Verbena officinalis (Common Vervain) – A Review on the Investigations of This Medicinally Important Plant Species

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ABSTRACT

Verbena officinalis (common vervain) is a medicinal plant species widely distributed in the world and commonly used in folk medicine of different countries, including traditional Chinese medicine. Monographs on "*Verbenae herba*" have been included in the European Pharmacopoeia since 2008, and in the Chinese Pharmacopoeia since 1995. This work presents botanical characteristics of this species. It reviews the current knowledge of its chemical composition, which is a rich source mostly of iridoids, phenylpropanoid glycosides, phenolic acids, flavonoids, terpenoids, and essential oil. A large part of this article summarizes traditional medicinal uses and professional pharmacological *in vitro* and *in vivo* studies that prove new important applications, e.g., antioxidant, antimicrobial, anti-inflammatory, neuroprotective anticancer, analgesic, or anticonvulsant of verbena herb extracts and individual metabolites. Moreover, emphasis is put on the use of *V. officinalis* in the food and cosmetics industries, especially due to its antioxidant, antibacterial, and anti-inflammatory properties, and the presence of essential oil with an attractive fragrance composition. This paper also presents the state of biotechnological studies of this species.

General Information

Verbena officinalis L. (common vervain) (Verbenaceae) is a well-known medicinal plant with an established position in European, Asian, and North American medicine. The raw material used in both traditional medicine and modern phytotherapy is the verbena herb – *Verbenae herba* [1–5].

The natural habitats of *V. officinalis* are widespread throughout the world – in Europe, the Americas, North and Central Africa, Asia, and Australia [6]. This species grows mainly in temperate climate zones. In Europe, it is common in the Mediterranean region [7–11]. This species prefers dry soils that hold moisture well and sunny locations. It is a ruderal species; it grows in fields, on stone rubble, roadsides, and wastelands, often near water reservoirs [12].

V. officinalis is a species with numerous synonymous names. In Latin, they are *Verbena sororia* D. Don and *V. spuria* L. Depending on the country, *V. officinalis* has numerous common names, e.g., vervain, common verbena, official vervain, simpler's joy, turkey grass, wild verbena (English), verveine officinale (France), echtes Eisenkraut (Germany), kumatsuzura (Japan), erva-de-ferro, ferraria, planta-da-sorte (Portugal and Brazil), järnört (Sweden), and ma bian cao (China) [13].

Identification of *V. officinalis* is most often based on morphological features and phytochemical analyses. However, recognition of the species based on morphological characteristics requires specialist knowledge, particularly in the case of fragmented pharmaceutical raw material. Phytochemical analysis is difficult due to the high variability of the chemical composition of the raw material depending on its origin. Currently, there are modern ways to uniquely identify the species using genetic markers [14].

V. officinalis is a perennial herbaceous plant growing from 75 cm to 1 m tall. Erect, branching at the top, woody stems with a quadrangular cross-section are covered with rough hairs. The upper leaves are sessile, serrated, and arranged opposite each other, while the middle ones are tripartite, and the lower ones are petiolate and pinnate. During the summer, this species produces small, pale lilac flowers gathered in spiky, loose, top inflorescences and some located in leaf axils. A single flower has a small, almost a two-lipped crown with a short, slightly bent tube that widens into a wreath. Inside the cup with four or five sharp serrations, there is an upper pistil with an ovary divided into chambers. There are 2, 4, or 5 stamens attached to the inside of a chamber. The fruit is an elongated, ribbed schizocarp [6, 15].

Brief Characteristics of the Genus Verbena

V. officinalis is the main species belonging to the genus *Verbena* of the family Verbenaceae (subfamily *Verbeneae*) [9, 16, 17]. In the professional scientific literature, there is conflicting information regarding the number of genera and species belonging to the family Verbenaceae. It is estimated that it includes about 30 genera, e.g., *Aloysia, Citharexylum, Lantana, Lippia, Phyla*, and *Verbena*. The entire Verbenacae family has about 1100 species. These are trees and shrubs, as well as herbaceous plants [9, 18]. The genus *Verbena*, according to various sources, comprises from 44 to 250 species. The species of the genus *Verbena* occur mainly in the Americas. Two species, *V. officinalis* L. and *Verbena supina* L., are found on all continents [19, 20]. Other popular species grown as ornamental plants are *Verbena hastata* L., *Verbena bonariensis* L., and *Verbena × hybrida* Groenl. & Rumpler. *Verbena × hybrida* is a hybrid of *Verbena incisa* Hook., *Verbena peruviana (*L.) Britto, *Verbena phlogiflora* Cham., and *Verbena teucroides* [21].

Vervain in Official Phytotherapy

Verbenae officinalis herba – the vervain herb although it has long been known as a traditional medicinal raw material. It appeared in official European medicine relatively recently. In 2008, a monograph on "Verbena herb" appeared in the European Pharmacopoeia (6th ed.). The raw material, in accordance with the requirements of the latest (10th) edition of the European Pharmacopoeia, should be standardized for verbenalin content [min. 1.5% DW (dry weight)] [22].

In addition, a monograph on "*Verbenae herba*" also appeared in the Chinese Pharmacopoeia, e.g., in the eighth (2005) edition. In the British Pharmacopoeia and German Pharmacopoeia, monographs on the *V. officinalis* herb had already been included earlier [3, 4, 16, 23]. *V. officinalis* has long been a well-known medicinal plant in the United States. A description of it is found, for example, in the Pharmacopoeia of the American Institute of Homeopathy from 1897.

What is interesting is that instead of a *V. officinalis* monograph, the European Pharmacopoeia contains a monograph of another *Verbena* species – "*Verbenae citriodorae folium*" – leaf of lemon verbena [*Aloysia citriodora* Palau; syn: *Aloysia triphylla* (L'Her.) Kuntze, *Verbana triphylla* L'Her.; *Lippia citriodora* Kunth, *Verbena citriodora* (Palau) Cav.]. *A. citriodora* is a species with a chemical composition different from that of *V. officinalis* (a typical oil raw material) and a different, limited, distribution of its natural habitats. *A. citriodora* is a shrub indigenous to South America, which was introduced into Europe at the end of the 17th century and has been widely used in infusions for its antispasmodic, antipyretic, sedative, and digestive properties [24–27]. Phenylpropanoids and their metabolites are the major compounds responsible for blood-cell protection against oxidative stress after administration of *L. citriodora* in rats [28]. According to the latest systematic studies, *A. citriodora*, in contrast to *V. officinalis*, which represents the subfamily *Verbeneae*, belongs to another subfamily of the family Verbenaceae, i.e., *Lantanae* [17].

Phytochemical profile

V. officinalis herba has a rich chemical composition (▶ **Table 1**). The main groups of secondary metabolites determining the biological activity profiles of the raw material are iridoid glycosides (▶ **Fig. 1**), including verbenalin (verbenaloside), aucubin (verbenin), and hastatoside, as well as phenylpropanoid glycosides, and caffeic acid derivatives (▶ **Fig. 1**) verbascoside (acteoside) and isoverbascoside (isoacteoside), and eukovoside. In addition, numerous flavonoids have been identified in verbena herb extracts, including compounds common in the plant kingdom such as kaempferol, luteolin, and apigenin, and specific flavonoids such as scutellarein and pedalitin. Also, noteworthy is the presence of phenolic acids: chlorogenic, ferulic, protocatechuic, rosmarinic, and dicaffeoylquinic acid derivatives (▶ **Table 1**). Preliminary research conducted by our team regarded the dynamics of accumulation of these metabolite groups during one vegetative cycle. We have proved the maximum content of iridoids, phenylpropanoid glycosides, flavonoids, and phenolic acids in the material harvested at full bloom. The main compounds were verbenalin (max. 6196 mg/100 g DW), verbascoside (max. 2264 mg/100 g DW), hastatoside (max. 582 mg/100 g DW), scutellarin (max. 248 mg/100 g DW), and isoverbascoside (max. 242 mg/100 g DW) [unpublished]. The terpenoid compounds found in the *V. officinalis* herb are monoterpenoids (citral, limonene, cineole, carvone), diterpenoids (carnosol, carnosic acid, rosmanol, isorosmanol), sesquiterpenoids (caryophyllene oxide, α-curcumane), and triterpenoids (ursolic acid and its derivatives) [29–36] (▶ **Tables 1** and **2**).

Phytochemical analyses have shown that methanolic extracts from *V. officinalis* stems contain sterols such as α-sitosterol, β-sitosterol, and daucosterol [29, 37].

The *V. officinalis* herb contains carbohydrates such as galacturonic acid, arabinose, galactose, rhamnose, xylose, mannose, and glucose, as well as large amounts of bioelements, mainly, potassium, phosphorus, calcium, magnesium, zinc, iron, manganese, and copper [38–40].

▶ **Table 1** Phytochemical composition of the *V. officinalis* herb.

Group of metabolites	Compounds	References
Iridoids	verbenalin and its derivative	[31, 50]
	3,4-dihydroverbenalin	[31]
	hastatoside and its derivative	[31, 84]
	7-hydroxydehydrohastatoside	[31]
	aucubin	[38, 49]
	verbeofflin	[31]
	verbenoside A	[85]
	verbenoside B	[85]
Phenylpropanoid glycosides	verbascoside and its derivatives: *6-acetyl-O-verbascoside, 4-acetyl-O-verbascoside, 2,4-diacetyl-O-verbascoside*	[50, 69]
	β-hydroxy verbascoside	[84]
	isoverbascoside and its derivatives	[37, 69]
	4-acetyl-O-isoverbascoside	[69]
	3,4-diacetyl-O- isoverbascoside	[69]
	β-hydroxy isoverbascoside	[84]
	eukovoside	[84]
	campenoside II	[69]
	isocampenoside II	[69]
	betonyoside A and its derivatives: *4,6-diacetyl-O-betonyoside A, 3,4-diacetyl-O-betonyoside A*	[69]
	cistanoside D	[86]
	leucosceptoside	[86]
Flavonoids	kaempferol	[87]
	luteolin and its glycosidic conjugates: *7-O-diglucuronide, 7-O-glucuronide, 7-O-glucoside*	[32, 37, 87]
	6-hydroxyluteolin glycoside	[88]
	luteolin-7-O-rutinoside	[89]
	apigenin and its glycosidic conjugates: *7-O-diglucuronide, 7-O-galactoside, 7-O-glucoside*	[32, 37, 38]
	6-hydroxyapigenin glycoside	[88]
	isoramnetin	[33]
	quercetol	[33]
	pedalitin and its glycosidic conjugates: *6-O-(2-O-feruloyl)-diglucuronide, 6-O-diglucuronide, 6-O-galactoside, 6-O-glucoside*	[38]
	scutellarein and its glycosidic conjugates: *7-O-(2-O-feruloyl)-diglucuronide, 7-O-diglucuronide, 7-O-glucuronide, 7-O-glucoside*	[29]
Phenolic acids	chlorogenic acid	[63]
	ferulic acid	[36]
	protocatechuic acid	[36]
	4,5-O-dicaffeoylquinic acid	[38]
	1,5-dicaffeoylquinic acid	[38]
	rosmarinic acid	[89]

continued

▶ **Table 1** *Continued*

Group of metabolites	Compounds	References
Terpenoids	**Diterpenoids**	
	carnosol	[89]
	carnosolic acid	[89]
	rosmanol	[89]
	isorosmanol	[89]
	Triterpenoids	
	ursolic acid and its derivatives	[34, 90, 91]
	3*α*,19,23-trihydroxyurs-12-en-28-oic acid	[34]
	2*α*,3*β*-dihydroxyurs-12-en-28-oic acid	[34]
	3*α*,24-dihydroxyurs-12-en-28-oic acid	[34, 35]
	3-epiursolic acid	[35]
Carbohydrates	arabinose, galactose, galacturonic acid, glucose, mannose, rhamnose, xylose	[40]
Sterols	daucosterol	[37]
	α-sitosterol	[91]
	β-sitosterol	[37]
Fatty acids	oleic acid	[91]
	3-epioleanolic acid	[35]
	3*α*,24-dihydroxy-olean-12-en-28-oic acid	[37]
Bioelements	potassium, sodium, iron, magnesium, calcium, phosphorus, copper, zinc, manganese	[39]

In the available literature, there are very few publications that deal with analyses of the constituents of root extracts [41]. Thin-layer chromatography and high-pressure liquid chromatography methods have been used to identify hastatoside (0.379%), verbascoside (0.317%), verbenalin (0.276%), and ursolic acid (0.057%) [41]. Our team's unpublished studies have proved the presence of verbascoside, isoverbascoside, verbenalin, loganin, and hastatoside in methanolic root extracts. The highest amount present was that of loganin (5557 mg/100 g DW) [unpublished].

The *V. officinalis* herb is also characterized by the presence of an essential oil, which consists of about 40 compounds, mainly, monoterpenoids (citral, limonene, cineole, carvone) [42] (▶ **Table 2**). The composition of *V. officinalis* essential oil depends on environmental conditions, *V. officinalis* chemotype tested, type of plant material, and the essential oil extraction method used. Hydrodistilled essential oil obtained from fresh plants grown in Italy revealed substantial amounts of citral (> 45%) and isobornyl formate (> 40%) [30, 42–45]. Steam-distilled essential oil from dried Moroccan plants contained mostly spathulenol (> 10%), limonene, and eucalyptol (7.5% each) [46]. The volatile fraction of dried *V. officinalis* harvested in Algeria was dominated by limonene (> 17%), carvone (> 14%), citral (> 14%), and caryophyllene oxide (> 12%) [47]. The volatile fraction obtained from the aerial parts of a *V. officinalis* intact plant collected in Poland was composed mainly of hexanoic acid (> 20%), linalool (> 8%), anethole (> 5%), and carvone (> 3%) [48] (▶ **Table 2**).

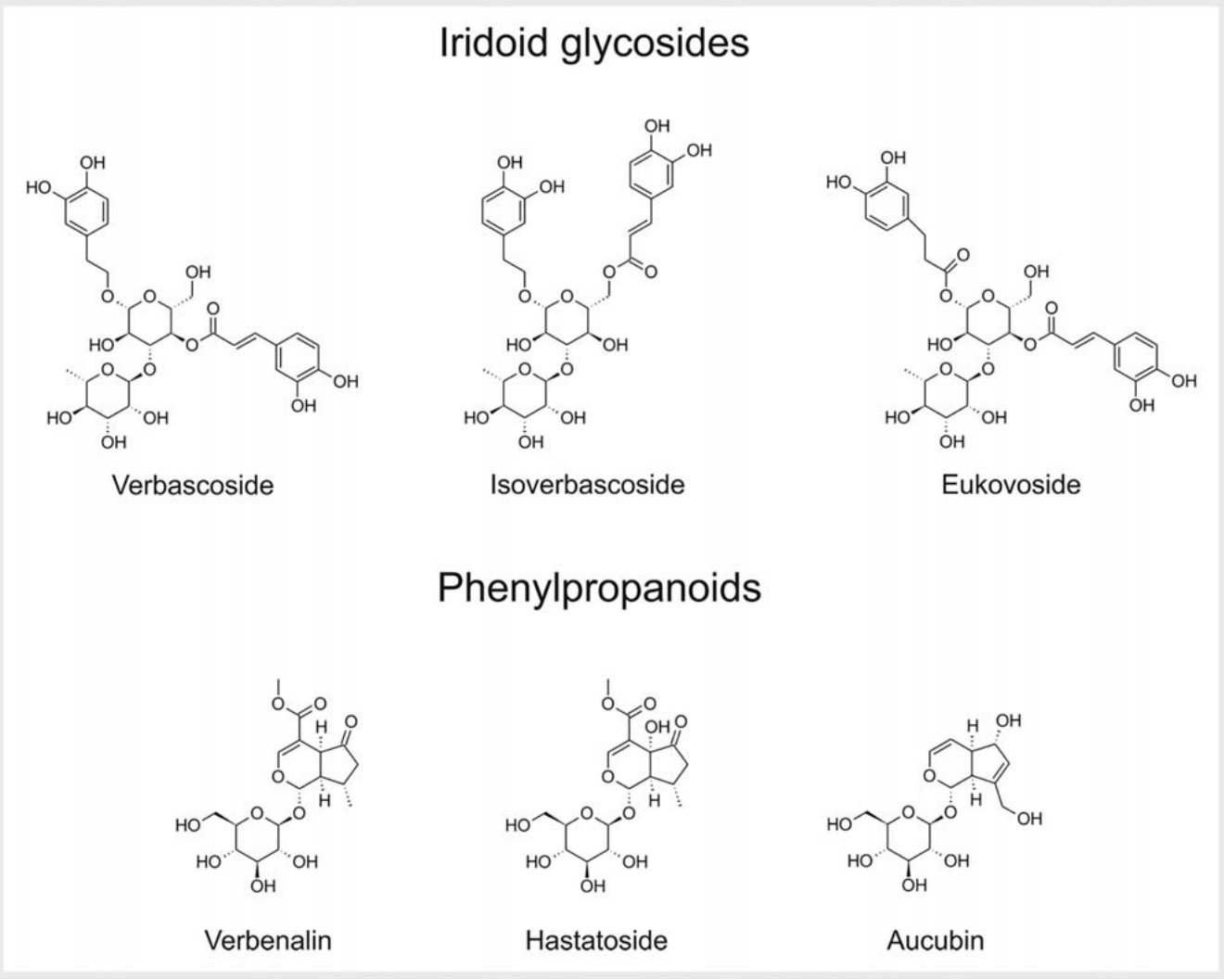
Therapeutic importance

V. officinalis herb extracts have long been used in traditional medicine, e.g., in European and North American, as well as traditional Chinese medicine.

Possible therapeutic applications based on centuries of use of this raw material are confirmed by modern scientific research on the chemical composition and action of active compounds [38, 49].

The *V. officinalis* herb is used as an antimicrobial, secretolytic, and expectorant raw material. *V. officinalis* is used in the treatment of upper respiratory tract diseases, mainly, in inflammation of the throat and sinuses, and in cases of cold, fever, tightness of the chest, bronchitis, asthma, pertussis, and sinusitis [50, 51]. Extracts from *V. officinalis* leaves are also known to help in the treatment of urinary tract disorders, such as urinary stones and urinary tract infections, and also have a diuretic effect. In women, the raw material is used to treat menstrual disorders, and in nursing mothers, to stimulate lactation [6, 29, 52]. *V. officinalis* has been successfully applied in disorders of the nervous system such as depression, insomnia, stress, anxiety, chronic fatigue syndrome, nervous exhaustion, sexual neurosis, and headache [29]. *V. officinalis* is also used in the treatment of digestive tract disorders, such as abdominal colic, jaundice, gallbladder inflammation, diarrhea, dysentery, stomachache, and intestinal worms [53–57]. Extracts from the *V. officinalis* herb are used to fight fever accompanying colds; they also have supportive properties in the treatment of malaria and rheumatism [29, 31, 58, 59].

In skin diseases, the *V. officinalis* herb is used as a softening, anti-inflammatory, and antibacterial agent, among others, in the treatment of difficult healing wounds and in gingivitis [6, 29, 49,



▶ **Fig. 1** Chemical structures of main *V. officinalis* metabolites.

60,61]. Moreover, *V. officinalis* extracts can be used topically in cases of wounds, bites, oral and throat inflammation, and muscle spasms [62].

Biological Activity of Extracts and Components Responsible, as Confirmed by Scientific Research

Antioxidant effect

Numerous scientific studies have confirmed the antioxidant effect of *V. officinalis* herb extracts, which is important in the prevention of cancer and heart disease [38].

Research on the antioxidant activity of a 50% ethanolic extract and an aqueous extract from the herb conducted at the Faculty of Pharmacy of the University of Navarra in Spain confirmed their beneficial effects in removing free radicals. The DPPH (2,2-diphenyl-1-picrylhydrazyl) tests showed high antiradical activity of both extracts tested: IC_{50} for the ethanolic extract was 21.04 ± 1.61 µg/mL, and for the aqueous one, 33.8 ± 0.43 µg/mL. The solutions also had an inhibitory effect on xanthan oxidase, an enzyme that induces the formation of active oxygen species (IC_{50} of 12.77 ± 1.65 µg/mL and 18.05 ± 3.80 µg/mL, respectively). Both extracts were fractionated using column chromatography, and the concentrations of active compounds were determined in the fractions obtained. The strongest antioxidant activity was shown by the fraction containing mainly verbascoside (which was most likely responsible for the properties studied) and small amounts of luteolin 7-glucoside, 1,5- and 4,5-dicaffeoylquinic acid, and isoverbascoside [38].

Antibacterial effect

In 2012, at Forman Christian College in Pakistan, research was conducted on the antibacterial activity of ethanolic extracts from the stems, leaves, and roots of *V. officinalis* using 13 bacterial strains that cause serious infections (including four methicillin-re-

▶ **Table 2** Main constituents of *V. officinalis* essential oil.

Compounds	% Essential oil	References
Monoterpenoids		
β-citral	14.8–45.5	[30, 46, 47, 70]
isobornyl formate	41.4–45.4	[30, 42, 44, 70]
citral (geranial)	3.3–44.5	[42, 44, 46]
limonene	2.3–17.7	[30, 42, 44, 46, 47, 70]
carvone	14.2	[47]
1.8-cineole	0.4–7.5	[30, 42, 44, 46, 70]
hepten-3-one	0.2–1.5	[30, 42, 44]
α-terpineol	0.2–1.4	[30, 42, 44, 46, 47, 70]
anethole	0.2–1.6	[30, 42, 44, 46]
β-pinene	1.5	[30, 42, 46, 47, 70]
thymol	1.5	[46]
methyl heptenone	1.3	[47]
carvacrol	1.2	[46]
trans-carveol	1.2	[46]
isopiperitone	1.0	[46]
α-pinene	0.2–0.8	[30, 42, 44, 46, 47, 70]
piperitone	0.8	[46]
cis-carveol	0.7	[46]
terpinen-4-ol	0.2–0.7	[30, 42, 44, 46, 70]
β-phellandrene	0.6–0.7	[30, 42, 44, 70]
geraniol	0.5	[46]
β-terpineol	0.5	[47]
sabinene	0.2–0.5	[30, 42, 44, 46, 70]
cinerone	0.4	[47]
p-cymene	0.4	[46]
nerol	0.4	[46]
linalol	0.1–0.4	[30, 42, 44, 47, 70]
(*E*)-*β*-ocimene	0.3	[30, 42, 44]
borneol	0.1–0.2	[30, 42, 44, 46]
iso-pinocamphone	0.2	[30, 42, 44]
trans-ocimene	0.2	[70]
o-cymene	0.1	[30, 42, 44, 70]
γ-terpinene	0.1	[30, 42, 44, 46, 70]
Sesquiterpenoids		
caryophyllene oxide	7.3–12.4	[46, 47]
spathulenol	1.2–10.8	[46, 47]
α-curcumane	6.8	[47]
β-caryophyllene	0.1–1.9	[30, 42, 44, 46, 47, 70]
trans-nerolidol	1.5	[47]
bicyclosesquiphellandrene	1.3	[47]
δ-cadinene	0.7–1.1	[46, 47]
β-bourbonene	0.6–1.1	[46, 47]
allo-aromadendrene	0.1–0.6	[30, 42, 44, 47, 70]
α-cubenene	0.6	[47]
γ-cadinene	0.5	[47]

continued

▶ **Table 2** *Continued*

Compounds	% Essential oil	References
germacene D	0.5	[47]
α-muurolene	0.5	[47]
bicyclogermacrene	0.1–0.5	[30,42,44,70]
cis-muurola-4(14).5-diene	0.2–0.5	[30,42,44,70]
isocaryophylene oxide	0.4	[46]
β-cedrene	0.4	[30,42,44,70]
α-copaene	0.2	[30,42,44,70]
β-elemene	0.2	[42,44,70]
β-cubenene	0.2	[46]
α-humulene	0.2	[30,42,44,70]
α-7-epi-selinene	0.1–0.2	[30,42,44]
isoledene	0.1	[30,42,44]

sistant *Staphylococcus aureus* strains and four multidrug-resistant *Salmonella typhi* strains). All the tested extracts showed a clear antibacterial effect on the strains used. The minimum inhibitory concentration (MIC) ranged from 0.02 to 0.15 mg/mL. Extracts from the stems showed, in general, higher antimicrobial activity than the leaf and root extracts. The *Acinetobacter baumannii* ATCC 29213 strain proved to be the most sensitive (lowest inhibitory concentration); its MIC for all leaf, stem, and root extracts was 0.02 mg/mL. Methicillin-resistant *S. aureus* strains and multidrug-resistant *S. typhi* strains were also sensitive to the tested extracts, with an MIC from 0.2 to 0.8 mg/mL. *Escherichia coli* ATCC 25922 bacteria proved to be the least sensitive to the action of the extracts, with an MIC of 0.15 mg/mL for all [39].

The antibacterial activity of *V. officinalis* herb extracts has also been tested at the Medical University of Dalian (China). A dry extract of *V. officinalis* was dissolved in 96, 60, and 30% ethanol in quantities of 25, 50, and 100 mg/mL. Antibacterial properties were compared by determining the zone in which bacterial growth was completely inhibited. The tests were carried out on strains of *E. coli, Proteus vulgaris*, and *Bacillus subtilis*. The potency was affected by the solvent concentration – the more diluted alcohol that was used, the more potent the extract was. Solutions containing 100 mg/mL extract in 30% ethanol showed the strongest antibacterial activity. The zone of complete growth inhibition was 21.12, 16.33, and 15.32 mm, respectively [63].

Antifungal activity

In 2008, researchers from the University of Navarra (Spain) published the results of their experiments on the antifungal properties of extracts from the leaves of *V. officinalis*. Extracts prepared using chloroform, methanol, and ethyl acetate were tested. In addition, fractions containing the main groups of compounds characteristic of verbena and compounds isolated from the extracts (luteolin 7-glucoside, luteolin 7-diglucuronide, apigenin 7-diglucuronide, chlorogenic acid, and verbascoside) were investigated. Antifungal activity was tested on the following fungi: *Alternaria alternata, Botrytis cinerea, Penicillium expansum*, and *Rhizopus stolonifer*, determined by measuring the size of the colonies, and was expressed as % inhibition (according to the formula: [(control – test sample)/control] × 100). None of the tested extracts showed significant activity against *A. alternata* and *B. cinerea* (% inhibition < 10%). However, a 50% methanolic extract showed antifungal activity against *P. expansum* and *R. stolonifer* (growth inhibition at a level of 32.55 and 28.98%, respectively). The percentage of inhibition was even higher for the fraction containing caffeic acid derivatives. For *P. expansum*, it was 87.45%, and for *R. stolonifera*, it was 79.11%. However, it was weaker than the action of the fraction containing chlorogenic acid and verbascoside. These studies allow for not only the determination of the antifungal activity of leaf extracts but also enable a more thorough understanding of the relationship between the effect and the concentration of individual active compounds in the plant [64].

Anti-inflammatory effect

In 2016, the research team from the University of Al-Mansura (Egypt) published the results of clinical studies on the use of a decoction from the *V. officinalis* herb in the treatment of chronic gingivitis. In a double-blind trial carried out at five dental centers on 260 patients with chronic gingivitis, the effect of a daily mouthwash with a *V. officinalis* decoction was analyzed. The patients used 10 mL of solution twice a day, 30 min after brushing their teeth. The condition of patients' gums and the amount of dental plaque were monitored on the 14th and 28th day of using the decoction and then compared with the condition on the day the experiment began. Observations showed a statistically significant difference in the condition of the oral cavity between the test and control groups. The beneficial effect of the decoction on the gums was confirmed. An anti-inflammatory effect of the decoction as well as its antibacterial effect (reduction in plaque) were demonstrated. It is worth emphasizing that the patients did not report any adverse effects [60].

The anti-inflammatory effect of *V. officinalis* herb extract has also been confirmed thanks to the collaboration of scientists from Italy (Università di Bologna, Università di Parma) and Austria

(Leopold-Franzens-Universität in Innsbruck). The research was aimed at testing and comparing the potency of various types of herb extracts (methanolic, derived by supercritical carbon dioxide, and flavonoid rich). The experiments were carried out on rats. Inflammation of the paw was caused by an injection of carrageenan in the animal's paw 30 min after oral administration of the herb extracts (in doses of 100 and 200 mg/kg). For comparison, indomethacin was used at a dose of 100 µg/kg. All of the extracts tested showed significant anti-inflammatory effects. The methanolic extract and the flavonoid-rich extract reduced inflammation by 38 and 34%, respectively. The extract obtained using carbon dioxide in a supercritical state exhibited the highest activity, reducing inflammation by 61%. The differences in the action of individual extracts result from their different chemical composition. The methanolic extract contained high amounts of verbascoside, verbenalin, hastastoside, luteolin diglucuronide, and apigenin diglucuronide, while the flavonoid-rich extract contained mainly two flavonoids, luteolin diglucuronide and apigenin diglucuronide, and verbascoside. The extract prepared using CO_2 had higher concentrations of lipophilic constituents such as linolic and linolenic acids [62].

Anti-inflammatory properties were also investigated by researchers at the University of Pamplona (Spain). They studied the effects of an ointment with the addition of 1 to 3% of dry extract from the leaves of *V. officinalis*. It was prepared by macerating 50 g of dry herb with 700 mL of 50% methanol. The chlorophyll-free solution was evaporated *in vacuo* to obtain a dry extract. Anti-inflammatory properties were determined by measuring paw volume using a plethysmometer immediately after carrageenan injection and after 1, 2, 3, and 4 h. [65]. Both routes of administration of *V. officinalis* extracts, oral [62] and topical [65], showed an anti-inflammatory effect in the rat model.

Local analgesic effect

Analgesic properties were examined *in vivo* by analyzing the number of paw licking episodes by rats after rubbing in ointments containing 1–3% verbena extract, 0.3 g ointment base, or an ointment containing 30% of methyl salicylate, and injecting 50 µL of a 2.5% formalin solution. The rats were observed for 60 min after administration of formaldehyde. The analgesic effect was also pronounced. The number of licking episodes was significantly reduced compared to the control [65].

Anticonvulsant, anxiolytic, and sedative effects

The use of verbena in traditional medicine to treat nervous system disorders had prompted researchers from Riphah International University, Islamabad (Pakistan), to study the anticonvulsant, anxiolytic, and sedative effects of *V. officinalis*. An extract from the *V. officinalis* herb was tested on mice. It was prepared by macerating the dried herb with 70% methanol and evaporating the solvent under reduced pressure. The extract was dissolved in an isotonic sodium chloride solution. Various methods were used to test the effect of the verbena extract on test animals: convulsions induced with pentetrazole (PTZ), elevated plus maze test, light-dark box test, open-field test, thiopental-induced sleep test, and acute toxicity test. The experiments confirmed the anticonvulsant, anxiolytic, and sedative effects of *V. officinalis*. They also demonstrated a dose-dependent delayed onset time of pentazocine-induced myoclonic and tonic-clonic seizures. The duration of tonic-clonic seizures was reduced. In the control group, which was given a solution of sodium chloride alone, the mortality rate was 100%. By comparison, in the group which was given a dose of 100 mg/kg of the *V. officinalis* extract, the mortality rate fell to 75%, and at doses from 300 to 500 mg/kg body weight (BW), it dropped to 0% (same as when 1 mg/kg diazepam was administered). In the open-field test, application of the extract at 50, 100, and 300 mg/kg BW significantly reduced the animals' ambulations from 121.75 (control group) to 109.5, 106.5, and 74.25, respectively, and rearing frequencies from 52.25 (control group) to 42.5, 39.5, and 24.25, respectively. The tested doses of the extract increased the number of central squares crossings from 6.25 (control group) to 11.25, 15.0, and 10.75 (for 50, 100, and 300 mg/kg BW of extract, respectively). The extract was also shown to affect the onset time and duration of sleep. In the control group, sleep occurred after 3.53 min, on average, and lasted 8.25 min. The applied doses of the *V. officinalis* extract, 50, 100, and 300 mg/kg, accelerated the onset time (to 2.61, 2.39, and 1.6 min, respectively) and extended the duration of sleep (up to 14.50, 66.35, and 523.65 min, respectively). The dose of 300 mg/kg BW produced effects comparable to the effects of 3 mg/kg BW diazepam (onset of sleep after 1.32 min, average duration 571.8 min). The acute toxicity experiments with doses of 3 g/kg BW and 5 g/kg BW did not cause mortality in the test rats, only a decrease in locomotor activity was observed, which proves the sedative effect of the tested solutions. The presented studies allow for approximation of the correlation between the activity of the plant extract and the activity of its individual active ingredients [29].

Research conducted at Tehran University of Medical Sciences (Iran) published in 2017 also confirmed the anticonvulsant effect of the ethanolic extracts from the *V. officinalis* herb. It was carried out on mice in which convulsions were induced by two methods: using electric current or using PTZ. The reference drug was diazepam. The dry extract was obtained by a 72-h extraction of dry *V. officinalis* herb under a reflux condenser with 95% ethanol. With the first method, it was demonstrated that the dry extract at doses of 100 and 200 mg/kg BW showed an anticonvulsant effect, and a dose of 400 mg/kg BW was as effective as 1 mg/kg BW diazepam. In the PTZ method, only a dose of 400 mg/kg BW delayed the onset of convulsions and shortened their duration. The acute toxicity test showed that the extract at 2 g/kg BW did not cause death, nor any visible adverse effects. Those experiments also shed light on the mechanisms of action of the compounds contained in extracts from the herb. The use of flumazenil and naloxone reversed the effect of the tested solutions, which may indicate that the substances contained in the *V. officinalis* herb act through both benzodiazepine and opioid receptors [66].

Sleep promoting activity

The traditional use of the infusion of *V. officinalis* in the treatment of insomnia has also been verified by scientific research. In Japan, two collaborating units, Central Research Institute, Mizkan Group Co., Ltd., Handa, Aichi and Department of Molecular Behavioral Biology, Osaka Bioscience Institute, studied the effects of the

components of an aqueous extract from *V. officinalis* on the quality of sleep *in vivo* on rat model. In the study, not only the herb (aerial parts of the plant in the flowering stage) was used to make the extract, but also the whole plant, including the roots. Hastatoside, verbenalin, and verbascoside were isolated from the obtained extract. Both the activity of the extract and the compounds themselves were investigated by monitoring the duration of the non-rapid eye movement (NREM) sleep phase, the so-called deep sleep, analyzing the activity of delta waves using EEG-EMG. The measured brain wave ranges were 0.5–30 Hz for EEG, and 20–200 Hz for EMG. The extract from *V. officinalis* and the solutions of extracted compounds were administered to rats directly into the stomach for 30 min (between 8:00 p.m. and 8:30 p.m.) at a rate of 200 µL/min. The doses given were 9 g/kg BW for the verbena extract; 0.32, 0.48, and 0.64 mmol/kg for hastatoside; 0.32, 0.64, and 1.28 mmol/kg for verbenalin; and 0.64 and 1.28 mmol/kg BW for verbascoside. EEG-EMG measurements were carried out 24 h after administration of the extracts.

The *V. officinalis* extract in the applied dose increased NREM sleep time, within 12 successive h of sleep, by 25.7% compared to the control group. The number of episodes of the NREM phase increased from about 138 to 149, and the duration of a single episode increased from 1.2 to 1.4 min.

Hastatoside did not significantly affect the duration of the NREM phase at a dose of 0.32 mmol/kg BW, whereas a dose of 0.48 mmol/kg BW increased this time by 25%. The strongest effect was demonstrated when hastatoside was administered at a dose of 0.64 mmol/kg (259 mg/kg BW). This dose initially reduced the overall duration of the NREM phase by 77% between 10:00 p.m. and 11:00 p.m., and then increased it by 20–320% between 2:00 a.m. and 8:00 a.m. The mean extension of the NREM phase between 3:00 a.m. and 8:00 a.m. was 81%.

Verbenalin had no effect on the duration of the NREM phase at the lowest dose used. After administering 0.64 mmol/kg BW, a 27% increase in NREM sleep was achieved. The strongest effect was observed when 1.28 mmol/kg BW (497 mg/kg BW) was used. Initially, the total duration of the NREM phase was shortened between 9:00 p.m. and 11:00 p.m. by 52%, and then there was a significant extension between 2:00 a.m. and 6:00 a.m. by 40–70%. The average duration of the NREM phase between 4:00 a.m. and 8:00 a.m. was 42%. On the next day of the experiment, the duration of the NREM phase returned to normal.

Verbascoside did not show any significant effects even at the highest dose used. Hastatoside and verbenalin acted similarly to benzodiazepines, extending the duration of the NREM phase; however, unlike these drugs, they did not adversely affect the activity of delta waves in the brains of the rats studied. Adverse effects included agitation and diarrhea for up to 2 h after administering the solutions. It was shown that hastatoside and verbenalin were compounds that could potentially be used in the treatment of sleep disorders, but further research is needed [67].

Neuroprotective effect

Experiments conducted at the University of Hong Kong (China) have demonstrated the neuroprotective effect of *V. officinalis* extracts. The studies were conducted *in vitro* on neurons of the cerebral cortex of rats. The *in vitro* cultured neurons were treated with an aqueous extract of the plant followed by various toxins such as β-amyloid$_{25–35}$ (25 µM for 24 h), tunicamycin (1 µg/mL for 16 h), dithiothreitol (0.5 and 1.0 mM for 16 h), hydrogen peroxide (50 µM for 16 h), and UV radiation (32 kJ/cm^2 for 2 h). The cells were then washed with a new medium. Cell lysates were subjected to a caspase activity assay. Cytotoxic activity was determined based on the level of lactate dehydrogenase (LD) in the harvested media. Measurements of the LD level showed that a verbena herb extract at a concentration of 100 µg/mL reduced the mortality of nerve cells exposed to β-amyloid (Aβ) by 9.1% and dithiothreitol by 9.8% (0.5 mM DTT) and 29.6% (1.0 mM DTT). However, the extract did not protect against the effects of tunicamycin, H_2O_2, and UV radiation, which may indicate a weaker effect of the extract against the agents directly damaging DNA.

Colorimetric caspase-3-like and caspase-2-like activity assays were used to measure the effect of a verbena extract in doses of 25 to 150 µg/mL. Aβ induced DEVD and VDVAD cleavage to the toxic effect of Aβ. The *V. officinalis* extract already significantly reduced Aβ-triggered activity at a dose of 75 mg/mL by 1.4 times compared to control conditions. This activity was dose dependent.

The results obtained prove that extracts from *V. officinalis* can potentially be used in the prevention of neurodegenerative diseases, with particular emphasis on Alzheimer's disease [40].

Antidepressant effect

The antidepressant effect of 50% water-methanol extracts from the leaves of *V. officinalis* has also been evaluated. Studies conducted with the cooperation of two Indian units, Hygia Institute of Pharmaceutical Education and Research and Integral University Lucknow, tested the effects of *V. officinalis* extracts on the behavior of mice. The rodents were divided into four groups: a control, which was given a saline solution, a reference group, which was given imipramine (15 mg/kg BW), and two study groups, which were given the extract at 100 and 200 mg/kg BW. The preparations were administered to mice for 7 days. Depressive behaviors were assessed in three types of tests: tail suspension test (TST), forced swim test (FST), and spontaneous locomotor activity test (SLMA). In the TST test on mice, a reduction in the duration of immobility was observed by 53 s (extract at 100 mg/kg BW) and by 73 s (at 200 mg/kg BW) compared to the control. In the FST test, the reduction in the duration of immobility was 36 and 46 s, respectively. In the SMLA test, however, the results were comparable among all study groups. The researchers also examined extract toxicity by giving the rodents the extract at 2000 mg/kg BW; this dose did not cause any of the test animals to die.

The TST and FST tests showed a dose-dependent, significant reduction in passive posture compared to the control, although lower compared to the reference group. *V. officinalis* extracts show antidepressant activity in animal models; however, the mechanism of the antidepressant effect of *V. officinalis* extracts is unknown and requires further research [68].

Cardiovascular effects

The *V. officinalis* herb contains large amounts of potassium, phosphorus, calcium, and magnesium. At the same time, the sodium content is relatively low, especially when compared to potassium.

The low Na/K ratio is beneficial when using preparations from *V. officinalis* in people with cardiovascular disorders [38].

Antiproliferative and anticancer effect

At the Faculty of Pharmaceutical Sciences, University of Salerno (Italy), research has been conducted on the cytotoxic effects of *V. officinalis* essential oil and citral (the main component of the oil), evaluating the apoptotic effect in chronic lymphocytic leukemia. The studies were carried out *in vitro* on cells from patients with untreated lymphocytic leukemia. The number of apoptotic cells that were labelled with the CD19-APC-Cy7 antibody was determined by flow cytometry. Analyses were carried out 4, 8, and 24 h after adding the oil/citral. The highest percentage of CD19-positive cells was found in samples incubated for 8 h. In the control sample, it was about 7%, after adding the oil, it was 68.2%, and after adding citral, it was 65.9%. The results showed high cytotoxic activity of both the oil and citral against tumor cells. However, further research is needed to thoroughly explain the mechanism of action [42].

The cytotoxic effect of the *V. officinalis* herb on liver cancer cells has been confirmed by scientific studies conducted at the Medical University of Henan (China). The experiment was conducted on 50 mice injected subcutaneously in a paw with tumor cells of H22 mice ascite hepatoma cell lines. Twenty-four hours after the procedure, the rodents were divided into five groups: control, three groups that were given a dry extract of *V. officinalis* at 10, 20, and 40 g/kg BW, and a group that was given cisplatin at a dose of 1 mg/kg BW. Each mouse was weighed and observed in terms of behavior, level of activity, and amount of food intake. To assess antitumor activity, the tumor inhibition rate (according to tumor and body weight) was measured. Measurements of the degree of swelling of the paw after immunization with sheep's blood and of the level of hemolysin in mice serum allowed for the assessment of the impact on the rodents' immune system. Effects on body weight and spleen index (ratio of spleen weight to body weight) were also assessed. Body weight increased in all experimental groups. All the increases were smaller compared with the model group, and the amounts of increase were generally negatively correlated with the dosage. Compared to the model group, the spleen indices for the groups treated with 20 and 40 g/kg BW were both increased, but the differences were not significant, which suggested that the tested extract had no significant effect on the spleen index. A relationship was demonstrated between the reduction in tumor mass and the amount of extract administered. The percentage of the tumor inhibition rate was 15.71, 28.20, and 38.78 for low, medium, and high dosages of verbena extract, respectively. For cisplatin at 1 mg/kg BW, the inhibition rate was 42.94%. Analyses of footpad swelling and hemolysin levels showed that the extract at the doses used did not cause significant changes in the functioning of the immune system [58].

Researchers from the University of Navarra in Pamplona (Spain) have investigated the antiproliferative effects of *V. officinalis* herb extract in the search for new drugs that could be used to treat colorectal cancer. They tested an aqueous extract, a purified extract, and the phenylpropanoid glycosides isolated. Antitumor activity was assessed after 24, 48, and 72 h by comparing the 50% inhibitory concentration (IC_{50}) with the control and a reference sample in which vinblastine sulfate was used. The antitumor effect was confirmed on two cell lines: DHD/K12/PROb (rat) and HCT-116 (human). From the structural viewpoint, several studies have indicated that the effect of ester-forming phenylpropanoic (e.g., caffeic acid and ferulic acid) and the o-dihydroxyl aromatic system within the molecule are necessary for antiproliferative activity. However, it seems that the activity of those compounds is not associated with the structure of the sugar moiety. Scientists have isolated 12 compounds that meet these requirements: verbascoside, isoverbascoside, campenoside II, isocampenoside II, 6′′-acetyl-O-verbascoside, 4′′′-acetyl-O-verbascoside, 4′′′O-acetyl-O-isoverbascoside, 2′′′,4′′′-diacetyl-O-verbascoside, 3′′′,4′′′-diacetyl-O-isoverbascoside, betanyoside A, 4′′′,6′′-diacetyl-O-betanyoside A, and 3′′′,6′,'-diacetyl-O-betanyoside A. The strongest action among the isolated compounds, comparable with vinblastine sulfate, was shown by 2′′′,4′′′-diacetyl-O-verbascoside, 3′′′,4′′′-diacetyl-O-isoverbascoside, 4′′′,6′′-diacetyl-O-betanyoside A, and 3′′′,6′,'- diacetyl-O-betanyoside A. A weaker, but also very strong, activity was shown by 6′′-acetyl-O-verbascoside, 4′′′-acetyl-O-verbascoside, and 4′′′O-acetyl-O-isoverbascoside. Research has shown that diacetylphenylpropanoids can be valuable chemopreventive compounds [69].

V. officinalis essential oil can be a source of natural compounds whose structure can lead to the development of new therapeutic agents. Research has been conducted on verbena essential oil as an apoptotic inductor in leukocytes of healthy and chronic myeloid leukemic patients. The study evaluated the proapoptotic activity of essential oil and of its main compound, citral (45.5% of analyzed oil), on neutrophil granulocytes collected from healthy patients and chronic myeloid leukemic patients. The control samples, after three different incubation times (6, 12, 24 h), did not show apoptosis, but only necrotic cellular elements. Cytometric analysis of cells treated with *V. officinalis* essential oil showed apoptotic elements (vs. controls), but there was no statistical difference. The vervain essential oil induced significant apoptosis (*vs* control) in granulocytes from both groups of donors. The percentage of apoptotic cells was greater in the blood from chronic myeloid leukemic patients (76% after 6 h) than in healthy blood (56% after 6 h). Non-treated granulocytes were necrotic in both groups. The exact molecular mechanism of the analyzed essential oil on cell cycle and apoptosis is still unclear. The apoptotic effect that is induced by *V. officinalis* essential oil could be related to the activation of caspase-3. The research was conducted at the Faculty of Pharmaceutical Sciences, University of Salerno [70].

Acceleration of wound healing

Research conducted with the cooperation of scientific units from Italy (Universities of Bologna and Parma) and Austria (University of Innsbruck) concerned the effect of *V. officinalis* on wound healing in rats. Gels were tested with the addition of one of three types of *V. officinalis* extracts: a methanolic extract (VoME), a flavonoid-rich extract (VoFE), and an extract prepared by carbon dioxide extraction ($VoCO_2$). The rodents under anesthesia had their skin cut with a scalpel over a length of 1.5 cm to the depth of adipose tissue. The wound site was smeared with 0.5 mL of gel containing 20 mg of extract and covered with an occlusive dressing. The condition of the wound was assessed after 24 and 48 h. After 24 h,

the length, color, and general aspect of the wound area were observed. Cicatrization was evaluated after 48 h by observing the morphology of the tissues (vascular caliber and presence of leukocytes). After 24 h, the extent of cicatrization was higher in the rats treated with the $VoCO_2$ extract. The histological results after 48 h showed the most significant wound reduction in the group treated with VoFE. Also, the VoME extract produced a marked reduction in damage, with the presence of new fibrous tissue, in comparison with the control group. There was clear evidence that topically applied extracts from the *V. officinalis* herb significantly increased wound healing [62].

Gastroprotective properties

In parallel with the research on accelerating wound healing, the cooperating units conducted research on the gastroprotective effect of VoFE and $VoCO_2$ extracts in doses of 100 and 200 mg/kg BW. As a reference test, misoprostol was used in a dose of 100 µg/kg BW. Thirty minutes after administration of the extracts and misoprostol, 96% ethanol was used as the damaging agent. After the next 2 h, the animals were sacrificed, and their stomachs were examined to establish the lesion index and assess gastric secretion (volume and pH). All the extracts showed gastroprotective effects, of which the most prominent was the effect of $VoCO_2$. The ulcer scores were significantly reduced after administration of the extracts at 100 or 200 mg/kg (1.83 and 1.33 for VoME, 1.38 and 1.00 for $VoCO_2$, and 1.62 and 1.12 for VoFE, respectively) in comparison with the control group (3.50). However, the reduction was lower than in the misoprostol group. No change in the volume of gastric juice and the value of gastric pH was observed in the rats pretreated with the verbena extracts [62].

Prevention against the spread of dangerous insect-borne diseases

Mosquitoes are a vector of transmission of serious infectious diseases such as malaria, dengue fever, and filariasis. To prevent the spread of an epidemic, it is important to reduce insect populations. To this end, toxic pesticides, which are not neutral to human and animal health, are used. In the search for new, safer insecticides, scientists from the University of Bab-Ezzouar (Algeria) have proven that the oil from the leaves of *V. officinalis* is lethal to water-borne mosquito larvae (*Culex pipiens*). Concentrations from 1 to 500 mg/L of the oil dissolved in water were tested. Mortality percentage was calculated after 3, 6, 12, and 24 h of exposure. After 24 h, the concentrations of 100 and 500 mg/L produced a 43 and 100% larvicidal effect, respectively. The results of these tests can be important in the design and synthesis of new, effective insecticides [47].

A summary of the most important results of the above-described studies together with an indication of the mechanisms of action and responsible metabolites are presented in ▶ **Table 3**.

Safety of Use

Verbena is recognized as "likely safe" for most people when taken orally in food amounts and "possibly safe" when taken orally in small amounts as part of a combination product containing gentian root, elderflower, sorrel, and cowslip flower. There is not enough information to know if verbena is safe when used in medicinal amounts other than as part of the combination product. The combination product can cause digestive system upset and occasionally allergic skin rash. Vervain should not be drunk with meals by vegetarians and vegans. Mills and Bone [71] state that phenylpropanoids interfere with non-haem iron absorption and include vervain in the list of herbs where this may be of concern. A study in Morocco using an *in vitro* model of digestion found that non-haem iron absorption was decreased by vervain, although vervain had one-third of the level of polyphenols in tea [72]. This was an *in vitro* study designed to estimate the effect of drinking tea, vervain, or mint teas on women weaning their babies, and so would require further confirmation [5]. There is not enough reliable information about the safety of taking *V. officinalis* by pregnant or breast-feeding women. Several sources suggest that this herb should not be used in pregnancy [2, 73], and it has been investigated in China as a possible herb to terminate early pregnancy [74].

The toxicity of vervain extracts in *in vivo* animal models was tested under some scientific researches. In the study conducted by Jawaid et al. on the antidepressant effect of *V. officinalis* leaf extracts, researchers examined extract toxicity. The toxic dose for mice was 2 g/kg BW. This dose of vervain extract didn't cause any deaths [68]. The study on anticonvulsant, anxiolytic, and sedative effects of *V. officinalis* performed by Khan et al. also showed the toxicity with even higher doses of extract (3 and 5 g/kg BW). Also, these doses didn't cause mortality of the tested rats, as a decrease in locomotor activity was observed [29].

Plant *In Vitro* Culture Studies

The aim of research in the field of plant biotechnology is to make use of the biochemical and morphotic potential of plant cells. The basic research objects are *in vitro* cultures conducted on special media, of protoplasts, cells, tissues, organs, and whole plants or their fragments. The main research areas are endogenous accumulation of secondary metabolites, biotransformation processes, plant micropropagation, and genetic engineering [75, 76].

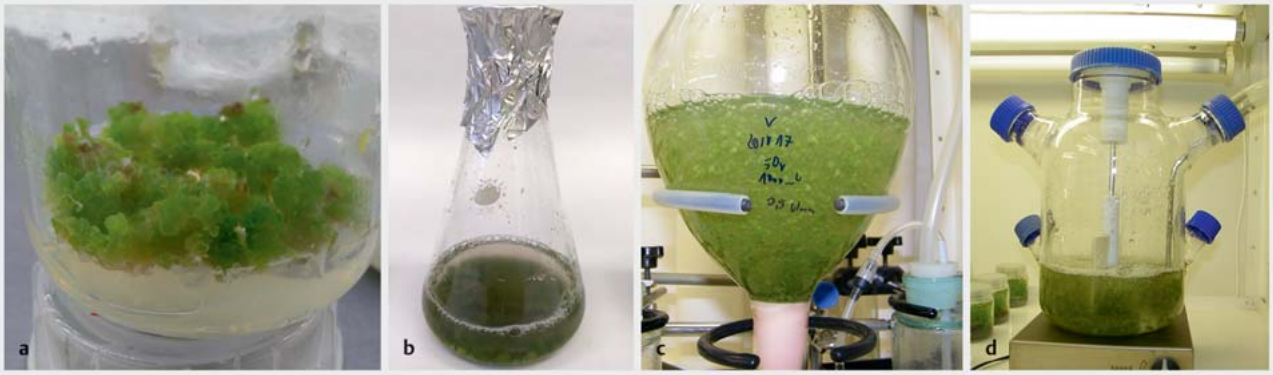
Scientific reports on biotechnological research concerned with *V. officinalis* are scarce and mainly concern the micro-reproduction of this species. An example is the research by a team from Abant Izzet Baysal University in Turkey, published in 2010, which describes an effective system of plant regeneration from the internodes, leaf blades, and petioles of *V. officinalis*. Explants were obtained from five-week-old seedlings cultured on MSMO (Murashige and Skoog Minimal Organics) medium [77]. Fragments of seedlings were placed on the medium with the addition of various types and concentrations of plant growth and development regulators (benzyloadenine – BA, thidiazuron – TDZ, indolyl-3-acetic acid – IAA). After a 3-week growth cycle, regenerated plants were transferred for shoot elongation onto the same media for the next 2 weeks. The next stage was a 3-week culture on the MSMO medium supplemented with various concentrations of IAA, indolyl-3-butyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D), or naphthyl-1-acetic acid (NAA) in order to induce root formation. Then, the plants were transplanted into sterilized soil. After 2 weeks, the best regeneration results were obtained with

▶ **Table 3** Mechanisms of action and the compounds responsible.

Therapeutic profile	Mechanism of action	Responsible compounds	References
Antioxidant effect	▪ high radical scavenging activity ▪ high reducing power ▪ inhibitory effect on xanthan oxidase, an enzyme that induces the formation of active forms of oxygen	flavonoids and caffeoyl derivatives	[38, 64]
Antibacterial effect	▪ inhibiting activity against gram-positive bacteria: *Streptococcus pyogenes, Streptococcus pneumoniae, Staphylococcus aureus*, methicillin-resistant *S. aureus, Bacillus subtilis* ▪ inhibiting activity against gram-negative bacteria: *Escherichia coli* ATCC 25922, MDR *Salmonella typhi, Acinetobacter baumannii* ATCC 29213, *Proteus vulgaris*	not recognized	[39, 89]
Antifungal activity	▪ inhibiting activity against *Penicillium expansum* and *Rhizopus stolonifer*	caffeoyl derivatives	[64]
Anti-inflammatory effect	▪ ameliorative effect for treating patients with chronic generalized gingivitis ▪ inhibition of the production of reactive radical species ▪ inhibition of edema development	iridoids, caffeoyl derivatives, flavonoid compounds	[60, 62, 65]
Analgesic activity	▪ analgesic effect possibly results from its central action	iridoids, caffeoyl derivatives, flavonoid compounds	[65]
Anticonvulsant effect	▪ anticonvulsant effect of possibly occurs through activation of the $GABA_A$ receptor ▪ anticonvulsant activity of *V. officinalis* may be due to increased GABA transmission caused by agonistic activation on BZDs and KOPr receptors	flavonoids, e.g., scutellarein and phenolic acid derivatives, e.g., verbascoside	[29]
Sleep-promoting activity	▪ significant increase in the amount of time spent in NREM sleep	hastatoside, verbenalin, verbascoside	[67]
Neuroprotective effect	▪ attenuates Aβ-activated PKR and JNK stress kinases ▪ defends oxidative stress for neurons ▪ inhibits Aβ-activated VDVAD and DEVE cleavage activity ▪ *V. officinalis* may inhibit the mechanism or execution of neuronal apoptosis	not recognized	[40]
Antidepressant effect	▪ increases serotonin, norepinephrine, and dopamine levels in the nerve terminals; an increase in all three neurotransmitters could be by inhibition of monoamine oxidase activity in the brain	not recognized	[68]
Anticancer effect	▪ apoptotic effect that is induced by essential oil could be related to activation of caspase-3	essential oil, phenylpropanoids	[42, 70]
Cicatrization	▪ neutrophil infiltration, as well as increase in the LPO level that characterizes the phlogistic process are reduced ▪ anti-inflammatory activity in the damaged tissue ▪ antibacterial activity	phenylpropanoids, flavonoids	[62]
Gastroprotective effect	▪ antioxidant properties related to antiulcer activity, since free radicals are developed in gastric mucosal lesions	flavonoids, polyphenols	[62]
Preventing diseases from spreading	▪ larvicidal activity of *V. officinalis* oil against mosquitoes	essential oil	[47]

explants derived from internodes. In the case of leaf explants, no regeneration of shoots was achieved. The highest number of explants producing new shoots was found on the medium supplemented with 13.32 µM BA in combination with 5.71 µM IAA. The addition of 4.92 µM IBA was the most favorable for root induction [78].

In recent years, our team has been conducting intensive research with *in vitro* cultures of *V. officinalis* initiated in 2014 (▶ **Fig. 2**). Cell metabolism in the conditions of *in vitro* cultures, as our research has shown, is geared towards the production of verbascoside and, in smaller amounts, isoverbascoside. Phenolic acids are also produced. The biotechnological work is focused on increasing the accumulation of bioactive compounds in the biomass of cultures by optimizing culture conditions [36, 79]. As a result of the research with callus agar cultures of *V. officinalis*, involving extensive optimization of culture medium composition (12 variants of the Murashige and Skoog (MS) medium [77], containing BA and IBA), conducted in the presence of light and in darkness, the optimal composition of the culture medium has been established, namely, the MS medium supplemented with 1 mg/L BA and 1 mg/L IBA [36]. This composition of plant growth and development regulators promoted high increases in biomass and the production of bioactive compounds, especially verbascoside (max. 2454.12 mg/100 g DW). The concentrations of phenolic acids were of a different order (max. 46.02 mg/100 g DW – free phenolic acids, 141.05 mg/100 g DW – bound phenolic acids) [36]. In the *in vitro* cultures, the concentrations of verbascoside, free phenolic acids, and bound phenolic acids, were, respectively, 3.28, 3.42, and 2.12 times higher than in the green parts of the parent plant.



▶ **Fig. 2** *V. officinalis in vitro* cultures: **a** callus cultures, **b** agitated cultures, **c** cultures in balloon bioreactor, **d** cultures in a stirred-tank bioreactor.

Testing of the lighting conditions under which *V. officinalis* callus cultures were grown was carried out in cooperation with a team from the Department of Ornamental Plants of the Agricultural University of Kraków, Poland. *In vitro* cultures were maintained on a solid MS medium enriched with 1 mg/L BA and 1 mg/L IBA under LED lights (red, blue, 70/30% red/blue) in darkness and under control white fluorescent lamps. The presence of verbascoside, isoverbascoside, and, additionally, seven phenolic acids (protocatechuic, chlorogenic, vanillic, caffeic, ferulic, o-coumaric, and m-coumaric acids) was confirmed in all extracts. Blue and red/blue lights stimulated the accumulation of verbascoside (max. 6716 and 6023 mg/100 g DW after a 4-week growth cycle) and isoverbascoside (max. 333 and 379 mg/100 g DW also after 4 weeks). The maximum amounts of verbascoside and isoverbascoside were, respectively, 1.8- and 7.0-fold higher than under the control conditions. Phenolic acids accumulated in different amounts, and the maximum total amounts ranged from 33 to 65 mg/100 g DW. LED lights also stimulated their accumulation in comparison with darkness and the control. Additionally, the quantities of photosynthetic pigments (chlorophyll a, b, and carotenoids) were estimated in acetonic extracts using spectrophotometry. Red/blue light stimulated the biosynthesis of pigments (max. total content 287 µg/g FW after 4-week growth cycles) [79].

In the next step of our research with *V. officinalis in vitro* cultures, we conducted optimization of the conditions for cultivating agitated and bioreactor cultures, with a view to nominating them as an alternative source of obtaining phenylpropanoid glycosides. In the agitated cultures (MS with 1 mg/L BA and 1 mg/L IBA, 4-week growth cycle), the estimated amounts of verbascoside and isoverbascoside were equal to 6857.23 and 374.64 mg/100 g DW. Our team, in cooperation with the scientific workers from the Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Gdańsk (Poland), performed studies on *V. officinalis* agitated cultures propagated in semi-industrial bioreactor systems. Balloon (▶ **Fig. 2 c**) and agitated and aerated (▶ **Fig. 2 d**) bioreactors were used. Higher verbascoside and isoverbascoside amounts were found in extracts from the biomass of cultures grown in the agitated and aerated bioreactor (9.18 g/100 g DW and 2.95 g/100 g DW, respectively). The amounts obtained in the extracts from the biomass grown in the balloon bioreactor were lower, but also high (7.68 and 2.25 g/100 g DW, respectively) [unpublished].

As part of the research conducted by our team on *V. officinalis*, we have also tested the biological potential of biomass from *in vitro* cultures, as well as extracts from parent plant material. Antioxidant assays were performed using the CUPRAC (cupric ion reducing antioxidant capacity) and QUENCHER-CUPRAC (quick, easy, new, cheap and reproducible treatment) methods, and demonstrated a higher antioxidant potential of suspension culture extracts in comparison with extracts from the callus or the green parts of the parent plant [unpublished]. Research on the antibacterial activity of methanolic extracts from callus agar and suspension cultures of *V. officinalis* against four strains of gram-positive bacteria (*Staphylococcus epidermidis, S. aureus, Bacillus cereus, Listeria monocytogenes*) and eight strains of gram-negative bacteria (*Yersinia enterocolitica, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Shigella sonnei, Salmonella enteritidis, Enterobacter aerogenes, Escherichia coli*) has shown very strong antibacterial activity. The highest antibacterial activity was found for, in the decreasing order, extracts from the verbena herb, suspension cultures, and biomass grown on agar medium. Gram-positive bacteria proved to be more sensitive to the tested extracts than gram-negative bacteria. The strain *E. coli* ATCC 25922 was the most resistant to the extracts tested [unpublished].

Studies on cytotoxic and antifungal activities were performed on extracts from the herb and the biomass from *in vitro* cultures, including callus and suspension cultures, and those cultured in the agitated and aerated bioreactor. Cytotoxic activity of extracts from the herb indicated no toxicity against *Artemia salina* (LC_{50} > 1000 µg/mL), extracts from the biomass from callus cultures showed low toxicity (LC_{50} = 698.67 µg/mL), and extracts from suspension cultures and from bioreactor grown cultures showed moderate toxicity (479.34 and 187 µg/mL, respectively). Extracts from the herb and biomass grown in the bioreactors showed strong antiproliferative activity, reducing cell viability by over 70% at 500 µg/mL after 24 h and by 90% at 750 µg/mL after 72 h. Antifungal activity was determined against *Candida albicans*

ATCC 10231. The MIC was determined for extracts in the concentration range from 1 to 0.0019 mg/mL. At the concentrations used, none of the tested extracts showed antifungal activity [unpublished].

The next stage of biotechnological research with *in vitro* cultures of *V. officinalis* will be initiation and optimization of cultures with a higher degree of organogenesis, i.e., shoot cultures. After initial optimization of culture conditions, the plan is to analyze the biomass for the concentrations of iridoids, phenylpropanoid glycosides, and phenolic acids [unpublished]. In addition, analyses of the essential oil content will be performed [unpublished].

Application in Food Production

In 2010, the European Food Safety Authority (EFSA) included *V. officinalis* in a scientific opinion – "justifications of health claims related to various food products/food ingredients regarding antioxidant activity, protection of cells from premature aging, antioxidant content, antioxidant properties, and protection of DNA". This opinion contains information indicating that *V. officinalis* can protect cells and tissues against damage caused by oxidative stress, and that it increases the body's physiological resistance [80]. In the food industry, vervain is recommended as a flavoring agent, especially in the production of beverages and alcoholic drinks [81].

Application in Cosmetology

Products from *V. officinalis* are used in cosmetology mainly due to the presence of essential oil with a characteristic scent. The following raw materials for use in the production of cosmetics are listed in the Cosmetic Ingredient Database (CosIng) [82] maintained by the European Commission: extract from the green parts and extract from the green parts and flowers – approved for use as emollients and as skin conditioners; extract from the flowers and floral water – for use in skin conditioning; water from the flowers and leaves – for use as a flavoring agent and for conditioning the skin; and oil and absolute (i.e., oil containing only volatile fragrances) from the leaves – approved as a component of perfumes.

There are many different cosmetics available on the global cosmetics market containing products from *V. officinalis*, including soaps, hair shampoos, body lotions, massage oils, and body scrubs.

Conclusions

The use of the *V. officinalis* herb in modern phytotherapy is grounded in its use in folk medicine of different countries and in traditional Chinese medicine. Professional research on the biological activity of extracts from this raw material conducted in recent years confirms its long-known use in traditional phytotherapy. However, the latest studies of the raw material report on its other potentially valuable applications, such as antioxidant, antimicrobial, anti-inflammatory, neuroprotective anticancer, analgesic, and anticonvulsant. This research is carried out in scientific centers around the world, for example, in Germany, Austria, Spain, Turkey, Egypt, Ethiopia, Pakistan, India, Japan, China, and Poland. The interest in *V. officinalis* results not only from the fact that this species is present on all continents, but mainly because of its medicinal values. The proven, very rich and diverse chemical composition of the raw material (iridoids, phenylpropanoid glycosides, flavonoids, terpenoids, phenolic acids, the presence of essential oil) determines its valuable therapeutic properties. Nowadays, officially, the *V. officinalis* herb is recommended in the treatment of respiratory tract disorders, such as colds, fever, tightness of the chest, bronchitis, asthma, pertussis, and sinusitis, nervous system disorders, such as stress, anxiety, depression, chronic fatigue syndrome, nervous exhaustion, and insomnia, sexual neurosis, and headache, digestive tract disorders such as abdominal colic, jaundice, gallbladder inflammation, and intestinal worms, and urinary tract disorders such as urinary stones, urinary tract infections, and reproductive system diseases, e.g. dysmenorrhea. Moreover, *V. officinalis* extracts could be used topically in cases of wounds, bites, oral and throat inflammation, muscle spasms, and rheumatic conditions.

The largest part of scientific research focused on *V. officinalis* is related to the phytochemical analyses. The chemical composition of extracts from various parts of the plant, and also the essential oil, have been widely analyzed [30, 35, 41–44, 46, 47, 70]. The various extracts: methanolic [62, 64, 65, 68], ethanolic [38, 39, 63, 66], ethyl acetate [64], chloroformic [64], and an extract prepared using supercritical carbon dioxide [62] have been studied. These studies have proved differences in the chemical composition depend on origin and the part of the studied plants, as well as applied extraction methods and use of solvents of different polarities.

Many authors have analyzed the important pharmacological properties of plant extracts (herb, leaf, root), such as antibacterial [39, 63], antifungal [64], and antioxidant [38, 64, 83, 84] (▶ **Table 3**). However, studies do not strictly indicate which component is responsible for a given activity. Further research should be focused on association of the mechanism of biological activities with specific compounds.

The biological activities of *V. officinalis* have been studied *in vitro* on cell lines. These studies deals with the neuroprotective effect on rat cortical neurons culture [40], selective apoptosis induction of neoplastic cells in leukocytes isolated from human blood [70], proapoptotic activity on lymphocytes from patients with untreated chronic lymphocytic leukemia [42], and antiproliferative activity on the rat colonic epithelial cell line DHD/K12/PROb and human colon adenocarcinoma cell line HCT-116 [69] (▶ **Table 3**). All mentioned studies concern mechanisms important in the treatment of civilization diseases (Alzheimer's disease and cancer treatment) but they are still a long way from *in vitro* experiments to use in human treatment.

There are also studies performed *in vivo* on mouse or rat models. Based on them, activities such as antitumor and immunostimulating [58], anti-inflammatory, cicatrization, gastroprotective [62], anxiolytic, sedative [29], analgesic [65], anticonvulsant [29, 66], sleep promoting [67], and antidepressant [68] were proven (▶ **Table 3**). From scientific literature reviews performed by us, we found only one publication regarding trials of vervain extracts

on humans. It was a wide multicenter clinical trial on patients with chronic generalized gingivitis [60].

The modern applications of *V. officinalis* are connected with its long-term traditional use. However, the effectiveness, potency and dosages of plant material and extracts require further evaluation. Moreover, some applications known from folk medicine still demand scientific confirmation, e.g., antispasmodic, diuretic and antipyretic activity, as well as applications on the respiratory system like expectorant and secretolytic, and of the digestive system disorders (e.g., diarrhea, dysentery, stomachache and intestinal worms).

In the view of performed review on *V. officinalis,* its chemical composition also determines an important position of this species in the food and cosmetics industries.

High hopes can also be placed in the research with *in vitro* cultures of *V. officinalis*. In the future, they may, after further optimization, be an alternative source for obtaining biologically active compounds valuable in medicine and in the production of food and cosmetics.

Contributors' Statement

Data collection: P. Kubica, A. Szopa, J. Dominiak; design of the study: P. Kubica, A. Szopa, M. Luczkiewicz, H. Ekiert; analysis and interpretation of the data: P. Kubica, A. Szopa, M. Luczkiewicz, H. Ekiert; drafting the manuscript: P. Kubica, A. Szopa, M. Luczkiewicz, H. Ekiert; critical revision of the manuscript: P. Kubica, A. Szopa, M. Luczkiewicz, H. Ekiert. All authors read and approved the manuscript in its final form.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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