REVIEW

The Therapeutic Effects of *Agrimonia eupatoria* L.

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Summary

Agrimonia eupatoria L. is an herb of the Rosaceae family, widely used in traditional (folk) medicine for its beneficial effects. Its water extracts (infusions and decoctions) are used in the treatment of airway and urinary system diseases, digestive tract diseases, and chronic wounds. Phytochemical analyses of *Agrimonia eupatoria* L. identified a variety of bioactive compounds including tannins, flavonoids, phenolic acids, triterpenoids and volatile oils possessing antioxidant, immunomodulatory and antimicrobial activities. The authors review the available literature sources examining and discussing the therapeutic and pharmacological effects of *Agrimonia eupatoria* L. at the molecular level *in vitro* and *in vivo.*

Key words

Agrimony • Antioxidant • Immunomodulatory • Antimicrobial • Therapeutic effect

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Agrimony in traditional medicine

Common agrimony (*Agrimonia eupatoria L.)* (also church steeples, stickwort, or liverwort, among other names) is a perennial herbaceous plant of the Rosaceae family with small yellow star-shaped flowers, a short rhizome and a hard, hairy stem inhabiting pasture lands across Europe. Written accounts of the beneficial effects of agrimony date back as far as the 4th to 5th centuries; the herb was also mentioned in the Old English Herbarium from the 10th century (Cameron 2006, Voights 1979, Watkins *et al.* 2012).

Agrimonia eupatoria L. has long been used in traditional medicine to treat:

- lung inflammation
- diarrhea
- liver diseases
- cholecystitis, cholestasis
- intestinal or bladder atony
- pyelonephritis
- bleeding disorders
- skin defects
- inflammatory conditions of the oral mucosa.

Young stems of agrimony, its leaves and flowers were used to prepare (by isolating the flavonoid quercitrin) a yellow pigment and to increase the storage stability of beer (Kresánek and Krejča 1977).

Indications linked to references and documents published by regulatory authorities:

- relief of diarrhea (EMA 2015, Opletal and Skřivanová 2010),

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- relief of minor inflammations of the mouth and throat (Bradley 1992, ESCOP 2005),
- relief of skin inflammations, promotion of wound healing (EMA 2015, Ghaima 2013, Jahodář 2010).

Methods

Relevant studies were identified using PubMed, Google Scholar and Science Direct. Reference lists of retrieved articles were also reviewed. The most relevant and up-to-date information was incorporated.

Relevance of agrimony in current therapeutic use

Agrimony in European documents

In a separate article, the European Medicine Agency (EMA) refers to agrimony as a plant with time-tested use and effects – though not adequately validated in clinical trials yet – and said to possess antimicrobial activity and improve digestion (EMA 2015, Opletal and Skřivanová 2010).

Infusions made from agrimony green tops (leaves) are taken internally by adults for their antidiarrheal effects (astringent action) in diarrheal diseases or as a gargle in oral cavity inflammation and pharyngitis (sore throat) (Bradley 1992, ESCOP 2005). Agrimony has been reported to exert antimicrobial actions and improve digestion (Opletal and Skřivanová 2010). Infusions are also recommended in inflammation of the kidney (pyelonephritis) and inflammation of the gallbladder (cholecystitis) as well as cholestasis (Jahodář 2010). Alternatively, agrimony applied in the form of compresses or added to ointments is already a common type of topical therapy that has been found to be helpful in treating different skin conditions, namely chronic eczema, purulent wound and psoriasis (EMA 2015, Jahodář 2010). Furthermore, it speeds up wound healing and exerts hemostatic effects (Jahodář 2010).

Generic drugs containing agrimony green tops (aerial parts) are available in liquid dosage forms to be applied either to the skin or used as bath additives. In documents published by EMA, the herb can be used to prepare ethanol extracts and water infusions. The former are available as tinctures (ratio of herbal substance to extraction solvent 1:5, extraction solvent, 45 % ethanol (V/V)) or water extracts (DER 1:1), extraction solvent, 25 % ethanol (V/V) (EMA 2015). Water infusions are taken internally as teas and applied externally as gargles, compresses and baths. The infusions for internal use are prepared by pouring 250 ml of boiling water on 1.5–4 g of the drug to be drunk twice or three times a day; with extracts, the amounts are 1–3 ml and, with tinctures, 1–4 ml to be taken three times per day. To prepare the infusion as a gargle, the dried herb is mixed with water at a 1:1 ratio. To treat superficial skin defects, compresses are applied, and baths taken twice a day. The herbal solutions for topical application are prepared by pouring 250 ml of boiling water on 3–10 g of the dried drug. The effect of topical application to reduce inflammation or treatment of minor superficial wound is assessed after one-week therapy (EMA 2015). No data are available about the effect of agrimony-based remedies in children below 12 years of age, and in pregnant and breastfeeding women (EMA 2015).

Taken together, more scientific data are needed to explain the favorable traditional effects of *Agrimonia eupatoria* L. Of note, closely related to the species are *A. pilosa* Ledeb. a plant traditionally grown in Japan and China (Park *et al.* 2020), and *A. procera* Wallr. (Granica *et al.* 2015) inhabiting various parts of the world and currently being intensively studied for their promising biological activities.

Antibacterial effects

Agrimonia eupatoria L. has been shown to have bactericidal action, in particular against *Staphylococcus aureus* in infected wounds (Watkins *et al.* 2012). Although wound colonization is most often polymicrobial, *Staphylococcus aureus* still seems to be among the most if not the most frequent causative agent of wound infections. It is therefore not surprising that *Agrimonia eupatoria* L. is broadly used in minor superficial wound healing. Wound healing is a complex multifactorial process. Quick wound healing depends both on the therapy used and on the patient (age, sex, tissue oxygenation, stress, hormones, diabetes, obesity, medications, alcoholism smoking, nutrition and infection) (Guo and DiPietro 2010). The presence of bacteria in wounds is not necessarily a disadvantage: to a certain degree, so called sub-infective, bacteria appears to increase neutrophil, monocyte and macrophage infiltration, raise prostaglandin E levels, boost collagen formation and promote granulation tissue formation. However, if a wound becomes locally infected or critically colonized, it will heal poorly: while chemotaxis becomes reduced due to high complement protein consumption whereas endotoxins, cytotoxic enzymes and free oxygen radicals increase tissue damage, release of vasoconstrictive metabolites induces localized thrombosis, dermal proteins are digested, fibroblast production decreases further interfering with collagen production (Edwards and Harding 2004). In clinical practice, topical agrimony is recommended to be applied to wounds for at least one week before the first assessment (EMA 2015).

Its antibacterial action against *Staphylococcus aureus* could also help explain its beneficial effect in the treatment of psoriasis and eczemas, especially atopic eczema. This highly prevalent condition (affecting up to 20 % of children worldwide) is characterized by abnormal microbial colonization with pathogenic organisms such as *Staphylococcus aureus* or *Malassezia furfur*. The latest European guidelines recommend patients to take baths with bacterial growth inhibiting formulas such as sodium hypochlorite. Topical compounds with antimicrobial actions have also been suggested as effective, especially in cases of superinfection (Ring *et al.* 2012).

A meta-analysis published in 2017 confirmed that psoriatic lesions also seem to be at increased risk for staphylococcal colonization compared with healthy individuals (Ng *et al.* 2017); in addition, an association between staphylococcal colonization and PASI (Psoriasis Area Severity Index) scores has been reported (Okubo *et al.* 2002, Tomi *et al.* 2005).

Root extracts have been found to have antimicrobial action against the *Bacillus subtilis* and *Escherichia coli* strains (Watkins *et al.* 2012). The effect of ethanolic agrimony extracts against *Escherichia coli* was superior to that of water agrimony extracts and was shown to have mild inhibitory activity against the *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains (Ghaima 2013). Other studies have reported that the inhibitory activity of an agrimony extract was the most effective against Gram-positive bacteria (Muruzović *et al.* 2016). Regarding Gram-negative bacteria, and *Escherichia coli* in particular, the additive effect of *Agrimonia eupatoria* L. may be an advantage when combined with antibiotics, which act synergistically and are effective against resistant strains (Muruzović *et al.* 2017). The herb's extracts are employed in the management of diarrhea. Experimental results reported by Komiazyk *et al.* suggested that an extract inhibited the binding of *Vibrio cholerae* to fibroblast cells through ganglioside GM1 receptor immobilization in the cell membrane (Komiazyk *et al.* 2019).

A significant antimicrobial effect of *Agrimonia eupatoria* was shown against *Helicobacter pylori* underlining the role of this herb as a potential supplement besides the currently used antibiotic treatments (Cardoso *et al.* 2018, Cwikla *et al.* 2010).

Antiviral effects

The antiviral activity of agrimony has been documented in several recent studies. An ethanolic extract of *Agrimonia eupatoria* L. exhibited an inhibitory effect on mengovirus, also known as Columbia SK virus, which belongs to the genus Cardiovirus and its genome is formed by a single-stranded positive-sense RNA molecule. The antiviral effect was also confirmed in negative-sense RNA viruses. Water and ethanolic extracts of *Agrimonia pilosa* Ledeb., an herb of the same genus, were tested against human influenza viruses in *in vitro* and *in ovo* experiments (Shin *et al.* 2010). Specifically, the plant's antiviral effect was confirmed for H1N1 influenza A virus (Spanish flu in 1918), H3N2 influenza A virus (Hong Kong flu in 1968) and for influenza virus B. Antiviral activity was also found against the avian strain of H9N2 subtype of influenza A virus. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to the group of RNA-related viruses. However, no scientific information is available regarding the effect of agrimony on SARS-CoV-2. To date, several large studies examining natural compounds and their potential antiviral activity for coronavirus disease-19 (COVID-19) have been published based on molecular docking or other high throughput screening methods (Tripathi *et al.* 2020). One reason for the absence of agrimony in these studies can be the still poor knowledge about its individual compounds and appropriate cellular pathways resulting in specific pharmacological effects or low quality of pharmaceutical preparations.

The Agrimonia species have been reported to possess antihepatitis activities. Aqueous extracts prepared from the aerial parts (stems and leaves) of *Agrimonia eupatoria* L. and *A. pilosa* Lebed. inhibited hepatitis surface antigen (HBsAg) secretion (Kwon *et al.* 2019). The inhibitory effect of the extracts against hepatitis B virus (HBV) (DNA virus) was measured in HepG2.2.15 cells and was found to be temperature-dependent, with the optimal temperature for substances heated in water to 60 °C (Kwon *et al.* 2005). In the case of hepatitis C virus (HCV), a single-stranded RNA virus, an antiviral action was documented with *A. pilosa* Ledeb. (Kwon *et al.* 2019). The importance of the study lies in the identification of several metabolites in an ethanolic extract and their evaluation for anti-HCV activities. The following compounds have been found to inhibit the expression of the HCV core 1b NS5A proteins in Huh7.5 cells: hyperin, luteolin, astragalin, afzelin, nicotiflorin, tiliroside, apigenin, procyanidin B2, dihydroquercetin, coumarin, rutin, quercetin, apigenin-7-O-glucuronide and baicalin.

Anti-inflammatory and immunodulatory effects

Nitric oxide plays a regulatory role in the development of inflammation. At low concentrations, NO acts protectively. As regards the physiological responses, the key players include constitutive NO production through enzymes of endothelial nitric oxide synthase (eNOS) involved in vascular regulation, and neuronal NOS (nNOS) involved in synaptic plasticity control, smooth muscle relaxation, blood pressure control and vasodilation in the central nervous system (CNS). eNOS has been shown to possess antioxidant and antiproliferative effects (Guzik *et al.* 2003). As a response to pathogen infection, inducible nitric oxide synthase (iNOS) expression is induced in macrophages resulting in increased NO synthesis and further activation of inflammatory responses. In the early stage of a bacterial infection, *Agrimonia procera* Wallr. stimulated the defense response by upregulating cytokine production in pigs (Gräber *et al.* 2018). Alcohol (butanol and methanol) extracts of *Agrimonia pilosa* Ledeb. have been shown to significantly suppress iNOS and, consequently, NO production without affecting COX-2 (Jung *et al.* 2010). On the other hand, high NO and nitrogen radical levels are toxic promoting oxidative stress and inducing apoptosis. *Agrimonia eupatoria* L. extracts significantly reduced macrophage-produced NO levels in murine cell line macrophages (RAW 264.7) without adversely affecting cell viability. Of note, toxicities of extracts measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay *in vitro* were found 770 µg/ml for a water extract and 276 µg/ml for a polyphenol-rich extract, while NO reduction was observed at concentrations of 382 and 138 µg/ml, respectively. The anti-inflammatory and analgesic effects of water agrimony extracts devoid of a toxic effect were also documented under *in vivo* conditions. Carrageenan-induced rat paw edema in rats was significantly reduced by administration of aqueous and polyphenol-rich extracts. The effect was comparable to that of diclofenac sodium, a standard anti-inflammatory drug (Santos *et al.* 2017). Ethanolic extracts of *Agrimonia pilosa* Ledeb., an herb of the same genus, was shown to have anti-inflammatory and anti-allergic activities inhibiting inflammatory cytokine (IL-1β, IL-4, IL-6) and interferon-β (INF-β) production in induced RAW 246.7 cells (Kim *et al.* 2012). Moreover, an ethanolic extract of *Agrimonia pilosa* Ledeb. was found to inhibit NO and PGE_2 production by downregulating iNOS and COX-2 expression. *In vitro* studies confirmed that administration of *Agrimonia eupatoria* L. extracts decreased the levels of tumor necrotic factor-α (TNF-α), IL-1β and IL-6 in murine microglial lines (Bae *et al.* 2010). In a study of Ivanova *et al.*, regular consumption of agrimony tea for 30 days resulted in a reduction of the pro-inflammatory cytokine IL-6 in healthy volunteers (Ivanova *et al.* 2013).

Antioxidant effects

The antioxidant activity of *Agrimonia eupatoria* L. extracts exhibited a significant ability to neutralize the 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl and superoxide anion radicals (Correia *et al.* 2007, Gurita (Ciobotaru) *et al.* 2018, Ivanova *et al.* 2001, Muruzović *et al.* 2016, Santos *et al.* 2017). The polarity of solvents used in the process of extraction affects the radical scavenging capacity with generally polar solvents being more potent (Venskutonis *et al.* 2007). A methanol extract of *Agrimonia eupatoria* L. was shown to have the highest antioxidant capacity among all herbs of the genus *Agrimonia* reflecting its highest bioflavonoid content (Kubínová *et al.* 2012). In the study of Ivanova *et al.*, agrimony tea consumption (1 g/200 ml twice a day) for 30 days significantly increased total antioxidant capacity of human blood plasma (Ivanova *et al.* 2013). Administration of an *Agrimonia procera* Wallr. extract to the roundworm (*Caenorhabditis elegans*) significantly reduced the magnitude of oxidative stress in these animals. Higher extract concentrations (100 and 200 µg/ml) extended the roundworm's mean lifespan, presumably related to forkhead transcription factor DAF-16 activation (mammalian FoxO orthologue) (Saier *et al.* 2018). In a study of Tupec and colleagues, the high antioxidant activity of an *Agrimonia eupatoria* extract correlated with the content of total phenolic compounds (Tupec *et al.* 2017).

Analgesic effects

Behavioral tests in rats documented an antinociceptive effect of *Agrimonia eupatoria* L. extracts

(200 mg/kg) in a cisplatin-induced neuropathic rat model, although the mechanism of action has not been conclusively established to date (Lee and Rhee 2016). Administration of the same dose (200 mg/kg) of an *Agrimonia pilosa* Ledeb. extract to mice was also found to produce an antinociceptive action (Park *et al.* 2012). Administration of *Agrimonia eupatoria* L. and *Agrimonia pilosa* Ledeb. extracts did not shorten the pain response in the hot plate test in rats suggesting that the extracts do not act centrally. A significant reduction in foot swelling and decreased paw licking time suggest peripheral analgesic effects of the extracts (Santos *et al.* 2017).

Phototherapic effects

Grieve referred to *Agrimonia eupatoria* L. as a photosensitive plant (Grieve 1931). While photosensitivity can be a source of possible skin adverse reactions (phototoxicity, photoallergy, phytophotodermatitis, photoaging, skin cancers) (Jain *et al.* 2011), photosensitizing agents are often used in the field of dermatology. Vitiligo, psoriasis and mycosis fungoides are conditions that are often treated by combining ultraviolet A-rays (UVA) or B-rays (UVB) with photosensitizing agents (Asim *et al.* 2013). The basic mechanisms of action behind phototherapy include DNA synthesis suppression, reduction of Langerhans cells, T lymphocytes and mast cells as well as cytokine and prostaglandin stimulation – these induce immune suppression, alterations in cytokine expression and cell cycle arrest (Bulat *et al.* 2011). Such actions, stimulated by the photosensitizing potential of Agrimonia, might help explain its therapeutic effect in inflammatory conditions such as eczema and psoriasis.

Hypotensive effects

Extracts (10–20 %) made of the underground part of *Agrimonia eupatoria* L. were administered intravenously to anesthetized cats. Doses ranging between 0.25 and 1 ml/kg were found to have a hypotensive effect with more than a 40 % decrease in blood pressure within 20 minutes of administration (Petkov 1979).

Diuretic activity and uricolytic effects

It was demonstrated that in rats, distilled water consumption resulted in loss of electrolytes via the kidneys. Administration of infusions at a concentration of 20 ml/kg and decoctions of *Agrimonia eupatoria* L. extracts at doses of 1, 2 or 3 g/kg with distilled water prevented losses of electrolytes, and K^+ ions in particular (Giachetti *et al.* 1989).

Antidiabetic effects

In a study using streptozotocin (STZ)-induced diabetic mice, agrimony decreased plasma glucose levels in these experimental animals (reducing hyperglycemia) and, generally, reduced polydipsia (Swanston-Flatt *et al.* 1990). Drinking of an agrimony decoction supplemented to potable water (2.5 g/l) in mice for 12 days resulted in decreased water and food intake and a significant decrease in hyperglycemia in STZ-induced diabetes. Likewise, administration of a water extract to the rat BRIN-BD11 pancreatic B-cell line had antidiabetic effects, with increased glucose uptake, glycogenesis, carbon dioxide (CO_2) production and lactate output. Unlike direct dried herb administration, agrimony decoctions at concentrations of 0.25–10 mg/ml stimulated insulin secretion without an adverse effect on BRIN-BD11 pancreatic cell viability (Gray and Flatt 1998, Swanston-Flatt *et al.* 1990). Of all species of the genus Agrimonia, *Agrimonia eupatoria* L. – due to its highest flavonoid content – inhibited most significantly α-glucosidase cleaving poly- and oligosaccharides to glucose, a process to be potentially employed in the treatment and management of diabetes (Kubínová *et al.* 2012, Kuczmannová *et al.* 2016). In an *in vitro* model, a water agrimony extract (decoction) was shown to inhibit glucose diffusion, a finding that suggested reduced rates of glucose absorption (Gallagher *et al.* 2003). In diabetic patients, hyperglycemia causes endothelial damage and deteriorates aortic elasticity. An *Agrimonia eupatoria* L. extract was reported to have significantly improved aortic relaxation (Kuczmannová *et al.* 2016). Diabetes combined with obesity poses a major health challenge associated as it is with increased expression of pro-inflammatory cytokines in adipose tissue and the liver and development of insulin resistance. In the mouse, a water *Agrimonia pilosa* Ledeb. extract alleviated metabolic syndrome symptoms while increasing the levels of adiponectin, an anti-inflammatory protein produced in adipose tissue (Jang *et al.* 2017).

Hepatoprotective effects

Elevated levels of alanine transaminase indicate damage to hepatic cells. In a randomized, double-blind, placebo-controlled study, twice-daily administration of capsules containing powdered *Agrimonia eupatoria* L. to subjects diagnosed with moderately elevated alanine transaminase levels for 8 weeks (160 mg/den) markedly decreased serum alanine transaminase, aspartate transaminase and triglyceride levels. Generally, *Agrimonia eupatoria* L. exerted a hepatoprotective action, improved lipid metabolism markers while having no adverse effects (Cho *et al.* 2018). Ethanolic extract administration to the rat caused liver damage at both macroscopic and microscopic levels upregulating toll-like receptor-4 (TLR-4), myeloid differentiation factor 88 (MyD88), iNOS, COX-2, and nuclear factor-κB (NF-κB) as well as causing liver steatosis and parenchymal inflammation with increased neutrophil infiltration and IL-6 and TNF-α cytokine production. Two-month administration of an *Agrimonia eupatoria* L. decoction was reported to be associated with a protective effect on the liver parenchyma, to alleviate the adverse effects of alcohol and to reduce the extent of steatosis and inflammation (Yoon *et al.* 2012).

Anticancer effects

Both water and methanol *Agrimonia eupatoria* L. extracts showed inhibitory activity on tumor (rhabdomyosarcoma [RD] and HeLa) cell lines, with the activity depending on the concentration (12, 24, 48 and 96 µg/ml) and incubation period (12, 24 and 48 h) *in vitro*. No effect of the extracts was seen on normal cells (mouse embryonic fibroblasts, MEF) (Ad'hiah *et al.* 2013). Application of an *Agrimonia pilosa* Ledeb. extract (50-400 µg/ml) on liver cancer Hep G2 cell lines for 24 hours decreased anti-apoptotic B-cell lymphoma protein (Bcl-2, Bcl-XL) expression, induced myeloid leukemia cell differentiation protein (Mcl-1) and activated the caspase-induced apoptotic pathway (caspases 3 and 9) (Nho *et al.* 2011). Inhibition of anti-apoptotic protein expression and enhanced pro-apoptotic protein expression were also observed in the human osteosarcoma U2-OS cell line. The extract's antitumor effect was confirmed in the BALB/c mouse (Huang *et al.* 2018). *In vitro,* conjugates of silver nanoparticles and an agrimony extract targeted at tumor cells inhibited the growth of A549 adenocarcinoma cells. The cytostatic effect of a combination of silver nanoparticles with agrimony was shown to be five times higher that of silver nanoparticles alone (Qu *et al.* 2014).

Effect on wound healing

According to a study of Ghaima (2013), topical application of an ethanolic (10 %) *Agrimonia eupatoria* L. extract ointment shortened wound healing time to 10–16 days, and its water (10 %) extract to 12 days in rats. Topical application of a water *Agrimonia pilosa* Ledeb. extract was found to improve epidermal permeability and maintain skin barrier function in mice. A water *Agrimonia pilosa* Ledeb. extract exhibited agonism to the transient receptor potential vanilloid 3 (TRPV3) cation channel in keratinocytes (Nam *et al.* 2017). While its function is primarily thermosensitive, TRPV3 plays a role in keratinocyte proliferation and differentiation as well as in stratum corneum formation (Cheng *et al.* 2010), hair follicle growth in the mouse (Imura *et al.* 2007) and maintenance of skin barrier function (Blaydon and Kelsell 2014). Upon activation, TRPV3 stimulates Ca^{2+} release, TNF-α production and epidermal growth factor receptor (EGFR) activation. The EGFR and TRPV3 subsequently form a signaling complex with eventual TRPV3 sensitization (Cheng *et al.* 2010). Agrimony's photosensitizing action might also contribute to wound healing by increasing the wound/ulcer bed sensitivity to ultraviolet radiation. Ultraviolet exposure induces cellular proliferation in the stratum corneum – this is a protective mechanism of the skin to fight further sunlight damage. UVC has been reported to induce fibronectin release, which promotes cell migration and wound contraction (Gupta *et al.* 2013).

Phytoestrogen effects

Agrimonia pilosa Ledeb. extracts were shown to act agonistically on estrogen receptors in MCF-7 breast cancer cells (*in vitro*) and to upregulate estrogen-responsive genes. Based on these findings, use of the extract is being considered to alleviate post-menopausal symptoms in women (Lee *et al.* 2012).

Anti-atherosclerotic effects

Thirty-day consumption of agrimony tea (1 g/200 ml twice a day) was reported to have significantly decreased high-density lipoprotein (HDL) cholesterol levels in healthy volunteers (Ivanova *et al.* 2013).

Antithrombotic effects

A hexane extract of *Agrimonia eupatoria* L. (500 µg/ml) was shown to prevent thrombus formation. A polyphenolic-polysaccharide complex of *Agrimonia eupatoria* L. acted on the heparin cofactor II (HC II) to inhibit thrombin (Tsigoris-Maniecka *et al.* 2019). The herb's anticoagulant effect occurred at concentrations as low as 80 µg/ml. Its water extract enhanced factor VII activity while decreasing factor VIII, IX and XI activity and inhibiting the intrinsic pathway (Fei *et al.* 2017).

Neuroprotective effects

A beneficial effect of *Agrimonia pilosa* Ledeb. was reported in the treatment of neurodegenerative diseases, dementia and Alzheimer's disease (Jung and Park 2007). In the rat, supplementation of the herb's water extract (2 %) to food prevented β-amyloid accumulation in the hippocampus (Park *et al.* 2017). Administration of a water *Agrimonia pilosa* Ledeb. extract appreciably mitigated the impact of ischemic brain damage, improved neurological function and Na^+/K^+ ATPase activity as well as neuronal energy metabolism (Zhu *et al.* 2017). Interesting data were brought by Kubínová *et al.* (Kubínová *et al.* 2016). The authors investigated anticholinesterase activity of aqueous extracts from five species of the genus Agrimonia. They confirmed inhibition of cholinesterase enzyme in all the extracts at tested concentrations of 100 µg/ml, with the best results obtained for *A. pilosa* Ledeb. In an *in vitro* model of glutamate excitotoxicity, an *Agrimonia eupatoria* L. extract enhanced hippocampal HT22 cell viability by free radical scavenging and oxidative stress reduction (Lee *et al.* 2010).

Potential adverse events

The lack of placebo-controlled clinical studies limits our knowledge on agrimony's possible adverse events. Nevertheless, the American Herbal Products Association (AHPA) considers it a class 1 drug: there is a potential for the occurrence of adverse events but these are unreported. As a photosensitive plant, Agrimonia is believed to cause different types of photodermatitis. (Grieve 1931).

Substances detected in agrimony and structure-related biological activities at a glance

Agrimonia eupatoria L. contains a large variety of biologically active substances (for details, see Table 1). It was documented that individual molecules can exert a combined, synergistic effect (Al-Snafi 2015, Bradley 1992, ESCOP 2005).

Agrimony eupatoria L.'s biologically active substances:

- polyphenols (tannins, phenolic acids, flavonoids and terpenoids),
- polysaccharides
- triterpenoids
- salicylic acid
- minerals, trace amounts of essential oils
- organic acids, ascorbic acid, nicotinamide complex, palmitic and stearic acids, ceryl alcohol and 0.2 % of volatile acids

According to previous phytochemical analyses, agrimony dried herb contains:

- tannins (2 %) (Correia *et al.* 2006, Granica *et al.* 2015)
- flavonoids (1,2–1.4 %) (Kurkina 2011, Lee *et al.* 2010, Shabana *et al.* 2003)
- phenolic acids (2.26 %) (Granica *et al.* 2015, Shabana *et al.* 2003)
- minerals (7.3–7.5 %) (Bradley 1992)
- triterpenoids (0.6 %) (Le Men and Pourrat 1955)
- volatile oils (Al-Snafi 2015, Feng *et al.* 2013)
- vitamins (EMA 2015)
- acids (EMA 2015)
- other constituents

Tannins

Agrimonia eupatoria L. contains at least 2 % of tannins. Its building block is pyrogallol ($C_6H_6O_3$, M_r 126.1). Three to 21 % of tannins are condensed tannins, in particular proanthocyanidins (procyanidin B3) (Correia *et al.* 2006, Cos *et al. 2012*). The proanthocyanidins are present mainly in the form of leuco-anthocyanins bioconverted by acid hydrolysis to cyanidin.

Tannin categories:

- hydrolysable tannins (gallotannin, agrimoniin)
- condensed tannins (proanthocyanidins)

Tannins have demonstrated antiseptic, astringent, antioxidant, anti-inflammatory, anti-mutagenic properties (Kocaçalişkan *et al.* 2006, Serrano *et al.* 2009, Sieniawska 2015).

Flavonoids

Agrimony comprises 1.2–1.4 % of flavonoids, which are phenylchromen derivatives (Kurkina 2011). Of these, the therapeutically important compounds include flavanones (hesperidin), flavones (apigenin, luteolin), flavonols (kaempferol, quercetin, quercitrin, myricetin, rutin), and isoflavans.

The most important members of the flavonoid family are listed below (Bilia *et al.* 1993, Kurkina 2011):

- hyperoside

- luteolin, its glucuronide isomers
- apigenin, its 7-O-glucoside, 7-O-β-D glucuronide
- quercetin,
- quercitrin, its 3-O-rhamnoside, rhamnoglucoside isomer, malonyl hexoside isomers
- isoquercitrin
- kaempferol, its 3-glucoside, 3-rhamnoside, 3-rutenoside, 7-O-β-D glucuronide, malonyl hexoside, hexoside, kaempferide, kaempferide 3-rhamnoside
- rutin
- isovitexin

Flavonoids have a significant antioxidant, anti-inflammatory, anti-allergic potential. Moreover, these compounds have been shown to exert diuretic, spasmolytic and vasodilator effects (Aslam *et al.* 2018, Tomko *et al.* 1999). Quercitrin is a quercetin glycoside shown to have higher bioavailability than quercetin (Guo and Bruno 2015). Topical application of isoquercitrin (isotrifoliin) (Bhatia *et al.* 2016), rutin (Almeida *et al.* 2012) and luteolin (Ozay *et al.* 2018) has proved beneficial in wound healing. Rutin has antihypertensive, anti-proliferative, antiseptic properties; it increases the efficacy of vitamin C, suppresses platelet aggregation thus preventing blood clot formation, promotes vessel wall elasticity while decreasing their fragility (Ganeshpurkar and Saluja 2017). Kaempferol and its glycosides possess antimicrobial and analgesic properties (Calderón-Montaño *et al.* 2011), with some of them directly inhibiting lipid peroxidation (Van Acker *et al.* 1996). Luteolin is a flavone reactive oxygen and nitrogen species scavenger (Xagorari *et al.* 2002).

Phenolic acids

Phenolic acids (2.26 %) are the most widely distributed plant non-flavonoid polyphenolic compounds (Granica *et al.* 2015, Shabana *et al.* 2003). Structurally, they are divided into hydroxybenzoic acid derivatives (benzoic, gallic, gentisic, salicylic, vanillic acids) and cinnamic acid derivatives (ferulic, caffeic, coumaric acids). They exert antioxidant activity by scavenging the hydroxyl radical and superoxide anion.

Polyphenolic compounds:

- chlorogenic acid
- caffeic acid
- ellagic acid
- p-hydroxybenzoic acid
- protocatechuic acid
- homoprotocatechuic acid
- genitisic acid
- vanillic acid
- salicylic acid
- p-coumaric acid
- ferulic acids
- 3-O-p-coumaroylquinic acid, 4-O-caffeoylquinic acid
- 5-O-caffeoylquinic acid

Caffeic acid, p-hydroxybenzoic acid, ellagic acid (Yuniarti *et al.* 2018), genitisic acid (Joshi *et al.* 2012), 3-O-p-coumaroylquinic acid and protocatechuic acid (Son *et al.* 2018) exhibit antioxidant and anti-inflammatory actions (Phan *et al.* 2001).

Caffeic acid inhibits myeloperoxidase activity, downregulates the inflammatory mediators nitric oxide synthase, COX-2 and TNF-α, and stimulates production of collagen polymers (Romana-Souza *et al.* 2018, Song *et al.* 2008).

Ellagic acid has demonstrated antiproliferative and antioxidant properties, and stimulates angiogenesis (Yuniarti *et al.* 2018).

Vanillic acid has antibacterial and anti-inflammatory effects (Torzewska and Rozalski 2014).

P-coumaric acid is a hydroxy derivative of cinnamic acid, has been shown to inhibit ROS (reactive oxygen species) production (Yue *et al.* 2019), downregulates the growth factors VEGF (vascular endothelial growth factor) and bFGF (basic fibroblast growth factor), has antiangiogenic, anti-infective, antioxidant, acts as a free radical scavenger, protects pancreatic islets, prevents myocardial and lung injury (Kong *et al.* 2013) and exerts hepatoprotective and hypolipidemic effects (Shen *et al.* 2019).

Ferulic acid has antibacterial, antiviral, anti-allergic, anti-inflammatory and antioxidant effects, inhibits melatonin production while reducing oxidative stress and thymine formation (Park *et al.* 2018, Zduńska *et al.* 2018).

Chlorogenic acid has been shown to attenuate oxidative stress. It has an anti-oxidant potential, inhibits lipid peroxidation, increases nitric oxide (NO) usage in vessels, improves endothelial function, and exerts antibacterial and anti-inflammatory actions (Suzuki *et al.* 2006, Tajik *et al.* 2017).

Rosmarinic acid has an antioxidant potential and antiviral, antibacterial and anti-allergic properties (Shekarchi *et al.* 2012).

Salicylic acid possesses analgesic properties, inhibits COX and reduces prostaglandin production while inhibiting uridin-5-diphosphoglucose (UDPG) oxidation and synthesis of mucosal polysaccharides (Lee and Spencer 1969).

Triterpenoids

Triterpenoids (triterpenoid saponins) are pentacyclic molecules synthesized from isoprene, and show anti-inflammatory, antioxidant and antimicrobial effects (Woźniak *et al.* 2015).

Table 1. Substances contained in agrimony

Group of compounds	Percentage content	Examples	References
Tannins	2 %	Proanthocyanidins (procyanidin B3) Leuko-anthocyanin Ellagitannins	(Correia *et al.* 2006, Granica *et al.* 2015)
Flavonoids	1.2–1.4 %	Hyperoside Luteolin Apigenin Quercetin Isoquercitrin Kaempferol	(Kurkina, 2011, Lee *et al.* 2010, Shabana *et al.* 2003)
Phenolic acids (Granica et al. 2015, Shabana et al. 2003)	2.26 %	Chlorogenic acid Ellagic acid p-Hydroxybenzoic acid Protocatechuicacid Homoprotocatechuic acid Genitisic acid Vanillic acid Salicylic acid p-Coumaric acid Ferulic acids 3-O-p-coumaroylquinic acid, 4-O-caffeoylquinic acid 5-O-caffeoylquinic acid	(Granica *et al.* 2015, Shabana *et al.* 2003)
Triterpenoids	0.6 %	Urosolic acid Euscapic acid 28-β-D-glucopyranosyl euscapic acid Tormentic acid esters	(Le Men and Pourrat 1955)
Acids		Palmitic acid Stearic acid Silicic acid	(EMA 2015)
Volatile oils	0.2 %	Cedrol α-Pinene Linalool α-Terpineol Bornyl acetate Eucalyptol	(Al-Snafi 2015, Feng *et al.* 2013)
Vitamins		Vitamin C Nicotinamide complex Thiamine	(EMA 2015)
Minerals	7.3–7.5 %	Silicon dioxide	(Bradley 1992)

The triterpenoids most abundant in agrimony include (EMA 2015):

- urosolic acid (0.6 %) (Le Men and Pourrat 1955)
- euscapic acid
- 28-β-D glucopyranosyl esters of euscapic acid
- tormentic acid

4.5. Minerals

Agrimony is relatively rich in silica (7.3–7.9 %), potassium (12.882 µg/g) and sodium (37.2 µg/g) (Bradley 1992, EMA 2015).

Vitamins

Agrimonia eupatoria L. contains a variety of vitamins (EMA 2015):

- ascorbic acid (vitamin C)
- nicotinamid complex (100–300 µg/g)
- thiamine (2 µg/g)
- vitamin K

Volatile oils

Agrimony contains volatile oils (0.2 %) (Al-Snafi 2015, Feng *et al.* 2013). Volatile oils are a mixture of hydrocarbon terpenes, sesquiterpenes, polyterpenes and their oxygenated derivatives. Volatile oils have carminative, antibacterial, antiviral and anti-inflammatory properties.

Volatile oils in agrimony:

- cedrol
- α-pinene
- linalool
- α-terpineol
- bornyl acetate
- eucalyptol

Cedrol has antiseptic, toning and antifungal effects and promotes extracellular matrix formation *via* increased ERK1/2, p38 and Akt phosphorylation (Jin *et al.* 2012).

α-Pinene exerts, by inhibition of prostaglandin E1 (PGE1), anti-inflammatory and antimicrobial effects (Tümen *et al.* 2018)

α-Terpineol has antiseptic, antiparasitic anti-inflammatory properties, and decreases the levels of TNF-α, GE2, and the interleukins IL-1β, IL-8 and IL-10 (Hart *et al.* 2000).

Eucalyptol (1,8-cineol) possesses anti-inflammatory activity, decreases myeloperoxidase concentrations and inhibits the cytokines TNF-α, IL-1β IL-4 and IL-5 (Juergens *et al.* 2003).

Conclusions

Agrimonia eupatoria L. is an herb with a wide range of effects. Results of experiments published to date have demonstrated its antimicrobial, antiviral, antioxidant, anti-inflammatory, hepatoprotective, neuroprotective, anti-cancer and anti-diabetic actions as well as a beneficial effect on wound healing. Besides, *Agrimonia eupatoria* L. exerts an immunoprotective effect, decreases the levels of pro-inflammatory cytokines while increasing those of anti-inflammatory cytokines. It has been shown to interfere with NO regulation, stimulate the expression and activity of the antioxidant enzymes superoxide dismutase, catalase and glutathione, and to scavenge free radicals. In the liver, the herb inhibits TLR-4 signaling and helps alleviate liver injury. In the intestines, its extracts inhibit α-glucosidase and, consequently, glucose absorption. Furthermore, it has been found to exert a cytostatic effect on tumor cells without affecting normal ones. The herb's extracts inhibit thrombus formation and the intrinsic pathway. This review thus brings a summary of the biological activities of agrimony together with mounting evidence of subcellular mechanisms of action of individual compounds. Clinical trials documenting and corroborating the beneficial actions of this medicinal plant in clinical practice are lacking. However, the currently available data on *Agrimonia eupatoria* L. and herbs of the same genus Agrimonia represent an interesting and affordable source of bioactive compounds with a remarkable potential for therapeutic purposes.

Conflict of Interest

There is no conflict of interest.

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