J Herbmed Pharmacol. 2021; 10(1): 14-30.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2021.02

Journal of Herbmed Pharmacology

A comprehensive review on phytochemical, pharmacological and therapeutic properties of *Agrimonia eupatoria* L.

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ARTICLE INFO

Article Type:
Review

Article History:
Received: 3 August 2020
Accepted: 10 October 2020

Keywords:
Agrimonia eupatoria
Agrimony
Flavonoid
Medicinal plant
Oxidative stress
Rosaceae

ABSTRACT

Agrimonia eupatoria is a perennial herb belonging to the Rosaceae family that all its parts are used to treat various diseases. In this article, we aimed to present a comprehensive review on the phytochemical, pharmacological, and therapeutic effects of this plant. We searched various databases and summarized the data documented in literature from 1976 to 2020. *Agrimonia eupatoria* has effects on various kinds of cancer, oxidative stress, diabetes mellitus, hepatitis B, and liver damage. It also has anti-adhesive, antibacterial, antimicrobial, and wound healing properties. It induces nitric oxide and inhibits pro-inflammatory cytokines production. Phytochemical studies related to this plant has led to isolation and identification of tannins, coumarins, and flavonoids as the most active chemicals with biological effects. Based on this comprehensive review about *Agrimonia eupatoria*, there will be more opportunities for investigators to search and discover ways to use bioactive agents of this herb to develop new Agrimony based medicines.

Implication for health policy/practice/research/medical education:
This review offers a comprehensive insight into the phytochemical, pharmacological, therapeutic activities, and safety of *Agrimonia eupatoria* L., and demonstrates that it can be used as a reliable source for preparation of new drugs..
Please cite this paper as: Ghobadi Pour M, Mirazi N, Moradkhani Sh, Rafieian Kopaei M, Rahimi-Madiseh M. A comprehensive review on phytochemical, pharmacological and therapeutic properties of *Agrimonia eupatoria* L. J Herbmed Pharmacol. 2021;10(1):14-30. doi: 10.34172/jhp.2021.02.

Introduction

Compared to conventional drugs, medicinal plants usually have low toxicity. Furthermore, in spite of the availability of all kinds of medicines and all health facilities, due to the high costs of hospital care or because of cultural beliefs and historical customs in most of the undeveloped countries, people still trust herbal medicines and some of the herbs still have kept their popularities (1).

Agrimonia eupatoria from the Rosaceae family, known as "Common Agrimony", is an erect perennial plant (30–60 cm in height). It is native to mainland Europe and found across Asia Minor and North Africa (2).

It is traditionally used as an antiadhesive, antibacterial (3,4), antioxidant, astringent (5,6), anti-inflammatory (7-9), and hepatoprotective (10) agent. It is also used for bed-wetting (11), treatment of hemorrhagic colitis, liver and urinary diseases (6,12), cancer (13), acute diarrhea, diabetes mellitus, inflammation of oral and pharyngeal mucosa (14,15), and the hepatitis B virus (16). In this article we aimed to present a comprehensive review on phytochemical, pharmacological, and therapeutic properties of this plant.

Botany

Scientific Classification

The scientific classification of *A. eupatoria* (Figure 1) is as follows:
Super kingdom: Eukaryota
Kingdom: Viridiplantae
Phylum: Streptophyta
Subphylum: Streptophytina
Class: Magnoliopsida

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Figure 1. *Agrimonia eupatoria.*

Order: Rosales
Family: Rosaceae
Subfamily: Rosoideae
Tribe: Sanguisorbeae
Subtribe: Agrimoniinae
Genus: Agrimonia
Species: *Agrimonia eupatoria*

Names of Aigremoine eupatoria in different languages

Names of *A. eupatoria* in different languages are as follows:
Anglo-Saxon: Garclive.
Arabic: Eufātorion, Shajarat ol barāghith, Shawkat Montenah, A'rang, Aghremun, Ghāfath, Hashisht ol ghāfath.
Chinese: Da Hua Long Ya Cao.
English: Common Agrimony, Cocklebur, Stickle wort, Liver Wort, Church, Steeples, Beggar's Ticks, White Tansy, Wild Tansy.
Farsi: Moshkaniyeh, Dava-ye- Jegar, Gole Khole, Gol Roghane Kaah, Gole, Chasbak, Alafe Geloo Dard.
French: Herbe d'eupatoire, Aigremoine commune, Eupatoire des anciens, Thé des bois, Souberiette, Aigremoine eupatoire, Herb de Saint Guillaume, Herb de la mère.
Germany: Kleiner odermenning, Leberkraut, Agremomen.
Greek: Eupatorios.
Italian: Erba vettonica, Santonia, Erba da andata, Agrimonia Eupatoria.
Russian: Repejniček Aptechnyĭ (17).
Solvak: Repík lekársky, Repík, repíček, starček, konopínec, útrobník, boží bič (18).
Spanish: Agrimonia, Hierba bacera, Hierba del podabor.
Swedish: Småborre.
Turkish: Egir otu, Kasik otu, Kizil yaprak, Koyun otu (17).

In Iranian traditional medicine (ITM), there are two plants that are called Ghafath; the amateur users usually do not notice this. Users should look for a description of the plant in each book before using it and should notice which one the writer means. The agrimonium sp. (Rosaceae) Ghafath has yellow flowers, but the second one has pink or reddish flowers with the scientific name of *Eupatorium cannabinum* L. (Asteraceae) (19). The second Ghafath named Ghafath e kanafi which means "hemp agrimony" in Persian (20), "hemp agrimony" is *Eupatorium cannabinum* common name in English.

Description

Agrimonia eupatoria is a plant with bear pinnate, toothed alternate, leaves with undersides like velvet and small pairs between larger pairs with erect, reddish and pubescent stems (50–150 cm high). It has basal leaves as a rosette, and on its long, slender spikes grow five small petals of bright yellow flowers. The fruits are small, cone-shaped, enclosed in a calyx-tube with bristle. The bristles with hook enable the dispersal of the seeds on animal fur. It also spreads vegetatively by stout, woody, deep-lying rhizomes (21).

Some insects associate only with this herb like an eriophyoid mite, *Aculus castriferrei* n. sp (22) and *Stigmella aeneofasciella* Larvae (23). This is why Agrimony is called "Shajarat ol barāghith" in Arabic, which means the tree of fleas in English.

Related species

Agrimonia pilosa and *A. viscidula* Bunge are used in China for comparable conditions (24). The German Commission E Pharmacopoeial Monograph enables the use of *A. procera* as a second precious source of Agrimoniae herba. Granica et al have shown, based on their collected data, that a legitimate source of plant materials for drug preparation is *A. procera* (Fragrant agrimony) (25).

Cultivation and habitat

Agrimony is a native European herb mostly found in marshes, on wasteland, and wet meadows (1). It is one of the most common species of dry grasslands in the ex-arable land of the SE Czech Republic (26) and Slovak Republic (18). Thus, it has been found in the Hindu Kush Mountains of Pakistan (27), as well as Western Himalaya, and India (28). It is harvested during its flowering stage in summer (1).

Agrimonia eupatoria does not reach the edge of its distribution. It displayed dynamic changes in leaf morphology in response to tree shade and in elongation of stems and inflorescences because of herbaceous shade. Because of *A. eupatoria's* high active plasticity, it can maintain constant shoot growth in a variety of light conditions (29). The normal distance in cultivation on seed yield is 4/m-2 (30). *A. eupatoria* best germination results obtained at 20°C at 12-hour daily photoperiod, 5.5 cm root cuttings develop stems and/or roots at 15°C and 25°C (31).

Table 1. Macroscopic characters of *A. eupatoria*

A. eupatoria		Characters
Inflorescence		Elongate raceme
		Terminal
		September-October
Stem structures	Length	30-60 cm high
	Surface	Villous
		Reddish
Leaves		Alternate
		Pinnatifid
	Segments	Oval-lanceolate
		Deeply dentate
		Irregular
	Underside villous	White
		Without glands
	Stipules	Purplish
		Clasping
		Incised dentate
Flowers		Small
		Yellow
	Receptacle	Concave
		A narrow opening
	Calyx tube	Furrowed
		Curved spurs at the tip
	Lobes	5
	Petals	5
		Small
		Imbricate
	Stamens	5-15
		Slender
	Carpel	A single at maturity
Fruit	An achene	Surrounded with the mithered receptacle
		Crowned with the persistent calyx
Taste	Bitter	
	Astringent	

Macroscopic Types

Agrimonia eupatoria's characters are given in Table 1 (24).

Microscopic characters

Agrimonia eupatoria's characters are given in Table 2 (32).

Ethnopharmacology

The plant has a "cool" and "drying" characteristics. All parts, mostly the aerial parts of the plant are used for various diseases (33).

History and folklore

Agrimonia eupatoria was named by Mithridates Eupator, a king of Pontus, a famous plant collector and botanical text author in Greek. Thus, Pliny records, 'it has gotten credit and reputation by a king, as may appear by the name'. Many centuries later, Fuchs named it 'Hepatorium', because of its protective effects on the liver. Until the 18th century Agrimony was known in the Linnaean classification under the title 'Eupatorion', so Dioscorides (IV 41) named it the same (21).

Traditional uses

Agrimonia eupatoria was worshiped in the Kysuce region (18). Since then, people have found various methods for using Agrimony for almost all parts of the body illnesses as shown in Table 3. *A. eupatoria* has been used since Saxon times. It was the primary ingredient of a battlefield cure for bullet wounds called "Arquebusade water" in the 15th century. Agrimony healing power is now credited with the herb's high silica content (33). *A. eupatoria,* known as Ghafath in Iranian traditional medicine (ITM), has been repeatedly used for liver strengthen and it was well documented as an outstanding liver tonic. It is very useful and extensively recommended in Iranian medical literature (34), and used mainly to treat liver disorders (35). It is one of the most important ingredients of ITM tablets like "Qurse Rewand" which means "Rhubarb tablet" in Iranian dialect. Its effects on rats have been recently documented as significant hepatocurative agent and showed signs of recovery and regeneration in damaged liver cells (36).

It is used as antibacterial, astringent, aggregant, antipyretic, diuretic, antidiabetic, anti-inflammatory, antiseptic, antiviral, candidicide, vermifuge, tonic, uricolytic, depurative, cholagogue, emmenagogue, fungicide, hemostat, litholytic, stomachic, sedative, and vulnerary remedy (37).

Arial parts/leaves applications of *A. eupatoria* are given in Table 3 (33). It has been reported that giving Tisane (cold) to lambs has an antidiarrhoeal effect and Tisane (hot) has laxative effects in lambs (38).

3.3 Recommendations on safety

It should be used with caution when there is a constipation (21,33). Side effects of nausea and constipation with excessive doses are likely (21).

Phytochemistry

The Agrimony plant contains tannins, volatile oil and coumarins (1), gum, a phytosterol (24), polysaccharides,

Table 2. Microscopic characters of *Agrimonia eupatoria*

Chromosomes Counts (X = 14)	Diploid (2n = 2x = 28)	
	Tetraploid (2n = 4x = 56)	Perfectly normal, high pollen fertility (94-100%)
	Pentaploid (2n = 5x = 70)	Abnormal meiotic course, high pollen sterility (74-88%). Did not produce seeds
	Hexaploid (2n = 6x = 84)	Perfectly normal, high pollen fertility (94-100%)

Table 3. Applications of *Agrimonia eupatoria*

Application		Method	For
Infusion		A gentle remedy, especially in infants and children. Can be taken by breastfeeding mothers.	Diarrhea
	Eyewash	A weak infusion (10 g herb to 500 mL water)	Conjunctivitis
	Gargle	Use the infusion	Sore throats
			Nasal catarrh
Tincture		More potent and drying than the infusion, and effective	Condition involves excess phlegm or mucus
			Cystitis urinary infections
			Bronchitis
			Heavy menstrual bleeding
Poultice		Of the leaves	Migraines
Wash			Wounds
			Sores
			Eczema
			Varicose ulcers

and flavonoids such as luteolin. From its dried aerial parts, the polyphenolic-polysaccharide complex was isolated with 55×10^3 g/mol molecular weight. It consisted mainly of pectin-like polysaccharides and polyphenolic moieties, composed of lignin-related units, with the dominance of dimethoxy phenyl structures. Agrimonia complex specific carbohydrate composition rich in highly esterified galacturonic acid, constituting thus highly methylated pectin network in which, besides arabinogalactan type II, the highly esterified homogalacturonan and rhamnogalacturonan type I are present, while some units being partially methylated (39). Investigated samples have been shown to contain about 8.2–10.9 mg/g of various flavonoids, 6.3–10.9 mg/g of various tannins (mostly agrimoniin, 2.6–5.4 mg/g), and 0.6–0.9 mg/g of phenolic acids (40). The value of flavonoids determined in the leaves of agrimony (*Agrimonia eupatoria*) was 1.05 RU (41). Total phenolic compounds of more than 19.61 mg galic acid equivalent (GAE)/g to 220.31 mg GA (gallic acid)/g, flavonoids of 20.58 mg RU (rutin)/g to 97.06 mg RU/g, total tannins of 3.06 mg GA/g to 207.27 mg GA/g, and pro-anthocyanidins of 4.15 CChE (Cyanidin chloride equivalent)/g to 103.72 CChE/g have been identified (42), claiming that phenol compounds were the major group of constituents of this plant (40).

The primary metabolites

From the primary metabolites, the amino acid composition has been determined. Seventeen amino acids and their respective amounts were identified in *A. eupatoria,* which is shown in Table 4 (43).

The second metabolites

Sixty-eight out of 87 separated constituents of the volatile oil were quantified, which were more than 87.03% of the total contents. Isolated compounds from the leaf and root of *A. eupatoria* are shown in Table 5, isolated compounds from the leaf of *A. eupatoria* are shown in Table 6 (44), and the phenolic and flavonoid compounds isolated from free or glycosides of the leaf of *A. eupatoria* are shown in Table 7.

Antioxidant potential and scavenging activity

Agrimony's anti-inflammatory ability may be clarified by its antioxidant activity. The plant has polyphenolic compounds capable of activating endogenous antioxidant defense mechanisms (51). The tests done on *A. eupatoria* extract and its polyphenol-enriched fractions against reactive species are shown in Table 8.

Table 4. Amino acid content of *Agrimonia eupatoria* herb

Substance	General formula	Content, mg/100 mg of the raw material	No.	Substance	General formula	Content, mg/100 mg of the raw material
Aspartic acid	C4H6O4N	0.93	10	Methionine	C5H10O2NS	0.31
Threonine	C4H9O2N	0.39	11	Isoleucine	C6H13O2N	0.46
Serine	C3H7O3N	0.62	12	Leucine	C6H13O2N	0.46
Glutamic acid	C5H8O4N	0.15	13	Tyrosine	C9H13O3N	0.23
Proline	C5H9O2N	0.47	14	Phenylalanine	C9H12O2N	0.33
Cysteine	C6H12N2O4S2	0.08	15	Histidine	C6H11O2N3	0.23
Glycine	C2H5O2N	0.93	16	Lysine	C6H13O2N2	0.53
Alanine	C3H7O2N	0.69	17	Arginine	C6H15O2N4	0.15
Valine	C5H11O2N	0.69				

Table 5. The compounds isolated from the leaf and root essence of *Agrimonia eupatoria*

No.	Compound Name	Molecule structure	No.	Compound Name	Molecule structure
1	1-(2-Furyl)-1-hexanone	C10H14O2	35	Geraniol acetate	C12H20O2
2	2,4-Dimethylbenzaldehyde	C9H10O	36	Geranyl acetone	C13H22O
3	2-Cyclopropylidene-1,7,7-trimethyl-bicyolo [2,2,1]heptane	C13H20	37	Hexanal	C6H12O
4	2-Methyl-4-hydroxyacetophenone	C9H20O2	38	L-Camphor	C10H16O
5	3,4-Dimethylbenzaldehyde	C9H10O	39	Linalool	C10H18O
6	3-Octanol	C10H18O	40	Longofolene	C15H24
7	4-Terpineol	C10H18O	41	Muurolol	C15H26O
8	6,10,14-Trimethyl-2-pentadecanone	C18H36O	42	Myristicin	C11H12O3
9	Acoradiene	C15H24	43	Neryl acetate	C12H20O2
10	Anethole	C10H12O	44	Nonanoic acid	C9H18O2
11	Aromadendrene	C15H24	45	Patchoulol	C15H26O
12	Bergamot oil	C12H20O2	46	P-Menth-1-en-4-ol	C10H18O
13	Borneol	C10H18O	47	Pulegone	C10H16O
14	Bornyl acetate	C12H20O2	48	Thymol	C10H14O
15	Camphene	C10H16	49	Torreyol	C15H26O
16	Carvacrol	C10H14O	50	Trans-Nerolidol	C15H26O
17	Caryophyllene	C15H24	51	α-Bisabolene	C15H24
18	Caryophyllene oxide	C15H24O	52	α-Cadinol	C15H26O
19	Cedrol	C15H26O	53	α-Campholenal	C10H16O
20	Cedryl acetate	C17H28O2	54	α-Cedrene	C15H24
21	Copaene	C15H24	55	α-Eudesmol	C15H26O
22	Cubenol	C15H26O	56	α-Guaiene	C15H24
23	Cuparene	C15H24	57	α-Himachalene	C15H24
24	Curcumene	C15H22	58	α-Longipinene	C15H24
25	Cymene	C10H14	59	α-Pinene	C10H16
26	Decanoic acid	C10H20O2	60	α-Selinene	C15H24
27	D-Limonene	C10H16	61	α-Terpineol	C10H18O
28	E-Cadinene	C15H24	62	α-trans-Ocimene	C10H16
29	Epi-Cedrol	C15H26O	63	β-Cedrene	C15H24
30	Epiglobulol	C15H26O	64	β-Pinene	C10H16
31	Eucalyptol	C10H18O	65	β-Selinene	C15H24
32	Eugenol methyl ether	C11H14O2	66	δ-Cadinene	C15H24
33	Farnesyl acetate	C17H28O2	67	δ-Guaiene	C15H24
34	Furan,2,5-dibutyl-	C12H20O	68	τ-Cadinene	C15H24

Table 6. The compounds isolated from the leaf essence of *Agrimonia eupatoria*

No.	Compound Name	Molecule structure	No.	Compound Name	Molecule structure
1	1-Hexanol	C6H14O	8	Germacrene D	C15H24
2	4,4-Dimethyladamantan-2-ol	C12H20O	9	Hexahydrofarnesyl acetone	C18H36O
3	4-Hydroxy-3-methylacetophenon	C9H10O2	10	Isomenthone	C10H18O
4	Cadala-1(10),3,8-triene	C15H22	11	Longipinocarvone	C15H28O
5	Carvone	C10H14O	12	Phenmethyl acetate	C9H10O2
6	Cis-7-Tetradecen-1-ol	C14H28O	13	Prenal	C5H8O
7	Costunolide	C15H20O2	14	β-Damascone	C13H18O

Table 7. The phenolic and flavonoid compounds isolated in a form of free or glycosidic from leaf of *Agrimonia eupatoria*

No.	Compound Name	Reference
1	1-O-Caffeoylquinic acid	(40)
2	3-O-Caffeoylquinic acid	(40)
3	3-O-p-coumaroylquinic acid	(25)
4	3-O-p-Coumaroylquinic acid	(40)
5	4-O-caffeoylquinic acid	(25, 40)
6	5-caffeoyl quinic acid	(46)
7	5-O-caffeoylquinic acid (chlorogenic acid)b	(25, 40)
8	Agrimoniin	(25, 40, 46)
9	Apigenin 7-O-glucoside (apigetrin)	(25, 40, 46)
10	Apigenin 7-O ß-D-glucuronide	(25, 40, 45, 47)
11	Apigenin derivative	(45)
12	Apigenin O-glucuronide	(46)
13	Astragalin (Kaempferol 3-O-glucoside)	(25, 40, 46-49)
14	Caffeoyl-hexoside	(45)
15	Catechin	(25, 40, 45, 46, 48)
16	Ellagic acid	(46)
17	Hexahydroxydiphenoyl-glucose	(50)
18	Isovitexin= Apigenin 6-C-glucoside	(25, 40, 46, 48)
19	Kaempferide	(49)
20	Kaempferide 3-rhamnoside	(49)
21	Kaempferide O-rhamnoside	(46)
22	Kaempferol	(49)
23	Kaempferol 3- O- (6”- O- p- coumaroyl)-glucoside, (tiliroside)	(46-48)
24	Kaempferol 3- O- beta- D- (2''- O- acetyl- 6''- (E)- p- coumaroyl)- glucopyranoside (2''-acetyl- tiliroside)	(47)
25	Kaempferol 3- O- ß- D- (2”- O-acetyl) glucopyranoside	(47)
26	Kaempferol 3-rhamnoside	(49)
27	Kaempferol 3-rutinoside	(49)
28	Kaempferol hexoside	(25, 40)
29	Kaempferol malonylhexoside	(25, 40)
30	Kaempferol O-acetylhexosyl-rhamnoside	(46)
31	Kaempferol Oacetyl-hexosyl-O-rhamnoside	(46)
32	Kaempferol O-(coumaroyl)-hexoside	(50)
33	Kaempferol O-malonylhexoside	(46)
34	Kaempferol-p-coumaroyl-hexoside	(45)
35	Keampferol 3-O-rutinosid	(50)
36	Luteolin 7-O-glucoside (cynaroside)	(25, 40, 46)
37	Luteolin 7-O-ß-D-glucopyranoside	(47)
38	Luteolin 7-O-ß-D-glucuronide	(25, 40, 45, 47)
39	Luteolin glucuronide isomer	(25, 40)
40	Luteolin malonylhexoside	(25)
41	Luteolin-acetyl-hexoside	(45)
42	*P*-Coumaric acid	(45, 46, 48)
43	p-Coumaroil quinic acid	(45)
44	p-Coumaroyl acid hexoside	(40)
45	Procyanidin B-1	(45)
46	Procyanidin B3= Procyanidin dimer B3= catechin-(4β→8)-catechin (B3)	(25, 40, 45, 48)
47	Procyanidin dimer B1= epicatechin- (4β→ 8)- catechin (B1)	(48)
48	Procyanidin dimer B2= epicatechin-(4β→8)-epicatechin (B2)	(48)
49	Procyanidin dimer B6= catechin-(4β→6) catechin (B6)	(48)
50	Procyanidin dimer B7= epicatechin-(4β→6)-catechin (B7)	(48)
51	Procyanidin tetramer	(46, 48)

Table 7. Continued

No.	Compound Name	Reference
52	Procyanidin tetramer-B	(45)
53	Procyanidin trimer	(25, 40, 46, 48)
54	Procyanidin trimer C1= epicatechin-(4→8)-epicatechin-(4→8)-epicatechin (C1)	(48)
55	Procyanidin trimer C2= catechin- (4→ 8)-catechin- (4→ 8)- catechin (C2)	(48)
56	Procyanidin trimer EEC= epicatechin- (4→ 8)- epicatechin- (4→8)-catechin (EEC)	(48)
57	Procyanidin-trimer-B	(45)
58	Protocatechuic acid	(48)
59	Quercetin 3-O-galactoside (hyperoside)	(25, 40, 48)
60	Quercetin 3-O-glucoside (isoquercitrin) (isoquercetin)	(25, 40, 45-48)
61	Quercetin 3-O-rhamnoglucoside (rutin)	(25, 40)
62	Quercetin 3-O-rhamnoside	(25, 40, 45)
63	Quercetin 6__-O-galloyl-3-O-glucoside	(40)
64	Quercetin malonylhexoside isomer	(25, 40, 46)
65	Quercetin O-galloyl-hexoside	(46)
66	Quercetin rhamnoglucoside isomer	(25, 40)
67	Quercetin-7-O-rhamnoside	(45)
68	Quercetin-acetyl-hexoside	(45)
69	Quercitin-acetil-glucoside	(45)
70	Quercitrin	(47)
71	Quinic acid	(45)
72	Rutin	(25, 45, 47)
73	Vitexin (Apigenin 8-C-glucoside)	(40, 46)

Table 8. *Ex vivo* measurements of antioxidant activity of *Agrimonia eupatoria*

Phytochemical test	Reagent	Result
Free radical scavenging activity or the antioxidant activity	DPPH (2,2-diphenyl-1-picrylhydrazyl) method	Indicating a general radical scavenging activity, with a dose-dependent ability (9.1-97.5%) (5,8), quite similar to ascorbic acid (EC_{50} = 17 µg) and much higher than Catechin (EC_{50} = 140 µg) (50). The MeOH extract and two Sep–Pak fractions (30 and 60%) showed prominent free radical scavenging activity. RC_{50}s were determined as 4.64×10^{-4}, 5.13×10^{-3} and 4.73×10^{-4} mg/mL (4). The antioxidant activity was highest for the acetone extract and ranged from 97.13% to 27.73% (42).
	Reducing power	Extracts of *A. eupatoria* showed moderate reducing power compared with the positive control, and showed activity in all examined concentrations. Hence, concluded that reducing power depends on concentration (42).
Superoxide anion scavenging effect	Detected by Nitro Blue Tetrazolium (NBT) reduction	Inhibited the NBT reduction in a dose-dependent manner
	Peroxyl and hydroxyl radicals	A very good scavenging capacity. The extract is an efficient scavenger of peroxyl radicals.
Oxidant species	Hydrogen peroxide (H_2O_2) assay	Exhibited H_2O_2 scavenging activity (8). Agrimony extract (AE) decreased both the superoxide dismutase (SOD) and catalase (CAT) expressions (45).
	Hypochlorous acid (Elastase (E) + α_1- antitrypsin (α_1) + Hypochlorous acid (HOCl)) and inhibition of Elastase	Extract abolished elastase activity; affected the α_1- antitrypsin activity, but promoted some inhibition of Elastase activity.
	Peroxynitrite	Inhibited the dihydrorhodamine 123 oxidation (8)
		S-nitroso-N-acetylpenicillamine (SNAP) assays (46)
2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (+) radical decolourisation reaction system		6.7-79.5% (5, 53) and RC_{50s} were determined as Agrimony = 0.79 mg/mL (45).
Trolox equivalent antioxidant capacity (TEAC)		Showed the scavenging activities (48), (TEAC 3.76+/-0.5mM/QE 702.29+/-6.82 microM) (6).
Thiobarbituric acid reactive substances (TBARS) method		The lipid peroxidation inhibition (48), 104.8±11.4 (% control) (53)

Table 8. Continued

Phytochemical test	Reagent	Result
The phenolic content		The relevant content of phenols and significant antioxidant activities were observed for the aqueous-alcoholic extract (49). Ascertained by High-performance liquid chromatography-diode array detection (HPLC-DAD) (55) ranged from 19.61 mgGA/g to 220.31 mgGA/g (43). Achieved by HPLC-PDA-ESI/MS^n (46).
The effect of in vitro digestion upon Caco-2 cells via LC-MS/MS		3,4,5-Trihydroxybenzaldehyde was stable throughout the digestive process, whereas Chlorogenic acid remained stable after the stomach phase, Rutin was decreased after the stomach phase (52).
Capacity to inhibit lipid peroxidation		Reacted
Xanthine oxidase activity	Uric acid formation	A dose-dependent inhibition (8)
Carbonyl content	Protein oxidation	86.8±3.7 (% control)
8-ohdg	DNA oxidation	149.6± 34.0 (% control) (53)
Oxidative damage	Plasmid DNA	Agrimony extract possesses better antioxidant properties to compare with rutin as standard.
Cytotoxicity test	24 h incubation on THP-1 cell line	No cytotoxic effect was observed.
Skin flap viability	On male Sprague-Dawley rats	*A. eupatoria* extract has the capacity to act on damaged skin (45).

EC_{50}: micrograms of freeze-dried samples required to scavenge 50% of the DPPH. Phytochemical tests were done on *A. eupatoria* extracts and its fractions.

Table 9. Used extraction techniques for Isoflavonoid's isolation and determination

Extraction technique	Diadzein	Genistein	Biochanin
Supercritical fluid extraction (SFE)	19.7 ± 0.8	N.d.	1.3 ± 0.1
Pressurized fluid extraction	4.8 ± 0.3	N.d.	0.6 ± 0.1
Matrix solid phase dispersion	2.8 ± 0.4	0.2 ± 0.1	N.d
Ultrasonic extraction in an ultrasonic bath (USE)	5.7 ± 0.1	N.d.	1.0 ± 0.1
Ultrasonic homogeniser (HOM)	12.8 ± 0.1	N.d.	0.6 ± 0.1
Soxhlet apparatus (SOX)	N.d.	N.d.	0.9 ± 0.2

N.d., concentration below the limit of detection.

Extraction techniques

Various extraction techniques have been used for the isolation and determination of *A. eupatoria*'s Isoflavonoids. The techniques are listed in Table 9 (54).

Pharmacological activities

Agrimonia eupatoria's physio-pharmacological activities are shown in Table 10.

Toxicity

The safety and efficacy of *A. eupatoria* have been confirmed during a very long period of its traditional application. Recent studies have shown that *A. eupatoria* aqueous extract (AEE) consumption by subjects was safe and generally well-tolerated without severe adverse events (65). Agrimony is an herbal medicine, which its safety is comparable with coffee (37). But, since Agrimony is an indication of geogenic contamination of flysch soils, elements like Ni, Co, Mn, Cu, Cr, V, and Mo typically concentrate in the roots and shoots of the plant. So, high uptake of these elements by Agrimony growing on similar soils is possible (69).

Conclusion

This review presents *A. eupatoria* description, history, and advances in phytochemistry and other aspects. Pharmacological studies carried out on its extracts and traditional uses revealed that it could be an important source for new drugs.

Authors' contributions

MGP and NM conceived of the presented idea; MGP developed the article, performed the computations, wrote, and prepared the manuscript; NM encouraged; while author NM and SHM supervised the research and critical revision of the article. MRK and MRM made the final version. All authors read the manuscript and confirmed the publication of the final version.

Conflict of interests

There is no conflict of interest

Ethical considerations

Ethical issues including text plagiarism, misconduct, manipulation or appropriation, data fabrication,

Table 10. Physio-pharmacological activities of *Agrimonia eupatoria*

Physio-pharmacological activities of *Agrimonia eupatoria*	Plant part/extract	Dose/model	Worked on	Standard drug	Result
Analgesic and anti-inflammatory	Aerial parts/Aqueous extract + extraction with ethyl acetate	For the aqueous extract single-dose of 199.18 mg/kg (mice) and 99.59 mg/kg (rats); for the fraction, single dose of 36.24 mg/kg (mice) and 18.12 mg/kg (rats)	Mice and rats		The anti-inflammatory and peripheral analgesic properties of agrimony were confirmed. No signals of renal or hepatic toxicities detected (55).
	Arial parts/water infusion and ethyl acetate fraction	The mouse carrageenan-induced paw edema model for *in vitro* anti-inflammatory activity (rats received 99.59 mg/kg, and 199.18 mg/kg doses of infusion and 18.12 mg/kg, and 36.24 mg/kg for the fraction). The acetic acid-induced writhing and hot-plate tests for peripheral and central analgesic potential (mice received 199.18 mg/kg, and 398.26 mg/kg, for infusion and 36.24 mg/kg, and 72.48 mg/kg for the fraction) and formalin assay to assess both activities (mice received 72.48 mg/kg of the fraction).	Male Wistar rats and male mice	Diclofenac sodium and morphine	*In vivo* anti-inflammatory and analgesic activities were verified (46).
Antiadhesive activity	Ethanol extract	Against *Campylobacter jejuni*	*Campylobacter jejuni*		No significant antiadhesion activity ($IC_{(50)}$ value >35 mg/mL) was found for *Agrimonia eupatoria* (56).
Antibacterial activities	Hydro ethanol extract	Modified microdilution assay	*Helicobacter pylori* and *Campylobacter jejuni*	Antibiotics	*Agrimonia eupatoria* was among the most active herbal extracts in inhibiting the growth of *Helicobacter pylori* (3).
	Seeds	n-Hexane, DCM and MeOH extracts and four Sep–Pak fractions of the MeOH extract.	*Bacillus cereus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella goldcoast,* and *Staphylococcus aureus*		The n-Hexane extract showed an inhibitory effect against *Bacillus cereus* and *Bacillus subtilis.* Sep–Pak fractions eluted with 30, 60, and 80% MeOH in water showed significant antibacterial activity (4).
	Areal parts/aqueous extract and crude ethanolic extract	10 mg/mL	*Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli*	Fucidin ointment	The ethanolic extract was more effective in inhibiting tested bacteria (57).
Antibiofilm activity	Aerial parts/ethanol, diethyl ether, water, and acetone extracts		*P. mirabilis* and *P. aeruginosa*	Tetracycline	Biofilm inhibitory concentration required to reduce biofilm coverage by 50% values for acetone extract was 4315 mg/mL for *P. mirabilis* and 4469.5 mg/mL for *P. aeruginosa* (42).
Anticholinesterase activity	Aerial flowering parts/ Aqueous extracts				Displayed inhibition of butyrylcholinesterase (BuChE), acetylcholinesterase (AChE), and inhibition of cholinesterases by apigenin, luteolin, and quercetin glycosides (50).

Table 10. Continued

Physio-pharmacological activities of *Agrimonia eupatoria*	Plant part/ extract	Dose/ Model	Worked on	Standard drug	Result
Anticoagulant activity	Dried aerial parts	Human plasma-derived from healthy donors			The *A. eupatoria* complex prevents the development of plasma clots, primarily in the intrinsic blood coagulation cascade pathway. It is primarily an indirect inhibitor of thrombin, mediated by antithrombin or by heparin cofactor II (39).
Anti-diabetic	Aqueous extract	Into the diet (62.5 g/kg) and drinking water (2.5 g/L), (1 mg/mL) on the abdominal muscle, 0.25-1 mg/mL on BRIN-BD11 cells/Streptozotocin (STZ)-diabetic	Mice, abdominal muscle, BRIN-BD11 pancreatic B-cell line		The presence of antihyperglycemic, insulin-releasing and insulin-like activity in *Agrimony eupatoria* demonstrated (15).
	Water infusion	Streptozotocin-induced diabetes mellitus (DM) rat model/200 mg/L	Male Wistar rats		In the avoidance and/or adjuvant treatment of developing cardiovascular problems linked to DM and sicknesses related to oxidative stress, *A. eupatoria* extract suggests its higher clinical potential (58).
Treatments for diabetes mellitus	Dried leaves	Supplied in the diet (6.25% by weight) or as decoctions or infusions (1 g/400 mL) in place of drinking water/ Streptozotocin (200 mg/kg i.p.) for 12 days	Mice		Treatment with agrimony reduced the level of hyperglycemia during the development of streptozotocin diabetes but did not reduce the rate of body weight loss. Certain traditional plant treatment for diabetes, namely Agrimony (14).
Anti-inflammatory Activity	Hydro-alcoholic extract and a polyphenol-enriched fraction	Against the reactive species/ 96-well microplate-based broth dilution assay			*Agrimonia eupatoria* anti-inflammatory activity mechanism could be its significant scavenging capacity of reactive species by its polyphenols (8).
		Enzyme-linked immunosorbent assay, nitric oxide assay, and Western blotting.	BV2 microglial cells		*Agrimoniae herba* suppressed lipopolysaccharide-induced nitric oxide production in BV2 microglial cells and lipopolysaccharide-induced production of proinflammatory cytokines such as interleukin 1 beta, tumor necrosis factor, and interleukin 6 in a dose-dependent manner had no cytotoxicity and inhibited the expression of inducible nitric oxide synthase. It may be used as a form of pharmaceutical acupuncture therapy in the treatment of brain inflammation (9).
	Tea	One-month	Healthy humans		Has potential in improving markers of lipid metabolism and inflammation (7).

Table 10. Continued

Physio-pharmacological activities of *Agrimonia eupatoria*	Plant part/extract	Dose/model	Worked on	Standard drug	Result
Antimicrobial activity	Extracts	From 2 to 0.004 mg/mL/ 96 well plate microdilution method (200, 40, and 8 µg/mL)	Selected gram-positive *Staphylococcus aureus and* gram-negative *Pseudomonas aeruginosa* and *Escherichia coli* bacteria of relevance in wounds		Moderate activity for *Potentilla reptans* L (59).
	Aerial parts/ Hot water, aqueous extract	Primary human skin fibroblasts (line C688)	*Escherichia coli* ATCC 25922, *E. coli* O44*, Vibrio cholerae* O395-tacCTB strain, and *Lactobacillus rhamnosus*		Displayed modest bacteriostatic potentials. Suppressed the binding of cholera toxin subunit B to the cell surface and immobilized GM_1 ganglioside (60).
Antimicrobial and antifungal activity	Aerial parts /ethanol, diethyl ether, water, and acetone extracts the acetone extract demonstrated the highest activity	The antimicrobial activity was tested by determining the minimum inhibitory concentration (MIC) using the microdilution method with resazurin	24 microorganisms including 18 strains of bacteria (probiotics strains: *Lactobacillus rhamnosus*, *Bacillus subtilis* IP 5832, and *Bifidobacterium animalis* subsp. *lactis*; standard strains: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *B. subtilis* ATCC 6633, and *P. aeruginosa* ATCC 27853; and clinical *isolates S. aureus, E. faecalis, Bacillus cereus, B. subtilis, E. coli, Salmonella enterica, Salmonella typhimurium, Klebsiella pneumoniae, P. mirabilis*, and *P. aeruginosa*) and six strains of fungi (*Aspergillus niger, Aspergillus flavus, Penicillium chrysogenu m, Penicillium italicum, C. albicans* ATCC 10231, and *Candida albicans*)	Tetracycline, ampicillin, amphotericin B, and itraconazole	The strongest antimicrobial activity was detected on G^+ bacteria, especially on probiotic species. The acetone extract demonstrated the highest activity (42).
Anti-nociceptive effect	Aerial part/ Ethanolic extract	200 mg/kg/ cisplatin-induced neuropathic pain model	Male Sprague-Dawley rats	Gabapetin	In the pin-prick test and plantar test, *A. eupatoria* extract displayed an antinociceptive property with a lower withdrawal time and a higher withdrawal threshold in the paw-withdrawal threshold test as compared to control animals. In the case of cold-allodynia, increased paw-withdrawal duration in the chemical test, and showed superior activity to gabapentin. *A. eupatoria* extract found to possess therapeutic potential for the treatment of neuropathic pain (61).

Table 10. Continued

Physio-pharmacological activities of *Agrimonia eupatoria*	Plant part/ extract	Dose/ Model	Worked on	Standard drug	Result
Antioxidant activity	Acetone, methanol, aqueous, acetone-hexane, acetone-t-butyl methyl ether, acetone-n- BuOH, acetone-aqueous extracts	In DPPH radical scavenging, ABTS(+) radical decolorization reaction systems			Radical scavenging capacity of agrimony extracts varied in a wide range and depending on the polarity of the solvent used to obtain the extract (9.1-97.5% in DPPH reaction and 6.7-79.5% in ABTS reaction) (5).
	Tea	Trolox equivalent antioxidant capacity (TEAC)		Honeybush, rooibos, black and green tea	As compared to the foreign plants examined, it can be considered a rich source of water-soluble antioxidants (6).
	Aerial flowering parts/Aqueous extracts				Antioxidant activity of Agrimonia extract can be affected especially by hexahydroxydiphenoyl (HHDP)-glucose and quercetin glycosides (50).
	Arial parts/water, acetone, ethanol, and diethyl ether extracts	In DPPH radical scavenging and Reducing power		Ascorbic acid	Higher concentrations of acetone extract and vitamin C operated in a similar way, confirming the high antioxidant activity of Agrimony. All tested extracts showed a concentration-dependent antiradical activity Reducing the power of acetone extract was found to be the most active, followed by ethanol, water, and diethyl ether extracts (acetone > ethanol > water > diethyl ether) (42).
	Arial parts/infusion and ethyl acetate fraction	DPPH, superoxide anion, hydroxyl radical, and SNAP assays			Showed a significant antiradical activity against all tested radicals. Decreased NO levels in vitro. Fraction being more active than infusion. They are potential sources of antiradical and anti-inflammatory polyphenols (46).
Anti-oxidative potential	Tea	One month	Healthy humans		Has potential in improving markers oxidative status in healthy adults (7).
Antioxidant status	0.1% & 0.2% agrimony extract	Supplied through drinking water	Broiler chickens		Can beneficially influence the antioxidant status of thigh meat thus improve meat quality (62).
	0.2% agrimony extract	Supplied with drinking water/(2:1000)	Broiler chickens at the age of 42 days	Clove (*Syzygium aromaticum* L.) Powder	It has a potential to increase the antioxidant status (63).

Table 10. Continued

Physio-pharmacological activities of *Agrimonia eupatoria*	Plant part/extract	Dose/Model	Worked on	Standard drug	Result
Anti-tumor	Aqueous and methanol extracts	6.0, 12.0, 24.0, 48.0 and 96.0 µg/mL	Human cervical cancer; Hela and Rhabdomyosarcoma (RD); RD cell lines and a primary cell culture; mouse embryo fibroblast; MEF		Five plant extracts concentrations showed concentration-dependent anti-tumor properties, and the methanol extract reported better growth inhibition percentage (PGI) values than aqueous extract in Hela and RD cell lines, while MEF cells, reported lower PGI values. Among these concentrations, for the three examined timespans, 96.0 µg/mL was the best in generating PGI in RD and Hela cancer cell lines (13).
Antiviral activity	Aerial parts (stems and leaves)/ Aqueous extract		Hepatitis B virus (HBV)		The extract prepared at 60 ºC has the greatest effect. The inhibitory activity was the highest at mid-July. *Agrimonia* genus plants contain potential antiviral activity against HBV (16).
Enzymatic and non-enzymatic antioxidants	Extracts extract from the crop tops	Administered in the water at a final concentration of 0.1% after 42 days of feeding the level of reduced glutathione was measured in the plasma and in liver, heart, and kidney mitochondria	120 one-day-old broilers COBB500		The activity of superoxide dismutase had a significant decrease. The application of agrimony extract appears to be suitable for the antioxidant effect against peroxidation of gamma-linolenic acid (63).
Growth performance, and selected indices of lipid profile	0.2% agrimony extract	Supplied with drinking water/ (2:1000)	Broiler chickens at the age of 42 days	Clove (*Syzygium aromaticum* L.) powder	It fails to influence either the selected lipid metabolism indices or the growth performance (64).
Hepatic oxidative stress.	Aqueous extracts (leaves)	For 4 weeks	Mice	Water	Led to a decrease in catalase activity produced a decrease in SOD activity. In general, agrimony appeared to be a promising extract, in protection; and was even slightly toxic (53).
Hepatoprotective effects	Water extract	10, 30, 100, and 300 mg/kg/d/ chronic ethanol-induced liver injury	Rats		*Agrimonia eupatoria* water extract enhanced chronic ethanol-induced liver damage, likely because of oxidative stress suppression and Toll-like receptor (TLR) -mediated inflammatory signals (10).
	Aqueous extract	(160 mg/d)/ two capsules twice a day for 8 weeks	Subjects aged between 20 and 70 years who were diagnosed with mild to moderately elevated ALT levels (between 45 and 135 IU/L).		A significant reduction in elevated alanine transaminase (ALT) and serum triglyceride (TG) was observed (65).

Table 10. Continued

Physio-pharmacological activities of *Agrimonia eupatoria*	Plant part/ extract	Dose/ Model	Worked on	Standard drug	Result
Irritable bowel syndrome (IBS)	Aerial parts	The diarrhea-predominant and alternating bowel habit IBS (DA-IBS) formula containing Agrimony	Patients who fulfilled the Rome II criteria for irritable bowel syndrome.		The DA-IBS formula was not effective in alternating bowel habit IBS or improving bowel habit in individuals with diarrhea-predominant but it improved a number of IBS symptoms significantly (66).
Neuroprotective	Methanolic extract	Glutamate-injured HT22 cells	HT22 hippocampal cells		Astragalin, isoquercitrin, quercitrin, and luteolin 7-O-ß-D-glucuronide compounds showed neuroprotective effects on glutamate-induced toxicity in HT22 cells (47).
Oxidative stability	0.1% & 0.2% agrimony extract	Supplied with drinking water	Broiler chickens		Can beneficially influence the oxidative stability of thigh meat thus improve meat quality (62).
Oxidative stress	Aqueous-alcoholic extract.	1%, 2.5%, 5%, 7.5%, 10%, 12.5% and 15% /24 h	Cell culture model 3T3-L1 pre-adipocytes		Pretreatment of cells with extract significantly reduced the stimulatory effect of the oxidizing agent on gene expression (6).
Wound healing activity	Areal parts/aqueous extract and crude ethanolic extract	10 mg/mL	*Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli*	Fucidin ointment	The wound healing was completed in l0 days by using the ethanolic extract ointment (57).
Wound healing scar formation inhibition	Pulverized in combination with three more herbs *Agrimonia eupatoria* (A) & *Nelumbo nucifera* Gaertn (N) & *Boswellia carterii* (B) and Pollen *Typhae angustifoliae* (P) (ANBP)		New Zealand white rabbits		ANBP plays dual roles, promoting wound healing and alleviating scar formation (68).

falsification, redundant publication as well as duplicate submissions have been carefully observed by authors.

Funding/Support

This research did not receive any specific grant from funding agencies in the public, commercial, or profit sectors. There has been no financial support for this work.

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