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Antiviral potential of Bulgarian medicinal plants

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Abstract Medicinal plants have been widely used to treat a variety of infectious and non-infectious diseases. Bulgarian flora includes 4,300 plant species, over 500 of which are rare or endemic to the country or the Balkan region. The aim of the present work is to summarize comprehensively the investigations on the antiviral activity of Bulgarian medicinal plants from the past three decades. The effect of different extracts derived from in vitro propagated plants has been examined as well. The phytochemical composition and its influence on specific steps of the viral life cycle have been discussed in this paper. The review includes the following families: *Amaryllidaceae*, *Fabaceae*, *Geraniaceae*, *Lamiaceae*, *Onagraceae*, *Ranunculaceae*, *Rosaceae*, *Scrophulariaceae* and *Rhodophyta*. Special attention has been paid to viruses as important human pathogens.

Keywords Bulgarian medicinal plants · Natural antiviral products · DNA and RNA viruses

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Abbreviations

ADV	Adenovirus
AIBDV	Avian infectious bursal disease virus
DHBV	Duck hepatitis B virus
DV-2	Dengue virus type-2
HBV	Hepatitis B virus
HCMV	Human cytomegalovirus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HSV-1	Herpes simplex virus type 1
HSV-2	Herpes simplex virus type 2
Influenza	Influenza virus
JEV	Japanese encephalitis virus
Para3	Paramyxovirus type 3
Polio	Poliomyelitis
RSV	Respiratory syncytial virus
SARSV	Severe acute respiratory syndrome virus

Introduction

Viral infections are among the most frequent causes of human diseases. More than 300 different types of viruses are associated with humans and cause diseases of varying levels of severity. Apart from its purely humanitarian aspect, this fact also has an important financial manifestation. A considerable part of all purchased drugs worldwide is intended for the treatment of viral infections (Monto 2003).

The most commonly used antiviral drugs in clinical practice are the chemotherapeutics. However, their widespread use is accompanied by the exhibition of a number of side reactions and emergence of resistant clinical isolates, some of which are two or even three times more resistant to whole classes of drugs (Piret and Boivin 2011). A good alternative for overcoming these problems is the use of natural products which have several major advantages over the currently applied chemotherapeutics. Firstly, medicinal plant extracts are more readily assimilated by the body due to their natural origin and have fewer side effects. Moreover, development of resistant strains to such antivirals is hampered due to their complex chemical structure and often to their multi-stage mode of action (Mukhtar et al. 2008).

The use of plants in medicine is a process that meets a number of constraints. The contents of the valuable active secondary metabolites in plants depend on the specific environmental conditions in their habitat. This leads to difficult standardization of the extracts and explains the observed variations in their activity. Another problem is that ethnopharmacologically valuable plants often have limited distribution, which renders their commercial use practically impossible. The cultivation of important medicinal plants under controlled conditions is a promising solution to these problems. The ability to improve the yield of active secondary metabolites by modifying the conditions of cultivation is an additional advantage of this approach.

Diseases are a part of our life. In search of ways for treatment, we have turned to the surrounding environment. Bulgarian folk medicine has progressed over the centuries based on the experience of the nation. Bulgarian culture emerged and developed embracing the heritage of Thracians, Slavs, Greeks and Romans who lived on the country's current territory during different historical periods. According to the legends, Orpheus is the founder not only of Thracian music and poetry, but also of Thracian medicine. The knowledge that our nation accumulated through the centuries has been passed from generation to generation, and today there are preserved sources, which describe how to use various plants to cure different diseases. More than 600 plants are used in folk medicine. In the 1930s, the treatment of postencephalitic parkinsonism with the herb *Atropa belladonna* L. or Nightshade ("ludo bile") gained popularity in Europe. It was introduced by the healer Ivan Raev and is known as "curra bulgara" or "Bulgarian treatment" (Vollmer 1940; Price and Merritt 1941). The treatment also proved effective for all other forms of parkinsonism. Even Queen Elena of Savoy (grandmother of the Bulgarian royal ancestor Simeon II), who suffered from this disease, sought Raev's help after all other means of treatment had failed and consequently, she made a full recovery. In the last decades of the 20th century, various Bulgarian medicinal products of plant origin gained international recognition—Nivalin (active component galanthamine from *Galantus nivalis* L. var. gracilis (Lilienfeld 2002; Darreh-Shori et al. 2013), suppresses the activity of cholinesterase and is used for the treatment of viral poliomyelitis, neuritis), Glauvent (antitussive medicine containing the alkaloid glaucine isolated from the plant *Glaucinum flavum*; it suppresses the activity of the cough center in the brain), Tribestan (prepared from the plant *Tribulus terrestris* L., increases the libido in fertility therapy and reduces total cholesterol and LDL), etc.

The varied landscape and geography of Bulgaria provide habitats for many different plants. The flora includes 4,300 species, over 500 of which are rare or endemic to the country or the Balkan region (Ivancheva and Stancheva 2001). Bulgaria is among the few countries in Europe where resurrection plants (reviving upon rehydration of the vegetative tissues even after prolonged periods of complete dehydration) grow in natural habitats. The native *Haberlea rhodopensis* Friv was documented in the middle of 19th century and was among the first species to be recognized as genuine resurrection plants (Ganchev 1950).

The approach to treatment of viral diseases using chemicals based on plant origin represents a blend between the experience and creativity of folk medicine and the techniques of modern pharmacology and phytochemistry. This review aims to summarize the results from antiviral experiments with Bulgarian medicinal plants.

Amaryllidaceae

Leucojum aestivum L.

Leucojum aestivum (Fig. 1) grows in damp areas mainly in South Bulgaria and along the Black Sea coast. The extract from this plant contains about 0.5 % alkaloids. Galanthamine (**1**) and lycorine (**2**) are the most abundant ones. The other alkaloids in the plant are lycorenine, tazettine, isotazettine, homolycorine (**3**)

Fig. 1 *A. mollis*, *E. hirsutum*, *L. album*, *L. cardiaca*, *V. densiflorum* were obtained from Wikimedia Commons under GNU free documentation license (http://en.wikipedia.org/wiki/GNU_Free_Documentation_License). Plants with antiviral activity

(Fig. 2) and estivin (chemical compounds with antiviral activity, derived from all mentioned in the current paper plants are shown on Table 1) (Georgieva et al. 2007). Galanthamine suppresses the activity of cholinesterase. This alkaloid enhances nerve impulse propagation, stimulates excitation processes in the spinal, bulbal and cortical centers, improves the tone and contraction ability of skeletal and smooth muscles and increases glandular secretion. It is used in the treatment of viral poliomyelitis, neuritis, radiculitis, various types of paralysis, myoatrophy, etc. and alleviates smooth muscles insufficiencies of the urinary bladder and the gastrointestinal tract (Stanilova et al. 1994).

Asteraceae

Calendula officinalis L.

Calendula officinalis (Fig. 1) is an annual plant, native to the Mediterranean region. In Bulgaria this

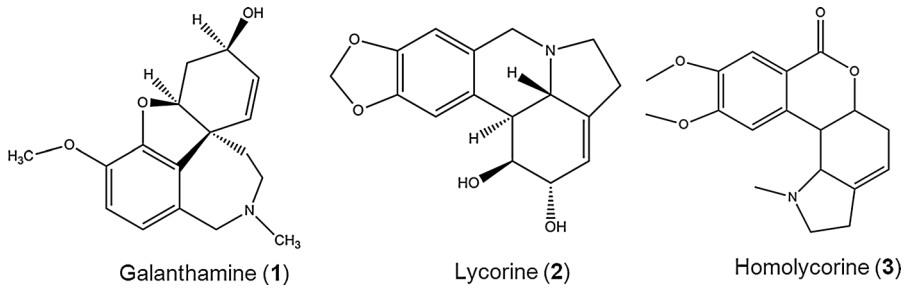


Fig. 2 Antiviral compounds isolated from *L. aestivum*

Table 1 Antiviral activity of chemical constituent from Bulgarian medicinal plants

Plant species	Chemical constituent	Virus	Concentration	References
L. aestivum L.	Galanthamine (**1**)	Poliovirus	No data	Stanilova et al. (1994)
	Lycorine (**2**)	Poliovirus	2.5 M	Hwang et al. (2008)
		SARSV	15.7	Li et al. (2005)
	Homolycorine (**3**)	HIV-1	0.4–7.3 μg/ml	Szlavik et al. (2004)
C. officinalis L.	Oleanolic acid (**4**)	HCV	0.8–3.5 μg/ml	Kong et al.(2013)
	Ursolic acid (**5**)	HPV-18	5–20 μM	Yim et al. (2006)
A. corniculatus Bieb.	Oleanolic acid (**4**)	HCV	0.8–3.5 μg/ml	Kong et al.(2013)
	Kaempferol (**6**)	HCMV	No data	Mitrocotsaet al. (2000)
	Quercetin (**7**)	HSV-1, poliovirus-1, RSV	200 μM	Kaul et al. (1985)
	Isorhamnetin-3-*O*-rutinoside (**8**)	HSV-1	No data	Kim et al. (1998)
	Orientin (**9**)	Para3	11.7 μg/ml	Li et al. (2002)
G. sanguineum L.	Kaempferol (**6**)	HCMV	No data	Mitrocotsaet al. (2000)
	Quercetin (**7**)	HSV-1, poliovirus-1, RSV	200 μM	Kaul et al. (1985)
	Myricetin (**10**)	HIV	100 μg/ml	Ko et al. (2009)
	Catechin (**11**)	RSV, HSV-1	25 μM	Kaul et al. (1985)
	Pelargonidin (**12**)	HSV-1	7–8 μg/ml	Danaher et al. (2011)
	Hyperoside (**13**)	DHBV	0.012–0.015 g/l	Wu et al. (2007)
L. album L.	Quercetin (**7**)	HSV-1, poliovirus-1, RSV	200 μM	Kaul et al. (1985)
	Verbascoside (**15**)	HSV-1, HSV-2	No data	Martins et al. (2009)
	Tiliroside (**14**)	HIV-1	No data	Seal et al. (2011)
	Lamiridosin A/B (**16**)	HCV	100 μg/ml	Zhang et al. (2009)
L. cardiaca L.	Ursolic acid (**5**)	HCV, HPV-18	5–20 μM	Yim et al. (2006)
	Quearcetin (**7**)	HSV-1, poliovirus-1, RSV	200 μM	Kaul et al. (1985)
	Hyperoside (**13**)	DHBV	0.012–0.015 g/l	Wu et al. (2007)
	Apigenin (**17**)	HSV-2	9.7 mg/l	Chiang et al. (2005)
	Rutin (**18**)	HIV-1	72.6 μM	Ahn et al. (2002)

Table 1 continued

Plant species	Chemical constituent	Virus	Concentration	References
M. officinalis L.	Oleanolic acid (**4**)	HCV	0.8–3.5 μg/ml	Kong et al.(2013)
	Ursolic acid (**5**)	HCV, HPV-18	5–20 μM	Yim et al. (2006)
	Pomolic acid (**19**)	HIV-1	1.4 μg/ml	Kashiwada et al. (1998)
	Rosmarinic acid (**20**)	HSV, JEV	No data	Petersen and Simmonds (2003)
	Protocatechuic acid (**21**)	AIBDV	20 mg/kg	Ou et al. (2012)
	Luteolin (**22**)	HPV-16	4.3 μM	Cherry et al. (2013)
	Caffeic acid (**23**)	HBV	3.9–109.3 μM	Wang et al. (2009)
	Linalool (**24**)	ADV-3,8,11	8–26.4 mg/l	Chiang et al. (2005)
T. simplex L.	(−)-Thalimonine (**25**)	Influenza	0.6 μM	Serkedjieva and Velcheva (2003)
		HSV-1	10 μM/ml	Varadinova et al. (1996)
A. mollis	Hyperoside (**13**)	DHBV	0.012–0.015 g/l	Wu et al. (2007)
	Gossypetin (**26**)	Influenza	36–43 μM	Jeong et al. (2009)
V. densiflorum Bertol. (*Verbascum thapsiforme* Schrad.)	Kaempferol (**6**)	HCMV	No data	Mitrocotsaet al. (2000)
	Verbascoside (**15**)	HSV-1, HSV-2	No data	Martins et al. (2009)
	Apigenin (**17**)	HSV-2	9.7 mg/l	Chiang et al. (2005)
	Rutin (**18**)	HIV-1	72.6 μM	Ahn et al. (2002)
	Protocatechuic acid (**21**)	AIBDV	20 mg/kg	Ou et al. (2012)
	Luteolin (**22**)	HPV-16	4.3 μM	Cherry et al. (2013)
	Caffeic acid (**23**)	HBV	3.9–109.3 μM	Wang et al. (2009)
	Aucubin (**27**)	HBV	1,000 μM	Chang (1997)
Z. prototypus	Thymol (**28**)	HSV-2	No data	Kamenarska et al. (2009)
	Carvacrol (**29**)	HSV-2	No data	

No data no data for exact concentration

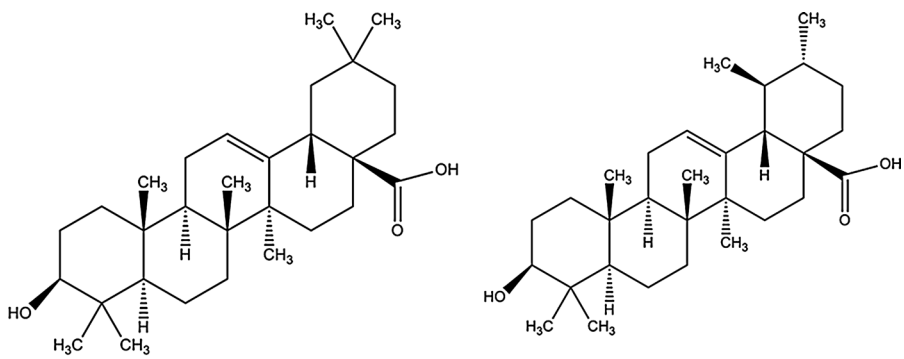


Fig. 3 Antiviral compounds isolated from *C. officinalis*

plant is also a popular ornamental flower. *Calendula* flowers were considered beneficial for their anti-inflammatory, wound-healing and antiseptic properties. This plant is used to treat various skin diseases, ranging from ulcerations to eczema. Oleanolic (**4**) and ursolic (**5**) acids (Fig. 3) are

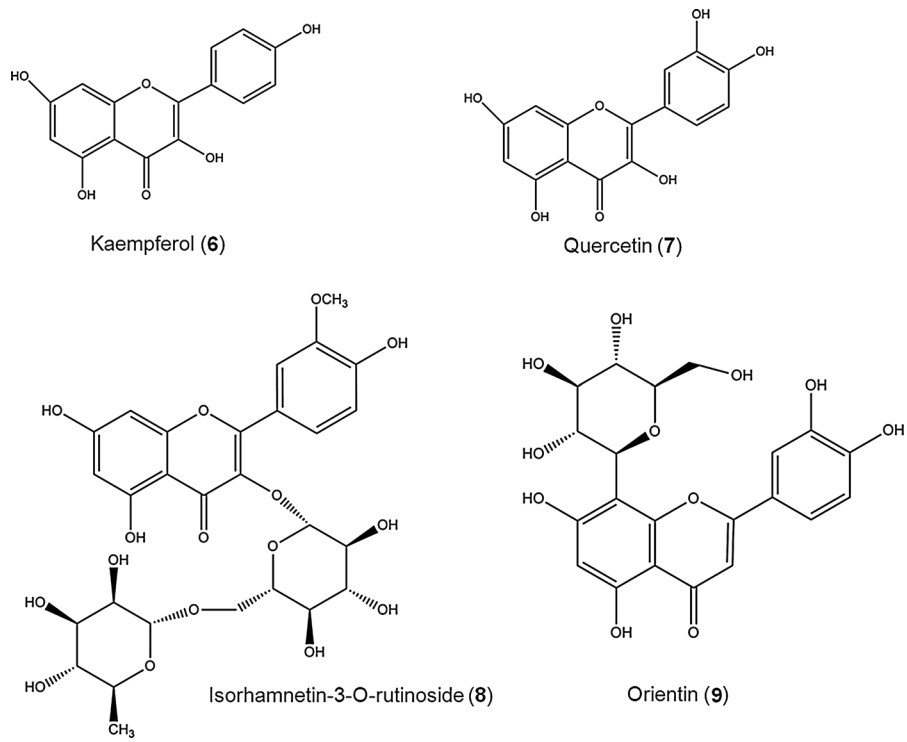


Fig. 4 Antiviral compounds isolated from *A. corniculatus*

well-known constituents with antiviral activity (Kowalski 2007).

Kalvatchev et al. (1997) showed that an organic extract from *C. officinalis* flowers in a concentration of 200 μg/ml has a significant dose- and time-dependent reduction effect of the human immunodeficiency virus type 1 (HIV-1) reverse transcription activity. An 85 % reverse transcription inhibition was achieved after 30 min of treatment of partially purified reverse transcriptase in a cell-free system. The authors also examined extracts of dried flowers of *C. officinalis* for their ability to inhibit the replication of HIV-1. Both the organic and aqueous extracts were nontoxic to human lymphocytic Molt-4 cells in a concentration of 1,000 μg/ml, but only the organic one exhibited potent anti-HIV activity in an in vitro MTT/tetrazolium-based assay. In the presence of the organic extract (500 μg/ml), the uninfected Molt-4 cells were completely protected for up to 24 h from fusion and subsequent death, caused by cocultivation with persistently infected U-937/HIV-1 cells. These results suggest that the organic extract of the flowers from *C. officinalis* possesses anti-HIV properties of therapeutic interest.

Fabaceae

Astragalus corniculatus Bieb., Astragalus vesicarius L., Astragalus ponticus Pall

Astragalus spp. are used in Bulgarian folk medicine as diuretics for the treatment of hypertension, renal disorder, nervous diseases and rheumatism (Ivancheva et al. 2006), and as diaphoretics. *A. corniculatus* (Fig. 1) is very rich in compounds with antiviral activity. It has been established that the plant contains the flavonoids kaempferol (**6**), quercetin (**7**), isorhamnetin-3-*O*-rutinoside (**8**) and orientin (**9**) (Fig. 4) (Krasteva and Nikolov 2008), as well as oleanolic acid (**4**) (Fig. 3) (Krasteva and Kalogan 2006). A pilot study of patients with hepatitis C showed improvement after 6 months of treatment with astragalus (Ivancheva et al. 2006). The presumed immune-boosting and antiviral effects of astragalus lead to its widespread use among people living with AIDS and other chronic conditions, including chronic fatigue syndrome, although the extract appears to be safe when used in limited amounts.

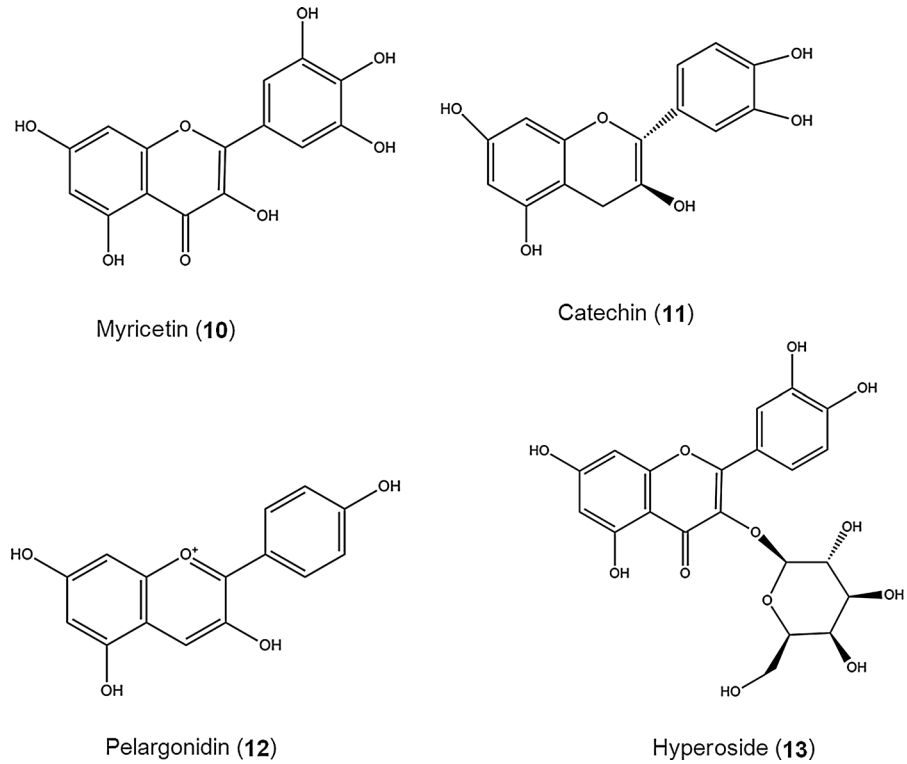


Fig. 5 Antiviral compounds isolated from *G. sanguineum*

Geraniaceae

Geranium sanguineum L.

Geranium sanguineum (Fig. 1) is a perennial herbaceous plant. In Bulgarian traditional medicine, the root system of this plant is used as astringent and because of its anti-inflammatory properties it is applied in the treatment of diarrhea, gastric-enteric catarrh and dysentery (Jordanov et al. 1973). The polyphenol complex obtained from the roots of *G. sanguineum* contains flavonoids such as kaempferol (**6**), quercetin (**7**) (Fig. 4), myricetin (**10**), catechin (**11**), pelargonidin (**12**), hyperoside (**13**) (Fig. 5) and tannins (Ivancheva and Wollenweber 1989). The complex was proved to have selective anti-influenza activity in vitro. The expression of hemagglutinin on the surface of cells infected with A/chicken/Rostock/34, the virus-induced cytopathic effect, the infectious virus yield and plaque formation were all reduced at non-toxic concentrations of the polyphenol complex. Synthesis of virus proteins was also selectively inhibited. A strong virucidal effect at high concentrations of the polyphenol complex (>200 μg/ml) was observed. The complex inhibited the early stage of the infection (within 3 h of infection). The selectivity of the antiviral action was confirmed by the variations in sensitivity to the polyphenol complex among different influenza viruses (Serkedjieva and Hay 1998).

A polyphenol complex obtained from the aerial roots of the medicinal plant *G. sanguineum* prevented mice mortality in an experimental influenza A/Aichi/2/68 (H3N2) virus infection. In order to establish how a maximum therapeutic benefit can be derived from this preparation, six different routes of inoculation were used (oral, intranasal, by aerosol, subcutaneous, intraperitoneal or intravenous). It was found that the aerosol application of the polyphenol complex was highly effective. When 5,400 μg/ml of the extract were applied according to a prophylactic–therapeutic schedule (−24, −2, +24, +48, +72 h post infection), a significant protective effect was observed. The protective index reached the value of 70.1 % and the mean survival time was prolonged with 3–5 days. The lung infectious virus titers and the lung consolidation of virus infected and polyphenol complex-treated animals were all reduced in comparison with the control group. The application of the polyphenol

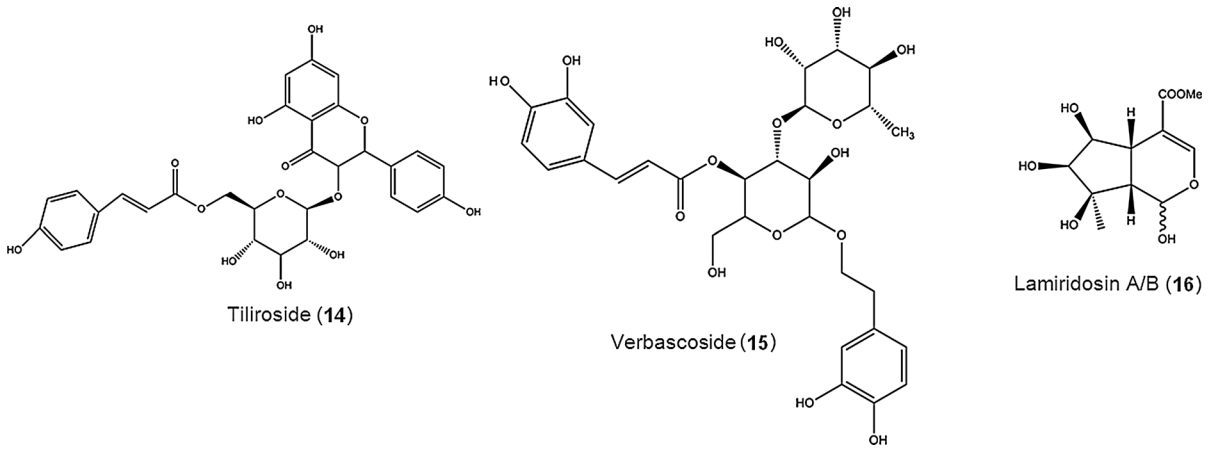


Fig. 6 Antiviral compounds isolated from *L. album*

complex according to schedules excluding pretreatment of mice proved that this condition was essential for protection (Serkedjieva et al. 2008). It was found that a polyphenol complex from *G. sanguineum* applied in doses of 12.5 and 25 μg/ml stimulate the phagocytic activity of peritoneal macrophages and blood polymorphonuclear leucocytes. The same doses do not affect significantly the phagocytic activity of alveolar macrophages, the migration of alveolar and peritoneal macrophages or the adherent activity of polymorphonuclear leucocytes. The polyphenol complex applied in concentrations of 3.1–25 μg/ml suppressed spontaneous nitric oxide production by peritoneal macrophages, while nitric oxide production, induced by lipopolysaccharide, Ifn-γ or lipopolysaccharide + Ifn-γ was not affected (Toshkova et al. 2004).

The polyphenol complex extracted from *G. sanguineum* also inhibited the reproduction of herpes simplex virus type 1 in vitro. The infectious titers were reduced with 2,5 log in presence of 100 μg/ml. In addition, newly synthesized virions with damaged protein envelopes were observed by electron microscopy (Serkedjieva and Manolova 1992).

Lamiaceae

Lamium album L.

The genus *Lamium* comprises of about 40 species of annual and perennial herbaceous plants distributed in Europe, Asia and Africa. *Lamium album* (Fig. 1) is known for its rich content of flavonoids and glucosides (quercetin (**7**) (Fig. 4) and tiliroside (**14**)), and phenylethanoid–verbascoside (**15**) (Fig. 6) (Budzianowski and Skrzypczak 1995). Extracts from the plant exhibit anti-inflammatory, astringent and antiseptic activity (Staneva et al. 1982). Anti herpetic properties were observed for methanol and chloroform extracts (Shishkov et al. 2008; Todorov et al. 2013). The extracts affected several stages of the herpes virus life cycle—adsorption, penetration and the first two stages of the viral replication cycle. Chloroform extracts of the plant at a concentration of 1,000 μg/ml blocked adsorption and penetration of the extracellular form of the virus up to 90 %. The replication of both HSV type 1 and type 2, was inhibited completely after application of the same concentration. The concentrations at which 50 % inhibition of viral replication was observed were around 600 μg/ml for both extracts and viral strains. The isomer lamiridosin A/B (**16**) (Fig. 6) present in the aqueous extract of the flowering tops of *L. album* (100 μg/ml) was found to inhibit significantly Hepatitis C virus entry (Zhang et al. 2009). When tested in a cell line *L. album* extracts also exhibited anticancer properties. At concentration of 5,000 μg/ml, the methanol extract from the plant demonstrated the strongest effect (Moskova-Doumanova et al. 2012).

Leonurus cardiaca L.

Leonurus cardiaca (motherwort) (Fig. 1) is the only species of the genus *Leonurus* found in Bulgaria. It is reported to possess antiallergenic, analgesic,

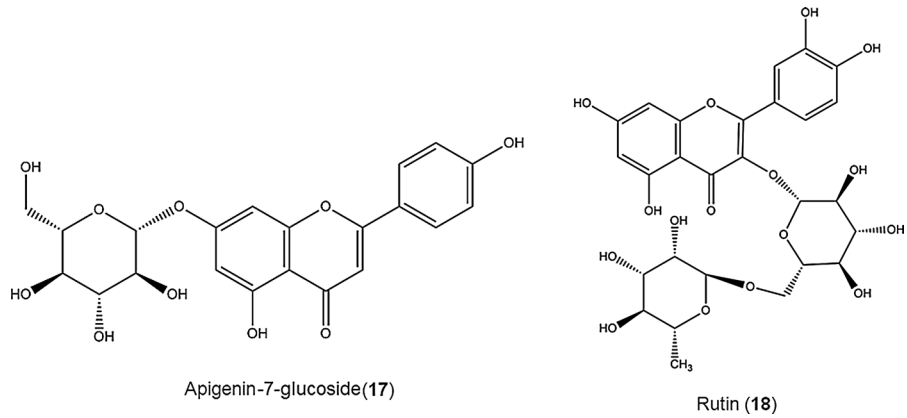


Fig. 7 Antiviral compounds isolated from in *L. cardiaca*

antiepileptic, anti-ischemic, antispasmodic, antitumor, astringent, cardiotonic, CNS antidepressant, diaphoretic, diuretic, expectorant, hypotensive, hypotonic, laxative, lipolytic, negative, chronotropic, nervine, oxytocic, sedative, stimulant, tonic and uterotonic activities (Morteza-Semnani et al. 2008). This diverse scope of effect is due to its complex chemical composition (Kuhn and Winston 2000). The plant contains several components with antiviral activity: ursolic acid (**5**) (Fig. 3) and the flavonoids quercetin (**7**) (Fig. 4), hyperoside (**13**) (Fig. 5), apigenin-7-glucoside (**17**) and rutin (**18**) (Fig. 7). Total chloroform and methanol extracts from plants collected from Bulgaria exhibit antiherpes activity against HSV-1 and -2 by influencing the viral replication cycle in concentrations between 200 and 800 μg/ml for different strains (Kostova et al. 2010).

Melissa officinalis L.

Melissa officinalis (Lemon balm) (Fig. 1) has a number of practical applications in medicinal science (Dimkov 2001). The activity of its aqueous extracts against herpes simplex virus and vaccinia virus was established for the first time back in 1967 (Herrmann and Kucera 1967). Then the caffeic acid (**19**) (Fig. 8) contained in the plant was acknowledged for its antiviral properties. The presence of caffeic, rosmarinic (**20**) (Fig. 8) and ferulic acids was demonstrated by thin-layer chromatography. The essential oil of *M. officinalis* contains linalool (**21**) (Fig. 8) (Patora et al. 2003), oleanolic acid (**4**), ursolic acid (**5**) (Fig. 3) (Maguire et al. 2013), pomolic acid (**22**), protocatechuic acid (**23**), and luteolin-7-glucoside (**24**) (Fig. 8) (Patora and Klimek 2002). A virucidal effect of the oil was established in cell medium within 3–6 h after treatment with a maximum tolerable concentration of 0.25 % (Dimitrova et al. 1993). The volatile oil components of *M. officinalis* were found to inhibit HSV-2 replication in cell cultures when applied in concentrations between 25 and 100 μg/ml (Allahverdiyev et al. 2004).

Hot-water extracts of *M. officinalis* were found to protect embryonated chicken eggs against the lethal action of Semliki Forest virus and Newcastle virus. It is suggested that the active moiety is a tannin or tannin-like polyphenol that perhaps acts at the cell surface (Cohen et al. 1964).

Onagraceae

Epilobium hirsutum L.

Epilobium hirsutum (Fig. 1) is a perennial plant that occurs in moist places at altitudes of up to 1,400 m on the entire territory of Bulgaria. The main biologically active components of the polyphenol mixture obtained from the plant are flavonoids and tannins. The water–alcohol extract and the four fractions of the polyphenol mixture of *E. hirsutum* have a significant inhibitory effect on the reproduction of influenza viruses in vitro, in ovo and in vivo (Ivancheva and Wollenweber 1989).

Ranunculaceae

Thalictrum simplex L.

Thalictrum spp. is known for their rich alkaloid content. They are used in traditional Tibetan and

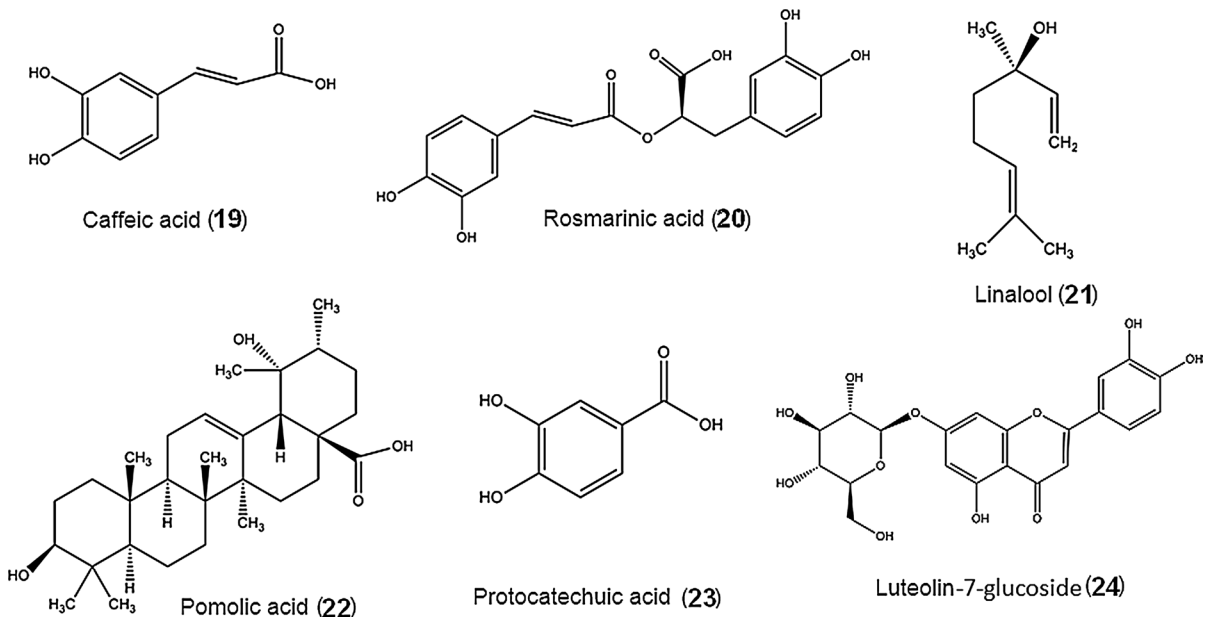


Fig. 8 Antiviral compounds isolated from *M. officinalis*

Mongolian medicine against acute and chronic infections, as blood purifiers and for wound healing. The alkaloid (−)-thalimonine (3,4-methylene-deoxy-2,8,9-trimethoxypavinan) (**25**) (Fig. 9) was isolated from *Thalictrum simplex* L (Fig. 1) and investigated for its effect against several viral agents (Velcheva et al. 1992). The alkaloid and its N-oxide proved to suppress significantly the viral replication. (−)-Thalimonine exhibited a highly selective antiviral effect against Influenza A/Waybridge. The selective index was 640 at a concentration of 0.1 μM. The alkaloid also completely blocked viral penetration at 99,999 % (Serkedjieva and Velcheva 2003). Furthermore, the same alkaloid affected the in vitro replication of HSV-1. The values of the effective concentration (0.007 μM) were similar to these of the referent drug Acyclovir (Varadinova et al. 1996).

Rosaceae

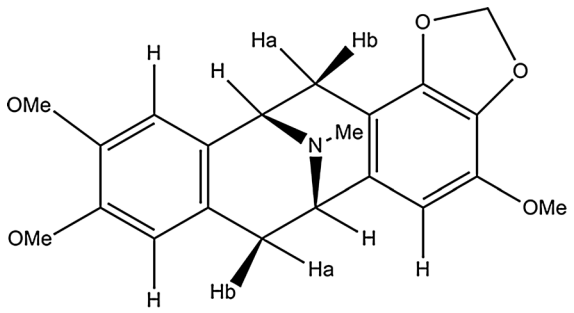
Alchemilla mollis

Alchemilla mollis (lady's mantle) (Fig. 1) is used in traditional Bulgarian medicine for different indications. Its extracts alleviate the symptom of sore throat, promote wound healing, arrest hemorrhages and relieve nausea and vomiting (Staneva et al. 1982). Various studies have indicated that *A. mollis* and other *Alchemilla* species have a potential free radical scavenging activity (Trendafilova et al. 2011) attributed to the phenolic compounds, tannins, and the flavonoid glycosides hyperoside (**13**) (Fig. 5) and gossypetin-3-glucoside (**26**) (Fig. 10) present in the plants (Trendafilova et al. 2012). It was found that the extract from *A. mollis* affected influenza virus particles directly and inhibited their infectivity. Plaque formation by the A/WSN/33 virus was significantly inhibited in the presence of 0.12 % extract in cell medium (Makau et al. 2013).

Scrophulariaceae

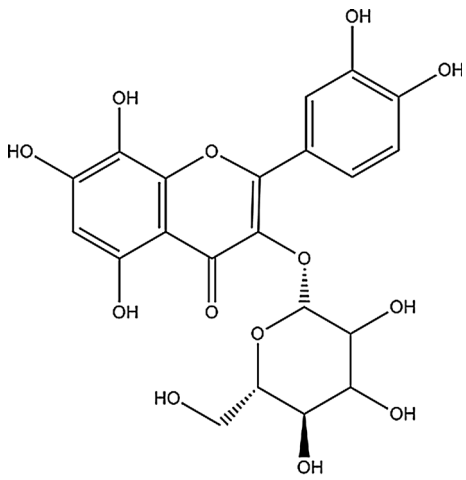
Verbascum densiflorum Bertol. (Verbascum thapsiforme Schrad)

Verbascum densiflorum (Fig. 1) is a biennial herbaceous plant, widespread in Bulgaria. *Verbasci* Flos is a traditional herb for treatment of sore throat and cough (Zgorniak-Nowosielska et al. 1991). The flowers are also used for the treatment of chills, dry coughs, and phlegm congestion due to the mild expectorant action of the saponins. Both the flowers and the leaves possess mildly demulcent, expectorant, and astringent properties. *Verbasci* Flos contains water soluble mucilage polysaccharides, which after hydrolysis yield mainly D-galactose, as well as arabinose, D-



(-)-Thalimonine (**25**)

Fig. 9 The alkaloid (−)-thalimonine with antiviral activity isolated from *T. simplex*



Gossypetin-7-glucoside (**26**)

Fig. 10 The antiviral compound gossypetin isolated from *A. mollis*

glucose, traces of D-xylose, L-rhamnose, D-mannose and L-fucose. Other components include uronic acids, flavonoids (apigenin (**17**) (Fig. 7), luteolin (**24**) (Fig. 8) and their 7-*O*-glucosides together with kaempferol (**6**) (Fig. 4) and rutin (**18**) (Fig. 7), caffeic acid (**19**), protocatechuic acid (**23**) (Fig. 8) and caffeic acid derivatives including ferulic acid and verbascoside (**15**) (Fig. 6), iridoid monoterpenes: aucubin (**27**) (Fig. 11), 6-β-xylosylaucubin, methylcatalpol, isocatalpol, and triterpenes. The lyophilized infusion from flowers of *V. thapsiforme* in a concentration of 300 μg/ml showed antiviral activity in in vitro studies against fowl plague virus, several influenza A strains and an influenza B strain. The Influenza virus titers showed reduction of 1 log–3 log units. The lyophilized infusion from flowers of *V. densiflorum* did not inactivate extracellular influenza viruses (Zgorniak-Nowosielska et al. 1991).

Rhodophyta

Ceramium rubrum Huds

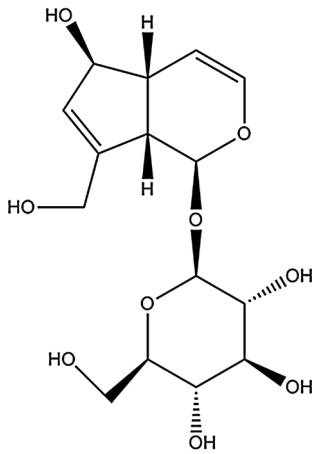
The chloroform extract from the red marine alga *C. rubrum* (Fig. 1) from the Bulgarian Black Sea coast shows considerable activity against influenza viruses A and B. The virus inhibition effect of the extract in the concentration range of 100–1,100 μg/ml is selective, dose-related and strain-specific, with selective indices in the range of 9.5–68.3. The extract reduces the virus-related cytopathogenic effect and hemagglutinin production in vitro and in ovo. It also inhibits HSV-1 and -2 replication in vitro and has a strong inactivation effect (Serkedjieva 2004).

Polysiphonia denudata, Gelidium spinosum, Zanardinia prototypus

In 2009, Kameranska et al. established that three species of red algae have considerable antiviral properties. A significant reduction in the replication of influenza and herpes simplex virus in cell cultures was observed. Replication of the influenza virus was inhibited by water and lypophilic extracts from *Polysiphonia denudata* (500 and 50 μg/ml, respectively) and *n*-butanol extracts from *Gelidium spinosum* (250 μg/ml). The values of the selective index were 16, 200 and 40 respectively. The propagation of the herpes simplex virus was reduced by the water extract of *P. denudata* (250 μg/ml) and the chloroform extract of *Zanardinia prototypus* (50 μg/ml) with selective indices of 10 and 14.4 respectively. The authors proposed that the biological activity of *Z. prototypus* (Fig. 1) was due to the presence of monoterpenes—thymol (**28**) and carvacrol (**29**) (Fig. 12).

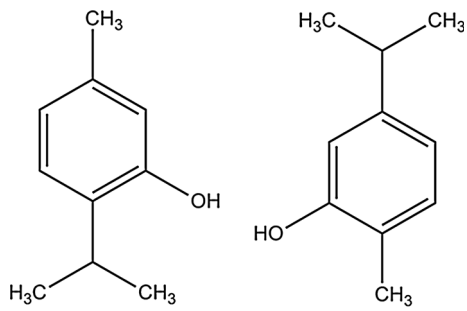
Conclusions and perspectives

Medicine has gone a long way from the application of the first infusions to the actual scientific proof that many plants have a remarkable healing potential. Due to the legacy of the wise folk healers who used the



Aucubin (**27**)

Fig. 11 The antiviral compound aucubin isolated from *V. densiflorum*



Thymol (**28**) Carvacrol (**29**)

Fig. 12 The antiviral compounds isolated from *Z. prototypus*

cures growing landing the soil, now the experience of long ages is embodied in old traditional recipes. Even though many species from the Bulgarian flora can also be found in other places around the world, the specific abiotic and biotic factors of the Bulgarian land contribute to a difference in the chemical composition of the plants. This is why it is important to examine their content in details. For the last 25 years many plants known from Bulgarian traditional medicine for their biological activity were studied as potential sources of substances with antiviral activity. More than 17 of these plants were found to possess inhibition activity against the life cycle of several DNA and RNA viruses or to inactivate their extracellular forms. The main metabolites for some of them were determined. Sadly, the mechanism of antiviral activity for the majority of them remains unknown. Hence, an on-going in depth research on the topic is needed. Such studies may lead to the discovery of new classes of compounds characterized by a previously unrecognized mechanism of antiviral action. For example, *Artemisia annua* gave the world artemisinin, a powerful weapon in the struggle with malaria with a new and unique mode of action.

An important problem in using drugs delivered by plant sources in industrial pharmacy is that the inconstant ecological factors lead to variation in content and volume of plant secondary metabolites. Due to this our future work must be pointed to in vitro cultivation of plant species in optimal constant (non-changing) environment which can eventually stimulates them to produce desirable metabolites. With this type of biotechnology methods we can overcome another problem in industrial plant utilization-limited areals. Some species which are endemic for specific region can't be used in industrial scale without proper way of propagation. Our collective from Sofia University has some pilot researches in this area.

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References

Ahn M, Kim C, Lee J et al (2002) Inhibition of HIV-1 integrase by galloyl glucoses from *Terminalia chebula* and flavonol glycoside gallates from *Euphorbia pekinensis*. Planta Med 68(5):457–459

Allahverdiyev A, Duran N, Ozguven M, Koltas S (2004) Antiviral activity of the volatile oils of *Melissa officinalis* L. against herpes simplex virus type 2. Phytomedicine 11(7):657–661

Budzianowski J, Skrzypczak L (1995) Phenylpropanoid esters from *Lamium album* flowers. Phytochemistry 38(4):997–1001

Chang I (1997) Antiviral activity of aucubin against hepatitis B virus replication. Phytother Res 11(3):189–192

Cherry J, Rietz A, Malinkevich A et al (2013) Structure based identification and characterization of flavonoids that disrupt human papillomavirus-16 E6 function. PLoS One 8(12):e84506

Chiang L, Ng L, Cheng P et al (2005) Antiviral activities of extracts and selected pure constituents of *Ocimum basilicum*. Clin Exp Pharmacol Physiol 32(10):811–816

Cohen R, Kucera L, Herrmann E (1964) Antiviral activity of *Melissa officinalis* (lemon balm) extract. Proc Soc Exp Biol Med 117(2):431–434

Danaher R, Wang C, Dai J et al (2011) Antiviral effects of blackberry extract against herpes simplex virus type 1. Oral Surg Oral Med Oral Pathol Oral Radiol Endodontol 112(3):31–35

Darreh-Shori T, Hosseini S, Nordberg A (2013) Pharmacodynamics of cholinesterase inhibitors suggests add-on therapy with a low-dose carbamylating inhibitor in patients on long-term treatment with rapidly reversible inhibitors. J Alzheimers Dis 39(2):423

Dimitrova Z, Dimov B, Manolova N et al (1993) Antiherpes effect of *Melissa officinalis* L. extracts. Acta Microbiol Bulg 29:65–72

Dimkov P (2001) Prirodolechenie. Astrala, Sofia

Ganchev I (1950) Anabiotic dry tenacity and other biological particularities of *Haberlea rhodopensis*. Rep Inst Bot Bulg Acad Sci 1(1):191–214

Georgieva L, Berkov S, Kondakova V et al (2007) Alkaloid variability in *Leucojum aestivum* from wild populations. Zeitschrift fur Naturforschung C 62(9–10):627–635

Herrmann E, Kucera L (1967) Antiviral substances in plants of the mint family (Labiatae). II. Nontannin polyphenol of *Melissa officinalis*. Proc Soc Exp Biol Med 124(3):869–874

Hwang Y, Chu J, Yang P et al (2008) Rapid identification of inhibitors that interfere with poliovirus replication using a cell-based assay. Antivir Res 77(3):232–236

Ivancheva S, Stancheva B (2001) Ethnobotany in Bulgaria. Plants Balk Penins Next Millenn 1:555–568

Ivancheva S, Wollenweber E (1989) Leaf exudate flavonoids in *Geranium macrorrhizum* L. and *G. lucidum* L. Indian Drugs 27(3):167–168

Ivancheva S, Nikolova M, Tsvetkova R (2006) Pharmacological activities and biologically active compounds of Bulgarian medicinal plants. Phytochem Adv Res Kerala Signpost 37/661(2):87–103

Jeong H, Ryu Y, Park S et al (2009) Neuraminidase inhibitory activities of flavonols isolated from *Rhodiola rosea* roots and their in vitro anti-influenza viral activities. Bioorg Med Chem 17(19):6816–6823

Jordanov D, Nikolov P, Boichinov A (1973) Phytotherapy. Medicina, Sofia

Kalvatchev Z, Walder R, Garzaro D (1997) Anti-HIV activity of extracts from *Calendula officinalis* flowers. Biomed Pharmacother 51:176–180

Kamenarska Z, Serkedjieva J, Najdenski H et al (2009) Antibacterial, antiviral, and cytotoxic activities of some red and brown seaweeds from the Black Sea. Bot Mar 52(1):80–86

Kashiwada Y, Wang H, Nagao T et al (1998) Anti-AIDS agents. 30. Anti-HIV activity of oleanolic acid, pomolic acid, and structurally related triterpenoids 1. J Nat Prod 61(9):1090–1095

Kaul T, Middleton E, Ogra P (1985) Antiviral effect of flavonoids on human viruses. J Med Virol 15(1):71–79

Kim H, Kang B, Park K et al (1998) Anti-herpes simplex virus type 1 (HSV-1) effect of isorhamnetin 3-O-D-glucopyranoside Isolated from *Brassica rapa*. J Pharm Soc Korea 42:607–612

Ko Y, Oh H, Ahn H et al (2009) Flavonoids as potential inhibitors of retroviral enzymes. J Kor Soc Appl Biol Chem 52(4):321–326

Kong L, Li S, Liao Q et al (2013) Oleanolic acid and ursolic acid: novel hepatitis C virus antivirals that inhibit NS5B activity. Antivir Res 98(1):44–53

Kostova K, Todorov D, Dimitrova M et al (2010). Efficacy of extracts from medtcal plant *Leonorus cardiaca* in herpes simplex-infected cells. In: Zeleva A (ed) Proceedings of the 20th international scientific conference on "Stara Zagora-2010", vol III, medical biology studies, 3–4 June 2010, Stara Zagora, Bulgaria, pp 50–54

Kowalski R (2007) Studies of selected plant raw materials as alternative sources of triterpenes of oleanolic and ursolic acid types. J Agric Food Chem 55(3):656–662

Krasteva I, Kalogan M (2006) Triterpenoid saponins from *Astragalus corniculatus*. Zeitschrift fur Naturforschung B 61(9):1166–1169

Krasteva I, Nikolov S (2008) Flavonoids in *Astragalus corniculatus*. Quim Nova 31(1):59–60

Kuhn M, Winston D (2000) Herbal therapy and supplements: a scientific and traditional approach. Wolters Kluwer Health, Philadelphia, pp 232–235

Li Y, Ma S, Yang Y et al (2002) Antiviral activities of flavonoids and organic acid from *Trollius chinensis* Bunge. J Ethnopharmacol 79(3):365–368

Li S, Chen C, Zhang H et al (2005) Identification of natural compounds with antiviral activities against SARS-associated coronavirus. Antivir Res 67(1):18–23

Lilienfeld S (2002) Galantamine—a novel cholinergic drug with a unique dual mode of action for the treatment of patients with Alzheimer's disease. CNS Drug Rev 8(2):159–176

Maguire M, Dvorkin L, Whelan J (2013) Boston healing landscape project. https://www.bu.edu/bhlp/Clinical/cross-cultural/herbal_index/herbs/Melissa%20Officinalis.html (cited Dec 2013)

Makau J, Watanabe K, Kobayashi N (2013) Anti-influenza activity of *Alchemilla mollis* extract: possible virucidal activity against influenza virus particles. Drug Discov Ther 7(5):189–195

Martins F, Esteves P, Mendes G et al (2009) Verbascoside isolated from *Lepechinia speciosa* has inhibitory activity against HSV-1 and HSV-2 in vitro. Nat Prod Commun 4(12):1693–1696

Mitrocotsa D, Mitaku S, Axarlis S et al (2000) Evaluation of the antiviral activity of kaempferol and its glycosides against human cytomegalovirus. Planta Med 66(04):377–379

Monto A (2003) The role of antivirals in the control of influenza. Vaccine 21(16):1796–1800

Morteza-Semnani K, Saeedi M, Akbarzadeh M (2008) The essential oil composition of Leonurus cardiaca L. J Essent Oil Res 20(2):107–109

Moskova-Doumanova V, Miteva G, Dimitrova M et al (2012) Methanol and chloroform extracts from *Lamium album* L. Affect cell properties of a549 cancer lung cell line. Biotechnol Biotechnol Equip 26:120–125

Mukhtar M, Arshad M, Ahmad M et al (2008) Antiviral potentials of medicinal plants. Virus Res 131(2):111–120

Ou C, Pang Q, Chen X et al (2012) Protocatechuic acid, a new active substance against the challenge of avian infectious bursal disease virus. Poult Sci 91(7):1604–1609

Patora J, Klimek B (2002) Flavonoids from lemon balm (*Melissa officinalis* L., *Lamiaceae*). Acta Pol Pharm 59(2):139–144

Patora J, Majda T, Gora J, Klimek B (2003) Variability in the content and composition of essential oil from lemon balm (*Melissa officinalis* L.) cultivated in Poland. Acta Pol Pharm 60(5):395–400

Petersen M, Simmonds M (2003) Rosmarinic acid. Phytochemistry 62(2):121–125

Piret J, Boivin G (2011) Resistance of herpes simplex viruses to nucleoside analogues: mechanisms, prevalence, and management. Antimicrob Agents Chemother 55(2):459–472

Price J, Merritt H (1941) The treatment of Parkinsonism: results obtained with wine of Bulgarian belladonna and the alkaloids of the USP belladonna. J Am Med Assoc 117(5): 335–337

Seal A, Aykkal R, Babu R, Ghosh M (2011) Docking study of HIV-1 reverse transcriptase with phytochemicals. Bioinformation 5(10):430–439

Serkedjieva J (2004) Antiviral activity of the red marine alga *Ceramium rubrum*. Phytother Res 18(6):480–483

Serkedjieva J, Hay A (1998) In vitro anti-influenza virus activity of a plant preparation from *Geranium sanguineum* L. Antivir Res 37(2):121–130

Serkedjieva J, Manolova N (1992) Plant polyphenolic complex inhibits the reproduction of influenza and herpes simplex viruses. Plant Polyphen 59:705–715

Serkedjieva J, Velcheva M (2003) In vitro anti-influenza virus activity of the pavine alkaloid (−)-thalimonine isolated from *Thalictrum simplex* L. Antivir Chem Chemother 14(2):75–80

Serkedjieva J, Gegova G, Mladenov K (2008) Protective efficacy of an aerosol preparation, obtained from *Geranium sanguineum* L., in experimental influenza infection. Pharmazie 63:160–163

Shishkov S, Dimitrova M, Kostova K et al (2008). Micropropagation and antiviral activity of extracts from *Lamium album* L. In: Proceedings of the international symposium on "New Researches in Biotechnology", USAMV, Bucharest, Romania, F, pp 31–37

Staneva D, Panova D, Rainova L, Asenov I (1982) Bilkite vuv vseki dom. Medicina I fizkultura, Sofia

Stanilova M, Ilcheva V, Zagorska N (1994) Morphogenetic potential and in vitro micropropagation of endangered plant species *Leucojum aestivum* L. and *Lilium rhodopaeum*. Delip. Plant Cell Rep 13(8):451–453

Szlavik L, Gyuris A, Minarovits J et al (2004) Alkaloids from *Leucojum vernum* and antiretroviral activity of Amaryllidaceae alkaloids. Planta Med 70(9):871–873

Todorov D, Dimitrova M, Shishkova K et al (2013) Comparative anti-herpes effects of the chloroform in vitro and in vivo extracts, derived from *Lamium album* L. Bulg J Agric Sci 19(2):190–193

Toshkova R, Nikolova N, Ivanova E et al (2004) In vitro investigation on the effect of a plant polyphenol extract with antiviral activity on the functions of mice phagocyte cells. Pharmazie 59:150–154

Trendafilova A, Todorova M, Nikolov M et al (2011) Flavonoid constituents and free radical scavenging activity of *Alchemilla mollis*. Nat Prod Commun 6:1851–1854

Trendafilova A, Todorova M, Gavrilova A, Vitkova A (2012) Flavonoid glycosides from Bulgarian endemic *Alchemilla achtarowii* Pawl. Biochem Syst Ecol 43:156–158

Varadinova T, Shishkov S, Ivanovska N et al (1996) Antiviral and immunological activity of a new pavine alkaloid (−)-thalimonine isolated from thalictrum simplex. Phytother Res 10(5):414–417

Velcheva M, Petrova R, Danghaaghiin S, Yasanghiin Z (1992) The structure of (−)-thalimonine. J Nat Prod 55:679–680

Vollmer H (1940) Bulgarian treatment of Parkinson's disease: pharmacologic aspects and clinical effects of alkaloids of belladonna root. Arch Neurol Psychiatry 43(6):1057–1080

Wang G, Shi L, Ren Y et al (2009) Anti-hepatitis B virus activity of chlorogenic acid, quinic acid and caffeic acid in vivo and in vitro. Antivir Res 83(2):186–190

Wu L, Yang X, Huang Z et al (2007) In vivo and in vitro antiviral activity of hyperoside extracted from *Abelmoschus manihot* (L.) medik. Acta Pharmacol Sin 28(3):404–409

Yim E, Lee M, Lee K et al (2006) Antiproliferative and antiviral mechanisms of ursolic acid and dexamethasone in cervical carcinoma cell lines. Int J Gynecol Cancer 16(6):2023–2031

Zgorniak-Nowosielska I, Grzybek J, Manolova N et al (1991) Antiviral activity of *Flos verbasci* infusion against influenza and Herpes simplex viruses. Archivum Immunologiae et Therapiae Experimentalis 39(1–2):103–108

Zhang H, Rothwangl K, Mesecar A et al (2009) Lamiridosins, hepatitis C virus entry inhibitors from *Lamium album*. J Nat Prod 72(12):2158–2162