (10, 19). There is evidence that diabetes type 2 is supported by low-grade inflammation and immune system activation, underlying insulin resistance (17). The agrimony extract was demonstrated to modify expression of pro-inflammatory factors and enzymes in glutathione metabolism in cell culture and animal models. In 3T3-L1 pre-adipocyte cell line aqueous-ethanolic extract stimulated expression of the rate limiting enzyme in *de novo* glutathione synthesis - γ-glutamate cisteine ligase (GCL) and decreased transcription of MCP-1 (macrophage contracting protein-1). Upon oxidative stimulation the same extract exhibited protective effects diminishing stimulatory activity of the oxidative treatment on GCL glutathione peroxidase-4 (GPx-4) and MCP-1 expression in 3T3-L1 pre-adipocytes. Elevated mRNA levels for GCL and lowered levels for MCP-1 mRNA in adipose tissue of rats on a standard diet and high fructose fed rats supplemented with *A. eupatoria* extract (12, 8) were established. Administration of *A. eupatoria* extract prevented body mass gain and fat accumulation in liver and adipose tissue and normalized serum lipid profile in high fructose fed rats (2, 12).

Recent investigation on *A. eupatoria* tea consumed by healthy volunteers confirmed the antioxidant properties of the herb which increasing total antioxidant capacity, as well as ferric reducing antioxidant potential of serum (20).

Conclusion. Many beneficial effects of *A. eupatoria* have been described. The herb is perspective therapeutic especially for social significant diseases such as diabetes and obesity. However, not all mechanisms contributing these effects are clarified yet. Antioxidant activity of *A.*

eupatoria, associated with high polyphenolic content, could explain its anti-inflammatory potential. Low-grade inflammation and oxidative stress are closely related by a network of signals: reactive oxygen species, nitric oxide and other reactive species production, which could damage DNA and contribute to the pathogenesis of many diseases. To overcome oxidative stress cell activates its antioxidant defense systems. Accumulated evidences suggest that antioxidant activity of *A. eupatoria* might be due to chemical structure of polyphenols and/or herb ability to activate the endogenous antioxidant defence systems. More aspects of *A. eupatoria* effects, as well as the fine mechanisms of its action are to be revealed by future studies.

REFFERENCES:

1. Bae H, Kim HJ, Shin M, Lee H, Yin CS, Ra J, Kim J., 2010. Inhibitory effect of Agrimoniae Herba on lipopolysaccharide-induced nitric oxide and proinflammatory cytokine production in BV2 microglial cells, Neurol Res., 1, 53-7

2. Bratoeva K., Bekyarova G., Kiselova Y., Ivanova D., 2010. Effect of Bulgarian herb extracts of polyphenols on metabolic disorders – induced by high fructose diet. Trakia Journal of Sciences. 8 (2), 56-60.

3. Copland A., Naharb L., Tomlinsona C., Hamiltona V., Middletona M., Kumarasamya Y., Sarkera S., 2003. Antibacterial and free radical scavenging activity of the seeds of Agrimonia eupatoria. Fitoterapia. 74, 133-135

4. Correia H., González-Paramás A, Amaral M., Santos-Buelga C., Batista M., 2006. Polyphenolic profile characterization of Agrimonia eupatoria L. by HPLC with different detection devices. Biomed Chromatogr. 20, 88-94.

5. Correia H., Batista M., Dinis T., 2007. The activity of an extract and fraction of Agrimonia eupatoria L. against reactive species. Biofactors. 29, 91-104.

6. Cwikla C, Schmidt K, Matthias A, Bone KM, Lehmann R, Tiralongo E., 2010. Investigations into the antibacterial activities of phytotherapeutics against Helicobacter pylori and Campylobacter jejuni, Phytother Res., 5, 649-56

7. Ivanova D., Gerova D., Chervenkov T., Yankova T., 2005. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. Journal of Ethnopharmacology. 96, 145-150.

8. Ivanova D., Kiselova-kaneva Y., Vankova D., Tasinov O. 2011. Protection by Agrimonia eupatoria extract of oxidant-challenged 3T3-L1 pre-adipocyte cells. IASO, Obesity reviews, 12 (Suppl. 1), 176–177.

9. Gião M., Pestana D., Faria A., Guimarães J., Pintado M., Calhau C., Azevedo I., Malcata F., 2010. Effects of extracts of selected medicinal plants upon hepatic oxidative stress. J Med Food.13, 131-136.

10. Gray AM, Flatt PR., 1998. Actions of the traditional anti-diabetic plant, Agrimony eupatoria (agrimony): effects on hyperglycaemia, cellular glucose metabolism and insulin secretion, Br J Nutr., 1, 109-14

11. Kiselova Y., Galunska B., Ivanova D., Yankova T., 2004. Total antioxidant capacity and polyfenol content correlation in aqueous-alcoholic plant extracts used in phytotherapy, Scripta Scientifica Medica, 36, 11-13

12. Kiselova Y., Nashar M., Ivanova D., 2011. Effects of dietary administration of Agrimonia eupatoria L. in experimental model of metabolic syndrome. IASO, Obesity reviews, 12 (Suppl. 1), 155.

13. Kwon DH, Kwon HY, Kim HJ, Chang EJ, Kim MB, Yoon SK, Song EY, Yoon DY, Lee YH, Choi IS, Choi YK., 2005. Inhibition of hepatitis B virus by an aqueous extract of Agrimonia eupatoria L., Phytother Res, 4, 355-8.

14. Lee K., Hwang L., Jeong E., Kim S., Kim Y., Sung S., 2010. Effect of neuroprotective flavonoids of Agrimonia eupatoria on glutamate-induced oxidative injury to HT22 Hippocampal cells. Biosci. Biotechnol. Biochem. 74 (8), 1704-1706.

15. Pamukov, Ahtardziev, 1989. Prirodna apteka, Zemizdat, Sofia

16. Petrova A., Ancheva M., Palamarev E., 1999. Kak da razpoznavame rasteniyata v nashata priroda, ekskurzionen opredelitel, Prosveta, Sofia

17. Pickup J., 2004. Inflamation and Activated Innate Immunity in the Pathogenesis of type 2 Diabetes. Diabetes Care. 27, 813-823.

18. Shabana M., Weglarz Z., Geszprych A., 2003. Phenolic constituents of agrimony (Agrimonia eupatoria L.) herb. Herba polonica Y. 49, 24-28.

19. Swanston-Flatt S., Day C., Bailey C., Flatt P., 1990. Traditional plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. Diabetologia. 33, 462-464.

20. Vankova D., Nashar M., Radanova M., Ivanova D., 2011. Effects of Agrimonia eupatoria on antioxidant potential in healthy volunteers. Nugoweek 2011, Measuring health: How to apply nutrigenomics for measuring metabolic health. 6-9 Sept., Wageningen, The Netherlands (accepted).

21. Venskutonis P., Škėmaitė M., Ragažinskienė O., 2007. Radical scavenging capacity of Agrimonia eupatoria and Agrimonia procera. Fitoterapia. 78, 166-168.

22. Venskutonis P., Skemaite M., Sivik B., 2008. Assessment of radical scavenging capacity of Agrimonia extracts isolated by supercritical carbon dioxide. The Journal of Supercritical Fluids. 45, 231-237.

23. Zhang J., Chen Y., 2009. Studies on the lowering blood sugar substances from agrimony. Journal of Chinese Medicinal Materials. 32, 1537-1539.