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A review of the traditional uses, phytochemistry, pharmacology, and clinical evidence for the use of the genus *Alchemilla* (Rosaceae)

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ABSTRACT

Ethnopharmacological relevance: The genus *Alchemilla* L. (lady's mantle) comprises 1000 species, of which more than 300 have been characterized from Europe. Notably, as folk medicines, *Alchemilla* species have long been prescribed for the treatment of dysmenorrhea, pruritus vulvae, menopausal complaints, and related diseases in women. This review summarizes the traditional uses, highlights promising plant species, and focuses on phytochemical and biological studies to highlight future areas of research.

Aim of the review: This literature review aims to provide a comprehensive overview of *Alchemilla* species, covering their botany, traditional uses, phytochemistry, and biological and pharmacological activities, and to summarize the current research status to better understand the application value of *Alchemilla* plants in modern phytotherapy.

Materials and methods: The search strategy utilized the major thematic platforms Reaxys, Web of Science, Google Scholar, Scopus, ScienceDirect, PubMed, the USDA Plant Database and Kew Science (Royal Botanic Gardens) and was performed with the term *Alchemilla*. These platforms were systematically searched for articles published from 1960 to 2023.

Results and discussion: Alchemilla species, as members of the Rosaceae family, produce tannins, phenolic acids, flavonoids, anthocyanins, coumarins, triterpenes and violet compounds. Effort has been made with this comprehensive review of *Alchemilla* plants to highlight the recent developments and milestones achieved in modern phytochemistry and phytotherapy, underlaying a broad spectrum of the activities of these plants, such as antioxidant, anti-inflammatory, neuroprotective, antimicrobial, antiobesity, cardiovascular, anticancer, and wound healing effects.

Conclusions: An increasing number of studies on the plants in the *Alchemilla* genus have provided data about the main constituents and their importance in modern medicine. Both *in vitro* and *in vivo* studies have indicated that *Alchemilla* plants possess an extensive spectrum of biological activities. Regardless of the remarkable medical potential of *Alchemilla* extracts, clinical studies are limited and need to be performed to produce safer and less expensive plant-based drugs.

1. Introduction

Alchemilla (syn. *Alchimilla* Mill., lady's mantle, bear's foot (eng.), Aslanpençesi (tr.), Aphanes L. (sp.), Lachemilla (Focke) Rydb., Zygalchemilla Rydb. (N. Amer. Fl.), Percepier Moench (nom. illeg.) is a genus of perennial or annual herbs or low shrubs in the family Rosaceae, with the common name lady's mantle (Graham, 1960; Royal Botanic Gardens,). These plants have traditionally been used as herbal infusions to treat gynaecological diseases, including *Alchemilla xanthochlora* Rothm., or in Central Europe, called *A. vulgaris*. The data provided in many papers clearly show that folk knowledge and the use of plant-based medicines are still active. Several ethnobotanical reports on *A. vulgaris* L. have pointed out its diverse biological properties against problems such as dysmenorrhea, pruritus vulvae, menopausal complaints, and related diseases in women (Jaradat and Zaid, 2019; Masullo et al., 2015). To prepare a single part of the infusion, 2–4 g of the dried herb is added to 150 mL of hot water and left for 10 min. The usual daily dose of lady's mantle herb is from 5 to 10 g. It is recommended to use 3 portions of the infusion during the day between meals (Czygan, 2004).

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Abbreviations

UV	ultraviolet
IR	infrared
TLC	thin-layer chromatography
HP-TLC	high-performance thin-layer chromatography
HPLC	high-performance liquid chromatography
SFC	supercritical fluid chromatography
LC-MS	liquid chromatography-mass spectroscopy
GC-MS	gas chromatography-mass spectroscopy
ABTS	diammonium 2,2′-azinobis[3-ethyl-2,3-dihydrobenzothiazole-6-sulphonate
DPPH 2	2-diphenyl-1-picrylhydrazyl
EtOH	ethanol
EtOAc	ethyl acetate
MeOH	methanol
CUPRAC	cupric ion reducing antioxidant capacity
FRAP	ferric reducing antioxidant power
TEAC	trolox equivalent antioxidant capacity
TE	trolox equivalent
EAA	equivalent ascorbic acid
AA	ascorbic acid
MDA	malonaldehyde
SNP	sodium nitroprusside
ORAC	oxygen radical absorbance capacity
HORAC	hydroxyl radical antioxidant capacity
TRAP	telomerase repeated amplification protocol
TBARS	thiobarbituric acid reactive substances
LOX	lipoxygenase
TRL4	toll-like receptor 4
HRBC	human red blood cell
MIC	minimum inhibitory concentration
Bs	*Bacillus subtilis*
Se	*Staphylococcus epidermidis*
Ef	*Enterococcus faecalis*
Sa	*Staphylococcus aureus*
Kp	*Klebsiella pneumoniae*
Pa	*Pseudomonas aeruginosa*
Ec	*Escherichia coli*
An	*Aspergillus niger*
Ca	*Candida albicans*
Sc	*Saccharomyces cerevisiae*
Pm	*Proteus mirabilis*
Sm	*Serratia marcescens*
Aj	*Acinetobacter johnsonii*
At	*Agrobacterium tumefaciens*
Rs	*Rhizoctonia solani*
Pi	*Penicillium italicum*
Fo	*Fusarium oxysporum*
Bc	*Branhamella catarrhalis*
Tm	*Trichophyton mentagrophytes*
Bm	*Bacillus mycoides*
Ml	*Micrococcus lysodeikticus*
St	*Salmonella typhimurium*
Ac	*Azotobacter chroococcum*
Tv	*Trichoderma viride*
Tl	*Trichoderma longibrachiatum*
Ab	*Aspergillus brasiliensis*
Ga	*Glaucus atlanticus*
Fo	*Fusarium oxysporum*
Aa	*Alternaria alternata*
Kr	*Kocuria rhizophila*
St	*Salmonella typhimurium*
Ea	*Enterobacter aerogenes*
Sp	*Streptococcus pyogenes*
Sd	*Shigella dysenteriae*
St	*Salmonella typhi*
MTT	2,5-diphenyl-2H-tetrazolium bromide
SRB	sulforhodamine B
LDH	lactate dehydrogenase
CV	crystal violet staining
MDBK	madin-darby bovine kidney
PTZ	pentylenetetrazole
IBAT	ileal bile acid transporter
MO2	muscle oxygen consumption
TNF-α	tumor necrosis factor alpha
VEGF	vascular endothelial growth factor
IL	interleukin
ALT	alanine transaminase
AST	aspartate aminotransferase
ALP	alkaline phosphatase
TBARS	thiobarbituric acid reactive substances
IND	indomethacin
PGE_2	prostaglandin E2
$PGF_{2\alpha}$	prostaglandin F2α

The main galenic form of the drug prepared from *Alchemilla* is infusions; however, to test their phytochemical profile and biological activity, apart from water extracts, alcoholic or deep eutectic solvents have also been used (Kovač et al., 2022). Several pharmacopoeias contain monographs on *Alchemilla* species, e.g., *Alchemillae herba* (the flowering, aerial parts of *A. vulgaris*) in the European Pharmacopoeia 10 (European Pharmacopoeia, 2019) or Polish Pharmacopoeia XII (Farmakopea Polska, 2020). As members of the Rosaceae family, the main phytochemicals in lady's mantle species are tannins, flavonoids, and phenolic acids. However, violet compounds, terpenes, and coumarins are also present in smaller amounts (Kanak et al., 2022). Despite the fact that traditional medicine is a powerful opportunity to exploit an unchanging knowledge, the modern medicine demanding to follow restrictive guidelines to preserve high quality of plant-based preparation. Therefore, using some knowledge about rich chemical composition of *Alchemilla* plants, supported by many studies, puts them high among potential therapeutics in the modern medicine. Following that, we presented research of extracts obtained from *Alchemilla* which display a broad spectrum of pharmacological properties. Based on the knowledge of their phytochemical composition, they have been studied as antioxidant, antimicrobial, anticancer, anti-inflammatory, and neuroprotective agents *in vitro* (Table 7) as well as therapeutics for obesity, convulsions, and endocrine and female diseases *in vivo* (Table 8). Additionally, *A. vulgaris* glycerine products were studied in clinical trials as drugs for the treatment of common mouth ulcers (Shrivastava and John, 2006). Thus, this review on the genus *Alchemilla* emphasizes the importance of plants as inexhaustible sources of biologically active compounds, highlights the possibilities for conducting new scientific research and points to the gaps in recent discoveries.

2. Methodology/search strategy

A literature review was used in this study. The Science Direct/ELSEVIER, Taylor & Francis Online, SCOPUS, PubMed/MEDLINE, Web of Science (SCI-EXPANDED), Wiley Online Library, Google Scholar, REAXYS, and EBSCO Discovery Service (EDS) databases were searched for integrative manuscripts published between 1960 and May 2023. Papers were included if they were published in English and used the

following combinations of the above keywords: *Alchemilla*, Rosaceae, phytochemistry, biological activity, flavonoid, tannin, phenolic compounds, secondary metabolites, violet compounds, phenolic acids, bioavailability, clinical trials, toxicology, traditional use, ethnopharmacology, extraction, isolation, *in vivo*, *in vitro*, *in silico*, genus distribution, and therapeutic agent. Search definitions were run in separate or restricted combinations that accounted for the requirements or limitations of the database used. A total of 169 publications were identified for inclusion. The chemical compounds in Figs. 2–4 were drawn using ChemSketch software. The all-plant names have been checked and finally correspond to the latest revision in The Plant List (www.theplantlist.org).

3. Botanical description and distribution

The genus *Alchemilla* is a group of perennial herbaceous plants that are commonly known as lady's mantle and comprise approximately 1000 species distributed across temperate regions of the Northern Hemisphere, mainly in low temperate and subarctic regions of Europe and Asia, although the native ranges of a few members of this genus are in temperate to tropical mountains (Africa and the Americas) (see Fig. 1) (Fröhner, 1995; Sepp and Paal, 1998; Shilpee et al., 2021; USDA, 2023). The botanical identification and taxonomy of this genus are overly complicated due to interspecific hybridization and facultative apomixis, resulting in high morphological variability among the species. The genus *Alchemilla* is divided into 18 sections, among which 5 are African (Fröhner, 1995). *Alchemilla* plants are well represented in the mountainous regions of Central and Southern Europe (Kurtto et al., 2007). In this latter region, the number of species diminishes as one goes further south, and the representatives of the genus are restricted to the more humid zones in the mountains, especially to those communities composed of elements with a boreal affinity. *Alchemilla* presents apomictic species that are not easy to identify. These macro- and micromorphological differences between taxa, due to apomixis, are persistent, and very few are dependent on the environmental conditions (Pihu et al., 2009).

Botanically, *Alchemilla* species share several characteristic features. They typically have basal rosettes of leaves arising from a central crown. *Alchemilla* plants typically grow in clumps and have a low, spreading growth habit. They vary in size, with most species ranging from 15 to 60 cm (6–24 inches) in height. The leaves of *Alchemilla* plants are one of their most distinctive features. They are palmate or lobed, resembling the shape of a fan or the palm of a hand. The leaf margins may be toothed or smooth. The leaves often have a slightly hairy or velvety texture and are typically green, although some species exhibit variations in coloration. *Alchemilla* plants produce small, inconspicuous flowers that are arranged in loose clusters or panicles. The flowers are usually yellowish-green, although they can also be white or reddish. The flowers do not have petals but are composed of sepals, which are the leaf-like structures surrounding the reproductive parts of the flower. The flowers are borne in branched clusters called cymes or panicles. The inflorescence arises from the leaf axils or terminal ends of the stems. The clusters of flowers create a delicate and airy appearance. *Alchemilla* plants reproduce both sexually, through seeds, and asexually, by forming clumps through rhizomes or stolon. The flowers are pollinated by insects, and after pollination, they develop small, dry fruits called achenes. *Alchemilla* species are renowned for their ability to capture and retain water droplets on the surface of their leaves. This phenomenon, known as guttation, is facilitated by specialized hair-like structures called trichomes. The water droplets on the leaves of *Alchemilla* plants are said to resemble very small pearls, which enhances their aesthetic appeal. Lady's mantle plants are adaptable and can thrive in various soil types, including moist, well-drained soils. They are commonly found in meadows, woodlands, and alpine regions, often growing in clumps or as groundcover. Some species of *Alchemilla* are also cultivated in gardens for their ornamental value. These characteristics, along with their historical uses and ornamental value, make *Alchemilla* plants a captivating and popular choice among gardeners and plant enthusiasts.

4. Traditional uses of *alchemilla* species

The use of plant extracts or plant-derived compounds to treat diseases is a healing method that has stood the test of time. Currently, many pharmacological classes of medicines, including prototypes of natural products (e.g., atropine and morphine), were originally discovered through explorations of traditional medicine and the sociocultural and

Fig. 1. Distribution of native and introduced *Alchemilla* species.

Table 1
Ethnopharmacological information of *Alchemilla* species ([A] – internal use; [B] – external use).

Alchemilla species	Traditional medicine uses	ICPC-2 category	References
A. vulgaris	infusion against menstrual pain and headache [A]	female genital	(Ginko et al., 2023; Jaradat and Zaid, 2019)
	weight loss, stomach and intestine pain and inflammation [A]	metabolic and nutritional, digestive	Said et al. (2002)
	wounds [B], diarrhoea, menorrhagia [A]	skin, digestive, female genital	Parthasarathy and Prince (2021)
	eczema, skin rashes [B]	skin	(Menković et al., 2011; Saad et al., 2005)
	weight loss [A]	metabolic and nutritional	(Alachkar et al., 2011; Said et al., 2002, 2011s
	antiseptic [B]	skin	Kiselova et al. (2006)
	astringent [B]	skin	Trouillas et al. (2003)
	diabetes [A]	metabolic and nutritional	Swanston-Flatt et al. (1989)
A. pedata	common cold, thyroid, anaemia, depression or anxiety [A]	digestive, respiratory, endocrine	Woldeamanuel et al. (2022)
A. mollis	women's illness, asthma, cough, bronchitis, and liver inflammation [A]	female genital, respiratory, skin	Parthasarathy and Prince (2021)
	wounds, skin diseases [B]		
	sore throat [A], arrest haemorrhages [B], relieve nausea and vomiting [A]	respiratory, general and unspecified, digestive	Todorov et al. (2014)
A. monticola	wounds and burns [B]	skin	Mladenova et al. (2021)
A. xanthochlora	ulcers [A]	digestive	Herbrechter et al. (2020)
A. hirsutiflora	gynaecological diseases [A]	female genital	Kalankan et al. (2015)
A. hessii	wounds [B]	skin	Kaval et al. (2014)
A. cryptantha	dysmenorrhea, lower abdominal pains [A]	female genital	Focho et al. (2009)
A. alpina	stomach ache (intestinal antalgic/anti-inflammatory), kidney stones (lithotriptic) [A]	digestive, urological	Rigat et al. (2007)

religious beliefs of native peoples (Gilani and Atta-ur, 2005). *A. vulgaris,* a well-known species from the genus *Alchemilla*, has been commonly used in folk medicine to heal gynaecological disorders, such as menorrhagia, dysmenorrhea, or menstrual pain (Tadić et al., 2020). In the ESCOP monograph, it is recommended that the aerial parts of the *A. vulgaris* be used as agents for pruritus vulvae, uterine bleeding, and menstrual pains (ESCOP, 2003). The ethnopharmacological applications of other lady's mantle species are outlined in Table 1. Most of the modern indications fall into the International Classification of Primary Care, 2nd edition (ICPC-2) disease categories of female genitalia (*A. vulgaris*, *A. mollis* Rothm., *A. hirsutiflora* Rothm., and *A. cryptantha* Steud. Ex A. Rich), skin (*A. vulgaris*, *A. mollis*, and *A. hessii* Rothm.) or digestion (*A. vulgaris*, *A. pedata* Hochst. Ex A. Rich., *A. mollis*, *A. xanthochlora*, and *A. alpina* L.).

5. Towards a modern approach to traditional use

Phytomedicine traditions are a potent opportunity to take advantage of unchanging knowledge. Ethnopharmacology is an evolutionary process to discover plant-based drugs or new techniques for semi-synthetic drugs. Often, the crucial role in this process plays the lack of scientific data to support therapeutic uses. Although, results of the newest studies of wound, gastrointestinal and gynaecological diseases healing, support folkloric use of *Alchemilla* species. At this point, to evaluate wound healing Tasić-Kostov and co-authors prepared a gel with *A. vulgaris* extracts and examined it topically on human skin sites pretreated with a patch consisting of sodium lauryl sulfate (SLS). They also performed a "scratch" test to explore the migration of fibroblasts and the extent of wound closure (Tasić-Kostov et al., 2019). Likewise, lesion diameter wound treatment for 7 consecutive days with fluid extract of *A. vulgaris* led to a reduction of lesion diameter (Shrivastava et al., 2007). Administration of the juice with *A. vulgaris* in rats with indomethacin-induced gastric ulcers pro-inflammatory mediators, ulcers index and score have decreased (Karaoglan et al., 2020). As mentioned in Table 1, *A. mollis* was used in female genitalia diseases. Studies from 2019 showed that its extract decreased cystic formation and reduced endometrioma (Bina et al., 2019). Furthermore, *in vivo* experiments, lady's mantle has confirmed the folk properties in metabolic, nutritional, and digestive disorders (weight loss, stomach and intestine pain, and inflammation). As mentioned above, there are indisputable trends to involve ethnopharmacology in modern phytotherapy.

6. Phytochemical constituents

Alchemilla species, as members of the Rosaceae family, produce tannins, phenolic acids, flavonoids, anthocyanins, coumarins, triterpenes and volatile (essential oils) compounds. Notably, literature surveys mainly show information concerning the total phenol, tannin, steroid or saponin content rather than a detailed chemical composition (Edrah, 2017).

6.1. Tannins and related compounds (see Fig. 2)

The abundance and widespread presence of tannins is a typical feature of the Rosaceae family (Augustynowicz et al., 2021; Tomczyk and Latté, 2009). *Alchemilla* species and the recent reports of their tannin contents are summarized in Table 2. The largest number of this type of phytoconstituent was found in *A. persica* Rothm. (14 compounds), *A. vulgaris* and *A. viridiflora* Rothm. (both 11 compounds). Ellagic acid, a dimeric hydrolysable molecule, is the product ellagitannin degradation and seems to be the most abundant tannin among all described *Alchemilla* species. Apart from ellagic acid, casuarictin has been reported in the genus *Alchemilla* as well as its derivative pedunculagin, formed via the loss of a gallate group. Several more phytochemical studies have demonstrated the presence of the antitumor tannin agrimoniin (a dimeric potentillin monomer linked via a dehydrogalloyl group) in *A. persica*, *A. xanthochlora*, *A. vulgaris*, *A. viridiflora* and *A. mollis* (Fedotcheva et al., 2021; Grochowski et al., 2017). Additionally, characteristic constituents isolated from *Alchemilla* species are sanguiins SH-6 and SH-10 as well as other hexahydrodiphenoyl (HHDP) derivatives.

Table 2
Tannins and related compounds isolated from *Alchemilla* species.

Compounds	*Alchemilla* species	References
ellagic acid	*A. vulgaris*	(Duckstein et al., 2012; Ibrahim et al., 2022; Ilić-Stojanović et al., 2018; Jelaća et al., 2022; Møller et al., 2009; Neagu et al., 2015; Yazici, 2021)
	A. mollis	(Duckstein et al., 2012; Ibrahim et al., 2022; Ilić-Stojanović et al., 2018; Møller et al., 2009)
	A. xanthochlora	Fraisse et al. (1999)
	A. glabra	Krivokuća et al. (2015)
	A. fissa	
	A. viridiflora	
	A. monticola	
	A. persica	Afshar et al. (2015)
pedunculagin	*A. persica*	(Öz et al., 2016; Özbilgin et al., 2019)
	A. vulgaris	Duckstein et al. (2012)
	A. mollis	
	A. viridiflora	Suručić et al. (2022)
	A. xanthochlora	Geiger et al. (1994)
pedunculagin isomers	*A. persica*	Afshar et al. (2015)
	A. viridiflora	Radović et al. (2022b)
laevigating F	*A. xanthochlora*	Geiger et al. (1994)
casuarictin	*A. persica*	(Öz et al., 2016; Özbilgin et al., 2019)
vescalagin/castalagin isomer	*A. mollis*	Duckstein et al. (2012)
	A. vulgaris	
	A. persicaria	(Öz et al., 2016; Özbilgin et al., 2019)
sanguiin H-6 isomers	*A. vulgaris*	(Duckstein et al., 2012; Gesek et al., 2021)
	A. mollis	
sanguiin H-10 isomers	*A. persicaria*	(Afshar et al., 2015; Öz et al., 2016; Özbilgin et al., 2019)
	A. vulgaris	(Duckstein et al., 2012; Gesek et al., 2021)
	A. mollis	
	A. viridiflora	Radović et al. (2022b)
catechin	*A. vulgaris*	(El-Hadidy et al., 2018; Ibrahim et al., 2022; Jurić et al., 2020; Møller et al., 2009; Valcheva-Kuzmanova et al., 2019; Yazici, 2021)
	A. barbatiflora	Renda et al. (2018)
	A. monticola	Mladenova et al. (2021)
	A. mollis	(Karatoprak et al., 2017; Kurtul et al., 2022)
	A. persica	(Afshar et al., 2015; Kurtul et al., 2022)
	A. caucasia	Karaoglan et al. (2020)
	A. glabra	Denev et al. (2014)
epicatechin	*A. vulgaris*	(Augšpole et al., 2018; El-Hadidy et al., 2018; Møller et al., 2009; Neagu et al., 2015; Valcheva-Kuzmanova et al., 2019)
	A. mollis	Karatoprak et al. (2017)
	A. persica	Afshar et al. (2015)
	A. glabra	Denev et al. (2014)
procyanidin B1	*A. persica*	Afshar et al. (2015)
procyanidin B3	*A. barbatiflora*	Renda et al. (2018)
pentagalloylglucose	*A. viridiflora*	Suručić et al. (2022)
tellimagrandin I		
tellimagrandin II		
brevifolin	*A. vulgaris*	Jelaća et al. (2022)
brevifolin carboxylic acid	*A. viridiflora*	Suručić et al. (2022)
	A. vulgaris	Jelaća et al. (2022)
methyl-gallate	*A. mollis*	Karatoprak et al. (2017)
	A. persica	Afshar et al. (2015)
galloyl-HHDP hexose	*A. viridiflora*	Suručić et al. (2022)
	A. persica	Afshar et al. (2015)
	A. vulgaris	Jelaća et al. (2022)
galloyl-bis-HHDP-glucose	*A. viridiflora*	Radović et al. (2022b)
digalloyl-galloyl galloside	*A. persica*	Afshar et al. (2015)
agrimoniin	*A. persica*	(Afshar et al., 2015; Öz et al., 2016; Özbilgin et al., 2019)
	A. viridiflora	Radović et al. (2022b)
	A. vulgaris	(Duckstein et al., 2012; Ghedira et al., 2012; Grochowski et al., 2017; Jelaća et al., 2022)
	A. mollis	Duckstein et al. (2012)
	A. xanthochlora	Geiger et al. (1994)

The condensed tannins of *A. persica* and *A. barbatiflora* Juz. Consist of a (−)-epicatechin and (+)-catechin or two (+)-catechin units joined by a bond between positions 4 and 8′, procyanidin B1 and procyanidin B3, respectively. In addition to procyanidins, their precursors, including catechin and epicatechin, were identified in several *Alchemilla* species. Some typical tannin molecules are depicted in Fig. 2. Beyond isolation and identification analyses, many authors have expressed the presence of tannins as their total content. For example, Oktyabrsky et al. revealed that the inflorescence of a. *vulgaris* does not contain tannins, while the leaves hold 6.6 mg/g dw (Oktyabrsky et al., 2009). The high content of tannins (4.6 ± 0.3%) in the whole herb of *A. vulgaris* was also determined by Maier et al. (2017). Using spectrophotometric methods, the total tannin contents in the aboveground parts of *A. kiwuensis* Engl. (Ngoupaye et al., 2022), *A. mollis*, *A. achtarowii* Pawl., or *A. jumrukczalica* Pawl. Were determined (Ilugin et al., 2016; Vitkova et al., 2013).

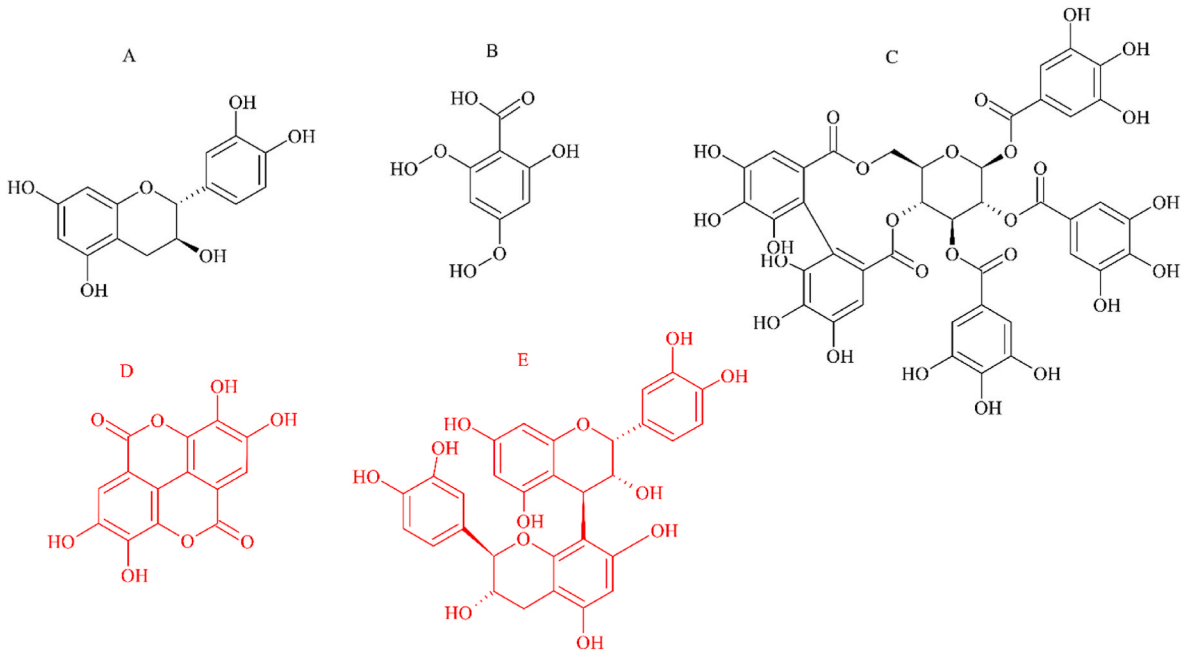

Fig. 2. Structures of tannins from *Alchemilla* species; A - catechin, B - brevifolin, C - tellimagrandin II, D - ellagic acid, E – procyanidin B1.

6.2. Phenolic acids and related compounds

Phenolic acids, the most prominent group of polyphenols, possess a phenolic moiety and a resonance-stabilized structure, resulting in the allocation of hydrogen atoms (Kumar and Goel, 2019). Due to their bioactive properties, phenolic acids from plant in the Rosaceae family and *Alchemilla* genus have been extensively studied. Altogether, 29 phenolic acids (Table 3) have been structurally identified in *Alchemilla* species. Many of these compounds (25) have been described from *A. vulgaris*. Additionally, gallic acid, caffeic acid and chlorogenic acid were detected in 4–6 varied species.

6.3. Flavonoids, anthocyanins, and their derivatives (see Fig. 3)

Flavonoids are the group of natural compounds with the largest number of structures altogether (61). Among them, 15 were identified as aglycones, 35 as mono- or di-*O*-glycosides and 6 as mono- or di-C-glycosides (Table 4). The most abundant *O*-glycosides contain quercetin as the aglycone combined with a saccharide at the C-3 position in the C-ring in the flavanol structure (e.g., hyperoside, guaijaverin, avicularin, and isoquercitrin) (Jelaća et al., 2022). On the other hand, the flavones apigenin and luteolin remain as aglycones among C-glycosides (vitexin, orientin) (Kaya et al., 2012a). In terms of the content of both phenolic acids and flavonoids, *A. vulgaris* seems to be the most thoroughly studied species among the entire genus; 30 flavonoids have been identified from this plant. Moreover, isoflavones, including genistein and daidzein, were isolated from *A. vulgaris*, representing their first instance of isolation from a species in this genus (Neagu et al., 2015). Among all reported compounds, the most widespread are quercetin 3-*O*-galactoside (hyperoside) (Fig. 3), quercetin 3-*O*-glucoside (isoquercitrin) and quercetin 3-*O*-rutinoside (rutin), which have been detected in 19, 18 and 17 species, respectively.

Aside from the flavonoids presented in Table 4, flavonoids are typically found in only Southern European species, such as *A. velebitica* Borb s ex Janch. (Juranović Cindrić et al., 2015). Although total bioactive compound content assays provide limited insight into the phytochemical composition, Smolyakova et al. attempted to develop extraction techniques and standardization methods for generating an extract using the aboveground parts of lady's mantle using the percent flavonoid content (Smolyakova et al., 2012). Among anthocyanins, only cyanidin derivatives have been found in *A. vulgaris* (Valcheva-Kuzmanova et al., 2019).

6.4. Essential oils and volatile compounds

As essential oils are isolated by distillation from flowers or leaves, they contain a diversity of volatile molecules—phenol-derived aromatic compounds, terpenes and terpenoids, and aliphatic components (Jakimiuk et al., 2022b). To date, the broad spectrum of these kinds of compounds has been detected in only seven *Alchemilla* species: *A. phegophila* Juz. (Dubel et al., 2022), *A. alpina* (Falchero et al., 2008), *A. xanthochlora* (Falchero et al., 2009), *A. persica* (Afshar et al., 2015) and *A. vulgaris* (Ahmed and Zhang, 2019) and *A. labellate*, *A. subrenata* (not occur in "The Plant List") (Dubel et al., 2022). All of the essential oil compounds that have been detected in *Alchemilla* species are summarized in Table 5.

6.5. Other compounds (see Fig. 4)

In addition to the compounds mentioned above, *Alchemilla* species contain fatty acids, sterols, coumarins, stilbenes and triterpenes (Fig. 4 and Table 6).

Olafsdottir et al. detected ursolic acid and its derivative oleanolic acid in *A. faeroënsis* Buser, *A. alpina* and *A. vulgaris* (Olafsdottir et al.,

Table 3
Phenolic acids and related compounds isolated from *Alchemilla* species.

Compounds	*Alchemilla* species	References
gallic acid	*A. vulgaris*	(Condrat et al., 2010; Duckstein et al., 2012; El-Hadidy et al., 2018; Ibrahim et al., 2022; Jelaća et al., 2022; Neagu et al., 2015; Yazici, 2021)
	A. mollis	(Duckstein et al., 2012; El-Hadidy et al., 2018; Ibrahim et al., 2022)
	A. jumrukczalica	Nikolova et al. (2011)
	A. acutiloba	Szewczyk et al. (2022)
	A. persica	Afshar et al. (2015)
	A. glabra	Denev et al. (2014)
gallic acid 4-glycoside	*A. persica*	Afshar et al. (2015)
gallic acid methoxy glycoside		
gallic acid-O-glycoside		
benzoic acid	*A. vulgaris*	(Ahmed and Zhang, 2019; El-Hadidy et al., 2018; Nikolova et al., 2011)
	A. jumrukczalica	Nikolova et al. (2011)
p-hydroxybenzoic acid	*A. vulgaris*	(Jurić et al., 2020; Nikolova et al., 2011; Yazici, 2021)
	A. jumrukczalica	Nikolova et al. (2011)
	A. acutiloba	Szewczyk et al. (2022)
m-hydroxybenzoic acid	*A. vulgaris*	Nikolova et al. (2011)
	A. jumrukczalica	
2,5-dihydroxybenzoic acid	*A. vulgaris*	Vlaisavljević et al. (2019)
3,4-dihydroxybenzoic acid	*A. vulgaris*	Valcheva-Kuzmanova et al. (2019)
	A. glabra	Denev et al. (2014)
salicylic acid	*A. vulgaris*	(El-Hadidy et al., 2018; Ibrahim et al., 2022; Nikolova et al., 2011)
	A. jumrukczalica	Nikolova et al. (2011)
	A. acutiloba	Szewczyk et al. (2022)
cinnamic acid	*A. vulgaris*	(El-Hadidy et al., 2018; Ibrahim et al., 2022)
	A. jumrukczalica	Nikolova et al. (2011)
3,4,5-methoxy-cinnamic acid	*A. vulgaris*	El-Hadidy et al. (2018)
chlorogenic acid	*A. mollis*	Duckstein et al. (2012)
	A. vulgaris	(El-Hadidy et al., 2018; Jelaća et al., 2022; Jurić et al., 2020; Møller et al., 2009; Neagu et al., 2015; Valcheva-Kuzmanova et al., 2019; Yazici, 2021)
	A. persica	Afshar et al. (2015)
	A. glabra	Denev et al. (2014)
neochlorogenic acid	*A. vulgaris*	Valcheva-Kuzmanova et al. (2019)
ferulic acid	*A. vulgaris*	(Jurić et al., 2020; Tasić-Kostov et al., 2019)
	A. acutiloba	Szewczyk et al. (2022)
iso-ferulic acid	*A. vulgaris*	El-Hadidy et al. (2018)
vanillic acid	*A. monticola*	Mladenova et al. (2021)
	A. vulgaris	(El-Hadidy et al., 2018; Nikolova et al., 2011)
	A. jumrukczalica	Nikolova et al. (2011)
	A. acutiloba	Szewczyk et al. (2022)
protocatechuic acid	*A. vulgaris*	(El-Hadidy et al., 2018; Ibrahim et al., 2022; Nikolova et al., 2011)
	A. jumrukczalica	Nikolova et al. (2011)
	A. acutiloba	Szewczyk et al. (2022)
caffeic acid	*A. vulgaris*	(El-Hadidy et al., 2018; Ibrahim et al., 2022; Jelaća et al., 2022; Jurić et al., 2020; Nikolova et al., 2011; Valcheva-Kuzmanova et al., 2019)
	A. jumrukczalica	Nikolova et al. (2011)
	A. acutiloba	Szewczyk et al. (2022)
	A. mollis	Karatoprak et al. (2017)
	A. glabra	Denev et al. (2014)
syringic acid	*A. vulgaris*	(Ibrahim et al., 2022; Jurić et al., 2020; Neagu et al., 2015; Renda et al., 2018)
	A. jumrukczalica	Nikolova et al. (2011)
	A. acutiloba	Szewczyk et al. (2022)
p-coumaric acid	*A. vulgaris*	(El-Hadidy et al., 2018; Jelaća et al., 2022; Jurić et al., 2020; Neagu et al., 2015; Nikolova et al., 2011)
	A. jumrukczalica	Nikolova et al. (2011)
	A. acutiloba	Szewczyk et al. (2022)
p-coumaroylquinic acid	*A. vulgaris*	Jelaća et al. (2022)
sinapic acid	*A. vulgaris*	(Jurić et al., 2020; Nikolova et al., 2011)
	A. jumrukczalica	Nikolova et al. (2011)
	A. acutiloba	Szewczyk et al. (2022)
β-phenylpyruvic acid	*A. jumrukczalica*	Nikolova et al. (2011)
	A. vulgaris	
gentisic acid	*A. jumrukczalica*	Nikolova et al. (2011)
	A. vulgaris	
	A. acutiloba	Szewczyk et al. (2022)
	A. mollis	Karatoprak et al. (2017)
β-resorcylic acid	*A. jumrukczalica*	Nikolova et al. (2011)
	A. vulgaris	
mandelic acid	*A. jumrukczalica*	Nikolova et al. (2011)
	A. vulgaris	
3,4,5-trimethoxymandelic acid	*A. jumrukczalica*	Nikolova et al. (2011)
	A. vulgaris	
rosmarinic acid	*A. acutiloba*	Szewczyk et al. (2022)

Table 4
Flavonoids, anthocyanins and their derivatives isolated from *Alchemilla* species.

Compounds	*Alchemilla* species	References
Flavonoids and flavonoid derivatives		
Apigenin	*A. vulgaris*	Jurić et al. (2020)
	A. caucasia	Karaoglan et al. (2020)
apigenin 7-*O*-glucoside (cosmosiin)	*A. vulgaris*	Tasić-Kostov et al. (2019)
	A. mollis	Karatoprak et al. (2017)
apigenin 8-*C*-glucoside (vitexin)	*A. monticola*	Mladenova et al. (2021)
	A. stricta	Kaya et al. (2012b)
	A. armeniaca	Kaya et al. (2012a)
	A. erzincanensis	
	A. orduensis	
	A. ikizdereensis	(Kaya et al., 2012a; Türk et al., 2011)
apigenin 6-*C*-arabinose-8-*C*-glucoside	*A. vulgaris*	El-Hadidy et al. (2018)
apigenin 6-*C*-rhamnose-8-*C*-glucoside		
apigenin 7-*O*-neohesperoside		
Acacetin		
aromadendrin glucoside derivative	*A. persica*	Afshar et al. (2015)
Luteolin	*A. vulgaris*	(Ibrahim et al., 2022; Neagu et al., 2015; Shrivastava and John, 2006)
	A. mollis	Shrivastava and John (2006)
luteolin 7-*O*-glucoside (cynaroside)	*A. vulgaris*	(Jelaća et al., 2022; Tasić-Kostov et al., 2019)
	A. speciosa	Felser and Schimmer (1999)
	A. mollis	Karatoprak et al. (2017)
luteolin 8-*C*-glucoside (orientin)	*A. procerrima*	Kaya et al. (2012b)
	A. stricta	
	A. armeniaca	Kaya et al. (2012a)
	A. cimilensis	
	A. orduensis	
	A. ikizdereensis	
	A. hirsutiflora	
	A. erythropoda	Türk et al. (2011)
	A. ikizdereensis	
luteolin 7-*O*-β-D-glucosyl-(2-*O*-α-L-rhamnoside) (lonicerin, scolymoside)	*A. speciosa*	Felser and Schimmer (1999)
luteolin 6-*C*-arabinose-8-*C*-glucoside	*A. vulgaris*	El-Hadidy et al. (2018)
luteolin 6-*C*-glucose-8-*C*-arabinoside		
Quercetin	*A. vulgaris*	(Mandrone et al., 2018; Neagu et al., 2015; Tasić-Kostov et al., 2019; Valcheva-Kuzmanova et al., 2019)
	A. speciosa	Felser and Schimmer (1999)
	A. monticola	Mladenova et al. (2021)
	A. acutiloba	Szewczyk et al. (2022)
quercetin 3-*O*-glucuronide	*A. vulgaris*	(Duckstein et al., 2012; Mandrone et al., 2018)
	A. mollis	Duckstein et al. (2012)
	A. monticola	Mladenova et al. (2021)
	A. xanthochlora	Lamaison et al. (1991)
	A. caucasia	Karaoglan et al. (2020)
	A. persica	Afshar et al. (2015)
quercetin 3-*O*-galactoside (hyperoside)	*A. vulgaris*	(Jelaća et al., 2022; Tasić-Kostov et al., 2019)
	A. barbatiflora	Renda et al. (2018)
	A. procerrima	Kaya et al. (2012b)
	A. hirtipedicellata	
	A. sericata	
	A. mollis	(Küpeli Akkol et al., 2015; Trendafilova et al., 2011)
	A. acutiloba	Szewczyk et al. (2022)
	A. speciosa	Felser and Schimmer (1999)
	A. achtarowii	Trendafilova et al. (2012)
	A. armeniaca	Kaya et al. (2012a)
	A. cimilensis	
	A. orduensis	
	A. oriturcica	
	A. bursensis	
	A. hirsutiflora	
	A. ikizdereensis	(Kaya et al., 2012a; Türk et al., 2011)
	A. erythropoda	Türk et al. (2011)
	A. oriturcica	
	A. persica	Küpeli Akkol et al. (2015)
quercetin 3-*O*-rhamnoside (quercitrin)	*A. orduensis*	Kaya et al. (2012a)
	A. hirsutiflora	
	A. trabzonica	Türk et al. (2011)
quercetin 3-*O*-α-L-arabinoside (guaijaverin)	*A. xanthochlora*	Fraisse et al. (2000)
	A. barbatiflora	Renda et al. (2018)
	A. pedata	Taddese et al. (2009)
	A. achtarowii	Trendafilova et al. (2012)
	A. vulgaris	(D'Agostino et al., 1998; Jelaća et al., 2022)
quercetin 3-*O*-α-L-arabinoside (avicularine)	*A. vulgaris*	Jelaća et al. (2022)
quercetin 3-*O*-β-D- sambubioside	*A. speciosa*	Felser and Schimmer (1999)

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Table 4 (*continued*)

Compounds	*Alchemilla* species	References
quercetin 3-*O*-*β*-D-sambubioside-7-*O*-*β*-D-glucoside		
quercetin 3-*O*-*β*-(2′-*O*-*α*-L-rhamnosyl)-glucoside uronic acid		
quercetin 3-*O*-glucoside (isoquercetin)	*A. vulgaris*	(D'Agostino et al., 1998; Jelaća et al., 2022; Neagu et al., 2015; Tasić-Kostov et al., 2019; Valcheva-Kuzmanova et al., 2019)
	A. monticola	Mladenova et al. (2021)
	A. speciosa	Felser and Schimmer (1999)
	A. stricta	Kaya et al. (2012b)
	A. hirtipedicellata	
	A. sericata	
	A. mollis	(Aslı et al., 2022; Küpeli Akkol et al., 2015; Trendafilova et al., 2011)
	A. acutiloba	Szewczyk et al. (2022)
	A. achtarowii	Trendafilova et al. (2012)
	A. erzincanensis	Kaya et al. (2012a)
	A. cimilensis	
	A. orduensis	
	A. bursensis	Kaya et al. (2012a)
	A. oriturcica	(Kaya et al., 2012a; Türk et al., 2011)
	A. viridiflora	Suručić et al. (2022)
	A. erythropoda	Türk et al. (2011)
	A. persica	Küpeli Akkol et al. (2015)
quercetin 3-*O*-arabinoside-7-*O*-glucoside	*A. vulgaris*	Jelaća et al. (2022)
quercetin 3-*O*-*α*-L-arabinosyl-(1–6)-*β*-D-glucoside (quercetin-3-*O*-vicianoside)		
quercetin 3-*O*-(6-*O*-acetyl-*β*-D-glucoside		
quercetin glucuronide methyl ether	*A. vulgaris*	Duckstein et al. (2012)
	A. mollis	
quercetin di-*O*-methyl ether	*A. viridiflora*	Suručić et al. (2022)
quercetin 3-(6″-ferulylglucoside)		
quercetin 3-methyl ether-7-glucuronide		
quercetin 3-*O*-rutinoside (rutin)	*A. vulgaris*	(Al-Osaj et al., 2016; D'Agostino et al., 1998; El-Hadidy et al., 2018; Ibrahim et al., 2022; Jelaća et al., 2022; Jurić et al., 2020; Mandrone et al., 2018; Neagu et al., 2015; Tasić-Kostov et al., 2019; Valcheva-Kuzmanova et al., 2019)
	A. speciosa	Felser and Schimmer (1999)
	A. monticola	Mladenova et al. (2021)
	A. procerrima	Kaya et al. (2012b)
	A. stricta	
	A. hirtipedicellata	
	A. sericata	
	A. acutiloba	Szewczyk et al. (2022)
	A. cimilensis	Kaya et al. (2012a)
	A. orduensis	
	A. bursensis	
	A. hirsutiflora	
	A. ikizdereensis	(Kaya et al., 2012a; Türk et al., 2011)
	A. oriturcica	
	A. mollis	Karatoprak et al. (2017)
	A. glabra	Denev et al. (2014)
quercetin 3-*O*-glucuronic acid (miquelianin)	*A. speciosa*	Felser and Schimmer (1999)
	A. mollis	(Kurtul et al., 2022; Trendafilova et al., 2011)
	A. barbatiflora	Renda et al. (2018)
	A. coriacea	(Fraisse et al., 1999; Trendafilova et al., 2012)
	A. filicauli	
	A. glabra	
	A. achtarowii	Trendafilova et al. (2012)
	A. persica	Kurtul et al. (2022)
	A. viridiflora	(Radović et al., 2022b; Suručić et al., 2022)
	A. vulgaris	Jelaća et al. (2022)
Kaempferol	*A. vulgaris*	(El-Hadidy et al., 2018; Filippova, 2017; Ibrahim et al., 2022; Jurić et al., 2020; Neagu et al., 2015; Tasić-Kostov et al., 2019; Vlaisavljević et al., 2019)
	A. monticola	Mladenova et al. (2021)
	A. acutiloba	Szewczyk et al. (2022)
kaempferol 3-*O*-glucuronide	*A. vulgaris*	Duckstein et al. (2012)
	A. mollis	
	A. speciosa	Felser and Schimmer (1999)
kaempferol 3-*O*-glucoside (astragalin)	*A. vulgaris*	(Jelaća et al., 2022; Tasić-Kostov et al., 2019)
	A. viridiflora	Radović et al. (2022b)
	A. achtarowii	Trendafilova et al. (2012)
	A. speciosa	Felser and Schimmer (1999)
	A. monticola	(Mladenova et al., 2021; Patel et al., 2022)
	A. acutiloba	Szewczyk et al. (2022)
kaempferol 3-*O*-*β*-D-xyloside	*A. barbatiflora*	Renda et al. (2018)
	A. vulgaris	Jelaća et al. (2022)

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Table 4 (*continued*)

Compounds	*Alchemilla* species	References
kaempferol 3-*O*-*β*-(2′-*O*-*α*-L-rhamnosyl)-glucoside uronic acid	*A. speciosa*	Felser and Schimmer (1999)
kaempferol 3-*O*-(6″-E-coumaroyl-*β*-D-glycoside) (tiliroside)	*A. vulgaris*	(D'Agostino et al., 1998; Jelaća et al., 2022; Tasić-Kostov et al., 2019)
	A. speciosa	Felser and Schimmer (1999)
	A. barbatiflora	Renda et al. (2018)
	A. mollis	Trendafilova et al. (2011)
	A. acutiloba	Szewczyk et al. (2022)
	A. achtarowii	Trendafilova et al. (2012)
	A. viridiflora	Suručić et al. (2022)
kaempferol 3-*O*-rutinoside (nicotiflorin)	*A. acutiloba*	Szewczyk et al. (2022)
	A. persica	Afshar et al. (2015)
	A. vulgaris	Jelaća et al. (2022)
kaempferol 3-*O*-(4″-E-*p*-coumaroyl)-robinobioside (variabiloside G)	*A. achtarowii*	Trendafilova et al. (2012)
kaempferol 7-*O*-glucoside	*A. viridiflora*	Suručić et al. (2022)
kaempferol 7-*O*-glucuronide		
kaempferol 3,7-*O*-dirhamnoside	*A. vulgaris*	El-Hadidy et al. (2018)
Morin	*A. vulgaris*	Tasić-Kostov et al. (2019)
Rhamnetin	*A. vulgaris*	El-Hadidy et al. (2018)
	A. acutiloba	Szewczyk et al. (2022)
isorhamnetin	*A. acutiloba*	Szewczyk et al. (2022)
isorhamnetin-3-*O*-glucoside	*A. acutiloba*	Szewczyk et al. (2022)
	A. viridiflora	Suručić et al. (2022)
isorhamnetin-3-*O*-rutinoside (narcissoside)	*A. acutiloba*	Szewczyk et al. (2022)
gosselin 7-*O*-*α*-rhamnoside (rhodiolgin)	*A. mollis*	Trendafilova et al. (2011)
gosselin-3-*O*-*β*-D-galactosyl-7-*O*-*α*-L-rhamnoside		
sinocrassoside D_2	*A. mollis*	Trendafilova et al. (2011)
Naringin	*A. vulgaris*	Ibrahim et al. (2022)
Naringenin	*A. acutiloba*	Szewczyk et al. (2022)
naringenin 7-*O*-glucoside		
Eriodictyol		
Hesperetin	*A. vulgaris*	El-Hadidy et al. (2018)
Myricetin	*A. vulgaris*	Neagu et al. (2015)
myricetin 3-*O*-glucuronide	*A. viridiflora*	Suručić et al. (2022)
chrysoeriol 7-*O*-glucuronide	*A. vulgaris*	Jelaća et al. (2022)
Genistein	*A. vulgaris*	Neagu et al. (2015)
Dadzein		
Anthocyanins and anthocyanins derivatives		
cyanidin 3-galactoside	*A. vulgaris*	Valcheva-Kuzmanova et al. (2019)
cyanidin 3-glucoside		
cyanidin 3-arabinoside		
cyanidin 3-xyloside		

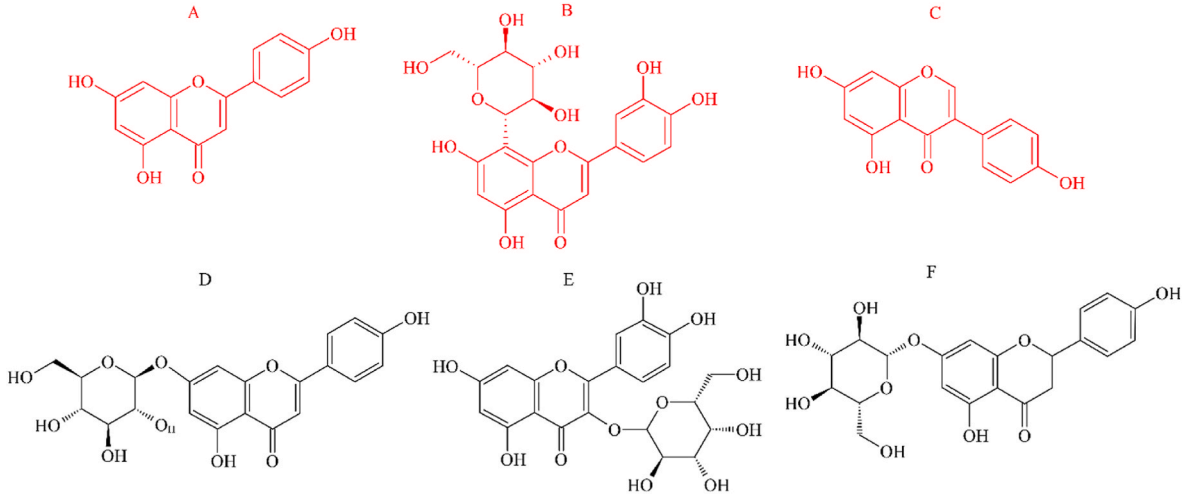

Fig. 3. Structures of flavonoids from *Alchemilla* species; A - apigenin, B - orientin, C – genistein, D - cosmosiin, E – hyperoside, F – naringenin 7-*O*-glucoside.

Table 5
Essential oils compounds detected in *Alchemilla* species.

Compound	*Alchemilla* species	References
phenylacetaldehyde	*A. flabellata*	Dubel et al. (2022)
	A. phegophila	
	A. subrenata	
	A. alpina	Falchero et al. (2008)
cis-linalool oxide	*A. phegophila*	Dubel et al. (2022)
	A. alpina	Falchero et al. (2008)
trans-linalool oxide	*A. phegophila*	Dubel et al. (2022)
	A. alpina	Falchero et al. (2008)
nonanal	*A. phegophila*	Dubel et al. (2022)
	A. subrenata	
linalool	*A. phegophila*	
	A. subrenata	
β-phenethyl alcohol	*A. flabellata*	
2-ethylcaproic acid	*A. flabellata*	
	A. phegophila	
	A. subrenata	
terpinene-4-ol	*A. phegophila*	
	A. alpina	Falchero et al. (2008)
p-cymene-8-ol	*A. phegophila*	Dubel et al. (2022)
α-terpineol	*A. flabellata*	
	A. phegophila	
	A. alpina	Falchero et al. (2008)
α-terpinyl acetate	*A. alpina*	
decanal	*A. flabellata*	Dubel et al. (2022)
	A. phegophila	
	A. alpina	Falchero et al. (2008)
caprylic acid	*A. flabellata*	Dubel et al. (2022)
myrtenol	*A. phegophila*	
	A. persica	Afshar et al. (2015)
geraniol	*A. phegophila*	Dubel et al. (2022)
	A. alpina	Falchero et al. (2008)
indole	*A. subrenata*	Dubel et al. (2022)
2-methoxy-4-vinylphenol	*A. flabellata*	
	A. phegophila	
	A. subrenata	
3-methyl-butanal	*A. alpina*	Falchero et al. (2008)
3-methyl-2-butenol		
2-methylbutanal		
pentanal		
3-penten-2-ol		
hexanal		
(E)-2-hexenal		
hexanol		
(Z)-3-hexenol		
furfural		
heptanol	*A. alpina*	Falchero et al. (2008)
nonanoic acid	*A. flabellata*	Dubel et al. (2022)
	A. phegophila	
	A. subrenata	
	A. alpina	Falchero et al. (2008)
γ-nonalactone	*A. flabellata*	Dubel et al. (2022)
eugenol	*A. flabellata*	
	A. phegophila	
	A. subrenata	
	A. alpina	Falchero et al. (2008)
vanillin	*A. alpina*	
2-dodecenal	*A. flabellata*	Dubel et al. (2022)
	A. phegophila	
capric acid	*A. flabellata*	
	A. phegophila	
	A. subrenata	
ethyl caprynate	*A. phegophila*	
dodecanal	*A. flabellata*	
	A. alpina	Falchero et al. (2008)
tridecanal	*A. alpina*	
2,3-dehydro-α-ionone	*A. phegophila*	Dubel et al. (2022)
β-caryophyllene	*A. flabellata*	
	A. persica	Afshar et al. (2015)
	A. alpina	Falchero et al. (2008)
geranylacetone	*A. flabellata*	Dubel et al. (2022)
	A. phegophila	
	A. subrenata	
β-farnesene	*A. subrenata*	

Table 5 (*continued*)

Compound	*Alchemilla* species	References
	A. persica	Afshar et al. (2015)
β-ionone epoxide	*A. flabellata*	Dubel et al. (2022)
	A. phegophila	
	A. subrenata	
β-ionone	*A. flabellata*	
	A. phegophila	
	A. persica	Afshar et al. (2015)
β-pinene	*A. alpina*	Falchero et al. (2008)
undecanal	*A. alpina*	
undecanoic acid	*A. flabellata*	Dubel et al. (2022)
tetradecanal	*A. flabellata*	
	A. phegophila	
	A. subrenata	
megastigmatrienon	*A. subrenata*	
spatulenol	*A. flabellata*	
caryophyllene oxide	*A. phegophila*	
	A. persica	Afshar et al. (2015)
lauric acid	*A. flabellata*	Dubel et al. (2022)
	A. phegophila	
	A. subrenata	
benzophenone	*A. flabellata*	
	A. phegophila	
	A. subrenata	
β-eudesmol	*A. flabellata*	
tridecanoic acid	*A. flabellata*	
	A. phegophila	
	A. subrenata	
hexadecanal	*A. flabellata*	
	A. phegophila	
	A. subrenata	
	A. alpina	Falchero et al. (2008)
11-tetradecenoic acid	*A. flabellata*	Dubel et al. (2022)
13-tetradecenoic	*A. phegophila*	
myristicin	*A. alpina*	Falchero et al. (2008)
myristic acid	*A. flabellata*	Dubel et al. (2022)
	A. phegophila	
	A. subrenata	
hexahydropharnesyl acetone	*A. flabellata*	
	A. phegophila	
	A. subrenata	
14-pentadecenoic	*A. flabellata*	
pentadecanoic acid	*A. flabellata*	
pentadecanal	*A. alpina*	Falchero et al. (2008)
	A. phegophila	Dubel et al. (2022)
	A. subrenata	
methyl palmitate	*A. subrenata*	
palmitoleic acid	*A. flabellata*	
	A. phegophila	
	A. subrenata	
palmitic acid	*A. flabellata*	
	A. phegophila	
	A. subrenata	
heptanoic acid	*A. vulgaris*	Ahmed and Zhang (2019)
octanoic acid		
hexanoic acid		
hexadecenoic acid		
	A. alpina	Falchero et al. (2008)
heptadecanoic acid	*A. flabellata*	Dubel et al. (2022)
	A. phegophila	
	A. subrenata	
methyl linolenate	*A. flabellata*	
	A. subrenata	
phytol	*A. flabellata*	
	A. phegophila	
	A. subrenata	
	A. persica	Afshar et al. (2015)
	A. alpina	Falchero et al. (2008)
phytone	*A. persica*	Afshar et al. (2015)
linoleic acid	*A. flabellata*	Dubel et al. (2022)
	A. phegophila	
	A. subrenata	
	A. alpina	Falchero et al. (2008)
stearic acid	*A. flabellata*	Dubel et al. (2022)
	A. phegophila	
	A. subrenata	

(*continued on next page*)

Table 5 (*continued*)

Compound	*Alchemilla* species	References
	A. vulgaris	Ahmed and Zhang (2019)
tricosane	*A. flabellata*	Dubel et al. (2022)
	A. phegophila	
	A. subrenata	
	A. persica	Afshar et al. (2015)
tetracosane	*A. phegophila*	Dubel et al. (2022)
	A. persica	Afshar et al. (2015)
pentacosane	*A. flabellata*	Dubel et al. (2022)
	A. persica	Afshar et al. (2015)
heptacosane	*A. flabellata*	Dubel et al. (2022)
	A. phegophila	
	A. subrenata	
	A. persica	Afshar et al. (2015)
squalene	*A. flabellata*	Dubel et al. (2022)
	A. phegophila	
	A. subrenata	
nonacosane	*A. phegophila*	
	A. subrenata	
docosane	*A. persica*	Afshar et al. (2015)
1,27-octacosadiene	*A. phegophila*	Dubel et al. (2022)
	A. subrenata	
triacontanol	*A. flabellata*	
	A. phegophila	
	A. subrenata	
tritriacontanol	*A. flabellata*	
	A. phegophila	
	A. subrenata	
camphor	*A. vulgaris*	Ahmed and Zhang (2019)
octanal	*A. alpina*	Falchero et al. (2008)
1,3,3-trimethyl-2- oxabicyclo [2.2.2] octane	*A. vulgaris*	Ahmed and Zhang (2019)
1,2-dihydro-1,1,6-trimethyl-naphthalene	*A. persica*	Afshar et al. (2015)
germacrene		
(E)-β-Damascenone		
1-octen-3-ol	*A. alpina*	Falchero et al. (2008)
myrcene		
perilla alcohol		
apiole		

2001). Other triterpenes that have been isolated from lady mantle species are betulinic acid and arjungenin (Jelača et al., 2022; Sokołowska-Woźniak and Krzaczek, 1993). *A. sericata* Rchb. Ex Buser and *A. vulgaris* are sources of fatty acids, while sterols were found in *A. caucasia* Buser and *A. pastoralis* Buser (Sezen Karaoğlan and Yılmaz, 2018; Shafaghat et al., 2017). Furthermore, the benzopyrone derivatives esculetin and aesculetin were identified in *A. speciosa* Buser and *A. vulgaris*, respectively (Borges et al., 2005; Jurić et al., 2020).

7. Phytochemical standardization and quality control of the extracts from *alchemilla* L

The quality of the herbal medicinal products, which are consumed by humans is principal since they are used for the well-being of humankind. There are guidelines for the quality control and standardization of the herbal medicines. Moreover, as in the recent times is a growing requirement for traditional herbal-based products, there is a necessity to provide their quality control (Balekundri and Mannur, 2020). The WHO set guidelines about quality control methods for medicinal plant materials based on organoleptic properties, ash values, moisture content, microbial contamination, and chromatographic and spectroscopic parameters (Kamble et al., 2018). The qualitative estimation of the herbal products is carried out mostly by UV, IR and TLC techniques, while the quantitative examination based on HP-TLC, HPLC, SFC, LC-MS or GC-MS methods (Balekundri and Mannur, 2020). The qualitative and quantitative evaluations are crucial to determine the authenticity of specific species as well as identify the false one. For example, Karaoglan and Yilmaz, contributed to the quality of the methanol extracts from *A. caucasica* by GC-MS fingerprint implying content of the fourteen phytocomponents (Karaoglan and Yilmaz, 2018). The aqueous ethanolic extracts from leaves of *A. hirtipedicellata*, *A. procerrima*, *A. sericata*, and *A. stricta* were standardized using TLC and HPLC techniques (Kaya et al., 2012b). The most valuable from the pharmacological point of view seems to be *Alchemilla vulgaris*. According to Polish Pharmacopoeia XII Alchemilla herbs should be standardized for tannins content (no less than 6 %) in conversion to pyrogallol (dry substance) (Farmakopea Polska, 2020). Furthermore, Kovač and co-workers perfected of deep eutectic solvent extraction of *A. vulgaris* for gallic acid and total tannins contents (Kovač et al., 2022). Also, to investigate *A. mollis* and *A. persica* chemical composition have been used HPLC analysis with some standards (hyperoside, isoquercitrin) (Küpeli Akkol et al., 2015).

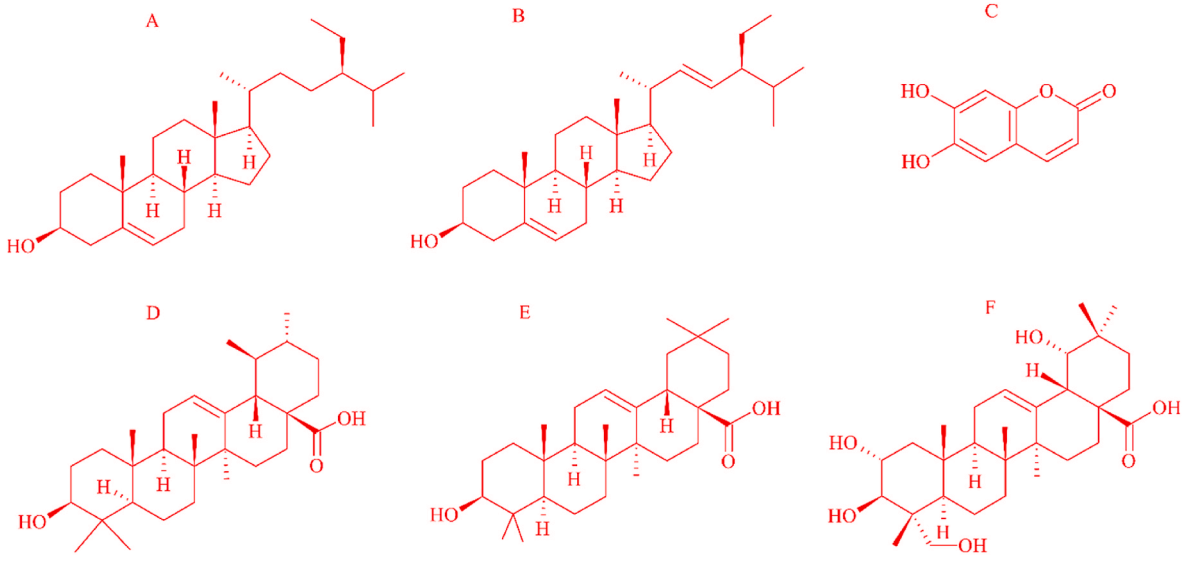

Fig. 4. Structures of other compounds found in *Alchemilla* species; A - β-sitosterol, B - stigmasterol, C - esculetin, D - ursolic acid, E – oleanolic acid, F - arjungenin.

Table 6
Other compounds found in *Alchemilla* species.

Compounds	*Alchemilla* species	References
Fatty acids and related compounds		
9-hexadecenoic acid, methyl ester hexadecanoic acid, methyl ester 9-octadecenoic acid(z), methyl ester heptadecanoic acid, methyl ester 1,2-benzenedicarboxylic acid, butylmethyl ester 9,12-octadecadienoic acid (z, z)-, methyl ester octadecanoic acid, methyl ester 11-eicosenoic acid, methyl ester docosanoic acid, methyl ester bis (2-ethylhexyl) phthalate	*A. sericata*	Shafaghat et al. (2017)
linoleic acid ethyl ester	*A. vulgaris*	Ahmed and Zhang (2019)
Sterols		
β-sitosterol	*A. caucasia*	Sezen Karaoğlan and Yılmaz (2018)
acetates *β*-sitosterol stigmasterol ergosterol	*A. pastoralis*	Sokołowska-Woźniak and Krzaczek (1993)
Coumarins		
esculetin	*A. speciosa*	(Borges et al., 2005; Schimmer and Eschelbach, 1997)
aesculetin	*A. vulgaris*	Jurić et al. (2020)
Stilbenes		
resveratrol	*A. vulgaris*	El-Hadidy et al. (2018)
Triterpens		
ursolic acid	*A. faeroënsis* *A. alpina* *A. vulgaris*	Olafsdottir et al. (2001)
2*α*-hydroxyursolic acid	*A. faeroënsis* *A. alpina* *A. vulgaris*	Olafsdottir et al. (2001)
2*α*,19*α*- dihydroxyursolic acid (tormentic acid)	*A. faeroënsis* *A. alpina* *A. vulgaris*	Olafsdottir et al. (2001)
2*α*,3*α*,19*α*-trihydroxyurs-12-en-28-oic acid (euscophic acid)	*A. faeroënsis* *A. alpina* *A. vulgaris*	Olafsdottir et al. (2001)
oleanolic acid	*A. faeroënsis* *A. alpina* *A. vulgaris*	Olafsdottir et al. (2001)
	A. pastoralis	Sokołowska-Woźniak and Krzaczek (1993)
betulinic acid	*A. pastoralis*	Sokołowska-Woźniak and Krzaczek (1993)
arjungenin	*A. vulgaris*	Jelaća et al. (2022)

8. Pharmacological profile

Lady's mantle plants are perennials with a confirmed, diverse spectrum of biological activities. The pharmacological effects of *Alchemilla* plants are similar to those of several other tannin- and flavonoid-containing herbs of the Rosaceae family (van Wyk and Wink, 2017). They possess a broad spectrum of pharmacological features, confirmed in both *in vitro* and *in vivo* studies, such as antioxidant, anti-inflammatory (Stephens et al., 2013), neuroprotective (Vlaisavljević et al., 2019), antimicrobial (Makau, 2013), antiobesity (Mladenova et al., 2021), cardiovascular (Pawlaczyk-Graja et al., 2009), anticancer (Trouillas et al., 2003) and wound healing (Tasić-Kostov et al., 2019) activities. All of the pharmacological effects of *Alchemilla* species are summarized in Tables 7 and 8.

8.1. In vitro assays

8.1.1. Antioxidant activity

One of the best-shown assets of plant extracts with high phenols content is their potential antioxidant activity. Excess free radicals can cause oxidative stress in normal cells, leading to an increase in pathological processes (Mainka et al., 2021). Due to the fact that preparations from *Alchemilla* species contain a broad spectrum of polyphenols (flavonoids, tannins, phenolic acids), a considerable number of studies have reported that they can be used as antioxidants. Most references mention an antiradical effect using DPPH and ABTS tests, chiefly extracts of *A. vulgaris*, *A. mollis*, *A. persica* and *A. alpina* and their fractions (Table 7). Additionally, a wide range of solvents for raw material extraction has been used, e.g., ethanol (10%–96%) (Nedyalkov et al., 2015), methanol (70%–100%) (Boroja et al., 2018), water (Karatoprak et al., 2018) and a mixture of ethanol and glycerine (Dzabijeva et al., 2018). The efficacy of individual extracts is given in Table 7, expressed as Trolox equivalents, percent inhibition or IC_{50} values. The most active extracts in the DPPH test seem to be ethanolic extracts of the roots (495.38 ± 19.32 mmol TE/g dry mass) rather than the aerial parts (366.55 ± 12.62 mmol TE/g dry mass), which is presumably associated with the higher content of tannins (Sapko et al., 2016). Moreover, Nedyalkov et al. using the ABTS assay, indicated that a fresh extract is a better antiradical agent than the preparation after 20 days of storage (Nedyalkov et al., 2015). The abovementioned methods of testing antiradical properties used spectrophotometric measurements; however, the ORAC assay measures a fluorescence signal from a probe that is quenched in the presence of reactive oxygen species (ROS). Extracts of *A. glabra* were found to have an activity of 1337 ± 68 μmol TE/g (Denev et al., 2014). Another valuable mechanism involved in antioxidant pathways is the reducing power of ions (Jakimiuk et al., 2022a). To this end, FRAP and CUPRAC assays have been performed. In one of these studies, Vlaisavljević et al. evaluated reducing ion potential of 80% methanol (MeOH), 70% ethanol (EtOH), ethyl acetate (EtOAc) and water extracts of *A. vulgaris*, and their activity followed the order of EtOAc> 80% MeOH> 70% EtOH > water (Vlaisavljević et al., 2019). Although the determination of *β*-carotene-linolenic acid is a common method by which antioxidant activity can be assessed, there are many difficulties when obtaining reliable results (low reproducibility, problematic quantification, complex preparation of reagents, temperature interference, and pH) (Prieto et al., 2012). Notably, only one study mentioned this type of activity from *A. vulgaris* extracts, pointing out their dose-dependent activity (Tasić-Kostov et al., 2019). Additionally, the preliminary TLC-DPPH screening method indicated the presence of antioxidant compounds in *n*-hexane, chloroform, ethyl acetate, methanol, and water extracts (Ondrejović et al., 2009). Said and co-workers tested antioxidant profile of tablets that contained *A. vulgaris* extract by measuring the lipid peroxidation induced by incubating the rat liver homogenate with ferrosulfate. They found out that even an incredibly low dosage (10 μg/mL) reduced MDA release to 0.53 nM/mg (from 0.89 nM/mg) (Said et al., 2011). To analyse NO• scavenging activity of *A. mollis*, as well as detected in this extract hyperoside and isoquercitrin, K562 cell line has been used. At the dosage from 62.5 to 3000 μg/mL A. *mollis* methanolic extracts exhibited dose dependent inhibition of nitrite levels (Aslı et al., 2022). A broad discussion of the antioxidant potential of *Alchemilla* was presented by Kanak et al. (2022).

8.1.2. Anti-inflammatory activity

Inflammation plays a crucial role in the development of various diseases, and since ancient times, inflammatory disorders have been cured with plants or plant-based products (Mueller et al., 2010). Additionally, research on three *Alchemilla* species (*A. vulgaris*, *A. persica*, and *A. mollis*) as anti-inflammatory agents has been performed (Table 7). Kurtul et al. used the human red blood cell (HRBC) test, which utilizes fresh human whole blood collected from healthy individuals who had not taken any anti-inflammatory or steroidal drugs for 14 days before

the study. Sample activity was determined by measuring its stabilization capacity against heat-induced hemolysis of the HRBC membrane. The extracts (*A. mollis* and *A. persica*, 80% methanol) significantly protected the HRBC membranes from hemolysis compared to the standard drug acetylsalicylic acid. The *A. mollis* aerial part extract ($IC_{50} = 1.22 \pm 0.07$ mg/mL) showed an elevated HRBC membrane stabilizing effect, while *A. persica* root extract displayed the lowest activity ($IC_{50} = 1.82 \pm 0.14$ mg/mL). Furthermore, after multistep separation process, the authors isolated ellagic acid and miquelianin from *A. mollis*. They found out that miquelianin possess similar activity to *A. mollis* ($IC_{50} = 1.23 \pm 0.02$ mg/mL) extracts while ellagic acid is much more effective anti-inflammatory agent ($IC_{50} = 0.57 \pm 0.01$ mg/mL). Thus, these results can be attributed to the higher tannin content, especially ellagic acid (Kurtul et al., 2022). The water and 70% ethanol extracts of *A. vulgaris* were investigated as potential products involved in the inflammatory response. The loss of soybean 15-lipoxygenase activity in the presence of the plant water extract was measured. The results are expressed as the IC_{50} value (0.52 mg/mL), which proves the high inhibitory activity of the transformation of arachidonic acid metabolites (Trouillas et al., 2003). Furthermore, a 70% ethanol *A. vulgaris* extract decreased LPS-induced IL-8 release and inhibited stimulation of the TLR2 and TLR4 signaling pathways. On the other hand, this extract was not effective against NF-κB p65 translocation (Schink et al., 2018).

8.1.3. Antimicrobial activity

The increasing number of drug-resistant pathogens creates an urgent need to identify plant-derived therapeutics that can provide alternatives to combat pathogenic bacteria (Vaou et al., 2021). An antimicrobial review of the *Alchemilla* literature revealed a broad spectrum of research activity in this field. Among 16 available publications, most found antimicrobial potential using agar well diffusion or broth microdilution methods (Table 7). A few investigations have compared many extracts using methods that are directly comparable. For example, *A. vulgaris* extracts (80% methanol, ethanol, and 50% ethanol/6% glycerine) in the agar well diffusion assay displayed dose-dependent activity against selected bacteria (Edrah, 2017; Ibrahim et al., 2022; Keskin et al., 2010; Usta et al., 2014). Comparing the activities of the *A. vulgaris* methanolic and the *A. sericata* hexane extracts, it was noticed that the inhibition zones for *Staphylococcus epidermidis* (12 mm and 11.1 mm, respectively) and *Pseudomonas aeruginosa* (11 mm and 10.1, respectively) were similar, while the ethanol extract possesses weaker inhibitory activity (7 mm) than the hexane extract (12.2 mm) against *Staphylococcus aureus*. (Ibrahim et al., 2022; Shafaghat et al., 2017). Quantitative analysis of the components of *A. vulgaris* methanolic root extract showed high salicylic and ellagic acids content, as well as quercetin and catechin contents. Synergic action of detected compounds may have crucial role in the antibacterial activity of the *Alchemilla* plants (Ibrahim et al., 2022). On the other hand, *A. mollis* alcoholic and water extracts did not exhibit activity against *S. aureus* (Usta et al., 2014). The effectiveness of medicinal plant extracts to inhibit bacterial growth may also be expressed as the minimum inhibitory concentration (MIC). To this end, *A. mollis* and *A. persica* 80% methanol extracts were tested employing the microdilution method against *S. aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Escherichia coli* and *P. aeruginosa*. The results showed that both the aerial parts and roots of *A. persica*, tested with serial twofold dilutions ranging from 10 to 0.078 mg/mL, displayed activity superior to that of *A. mollis* (Kurtul et al., 2022). Moreover, data obtained from *A. vulgaris* analyses indicated only slight differences between the inhibition of bacterial growth exhibited by its aboveground parts and root methanolic extract (40 mg/mL) (Boroja et al., 2018). Said and coworkers conducted a study to evaluate the efficacy of a topical cream (HPC) consisting of water-ethanol extracts of the combination of *Nigella sativa*, *A. vulgaris* and *Conyza canadensis* (1:0.6:0.6 *w/w*) *in vitro*. In this study, the disc diffusion method with *E. coli* was used with HPC concentrations of 1, 2 and 5 mg/disc. The plant-based formulation showed dose-dependent antibacterial activity, expressed as 60% inhibition, in comparison to the reference antibiotic ampicillin at 5 mg/disc (Said et al., 2022).

8.1.4. Cardiovascular activity

Cardiovascular diseases involve the cardiovascular system, which comprises the heart and veins, and are known as the most frequent causes of death worldwide (Michel et al., 2020). Bearing in mind that plants are widely used for the treatment of hypertension, Radović et al. hypothesized that methanol extracts of *A. viridiflora* may be angiotensin I-converting enzyme (ACE) inhibitors. The enzymatic assay showed that the inhibitory activity of *A. viridiflora* (IC_{50} value) was 2.51 ± 0.00 μg/mL. The authors pointing out that this activity is highly probable connected with the tiliroside, tellimagrandin I, galloyl-HHDP and ellagic acid pentose contents (Radović et al., 2022). To investigate the vascular effects of methanol extract and aqueous extract of *A. vulgaris* (0.01–10 mg/mL) isolated rat aorta has been used. Interestingly, methanol and aqueous extracts of *A. vulgaris* displayed opposite vascular effects. After administration of an aqueous extract the contraction of the rings has been increased, while methanol extract caused their relaxation (Takir et al., 2014).

8.1.5. Neuroprotective activity

Herbrechter and coauthors investigated the effect of 158 herbal remedies, including *A. xanthochlora*, on human TRPV1 and the two-pore domain potassium channels KCNK2, KCNK3 and KCNK9. They proved that the ethanol extract of the aerial parts of this plant is a positive modulator of the TRPV1 channel. TRPV1 is a pain detector for noxious heat and has been a target in the development of pain reducers (Herbrechter et al., 2020).

8.1.6. Anticancer activity

Numerous phytochemicals have been identified as potential candidates that can block or slow the growth of cancer cells with a lack of side effects (Iqbal et al., 2017). Previous studies have implied that herbs with antioxidant and anti-inflammatory potential, especially phenolic-rich plants, inhibit tumor promotion and cell proliferation (Huang et al., 1994). On several occasions, the anticancer characteristics of *Alchemilla* extracts have been investigated (Table 7). The abovementioned studies mainly involve MTT and sulforhodamine B (SRB) assays. An SRB colorimetric assay for cytotoxicity screening was used to investigate *A. mollis* extracts against the breast cancer cell line MCF-7. Karatoprak et al. claimed that the water and deionized water extracts of *A. mollis* inhibit the viability of cells by 70% at 125 μg/mL and may contain cytotoxic agents for medical use (Karatoprak et al., 2018). Notably, the MTT assay is more commonly used to determine the viability of cancer cells (Strawa et al., 2022). Using the MTT assay, Ibrahim and coworkers revealed that the *A. vulgaris* 80% MeOH extract displays moderate cytotoxic activity against MCF-7 ($IC_{50} = 92.25$ μg/mL), PC-3 ($IC_{50} = 88.60$ μg/mL) and Caco-2 ($IC_{50} = 110.51$ μg/mL) cancer cell lines (Ibrahim et al., 2022). Additionally, the hexane and chloroform fractions of *A. vulgaris* in a polyherbal formula (PHF6) containing *Cichorium pumilum*, *Crataegus azarolus*, *Eruca sativa*, *Ferula hermonis*, and *Hypericum triquetrifolium* extracts were investigated against the MDA MB-231 and MCF-7 cell lines. PHF6 decreases the viability of cancer cells, causes significant LDH release and induces apoptosis in both MDA MB-231 and MCF-7 cells (Al-Zharani and Abutaha, 2023). In addition, Vlaisavljević et al. studied the viability of A2780, HeLa, MCF-7, and PC-3 cells via CV and MTT assays. According to the obtained results, the 70% ethyl acetate fraction possessed the strongest toxicity to HeLa, MCF-7, and PC-3 cells, while the 80% methanol fraction had the strongest toxicity to A2780 cells (Vlaisavljević et al., 2019). Among 16 water plant extracts investigated by Trouillas et al., *A. vulgaris* seems to be one of the most potent anticancer extracts against the melanoma cell line B16. The antiproliferative effect ranged from 15% to 60% at concentrations of 0.0125–0.1 mg/mL (Trouillas et al., 2003). In addition to *A. vulgaris*, the *A. mollis* methanolic extract and *A. smirnovii* 96% ethanolic extract were

tested in MTT assays using the K562 and A549 cell lines, respectively. The methanol extract of *A. mollis* decreased cell viability at a concentration of >0.02 mg/mL, while the *A. smirnovi* ethanol extract exhibited a strong cytotoxic effect at the lowest concentration used, 0.125 mg DW/mL (Aslı et al., 2022; Ginovyan et al., 2022). Immunocytochemical staining via the caspase-3 method was used to determine the apoptotic effect in HeLa cells using *A. erythropoda*, *A. ikizdereensis*, *A. oriturcica*, and *A. trabzonica* extracts. The lowest apoptotic activity at the highest tested concentration (200 μg/mL) was found with *A. oriturcica* (14%), while the strongest effect was found with *A. trabzonica* (24%) (Türk et al., 2011).

8.1.7. Anti-overweight activity

Because obesity has become a civilization disease, Mladenova and coworkers attempted to translate traditional knowledge into modern therapeutic applications (Table 7). Therefore, they hypothesized that the *A. monticola* water-methanol extract had anti-adipogenic potential. The results of the *in vitro* analysis showed that the expression of the adipogenic genes CCAAT/enhancer-binding protein alpha (CEBPA) and PPARG was downregulated upon treatment with the plant product. Furthermore, the obtained nuclear magnetic resonance (NMR) based metabolomics data showed that the most abundant signals corresponded to kaempferol 3-*O*-glucoside and quercetin 3-*O*-rhamnoside, implying their responsibility for pharmacological activity of prepared extracts (Mladenova et al., 2021).

8.1.8. Enzyme inhibitory activity

Enzyme inhibitory factors seem to be attractive because of their application in curing various diseases. The secondary metabolites of plants may be enzyme inhibitors that block their activity and can destroy a pathogen or restore metabolic imbalances (Omar et al., 2023). For example, the 80% methanol, 70% ethanol, 70% ethyl acetate and water extracts *of A. vulgaris* were studied as potential amylase, acetylcholinesterase and butyrylcholinesterase inhibitors (Table 7). Based on the obtained results, the 70% ethanol extract possessed the highest anti-acetylcholinesterase potential (5.14 mg GALE per g of extract), and the 80% methanol extract maintained the most potent butyrylcholinesterase inhibition (9.59 mg GALE per g of extract), while the extracts that acted as amylase inhibitors ranged from highest to lowest activity as follows: water >70% ethanol >80% methanol > ethyl acetate. The extracts with the highest content of gallic acid, caffeic acid, catechin and quercetin also display the highest activity (Vlaisavljević et al., 2019). Additionally, lady mantle herbs may be attractive targets for cosmeceutical research due to their inhibition of tyrosinase activity. The available research points out the strong activity of the 70% ethanol extract of *A. vulgaris* with 71.55 ± 4.39% inhibition of tyrosinase at 3 mg/mL (Neagu et al., 2015). The bioassay-guided fractionation of the water-methanolic extract of *A. vulgaris* led to the identification and isolation of flavonoids with collagenase inhibitory activity (Mandrone et al., 2018).

8.1.9. Wound healing activity

Ethnopharmacological studies have suggested that *A. vulgaris*, *A. mollis* and *A. hessii* possess wound healing properties (Kaval et al., 2014; Parthasarathy and Prince, 2021). Additionally, lady's mantle species exhibit a broad spectrum of antiradical characteristics, and it has been proven that wound-healing properties and antioxidant activity coexist in many plant species (Süntar et al., 2012). Thus, Tasić-Kostov and coworkers performed a scratch test, which revealed the wound healing effects of the 70% ethanol, 80% propylene glycol and water extracts of *A. vulgaris*. The most beneficial effect on wound healing was observed with hydrogels of the propylene glycol extract at 250 μg/mL. Likewise, the ethanolic extract (at 50 μg/mL) displayed activity on fibroblast migration and the extent of wound closure (Tasić-Kostov et al., 2019). On the other hand, Shrivastava et al. demonstrated wound healing activity of the 1% *A. vulgaris* hydroglycerinated fluid extract using the Chang liver and Madin–Darby bovine kidney (MDBK) epithelial cell lines and myofibroblasts, attaining 21.3%, 10.6% and 15.5% increases in the cell numbers, respectively (Shrivastava et al., 2007).

8.2. In vivo assays

An in-depth overview of the literature concerning the *in vivo* activity of *Alchemilla* plants includes to 22 different studies, describing anticonvulsant, hormone regulation, anti-overweight, neuroprotective, antitoxic, hepatoprotective, gastroprotective, wound healing, antiaging, anti-inflammatory, cardiovascular activities as well as healing activity in female diseases (Table 8).

8.2.1. Anticonvulsant activity

According to a Ngoupaye study from 2022, scientists reported the anticonvulsant effect of the *A. kiwuensis* water extract. In this study, acute epileptic seizures were provoked in mice aged 2.5–3 months with pentylenetetrazole (PTZ), picrotoxin (PIC) and strychnine (STR). For this test, animals of both sexes were randomly distributed into 5 groups, where group 1 received distilled water, group 2 received clonazepam (as a positive control) and groups 3, 4, and 5 received 20.63 mg/kg, 41.25 mg/kg, and 82.5 mg/kg plant extract, respectively. One hour after the oral administration of various therapeutics, clonic seizures were induced. Factors estimated in this investigation were protection against seizures and seizure onset time. Mice that did not convulse after 10 or 15 min of observation were classified as protected. The results showed that after PTZ-induced seizures, mice were protected by administrating 82.5 and 41.25 mg/kg A. *kiwuensis* with efficacies of 71% and 86%, respectively. The plant extract protected against seizures at a dose of 82.5 mg/kg and had 57.17% activity after PIC-induced convulsions. On the other hand, the plant extract did not have an effect on STR-induced seizures, including death. Furthermore, *A. kiwuensis* extract at a dose of 5000 mg/kg was declared nontoxic (Ngoupaye et al., 2022).

8.2.2. Hormone regulation activity

In another study, scientists surveyed the effects of polyphenol compounds from a. *vulgaris* on the morpho-functional state of the thyroid gland in rats exposed to low temperatures. Two weeks of treatment with water herb extract (10 mg/kg/day) resulted reduced T4 levels in the blood and an increased deiodination ratio. Additionally, the contents of thyroid hormones and thyroglobulin in the thyroid gland (TG) increased compared to the baseline values (Borodin et al., 1999).

8.2.3. Anti-overweight activity

A formulation containing 60 mg of *A. vulgaris* leaves, 50 mg of *Olea europaea* leaves, 20 mg of *Mentha longifolia* leaves, 25 mg of *Cuminum cyminum* seeds, 7 mg of vitamin C and 148 mg of tricalcium phosphate was used as slimming pills ("Weightlevel") in chickens and rats. The antiobesity properties of weight level studies in chickens pointed out that the group fed normal food enriched with 3% product extract weekly displayed reduced weight (to 815 ± 10 g) in the 4th week, where the baseline body weight of the chickens was 1000 ± 15 g. Notably, safety analysis, defined by the LD_{50}, in rats indicated toxicity at a high concentration of approximately 5 g/kg (Said et al., 2011).

8.2.4. Healing activity in female diseases

Following ethnopharmacological reports on *A. mollis* and *A. persica* used for women's illnesses, Küpeli Akkol and coworkers evaluated the treatment potential of 80% methanol extracts of both *A. mollis* and *A. persica* in experimentally induced endometriosis in rats. In this study, six-week-old female rats were surgically auto transplanted with endometrial tissue into the abdominal wall. For this test, animals were randomly divided into groups, one of which was the control group, the second group took a reference drug (20 mg of Receptal®), and 100 mg/kg extract doses were administered to the study groups. After the end of

Table 7
Bioactivities of *Alchemilla* species reported *in vitro* experimental models.

Activity	Tested material	Experimental model	Concentration used	Efficacy	References
Antioxidant	*A. mollis* 10–90% EtOH extracts	ABTS test	not given	96.61 ± 1.54 to 308.44 ± 6.74 mmol TE/dm^3 (for fresh extracts) and 27.10 ± 0.22 to 212.40 ± 2.24 mmol TE/dm^3 after 20 days of storage	Nedyalkov et al. (2015)
	A. mollis 50% EtOH extract	ABTS test	not given	IC_{50} = 7.8 μg/mL	Hwang et al. (2018)
	A. vulgaris H_2O extract	ABTS test	not given	4.79 ± 0.14 mM TEAC	Kiselova et al. (2006)
	A. vulgaris MeOH extracts	ABTS test	not given	IC_{50} (μg/mL): Aerial parts: 14.80 ± 2.15 Roots: 32.49 ± 1.95	Boroja et al. (2018)
	A. vulgaris 80% MeOH 70% EtOH 70% EtOAc, H_2O extracts	ABTS test	not given	MeOH: 143.55 ± 3.65 EtOH: 119.62 ± 3.20 EtOAc: 174.05 ± 0.90 H_2O: 37.50 ± 0.39 mg TE per g extract	Vlaisavljević et al. (2019)
	A. vulgaris 70% EtOH extract	ABTS test	plant material–solvent ratio of 1/50 (*g/v*)	roots: 495.38 ± 19.32 aerial parts: 366.55 ± 12.62 mmol TE/g of dry mass	Sapko et al. (2016)
		DPPH test	plant material–solvent ratio of 1/50 (*m/v*)	roots: 1535.5 ± 72.15 aerial parts: 841.84 ± 36.41 mmol TE/g of dry mass	Sapko et al. (2016)
	A. alpina 96% MeOH, EtOH and $ChCl_3$ extracts	DPPH test	1.25, 2.5, 5, 10 mg/mL	Percent of inhibition (%): 1.25 mg/mL - 45.4 ± 0.440 2.5 mg/mL - 67.8 ± 0.978 5 mg/mL - 84.8 ± 1.348 10 mg/mL - 94.4 ± 1.301	(İnci et al., 2021)
	A. mollis 50% EtOH extract	DPPH test	not given	IC_{50} = 42.4 μg/mL	Hwang et al. (2018)
	A. sericata hexane extract	DPPH test	not given	IC_{50} = 185 μg/mL	Shafaghat et al. (2017)
	A. smirnovii 96% EtOH extract	DPPH test	not given	dose-dependent antiradical activity	Ginovyan et al. (2022)
	A. mollis MeOH, EtOAc, $ChCl_3$ extracts	DPPH test	not given	IC_{50} (μg/mL): $CHCl_3$ >200 EtOAc - 9.8 ± 1.8 MeOH - 31.7 ± 4.9	Trendafilova et al. (2011)
	A. mollis 10–90% EtOH extracts	DPPH test	not given	76.17 ± 1.53 to 247.58 ± 2.26 mmol TE/dm^3 (for fresh extracts) and 21.50 ± 0.10 to 97.53 ± 0.59 mmol TE/dm^3 after 20 days of storage	Nedyalkov et al. (2015)
	A. vulgaris 70% EtOH (AE), 80% propylene (AP) glycol, H_2O extracts (AW)	DPPH test	H_2O: 10–160 μg/mL, 70% EtOH: 0.1–80 μg/mL, 80% propylene glycol: 0.5–20 μL/mL	IC_{50} (μg/mL): AE: 0.11 ± 0.07 AW: 27.22 ± 1.14 AP: 2.88 ± 0.21	Tasić-Kostov et al. (2019)
	A. vulgaris MeOH extracts	DPPH test	serial dilutions: started from 0.25 mg/mL	IC_{50} (μg/mL): Aerial parts: 5.96 ± 0.21 Roots: 11.86 ± 0.56	Boroja et al. (2018)
	A. vulgaris EtOH extract	DPPH test	not given	18.02 ± 0.02 μg/gsr trolox	Jelaća et al. (2022)
	A. persica MeOH:H_2O (8:2) extract	DPPH test	1 to 0.015 mg/mL	root: IC_{50} = 0.055 M aerial parts: IC_{50} = 0.151 M	Ergene et al. (2010)
	A. vulgaris	DPPH test	not given	TLC: white colored bands on purple background	Simion et al. (2018)
	A. vulgaris H_2O extract	DPPH test	not given	IC_{50} = 0.09 mg/mL	Trouillas et al. (2003)
	A. vulgaris H_2O extract		0.02 g/mL	no clear data	Dimiņš et al. (2013)
	A. vulgaris 50% EtOH/ 6% glycerin solution	DPPH test	plant material–solvent ratio of 1/10 (*m/v*)	IC_{50} = 0.2 mg/mL	Dzabijeva et al. (2018)
	A. vulgaris hexane and $ChCl_3$ extracts	DPPH test	1 mg/mL of polyherbal formula (PHF6): *A. vulgaris, C. pumilum, C. azarolus, E. sativa, F. hermonis, and H. triquetrifolium*	hexane: 35.1% ± 0.01 $ChCl_3$: 36.9% ± 0.006	Al-Zharani and Abutaha (2023)
	A. vulgaris EtOAc and MeOH extracts	DPPH test	20–100 μg/mL	IC_{50}: 66.71% (EtOAc) and 23.47% (MeOH)	Shilpee et al. (2021)
	A. vulgaris 10% mass H_2O and 10% mass 70% EtOH extracts	DPPH test	not given	EtOH extracts: 87.95% (at 3 mg/mL) and 80.71% (at 1.5 mg/mL)	(Neagu et al., 2015)

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Table 7 (*continued*)

Activity	Tested material	Experimental model	Concentration used	Efficacy	References
	***A. jungarica, A. vulgaris*, 80% MeOH extract**	**DPPH test**	**not given**	**IC_{50}: 19.69 μg/mL**	**Nikolova et al. (2011)**
	A. mollis H_2O (WE), deodorized H_2O (DWE), 50 % MeOH (ME) extracts	DPPH test	not given	IC_{50} (mg/mL): WE: 0.264 ± 0.028 DWE: 0.146 ± 0.015 ME: 0.161 ± 0.018	Karatoprak et al. (2018)
	A. vulgaris 80% MeOH 70% EtOH 70% EtOAc, H_2O extracts	DPPH test	not given	MeOH: 153.30 ± 0.013 EtOH: 95.99 ± 0.005 EtOAc: 502.56 ± 0.01 H_2O: 89.25 ± 0.02 mg TE per g extract	Vlaisavljević et al. (2019)
		CUPRAC test	not given	MeOH: 216.14 ± 6.86 EtOH: 203.53 ± 9.29 EtOAc: 283.16 ± 12.36 H_2O: 78.56 ± 0.26 mg TE per g extract	Vlaisavljević et al. (2019)
	A. mollis 10–90% EtOH extracts	CUPRAC test	not given	125.10 ± 0.48 to 363.79 ± 0.74 mmol TE/dm^3 (for fresh extracts) and from 79.72 ± 1.12 to 226.52 ± 1.22 mmol TE/dm^3 after 20 days of storage	Nedyalkov et al. (2015)
	A. mollis 10–90% EtOH extracts	FRAP test	not given	214.16 ± 1.58 to 382.78 ± 1.16 mmol TE/dm^3 (for fresh extracts) and 67.91 ± 0.31 to 275.55 ± 0.96 mmol TE/dm^3 after 20 days of storage	Nedyalkov et al. (2015)
	A. vulgaris 10% mass H_2O and 10% mass 70% EtOH extracts	FRAP test	not given	H_2O extract: above 1.5 EtOH extract: above 1.7	Neagu et al. (2015)
	A. vulgaris MeOH extracts	FRAP test	not given	Aerial parts: 632.99 ± 10.26 Roots: 607.52 ± 10.01 mg Trolox/g of extract	Boroja et al. (2018)
	A. vulgaris 70% EtOH extract	FRAP test	plant material–solvent ratio of 1/50 (*g*/*v*)	roots: 988.92 ± 49.76 aerial parts: 963.17 ± 43.00 mmol Fe^{2+}/g of dry mass	Sapko et al. (2016)
	A. vulgaris 80% MeOH 70% EtOH 70% EtOAc, H_2O extracts	FRAP test	not given	MeOH: 7899.45 ± 0.49 EtOH: 6405.75 ± 0.08 EtOAc: 8745.31 ± 0.04 H_2O: 3240.09 ± 0.08 mg EAA per g of extract	Vlaisavljević et al. (2019)
		metal chelating	not given	MeOH: 42.58 ± 0.26 EtOH: 42.32 ± 0.05 EtOAc: 37.96 ± 1.29 H_2O: 39.23 ± 0.32 mg EDTAE per g extract	Vlaisavljević et al. (2019)
	A. vulgaris 80% MeOH 70% EtOH 70% EtOAc, H_2O extracts	phosphomolybdenum assay	not given	MeOH: 1.77 ± 0.01 EtOH: 1.57 ± 0.02 EtOAc: 2.22 ± 0.07 H_2O: 0.53 ± 0.03 mg TE per g extract	Vlaisavljević et al. (2019)
	A. vulgaris MeOH extracts	phosphomolybdenum assay	not given	Aerial parts: 265.62 ± 12.10 Roots: 316.47 ± 18.71 mg AA/g of extract	Boroja et al. (2018)
	A. vulgaris H_2O extract	fenton reaction	10 mM	IC_{50} = 0.18 mg/mL	Trouillas et al. (2003)
	A. vulgaris H_2O extract	superoxide radical scavenging test	not given	IC_{50} = 0.95 mg/mL	Trouillas et al. (2003)
	A. vulgaris	rat liver homogenates	tablet contained 60 mg A. *vulgaris* L., 50 mg *O. europaea* L., 20 mg M. *longiforia* L., 25 mg C. *cyminum* L., 7 mg vitamin C and 148 mg tricalcium phosphate	10 μg/mL of product reduces MDA release from 0.89 ± 0.05 to 0.53 ± 0.03 nM/mg protein, at 50 μg/mL to 0.28 ± 0.03 nM/mg protein	Said et al. (2011)
	A. mollis MeOH extract	SNP induced NO• production	62.5, 125, 250, 500, 1000, 3000 μg/mL	significant dose dependent inhibition of nitrite levels	Aslı et al. (2022)
	A. glabra	ORAC assay HORAC assay TRAP assay	not given	1337 ± 68 μmol TE/g 1999 ± 70 μmol TE/g 1815 ± 38 μmol GAE/g	Denev et al. (2014)
	A. persica MeOH: H_2O (8:2) extract	TBARS assay	not given	MDA level (nmol/mL): aerial parts: 5.9, roots: 19.08	Ergene et al. (2010)
	A. vulgaris 70% EtOH, 80% propylene glycol, H_2O extracts	β-carotene–linoleic acid assay	12.5–200 μg/mL	Dose dependent activity: 70 % EtOH: 60.20 ± 1.74–69.86 ± 0.67 % H_2O: 68.08 ± 0.44–78.95 ± 1.13 %	Tasić-Kostov et al. (2019)

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Table 7 (*continued*)

Activity	Tested material	Experimental model	Concentration used	Efficacy	References
				80% propylene glycol: 65.33 ± 0.29–79.57 ± 0.96 %	
Neuroprotective	*A. xanthochlora* EtOH extract	*Xenopus laevis* oocytes, TASK-1 and TASK-3 channels	0.2 g/mL	positive modulators of the channels	Herbrechter et al. (2020)
Anti-inflammatory	*A. vulgaris* H_2O extract	15-LOX	2, 1.25, 0.625 and 0.1 mg/mL	IC_{50} = 0.52 mg/mL	Trouillas et al. (2003)
	A. vulgaris 70% EtOH extract	THP-1 monocytes and HeLa-TLR4 transfected reporter cells	0.2 g/mL	decrease of LPS-induced IL-8 release, inhibitory effects on stimulated signaling pathways of both TLR2 and TLR4, no effect on NF-κB p65 translocation	Schink et al. (2018)
	A. mollis MeOH: H2O (8:2) extracts *A. persica* MeOH: H2O (8:2) extracts	HRBC test	Stock solution: 20 mg of extract/acetylsalicylic acid with 1 mL of their solvents	IC_{50} (mg/mL): Aerial parts: 1.22 ± 0.07; Roots: 1.34 ± 0.08. IC_{50} (mg/mL): Aerial parts: 1.52 ± 0.09; Roots: 1.82 ± 0.14.	Kurtul et al. (2022)
Cardiovascular	HPC (*N.sativa, A. vulgaris, C. canadensis*, 1:0.6:0.6 *w/w*)	blood vessels from Sprague–Dawley male rats	10 mg/mL	vasoconstriction effect on intestinal vein rings (40% increase compared to phenylephrine)	Said et al. (2022)
	A. vulgaris MeOH (ME) and H_2O (AE) extracts	isolated rat aorta	0.01–10 mg/mL	ME: concentration-dependent relaxations in rat aortic rings; AE: increased contractions in aortic rings.	Takir et al. (2014)
			10 mg/mL, 20 min	ME: reduced the maximal contractility to NA and K^+; AE: increase contractions induced by NA and K^+	
	A. viridiflora MeOH extract	angiotensin I-converting Enzyme (ACE) Inhibitory Activity	0.0016–5.00 mg/mL	IC_{50} = 2.51 ± 0.00 μg/mL	Radović et al. (2022)
Antimicrobal	*A. vulgaris* EtOAc and EtOH extracts	vaccinia and ectromelia viruses	12.5–200 μg/mL	neutralization index for: vaccinia – 4.0 l g ectromelia - 3.5 l g	Filippova (2017)
	A. glabra, A. fissa, A. viridiflora, A. monticol MeOH, CH_2Cl_2, C_6H_6 extracts	*Helicobacter pylori*	not given	ranges of concentrations 4 μg/mL for MeOH extracts of *A. viridiflora*, *A. glabra*, *A. monticola*, and 256 μg/mL for C_6H_6 extracts of *A. viridiflora*, *A. glabra*, *A. fissa*. The best overall activity possesses *A. monticola* extracts	Krivokuća et al. (2015)
	A. sericata hexane extract	agar diffusion test with: *B. subtilis* (Bs), *S. epidermidis* (Se), *E. faecalis* (Ef), *S. aureus* (Sa), *K. pneumoniae* (Kp), *P. aeruginosa* (Pa), *E. coli* (Ec), *A. niger* (An) *C. albicans* (Ca), *S. cerevisiae* (Sc)	30 μL of the hexane extracts	Zone of inhibition (mm): Bs: 12.9 ± 0.15; Se: 11.1 ± 0.11; Ef: NA; Sa: 12.2 ± 0.14; Kp: 9.2 ± 0.21; Pa: 10.1 ± 0.12; Ec: NA; An: 10.7 ± 0.21; Ca: 8.9 ± 0.14; Sc:10.1 ± 0.11	Shafaghat et al. (2017)
	A. vulgaris H_2O: EtOH extracts	microdilution method with: *S. aureus* (Sa), *E. coli* (Ec), *P. mirabilis* (Pm)	0.015–2 mg/mL	MIC, mg/mL: Sa >2; Pm = 1; Ec = 1.	(Đukanović et al., 2021)
	A. vulgaris MeOH: H_2O (8:2, *v/v*) extracts	agar diffusion test with: *S. marcescens* (Sm), *A. johnsonii* (Aj), *A. tumefaciens* (At), *R. solani* (Rs), *P. italicum* (Pi), *F. oxysporium* (Fo)	15.6–1000 μg/mL	Inhibition zone (mm): Sm: 6–10; Aj: 6–12.33; At: 6–11; Rs: 7–8.23; Pi: 6.97–8.30; Fo: 4.90–6.17; Growth Inhibition (%): Sm: 0–83.33; Aj: 0–105.56; At: 0–83.33; Rs: 8.52–22.22; Pi: 7.97–22.59; Fo: 31.48–45.56;	Ibrahim et al. (2022)
	A. vulgaris EtOH extract	disc diffusion method with: *S. aureus* (Sa), *S. epidermidis* (Se), *E. coli* (Ec), *K. pneumoniae* (Kp), *P. aeruginosa* (Pa), *C. albicans* (Ca)	10 mg/mL, 50 μL/disc	Inhibition zone (mm): Ec: 10, Kp: 9, Pa: 11, Sa: 7, Se: 12, Ca: 15	Edrah (2017)
	A. mollis H_2O (WE), deodorized H_2O (DWE), 50 % MeOH (ME) extracts	agar dilution method with: *S. aureus* (Sa), *E. faecalis* (Ef), *E. coli* (Ec), *P. aeruginosa* (Pa), *C. albicans* (Ca), *K. pneumoniae* (Kp)	0.1–10.0 mg/mL	MIC (mg/mL): Sa: 0.5 for WE, DWE, ME; Ec: 5.0 for WE, DWE; Pa: 2.0 for WE, DWE, ME Ef: 5.0 for ME, 7.5 for DWE, WE; Kp: .0 for ME, 7.5 for DWE, WE; Ca: no activity	Karatoprak et al. (2018)
	A. mollis MeOH: H2O (8:2) extracts *A. persica* MeOH: H2O (8:2) extracts	microdilution methods with: *S. aureus* (Sa), *E. faecalis* (Ef), *B. subtilis* (Bs), *E. coli* (Ec), *P. aeruginosa* (Pa), *C. albicans* (Ca)	Serial two-fold dilutions ranging from 10 to 0.078 mg/mL	Aerial parts: (MIC, mg/mL): Sa = 5; Ef = 5; Pa = 10; Roots: (MIC, mg/mL): Sa = 5; Bs = 10; Pa = 10; MIC (mg/mL): aerial parts: Sa = 5; Ef = 5; Pa = 5; Ca = 10; roots: Sa = 5; Ef = 10; Bs = 2.5; Pa = 10; Ca = 10	Kurtul et al. (2022)

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Table 7 (*continued*)

Activity	Tested material	Experimental model	Concentration used	Efficacy	References
	A. rizeensis $CHCl_3$ and H_2O extracts	agar well diffusion and broth microdilution methods with: *E. coli* (Ec), *B. catarrhalis* (Bc), *S. aureus* (Sa), *B. subtilis* (Bs), *H. pylori* (Hp), *C. albicans* (Ca) *T. rubrum* (Tr)	not given	MIC (mg/mL): Bc > 1000; Sa = 0.312; Hp > 1000; Tr = 0.625	Buruk et al. (2006)
	A. pedata	agar well diffusion with: *E. coli* (Ec), *S. aureus* (Sa), *C. albicans* (Ca) *T. mentagrophytes* (Tm)	25 and 5 mg/mL	MIC (mg/mL): Ec = 0.125; Sa = 0.125; Ca = 10; Tm = 10.	Taddese et al. (2009)
	A. vulgaris MeOH extracts	microdilution method with: *M. lysodeikticus* (Ml), *S. typhimurium* (St), *B. subtilis* (Bs), *E. faecalis* (Ef), *E. coli* (Ec), *K. pneumoniae* (Kp), *P. aeruginosa* (Pa), *B. mycoides* (Bm), *A. chroococcum* (Ac), *P. fastigiate* (Pf), *P. canescens* (Pc), *T. viride* (Tv), *T. longibrachiatum* (Tl), *A. brasiliensis* (Ab), *A. glaucus* (Ag), *F. oxysporum* (Fo), *A. alternata* (Aa), *D. stemonitis* (Ds), *C. albicans* (Ca)	40 mg/mL	MIC (mg/mL): aerial parts: Ml = 0.156; St = 0.625; Bs = 2.5; Ef = 0.625; Ec = 1.25; Kp = 5; Pa = 2.5; Bm = 0.625; Ac = 5; Pf, Fs = 10; Pc, Ab, Aa = 20; Ag = 5; Ds = 2.5; Tv, Tl, Ca >20. roots: Ml = 0.156; St = 0.625; Bs = 1.25; Ef = 0.156; Ec = 1.25; Kp = 10; Pa = 5; Bm = 0.156; Ac = 2.5; Pf, Pc, Tv, Tl, Fo = 20; Ag = 10; Ds = 5; Ab, Aa, Ca >20.	Boroja et al. (2018)
	A. vulgaris EtOH extract	well diffusion with: *S. aureus* (Sa), *E. coli* (Ec), *K. rhizophila* (Kr), *B. cereus* (Bc), *B. subtilis* (Bs), *S. typhimurium* (St), *P. vulgaris* (Pv), *E. faecalis* (Ef), *E. aerogenes* (Ea), *C. albicans* (Ca)	4 mg/well	Inhibition zone (mm): Sa: 12, Kr: 14, Pv: 10, Ef: 12, Ca: 10, Ec, Bc, Bs, St, Ec, Ea: equal to negative control inhibitions or under recorded	Keskin et al. (2010)
	A. mollis EtOH, MeOH, H_2O extracts	well diffusion with: *S. aureus* (Sa), *S. epidermidis* (Se), *S. pyogenes* (Sp), *P. aeruginosa* (Pa), *K. pneumonia* (Kp), *E. coli* (Ec)	stock: 100 mg/mL	Inhibition zone (mm): Sa, Sp, Kp: not active; Se: MeOH 10.00; Pa: EtOH 22.67, MeOH 22.67; Ec: H_2O 9.33, EtOH 15.33, MeOH 9.33.	Usta et al. (2014)
	A. vulgaris 50% EtOH/6% glycerin solution	well diffusion with: *S. epidermis* (Se), *P. acnes* (Pa), *P. granulosum* (Pg)	plant material–solvent ratio of 1/10 (*m/v*)	Inhibition zone (mm): Se: 0, Pa: 10, Pg: 13.	Dzabijeva et al. (2018)
	A. diademata MeOH: H_2O (*v/v*): 1:0, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6	disk diffusion method with: *E. coli, Proteus* sp., *P. aeruginosa, S. dysenteriae, S. enteritidis, S. typhi, S. aureus, S. faecalis, C. albicans*	10 and 20 μL extract per disk	10 μL: 22.2 % 20 μL: 55.5 %	Barbour et al. (2004)
	HPC cream (*N. sativa, A. vulgaris, C. canadensis*, 1:0.6:0.6 *w/w*)	disk diffusion method with *E. coli*	1, 2 and 5 mg/disc	dose-dependent antibacterial activity (60% inhibition compared to ampicillin at 5 mg/disc)	Said et al. (2022)
Anticancer	*A. erythropoda A. ikizdereensis A. oriturcica A. trabzonica*	MTT test using HeLa cells, immunocytochemical staining of caspase-3	0–200 μg/mL	150–200 μg/mL caused the increase of necrotic effect, lowest apoptotic effect was found in *A. oriturcica* (14%) and the highest apoptotic effect found in *A. trabzonica* was (24%)	Türk et al. (2011)
	A. vulgaris H_2O extract	B16 melanoma cell line, after 2 days of growth	0.0125, 0.025, 0.05 and 0.1 mg/mL	~15%, 25%, 35%, 60% antiproliferative effect	Trouillas et al. (2003)
	A. vulgaris MeOH: H_2O (8:2, *v/v*) extracts	MTT test using MCF-7, PC-3, Caco-2 cells	not given	IC_{50} (μg/mL): MCF-7: 92.25, PC-3: 88.60, Caco-2: 110.51	Ibrahim et al. (2022)
	A. mollis MeOH extract	MTT test using K562 cells	not given	cell viability decreased at concentrations >0.02 mg/mL	Aslı et al. (2022)
	A. mollis MeOH, DDW and H_2O extracts	SRB test using MCF7 cells	62.5–1000 μg/mL	IC_{50} (μg/mL): H_2O: 59.34 ± 3.41, DDW:87.37 ± 25.15 MeOH: 68.18 ± 6.12	(İlgün et al., 2017)
	A. vulgaris extracts	MTT test using B16 and B16F10 cells	not given	dose-dependent decrease of cell viability after 72 h- treatment	Jelača et al. (2021)
	A. vulgaris EtOH extract	breast cancer cells 4T1	not given	decreased viability of cancer cells and apoptosis detection	Jelača et al. (2022)
	A. vulgaris hexane and $ChCl_3$ extracts	MTT and LDH assays using MDA MB-231 and MCF-7 cells, apoptosis assays	0–250 μg/mL of polyherbal formula (PHF6): *A. vulgaris, C. pumilum, C. azarolus, E. sativa, F. hermonis, H. triquetrifolium*	MTT - hexane: 48.7 μg/mL (MCF7), 82.8 μg/mL (MDA-MB-231) and $ChCl_3$: 44.4 μg/mL (MCF7 and MDA-MB-231), hexane and $ChCl_3$ extracts caused a significant LDH release at 250 and 125 μg/mL, extract induced apoptosis in MCF7 and MDA-MB-231	Al-Zharani and Abutaha (2023)
	A. vulgaris 80% MeOH 70% EtOH 70% EtOAc, H_2O extracts	MTT assay using A2780, HeLa, MCF7, PC-3 cells	not given	IC_{50} (μg/mL): A2780: MeOH 27.9 ± 1.9, HeLa: EtOAc 46.4 ± 5.1, MCF7: EtOAc 31.3 ± 1.5, PC-3: EtOAc 18.7 ± 0.9	Vlaisavljević et al. (2019)

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Table 7 (*continued*)

Activity	Tested material	Experimental model	Concentration used	Efficacy	References
		CV assay using A2780, HeLa, MCF7, PC-3 cells	not given	IC_{50} (μg/mL): A2780: MeOH 38.3 ± 1.1, HeLa: EtOAc 53.8 ± 3.9, MCF7: EtOAc 37.5 ± 5, PC-3: EtOAc 31.7 ± 0.3	Vlaisavljević et al. (2019)
	A. smirnovii 96% EtOH extract	MTT assay using HeLa and A549 cells	0.5, 0.25, and 0.125 mg DW/mL	strong cytotoxicity at the lowest tested concentration 0.125 mg DW/mL	Ginovyan et al. (2022)
	A. mollis H_2O (WE), deodorized H_2O (DWE), 50% MeOH (ME) extracts	SRB viability assay on MCF7 cells	15.625, 31.25, 62.5, 125, 250, 500, 1000 μg/mL	WE and DWE inhibited the viability cells by 70% at a 125 μg/mL	Karatoprak et al. (2018)
Anti-Overweight	*A. monticola* 50% MeOH extract	human adipocytes	5, 10 and 25 μg/mL	downregulated genes: CCAAT/enhancer-binding protein alpha (CEBPA) and PPARG, inhibitory effect on protein kinase B (AKT), PI3K and PPARγ in dose-dependent manner	(Mladenova et al., 2021; Patel et al., 2022)
Enzymes inhibition	*A. vulgaris* 80% MeOH 70% EtOH 70% EtOAc, H_2O extracts	amylase inhibition	not given	MeOH: 0.34 ± 0.04 EtOH: 0.32 ± 0.03 EtOAc: 0.41 ± 0.03 H_2O: 0.22 ± 0.03 mmol ACAE per g extract	Vlaisavljević et al. (2019)
	A. vulgaris 10% mass H_2O and 10% mass 70% EtOH extracts	tyrosinase inhibition	3, 1.5, 0.75 mg/mL	H_2O (%): 60.00 ± 2.78 (3 mg/mL), 53.21 ± 6.78 (1.5 mg/mL), 39.21 ± 5.29 (0.75 mg/mL); EtOH (%): 71.55 ± 4.39 (3 mg/mL), 59.12 ± 2.51 (1.5 mg/mL), 45.12 ± 4.26 (0.75 mg/mL).	Neagu et al. (2015)
	A. vulgaris 80% MeOH 70% EtOH 70% EtOAc, H_2O extracts	acetylcholinesterase inhibition	not given	MeOH: 5.17 ± 0.02 EtOH: 5.14 ± 0.01 EtOAc: 5.21 ± 0.05 H_2O: 5.17 ± 0.02 mg GALAE per g extract	Vlaisavljević et al. (2019)
	A. vulgaris 10% mass H_2O and 10% mass 70% EtOH extracts		100 μL of sample solution at 3, 1.5, 0.75 mg/mL	H_2O (%): 84.56 ± 5.64 (3 mg/mL), 71.36 ± 3.54 (1.5 mg/mL), 50.76 ± 5.78 (0.75 mg/mL); EtOH (%): 96.50 ± 4.93 (3 mg/mL), 78.56 ± 2.45 (1.5 mg/mL), 56.83 ± 3.25 (0.75 mg/mL).	Neagu et al. (2015)
	A. vulgaris 80% MeOH 70% EtOH 70% EtOAc, H_2O extracts	butyrylcholinesterase inhibition	not given	MeOH: 9.59 ± 0.14 EtOH: 9.71 ± 0.09 EtOAc: 9.61 ± 0.02 H_2O: 10.19 ± 0.16 mg GALAE per g extract	Vlaisavljević et al. (2019)
Wound healing	*A. vulgaris* 70% EtOH, 80% propylene glycol, H_2O extracts	"scratch" test	50 μg/mL for AW (H_2O) and AE (70% EtOH); 250 μg/Ml for AP (80% propylene glycol)	Effect of AE and AP extracts on migration of fibroblasts, extent of wound closure, wound healing process were the most pronounced.	Tasić-Kostov et al. (2019)
	A. vulgaris hydroglycerinated fluid extract	MDBK epithelial cells primary smooth muscle cell myofibroblast cultures Chang liver cells	1 % of extract	increase in cells number 21.3 ± 2.1% increase in cells number 15.5 ± 2.2% increase in cells number 10.6 ± 0.6%	Shrivastava et al. (2007)

[a]MeOH -methanol; EtOH – ethanol; EtOAc - ethyl acetate.

treatment, endometriotic foci areas and intraabdominal adhesions were estimated and compared with the former findings. The results indicated that the aerial parts of *A. mollis* and *A. persica* caused cystic formation and that TNF-α, VEGF and IL-6 levels decreased. The root extracts did not display any difference between pre- and posttreatment (Küpeli Akkol et al., 2015).

8.2.5. Neuroprotective activity

To study the neuroprotective effect of *A. vulgaris*, the plant water infusion was administered to animals at doses of 5 or 25 mL/kg daily for 5 days. The positive control was piracetam (400 mg/kg Nootropil®). At a concentration of 5 mg/mL, the hypoxia latent time remained 29.6 min, while at 25 mg/mL, the hypoxia latent time was 32.5 min. Administration of the infusion also affected the latent time for entry into the dark arm during reflex training, which was 15.7 min and 28.0 min at 5 and 25 mL/kg, respectively. On the other hand, neither studied dose significantly influenced the latent survival time of mice under hermetic chamber conditions (Shilova et al., 2020).

8.2.6. Antitoxic activity

To evaluate a protective effect of methanolic extracts of aerial parts (AVA) and roots (AVR) of *A. vulgaris* against cisplatin-induced toxicological alterations in rats Jurić and coworkers treated them with extracts for 10 days (at three different dosages: 50, 100, 200 mg/kg). According to their results treatments with both AVA and AVR decreased levels of serum parameters of liver (TB, TP, ALT, ALP, GGT) and kidneys (UR, CRE, UA) impaling that these extracts may be used in preventing cisplatin-induced toxicity during chemotherapy (Jurić et al., 2020).

Table 8
Bioactivities of *Alchemilla* species reported in *vivo* experimental models.

Activity	Tested material	Experimental model	Concentration	Efficacy	References
Anticonvulsant	*A. kiwuensis* H_2O extract	mice	20.63 mg/kg, 41.25 mg/kg and 82.5 mg/kg	PTZ-induced seizure: protected mice against seizures at 82.5 and 41.25 mg/kg with 71% and 86%, respectively; PTZ-induced seizure onset time: increased at the 82.5 and 41.25 mg/kg; PIC-induced seizures: protected against seizures at 82.5 mg/kg with 57.17%; PIC-induced seizure onset time: increase in a dose dependent manner; STR-induced seizures: had no effect seizure and death	Ngoupaye et al. (2022)
Hormone regulation	*A. vulgaris* H_2O extract	rats	10 mg/kg/day	lowered the blood level of T_4, increased the deiodination ratio, activate proliferative processes in the interfollicular islets, content of thyroid hormones and thyroglobulin in the TG increased compared to the baseline values	Borodin et al. (1999)
Anti-overweight	*A. vulgaris*	chickens	tablet contained 60 mg A. *vulgaris*, 50 mg *O. europaea*, 20 mg M. *longiforia*, 25 mg C. *cyminum*, 7 mg vitamin C and 148 mg tricalcium phosphate	administration the product during 4 weeks of study reduces body weight in the study group	Said et al. (2011)
		rats		weigh level extracts stimulate IBAT respiration rate, in a dose-dependent manner up to > 3-fold higher than basal MO2 values.	
Female diseases	*A. mollis* *A. persica* MeOH: H_2O (8:2) extracts	rats	100 mg/kg doses in 0.5% CMC suspension in distilled water	*A. mollis*: the cystic formation decreased from 101.35 to 11.87 mm^3, TNF-α, VEGF and IL-6; *A. persica*: reduction in the endometrioma	(Bina et al., 2019; Küpeli Akkol et al., 2015)
Neuroprotective	*A. vulgaris* H_2O extract	mice	5 and 25 mL/kg daily for 5 days	hypoxia latent time: 5 mL/kg - 29.6 ± 2.5 min, 25 mL/kg - 32.5 ± 2.9 min; latent time for entry into dark arm during reflex training: 5 mL/kg - 15.7 ± 1.6 min, 25 mL/kg - 28.0 ± 2.9 min	Shilova et al. (2020)
Antitoxic	*A. vulgaris* MeOH extract	rats	extracts: 50, 100, and 200 mg/kg for 10 days	decreased levels of serum parameters of liver, kidneys and testicles injury, tissue's morphology and parameters of oxidative stress	Jurić et al. (2020)
Antidiabetic	*A. mollis* MeOH: H_2O (8:2, *v/v*) extract	alloxan-induced rats	100 mg/kg and 200 mg/kg	none of the extracts induced significant reduction on levels of blood sugar	(Ozbek et al., 2017; Parthasarathy and Prince, 2021)
	A. persica MeOH: H_2O (8:2, *v/v*) extract	alloxan-induced diabetic mice	100 mg/kg and 200 mg/kg	none of the extracts exhibited a significant lowering effect on blood glucose levels	Özbilgin et al. (2019)
	A. viridiflora MeOH extract	streptozotocin-induced diabetic rats	50, 100 and 200 mg/kg, *p.o*	200 mg/kg: decreased blood glucose level after 10 (32.2%) and 20 days (38.3%); 50 mg/kg had no statistically significant effect	Radović et al. (2022)
Hepatoprotective	*A. mollis* MeOH: H_2O (8:2, *v/v*) extract	CCl_4 - induced toxity rats	100 mg/kg, 200 mg/kg	ALT levels were lowered, significant differences in AST levels were not found	Ozbek et al. (2017)
	A. vulgaris 80% EtOH and H_2O extract	rats	50, 100 ppm of EtOH and 50, 100 ppm of H_2O	decrease in liver enzymes (AST, ALT, ALP) after administration 100 ppm of EtOH extract	El-Hadidy et al. (2018)
Gastroprotective	*A. caucasica* MeOH extract	IND-induced ulcer in rats	50, 100, and 200 mg/kg	200 mg/kg dose was the most effective, all doses reduced MDA level and enhanced SOD activity and GSH level	Karaoglan et al. (2020)
	juice: 20 g of the *A. vulgaris* with 1 kg A. *melanocarpa* fruit	IND-induced gastric ulcers in rats	10 mL/kg was administered for 10 days	ulcer score: 1.40 ± 0.54 ulcer index: 1.40 percentage of protection: 68.25% TBARS: above 5 nmol/g PGE_2: approx. 1500 pg/g	Valcheva-Kuzmanova et al. (2019)
Wound healing	*A. mollis, A.persica* MeOH: H2O (8:2, *v/v*) extracts	male Swiss albino mice and Sprague-Dawley rats	not given	tensile strength values on the incision wound model: 39.3% (*A. mollis*), 33.3% (*A.persica*), contraction values: 51.4% (*A. mollis*), 43.5% (*A.persica*)	Öz et al. (2016)
	Gels with *A. vulgaris* 70 % EtOH (GAE), 80% propylene glycol (GAP), H_2O (GAW) extracts	human skin sites pretreated with patch with SLS	1/200 for all examined gels	satisfying barrier repairment potential of investigated samples after 3 (GAE and GAW gels) and 7 (GAP gel) days of treatment compared to basal parameters	Tasić-Kostov et al. (2019)
	A. vulgaris (20%), *M. tenuiflora* (20%) extracts, glycerol (42%),	7-week-old male BALB/c mice	0.1 g of ointment containing herbal mixture every 12 h for 12 days	herbal mixture promoted re-epithelialization, collagen synthesis, and regeneration of skin appendages	Choi et al. (2018)

(*continued on next page*)

Table 8 (*continued*)

Activity	Tested material	Experimental model	Concentration	Efficacy	References
	honey (10%), xanthan gum (8%)				
	A. vulgaris glycerinated fluid extract	rats	3% in glycerin, once a day for 7 consecutive days	Lesion diameter (mm): Day 1: 8.0 ± 0.1 Day 7: 0.1 ± 0.1	Shrivastava et al. (2007)
Anti-inflammatory	*A. mollis, A. persica* MeOH: H_2O (8:2, *v/v*) extract	mice and rats	0.2 mL/20 g	at 200 mg/kg dose 30.6% (*A. mollis*) and 26.6% (*A. persica*) anti-inflammatory activity	Öz et al. (2016)
Cardiovascular	*A. vulgaris* MeOH and H_2O extracts	rats	10 mg/mL	E_{max}: mN/mm $PGF_{2\alpha}$: MeOH: 1.39 ± 0.43; H_2O: 1.67 ± 0.41 K^+: MeOH: 1.14 ± 0.13; H_2O: 2.05 ± 0.11	Takir et al. (2015)
			300 mg/kg/day for 5 weeks	Blood pressure (mm/Hg) at 5th week: MeOH: 119.60 ± 2.50; H_2O: 138.50 ± 2.14	
	A. vulgaris extract	rats	300 mg/kg for 10 days	lysophospholipids decrease, increased lipid content and normalizes phospholipid composition of erythrocyte membra- nes, improvement of erythrocyte deformability	Plotnikov et al. (2006)
Anti-photoaging	*A. mollis* 50% EtOH extract	mice	exposure on UV + diet containing 0.1% or 1 % of extract	prevention of wrinkle formation, skin thickening, water loss, and erythema	Hwang et al. (2018)

8.2.7. Antidiabetic activity

Diabetes mellitus is a metabolic disorder affecting nearly 10% of the population worldwide, and is incidence is increasing daily. The use of drugs of natural origin allows for a delay in the progression of this disease or a reduction in the dose of antidiabetic drugs needed (Ahad Hussain et al., 2014; Sabu and Kuttan, 2002). *A. viridiflora* methanol extracts possess moderate antidiabetic activity. When given *per os*, 200 mg/kg A. *viridiflora* extract decreased the blood glucose levels of streptozotocin-induced diabetic rats after 10 and 20 days by 32.2% and 38.3%, respectively (Radović et al., 2022). On the other hand, Ozbek and co-authors evaluated the antidiabetic activity of the aerial parts and roots methanolic-water extracts of *A. mollis* on alloxan-induced diabetic rats. They proved that the intake of extracts at doses of 100 mg/kg and 200 mg/kg did not induce a significant reduction in blood sugar levels (Ozbek et al., 2017). Similar conclusions were presented by Swanston-Flatt et al., in 1989. After 12 days of administration of *A. vulgaris* infusion to streptozotocin-induced diabetic mice, no significant changes in plasma glucose or insulin concentrations were observed (Swanston-Flatt et al., 1989). In another study, alloxan-induced diabetic mice treated with 100 and 200 mg/kg 80% methanol extract of the aerial parts and roots of *A. persica* did not exhibit a significant blood glucose level lowering effect (Ozbek et al., 2017). Because the data presented in these publications indicate that preparations from *Alchemilla* plants have low antidiabetic activity, it seems unreasonable to examine them as future antidiabetic drugs of natural origin.

8.2.8. Hepatoprotective activity

To characterize the hepatoprotective activity of aerial parts and roots of *A. mollis*, Ozbek and coworkers used rats with CCl_4-induced toxicity. After 2 days of intraperitoneal administration of CCl_4 (0.8 mL/kg), two groups received 100 mg/kg aerial parts or roots 80% methanol extract of *A. mollis* and two other treatment groups received 200 mg/kg the same samples once a day. The study was conducted for 7 days. The downside of the experiment was the lack of a positive control. However, the results of this test showed that extracts of both aerial parts and roots of *A. mollis* lowered alanine transaminase (ALT) levels, although aspartate transaminase (AST) levels were not significantly different between the treatment groups and the CCl_4 group. Furthermore, histopathological examinations displayed prominent recovery after the administration of all dosages except 100 mg/kg A. *mollis* aerial part extract (Ozbek et al., 2017). Similar experiments were conducted by El-Hadidy et al. using 80% ethanolic and aqueous *A. vulgaris* extracts (50 or 100 ppm), as well as its dried leaves (1% and 2%). In this study, AST, ALT, and alkaline phosphatase (ALP) levels were evaluated in rats induced by CCl_4. Significant reductions in AST, ALT, ALP, and bilirubin levels were seen after *A. vulgaris* treatment in all groups except for the 50-ppm water extract group. No changes were observed in albumin levels compared to the normal group (El-Hadidy et al., 2018).

8.2.9. Gastroprotective activity

Efforts have been made to examine the gastroprotective activity of *A. caucasica* extract at doses of 50, 100, and 200 mg/kg against indomethacin-induced ulcers in rats. Rats were divided into 6 groups: group 1 contained healthy animals, group 2 received 25 mg of indomethacin (IND), group 3 received 25 mg of IND and 40 mg of famotidine as a positive control, and groups 4, 5 and 6 received 25 mg of IND with 50 mg/kg extract, 25 mg of IND with 100 mg/kg extract and 25 mg of IND with 200 mg/kg extract, respectively. After biochemical estimation, the 200 mg/kg dose was determined to be the most effective and similar to the healthy group. Nevertheless, *A. caucasica* reduced the malondialdehyde (MDA) level, increased the glutathione (GSH) level, and enhanced superoxide dismutase (SOD) activity, proving its gastroprotective activity (Karaoglan et al., 2020). Another study aimed to investigate the effect of the juice produced from 1 kg of *Aronia melanocarpa* fruits and 20 g of *A. vulgaris* herbs. Over a period of 10 days, the treatment group was pretreated with the juice at a dose of 10 mL/kg. On the 10th day, 1 h after pretreatment, 30 mg/kg IND was subcutaneously injected into rats, and after 4 h, gastric ulcer formation was evaluated. According to the scores obtained, pretreating rats with the juice caused a reduction in the rawness of IND-induced gastric lesions (ulcer score and index) and antagonized the impact of IND on apoptosis and lipid peroxidation processes (TBARS above 5 nmol/g) (Valcheva-Kuzmanova et al., 2019).

8.2.10. Wound healing activity

Within *in vitro* scratch assays, Tasić-Kostov et al. studied wound healing with *A. vulgaris* extract gels on human skin irritated with a sodium lauryl sulfate (SLS) patch, which exerted a significant effect on trans epidermal water loss (TEWL). The change seemed to be reversible after 3 days of curing with gels containing water and the ethanol *A. vulgaris* extract and after 7 days of treatment with the gels with the 80% propylene glycol plant extract. Based on the results, the authors

suggested that topical use of gels with lady mantle extracts provokes epithelial cell proliferation, inducing wound healing (Tasić-Kostov et al., 2019). Herbal extracts with wound healing properties for topical application are traditionally incorporated into ointments that consist of waxes, Vaseline, or lanolin (Eastman et al., 2014). In one study, 0.1 g of ointment composed of *A. vulgaris* (20%) and *Mimosa tenuiflora* (20%) extracts, glycerol (42%), honey (10%) and xanthan gum (8%) were studied on 7-week-old mice with a 1 cm incisional wound. The topical application of the herbal ointment as well as the fusidic acid ointment was repeated every 12 h. After 12 days, treatment with the herbal mixture resulted in reepithelialization, an increase in collagen synthesis, and the regeneration of skin appendages (Choi et al., 2018). Additionally, aqueous-methanol extracts of both *A. mollis* and *A. persica* incorporated into an ointment were estimated for their wound healing potential. The herbal ointments, reference drug and ointment base were topically applied to the dorsal wounds of each group of mice once daily for 9 days. According to the obtained results, the tensile strength values of the incision wound model were 39.3% and 33.3% for *A. mollis* and *A. persica*, respectively. The contraction values reached 51.4% (*A. mollis*) and 43.5% (*A. persica*) (Öz et al., 2016).

8.2.11. Anti-photoaging activity

In addition to the wound healing properties of *A. mollis* extracts or their preparations, the ethanol-water extract of *A. mollis* has been tested for its antiphotoaging activity. In this study, sixteen hairless mice were UVB-irradiated with sunlamps. The exposure during the first week was 100 mJ/cm^2 seven times per week and 200 mJ/cm^2 twice a week for the following nine weeks. One of the experimental groups of mice was fed a diet containing 0.1% *A. mollis* extract, while the second group was fed a diet containing 1% extract. It was found that in the treated groups, the mouse skins appeared to be smoother and thinner than those in the control group, and the effect was dose dependent. Furthermore, the density of the collagen fibers treated with *A. mollis* extract was significantly higher (Hwang et al., 2018).

8.2.12. Anti-inflammatory activity

Anti-inflammatory activity of the 80% methanolic extracts from the aerial parts of *A. mollis* and *A. persica* was studied using acetic acid-induced increase in capillary permeability. It was found that extracts from both plants possess anti-inflammatory activity at dose 200 mg/kg. *A. mollis* exhibited activity with the values of 30.6% and *A. presica* of 26.6%.

8.2.13. Cardiovascular activity

To investigate the blood pressure-lowering properties of *A. vulgaris* methanol and water extracts, Takir and co-authors fed rats with a dose of 300 mg/kg/day of each of the extracts for 5 weeks. At the end of the curation, blood pressure was measured. After administration of the methanol extract, the blood pressure reached 119.60 mmHg, and the blood pressure was 138.50 mmHg after water extract treatment, compared to that in the control group (156.50 mmHg). In addition, both extracts produced relaxations in $PGF_{2\alpha}$ (MeOH: 1.39 and H_2O: 1.67), while the opposite vascular influence was noticed when the extracts were applied in K^+ precontracted arteries (Takir et al., 2015). Plotnikov et al. showed that treating rats for 10 days with 300 mg/kg extract increased the lipid content and normalized the phospholipid composition of erythrocyte membranes, which favors decreases in the levels of irreversibly modified erythrocytes and an improvement in erythrocyte deformability (Plotnikov et al., 2006).

Place Table 8 here

8.3. In silico assays

Recent developments in computational (*in silico*) approaches have provided essential information on natural compounds and methods to examine their pharmacological profiles. It is a tool that allows the prediction of potential biological activity and supplies a better indication of how the structure of a plant-based compound can influence its targets (Fang et al., 2017).

8.3.1. Antiviruses activity

Suručić et al. supplied information about the inhibitory effects of *A. viridiflora* polyphenols on SARS-CoV-2 internalization *in silico*. According to their results, ellagitannins most likely blocked S-glycoprotein interactions with ACE2, whereas the NRP1 receptor interacted with flavonoid compounds (Suručić et al., 2022).

8.3.2. Cardiovascular activity

Also, Radović and co-workers performed the molecular docking simulation study of ACE inhibitory activity of compounds isolated from methanolic extract of *A. viridiflora*. Among them tiliroside, tellimagrandin I, ellagic acid pentose and galloyl-HHDP-glucose showed high affinity for ACE binding site (Radović et al., 2022).

8.3.3. Anti-overweight activity

To evaluate mechanism of action of kaempferol 3-*O*-glucoside and quercetin 3-*O*-rhamnoside detected in the *A. monticola* docking runs were performed using C/EBPα, PPARγ, AKT and PI3K proteins. The strong binding free energies suggested a potential envolve of PI3K and PPARγ proteins in the mechanism of action of these compounds in the adipocytes (Mladenova et al., 2021).

8.4. Clinical trials

Besides *in vitro* and *in vivo* studies there are some reports of the use of the *A. vulgaris* herb for traditional therapeutic indications as a raw material in certain clinical trials.

A clinical trial was conducted on 341 girls aged 11–17 suffering from menstrual disorders. The patients selected for the study were orally administered 50–60 drops of a liquid *A. vulgaris* extract containing 5.8% tannin compounds and 2.2% flavonoid glycosides three to five times a day. It was observed that as a result of treatment, menstrual bleeding lasted no longer than three to five days. The extract also reduced the intensity of menstrual bleeding when administered prophylactically for a period of 10–15 days before menstruation. During the studies, no adverse effects were observed, and the extract was considered safe for use (Bradley, 2006).

The purpose of another clinical study using lady's mantle was to evaluate the efficacy of a 3% glycerine preparation containing an *A. vulgaris* extract in the treatment of aphthous ulcers, one of the most common types of recurrent ulcers in the oral mucosa. An open-label study was conducted with 48 patients aged 4–44 years, excluding patients with herpes ulcers. The trial tried to determine the healing properties and patient tolerance to the recommended preparation. The preparation was applied topically three times a day. The results showed that the preparation relieved discomfort and caused complete recovery in most patients within two days and in 75% of patients within three days, which was a significantly better result than the number of untreated patients with complete recovery (10.4% and 33.3%, respectively) and those treated conventionally (15% and 40%, respectively). The preparation used was well tolerated by patients, which suggests that the tested agent containing *A. vulgaris* extract is safe and highly efficacious in the treatment of aphthous oral ulcers (Shrivastava and John, 2006).

In another study, the authors' aim was to investigate the effects of spraying a glycerine extract of *A. vulgaris* into the oropharynx to prevent sore throat resulting from intubation following general anesthesia. The study included 94 patients (aged ≥18 years) who qualified for thoracic surgery using a double-lumen tube. Prior to intubation, 0.2 mg/kg dexamethasone was administered to all patients intravenously, and 2 mL of saline was sprayed into the oropharyngeal cavity ($n = 45$), or 0.04 mL/kg saline was administered intravenously, and 1 g of Neo Mucosal

Activator mixed with 1 mL of normal saline was sprayed into the oropharyngeal cavity ($n = 43$). The study was performed in a double-blind, prospectively randomized manner. Postoperative sore throat and hoarseness were recorded for each patient using a numerical rating scale and a 4-point scale to detect changes in voice quality after tracheal extubation at 1, 6 and 24 h. The incidence of sore throat 24 h after surgery was assessed first. Secondary endpoints were the incidence and severity of sore throat and hoarseness. There were no significant differences between groups in the incidence of sore throat 24 h after surgery (57.8% vs. 46.5%; $p = 0.290$) or in the incidence and severity of sore throat and hoarseness 1, 6 and 24 h after surgery. In conclusion, it was determined that the application of *A. vulgaris* extract to the oropharyngeal mucosa had no significant effects on preventing intubation-induced sore throat or hoarseness compared with intravenous injection of low-dose dexamethasone as a positive control. It is thus justified to conduct further research on the method of application and selection of the best dose of the extract by comparisons with placebo and other drugs, as well as a more thorough verification of the preventive effect of the tested formulation with *A. vulgaris* extract. Unfortunately, this study had many limitations. First, it did not include a control group of patients who did not receive prophylactic analgesics, which prevents determination of the extent to which the *A. vulgaris* preparation reduced the incidence of sore throat. In the study, the authors also did not describe how the pharmaceutical formulation was prepared, and there was no information on the full phytochemical standardization of the extract necessary to describe its main active ingredients (Chung et al., 2021).

One of the most common diseases of the digestive tract is hemorrhoids. Numerous conventional therapeutic methods for hemorrhoids are very often associated with serious complications. Currently, the search for traditional medicines, including those based on plant ingredients, is underway. For this purpose, a randomized, double-blind clinical trial was conducted to determine the efficacy and safety of a topical cream containing three plant extracts, including *A. vulgaris* extract (0.3 g/50 mL). This study, with 77 patients enrolled, was intended to determine the efficacy and safety of the formulation created to treat hemorrhoids. This clinical trial included patients suffering from symptomatic hemorrhoids at various stages of disease progression. The preparations were applied twice a day for six days. Each patient attended five visits and a follow-up visit: baseline, at days 2, 4, 6, and at the follow-up endpoint of 30 days. The results indicated that patients showed significant clinical improvement in all parameters of disease severity compared to those in the placebo group (Said et al., 2022).

The purpose of another clinical study using the aerial parts of *A. vulgaris* was to evaluate the effects of plant extracts rich in tannins (procyanidins) on the growth of fibroblasts and epithelial cells in an *in vitro* model and then to assess their efficacy in healing deep wounds. As noted, the growth of epithelial cells and fibroblasts applies to all types of deep wounds; however, a limitation of the study was that the origin of the wounds was not accounted for. The study included 93 adult patients who were randomly divided into two groups. Forty-one patients were randomized to the placebo group (AS-22) and 52 to the active treatment group (AS-21) over a period of six weeks. A statistically significant difference was observed between the placebo and AS-21 group regarding reductions in wound area (33.37% vs. 97.87%) and wound volume (29.45% vs. 94.17%) after six weeks of treatment. During the study, significant decreases in average wound moisture and pain sensation were also observed. The results of this study also showed that the healing time was significantly shorter in the group of patients treated with the formulated topical product containing 1.5% dry procyanidin extract of *M. tenuiflora* (13.5% polyphenols) and 1.5% dry extract of *A. vulgaris* (12% polyphenols) compared to the placebo group. In addition, 64% glycerol and 33% honey were present in the product formulation. The results of this study indicate that the fraction of procyanidins from *A. vulgaris* and *M. tenuiflora* neutralized excess metalloproteinases in the deep wound, thus stopping the degradation of the intercellular matrix and creating a favorable environment for the growth of fibroblasts found deep in the wound and epithelial cells. It was also observed that gradual decreases in wound depth due to fibroblast cell proliferation and wound surface area due to epithelial cell growth accelerated the healing process (Shrivastava, 2011).

Another study prepared a blend of extracts from four plants used in traditional Arabic and Islamic medicine as well as in European herbalism and evaluated its safety and efficacy in weight loss. The study used plant raw materials, including the leaves of *A. vulgaris*, *O. europaea* and *M. longifolia* and the seeds of *C. cyminum*. In the first stage of the study, no signs of toxicity were observed when cultured human fibroblasts were treated with a mixture of the raw materials, as shown by the release of lactate dehydrogenase. These results were confirmed in experimental studies in rats, in which an LD_{50} of 15.3 g/kg was determined. The antioxidant properties at exceptionally low concentrations (10 μg/mL), as determined by the lipid peroxidation method, were also evaluated. Studies conducted on chickens given this mixture weekly for four weeks showed that the animals had progressive and significant weight loss compared with the chickens in the control group. The promising results from preclinical studies became the basis for a clinical trial including 80 volunteers with a body mass index (BMI) of 30.67 ± 2.14 kg/m^2. All 80 subjects were asked to continue their usual diet, eating only three main meals a day, and take one tablet of a product containing a mixture of raw materials, including the leaves of *A. vulgaris*, exactly 30 min before each meal. Fourteen patients were excluded due to noncompliance with the protocol, and the remaining 66 patients were evaluated for treatment efficacy and weight level tolerance every month for three months. The ingested herbal product was well tolerated by all subjects, and no side effects were reported. Progressive and significant weight loss was observed in these participants throughout the study period. Greater weight loss was seen in those with a BMI of 25–30 kg/m^2 (overweight) compared to those with a BMI >30 kg m^2 (obese). The BMIs decreased after three months from 28.5 ± 1.2 and 32.1 ± 1.8 kg/m^2 to 24.5 ± 1.4 and 27.5 ± 2.2 kg/m^2 in the overweight and obese groups, respectively (Said et al., 2011).

9. Toxicity

A. vulgaris is regarded as safe by the German Commission even at large doses without known adverse effects (Said et al., 2011). For example, in studies involving the administration of an extract of *A. vulgaris* to teenage female patients at regular intervals over 6 years, no significant changes in the monitored biochemical parameters were found (Miętkiewska et al., 2018). Countless numbers of *A. vulgaris* extracts were tested for their toxic effects to normal cells using MTT or SRB tests. Tasić-Kostov et al. used a fibroblast cell line and administered different concentrations of different *A. vulgaris extracts* that were proven in a viability assay (MTT) to be noncytotoxic: 50 μg/mL water and 70% ethanolic extracts and 250 μg/mL 80% propylene glycol extract (Tasić-Kostov et al., 2019). Additionally, to prove the safety of this plant, the Ames test was performed using *Salmonella typhimurium* strains TA 98 and TA 100. This research proved that the commercial 70% ethanol tincture of *Alchemillae herba* is not mutagenic (Schimmer et al., 1993, 1994). Moreover, it was found that the contents of tannins in the extracts did not correlate with the antimutagenic properties; however, tannin-free fractions did not inhibit the mutagenic activity. On this basis, it was assumed that the tannin fraction is involved in the antimutagenic extracts tested (Schimmer and Lindenbaum, 1995).

10. Conclusions

A comprehensive review was conducted to gather information about the traditional uses, botany, phytochemistry, pharmacology, and toxicology of *Alchemilla* plants. A continually increasing number of compounds isolated and identified from species in the *Alchemilla* genus have provided substantial information about the main constituents

underlying their usefulness in modern medicine. Although remarkable progress has been made toward their development in science and medicine, many people worldwide do not have access to present day healthcare. Thus, we noticed a need for extensive scientific investigations aiming to rationalize the ethnopharmacological application of bear's foot plants in the treatment of many disorders, such as wounds, gynecological, gastric, and cardiovascular diseases, as well as chronic inflammation or infection. Both *in vitro* and *in vivo* study results have indicated that *Alchemilla* species have a broad spectrum of biological activities and could be considered useful in phytotherapy and the production of safer and less expensive plant-based drugs. Although *A. vulgaris* is a safe herb, the pharmacokinetics and pharmacodynamics of the individual compounds, as well as estimations of their interactions with dietary molecules and the most common drugs, need to be determined. Additionally, clinical study data are extremely limited. In summary, *Alchemilla* species, especially *A. vulgaris*, can open doors in the development of many substantial remedies with various applications to resolve several health aliments.

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CRediT authorship contribution statement

Katarzyna Jakimiuk: Conceptualization, Data curation, Investigation, Writing – original draft, Writing – review & editing, Project administration. **Michał Tomczyk:** Data curation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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