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4 Pharmacological activities and biologically active compounds of Bulgarian medicinal plants

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Abstract

Bulgarian medicinal plants, which have been studied during the last years, are reviewed. The review includes the following families: Amaryllidaceae, Asteraceae, Berberidaceae, Boraginaceae, Fabaceae, Geraniaceae, Lamiaceae, Oleaceae, Onagraceae, Scrophulariaceae, Solanaceae, Ranunculaceae, Rosaceae, Rutaceae, Valerianaceae, Zygophyllaceae. Main pharmacological properties are antiviral, antimicrobial, antioxidative, anti-inflammatory, antiseptic, spasmolytic, sedative and hypotensive.

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Introduction

Bulgaria is situated in the Balkan peninsula, South-East Europe, between 22˚ 21’ 40” and 28˚ 36’ 35” E longitude, and 41˚ 14’ 05” and 44˚ 12’ 45” N latitude, occupies the area of 110 912 km^2 with elevations ranging from 0 to 2925 m and has corresponding subalpine, Mediterranean and continental climates. The relief of the country is quite diverse ranging from plains to low hills and high mountains. The climate is moderate continental to modified continental, but in southern regions reflects rather a strong Mediterranean influence. As a result of this climatic conditions the Bulgarian flora is remarkable for its diversity (3500 plant species including 600 known medicinal plants) [1]. Bulgarian Flora has become very famous for the treatment of Parkinson disease with *Atropa belladonna* L. (Solanaceae), for medication on the Alzheimer’s disease with *Galanthus nivalis* L. (Amaryllidaceae) as well as for the cure for cardio-vascular disorder with *Geranium sanguineum* L. (Geraniaceae).

The aim of the present work is to summarize examinations about biologically active substances and pharmacological activity of Bulgarian medicinal plants in last decades.

Amaryllidaceae

Galanthus nivalis L.

G. nivalis has attracted considerable attention due to synthesis of pharmaceutically important alkaloids [2]. The main alkaloid in this plant is galanthamine; it has shown cholinesterase inhibitory activity and it has found an application in medicine for the treatment of the Alzheimer’s disease [3]. Further, natural and synthetic galanthamine have been found to be as effective as analgetic agents as morphine [4]. In the aerial parts of *G. nivalis* some phenolic acids have been detected. Ferulic acid reached 74% of the phenolic acid mixture, vanillic and syringic acids were observed too, *p*-coumaric, caffeic and protocatechuic acids were found in trace amounts [5].

Leucojum aestivum L.

This plant grows on damp places mainly in south of Bulgaria and over the Black Sea coast. The drug contains about 0,5% alkaloids. Galanthamine and lycorine are the main alkaloids; other alkaloids in the plant are lycorenine, tazettine, isotazettine, homolycorine and estivin. Aerial parts of *L. aestivum* together with its cortex are used as source for industry obtaining galanthamine, which under different forms is widely used in the therapy (Nivalin). Galanthamine is with anti-cholinesterase activity – depress activity on the cholinesterase, to steady acetyl-cholinesterase; this alkaloid enhances the nerve signals propagation, stimulates the excitation processes in the spinal, bulbal and cortical centers, improves the skeletal and smooth muscles tonus

and contraction ability, increases the glandular secretion. Galanthamine is an atropine antagonist, and it's with anticurare activity. In addition it is used in the treatment of poliomyelitis, neuritis, radiculitis, various types of paralysis, myoatrophy etc. and exerts improving effect on the smooth muscles insufficiency of the urinary bladder and gastrointestinal tract [6].

Asteraceae

Achillea millefolium L.

A. millefolium is a perennial herbaceous plant, widespread in Bulgaria. The aerial parts of the plant (Herba et Flores Millefolii) are used to treat inflammation, especially in the intestinal and female reproductive tracts as well as to help the stop of minor bleeding and to treat wounds. In Bulgarian folk medicine the herb is applied in hepatic and urinary diseases, jaundice [7].

Chemical analyses of *A. millefolium* show the presence of essential oil, tannins, flavonoids, sesquiterpene lactones, alkamides, inulin and ascorbic acid [9]. Essential oil of *A. millefolium* possesses antioxidant and antimicrobial properties *in vitro* against *Streptococcus pneumoniae, Clostridium perfringens, Candida albicans, Mycobacterium smegmatis, Acinetobacter lwoffii* and *Candida krusei* while water-insoluble parts of the methanolic extracts exhibited slight or no activity [9].

Calendula officinalis L.

C. officinalis is an annual plant, native of the Mediterranean countries. In Bulgaria this plant is spread as cultivar. Calendula flowers were considered beneficial for reducing inflammation, wound healing, and as an antiseptic. *C. officinalis* is used to treat various skin diseases, ranging from skin ulcerations to eczema. Internally, the soothing effects of Calendula have been used for stomach ulcers and inflammation. Infusion of *C. officinalis* is characterized with low toxicity and hypno-sedative properties [7].

Flavonoids (isoquercitrin, narcissin, and rutin) found in high amounts in *C. officinalis* are thought to account for much of its anti-inflammatory activity [10]. Calendula's high-molecular weight polysaccharides stimulate immune system activity. Organic extract from *C. officinalis* flowers caused a significant dose- and time-dependent reduction of the human immunodeficiency virus type 1 (HIV-1) reverse transcription (RT) activity [11]. Other potentially important constituents include terpenoids (α- and β-amyrin, lupeol, longispinogenin, and sterols), volatile oils, arvoside A and carotenoid pigments.

Carlina acanthifolia All.

C. acanthifolia All. is a perennial herbaceous species growing from 800 to 2000 m asl. Radix Carlinae contains essential oil, tannins, inulin, resins, etc.

The tincture of roots has been used as a diuretic and anti-inflammatory remedy for urinary tract [1,12]. The diuretic activity may be connected to high content of unulin. The plant has also strongly express hypotensive effect. The water and water-ethanolic extracts are applied in gastrointestinal disorders and as antihemorrhoid agent. Externally it is used at skin rash. Sometimes the herb is given in case of faintness, overtired, brain-fag [7,13].

Carthamus lanatus L.

It is a biennial plant growing in the Mediterranean area, which possesses sedative, antitumor and interferon-inducing activities. Sesquiterpene glycosides, flavonoids, triterpenes, sterols, serotonins, lipids, amino acids and carbohydrates are the main biologically active compounds in this plant.

Fractions of methanol, dichloromethane, water extracts and volatiles of *C. lanatus* aerial parts given by oral route at a dose of 2 mg/kg showed significant anti-inflammatory activities in rats. On the contrary, only the water fraction of MeOH extract possesses a significant analgesic activity [14]. The dichloromethane extract exhibited a considerable clastogenic effect and the water extract a negligible one [15]. Volatiles, sterols (sitosterol and stigmasterol) and a fraction of the dichloromethane extract showed strong cytotoxicity (*Artemia salina* assay) [16]. The ethyl acetate fraction of the methanol extract appeared to possess promising natural antioxidant and cytotoxic activities [17]. The dichloromethane extract of *Carthamus lanatus* and its water-alcoholic part exhibited the most significant anti-inflammatory activity [18]. The H_2O/MeOH fraction of the CH_2Cl_2 extract exhibited the highest rates of antibacterial activity; it was well demonstrated both against *S. aureus* and *E. coli*. Interestingly, the same fraction and the volatiles showed significant cytotoxic activity being in correlation with the observed antibacterial activity [19].

Chamomilla recutica (L) Rausch

C. recutica is the most well known and widely used medicinal plant in herbal medicine as an anti-inflammatory, antiseptic, bactericide, spasmolytic, sedative and capillary strengthening drug [1,12].

The plant contains essential oil (bisabolol oxides, farnesene and spiro-ether) that has anti-inflammatory and spasmolytic actions. In Bulgarian chamomile important flavonoids, including apigenin, luteolin and quercetin have been identified [20]. The *C. recutita* flavonoids possess a marked hepatoprotective effect and stimulate the erythroid germ of bone marrow when the rat liver is affected by a toxic carbon tetrachloride action. Apigenin, isolated from the aqueous extract of *C. recutica*, has a clear anxiolytic activity and slight sedative effects but not being anticonvulsant or myorelaxant. The plant contains coumarins (herniarin and umbelliferone); these compounds have

anti-inflammatory properties. In addition, *C. recutica* contains phenolic carboxylic acids such as vanillic, anisic, syringic and caffeic acid. Other relevant constituents are anthemic acid, anthemidine, tannins and matricarin. Six triterpene alcohols and five sterols have been isolated from the flowers of *C. recutica* [20,21]. Extracts of *C. recutita* showed highly significant acaricidal activity [22].

Solidago virgaurea L.

S. virgaurea is a perennial herbaceous plant, growing up to 2200 m asl in Bulgaria. The Herba Virgaureae has been used as an anti-inflammatory means for the treatment of urinary and prostatic diseases. The plant has also been reported to have antibacterial, antimycotic, analgesic spasmolytic, hypotensive and carminative activities [1]. The crude ethanolic and methanolic extracts of *S. virgaurea* showed a moderate bactericidal activity [23].

The plant contains triterpene saponins, flavonoids (derivatives of quercetin, kaempferol and apigenin), polyphenolic acids (ferulic and chlorogenic), tannins, essential oil and polysaccharides [24].

Berberidaceae

Berberis vulgaris L.

It is a shrub, reaching up to 3 m in height. The roots of the plant (Radix Berberidis) as well as root bark and stem bark (Cortex Berberidis) are used in Bulgarian folk medicine to treat chronic liver and bile disorders and kidney inflammations. Berberine that is obtained from *B. vulgaris* roots is applied in official medicine for treatment of cholecystopathiae. Chemical investigations of *B. vulgaris* showed the presence of isoquinoline alkaloids in all parts of the plant [25]. *B. vulgaris* was used also to treat infections and stomach problems. It has also been used internally to treat skin conditions. The alkaloid, berberine, receives the most research and the widest acclaim as the active component of barberry and its relatives. Berberine and its related constituents (such as oxyacanthine) are antibacterial and have been shown to kill amoebae in a test tube study. Berberine inhibits bacteria from attaching to human cells, helping to prevent infections. This compound treats diarrhea caused by bacteria, such as *E. coli.* Berberine also stimulates some immune system cells to function better. Berbamine is another alkaloid found in barberry: it may help to reduce inflammation and is an antioxidant. The bitter compounds in barberry, including the above mentioned alkaloids, stimulate digestive function following meals.

In traditional folk medicine, barberry has been used to treat diarrhea, reduce fever, improve appetite, relieve upset stomach, and promote vigor as well as a sense of well being.

Berberis vulgaris is used to ease inflammation and infection of the urinary, gastrointestinal, and respiratory tracts (such as pharyngitis (sore throat), sinusitis, rhinitis (nasal congestion), bronchitis and traditionally tuberculosis) as well as candida (yeast) infections of the skin or vagina. Barberry extract may also improve symptoms of certain skin conditions including psoriasis, but further studies are needed to confirm these findings [26-28].

Boraginaceae

Alkanna tinctoria (L.) Tausch

Alkanna tinctoria L. is a perennial plant that grows in Southwest and South of Bulgaria.

The roots of *A. tinctoria* contain the naphthoquinone alkannin or shikonin (they are optics antipodes with common formula $C_{16}H_{16}O_5$), alkanan and alkane's esthers. The active compound alkannin (shikonin) has antiviral, antibacterial, anti-inflammatory activity and shows certain anticancer activity. The content of alkannin was studied by spectrophotometric assay in the roots of *A. tinctoria*. The quantity of this compound varies significantly: from 3.40 to 13%. The highest percentage of this compound has been found in the crust of the roots (10.06 - 12.85%) [29].

Fabaceae

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

Astragalus species are used by Bulgarian folk medicine as a diuretic for treatment of hypertension, renal disorder, nervous diseases and rheumatism. It is also used as a diaphoretic. Flavonoid mixture of some species revealed strong antioxidant activity. The antimicrobial effect of astragalus flavonoids has been studied as well. Astragalus may improve the activity of existing immune cells and also may increase the production of new immune cells. In addition, it has been shown that oral astragalus has mild antiviral activity. Therefore, it may help to prevent colds and other respiratory infections when it is taken consistently over long time periods. However, it is not very useful for treating cold or flu. Both the possible immune-boosting and antiviral effects of astragalus make its use widespread among people living with AIDS and other chronic conditions, including chronic fatigue syndrome; although it appears to be safe when used in limited amounts, little scientific information supports its effectiveness as an antiviral agent. Astragalus has been used to treat high blood pressure because it may have diuretic effects that promote the elimination of excess water from the body. It may also have other cardiovascular effects that may benefit individuals with heart failure. In addition, astragalus may protect kidney tissue from damage when blood flow to the kidneys is blocked and then re-started. A small study of patients with hepatitis C showed improvement after 6 months of treatment with

astragalus. It is believed that astragalus may help to reduce the side effects of radiation and drug treatments for cancer although this possible effect is not completely understood. Small studies on animals show that astragalus may increase sperm production as well as its activity (motility). However much more research is needed before astragalus may be recommended for oral use to treat any of these conditions.

A purified mixture containing mostly saponins (PMS) from *A. corniculatus* Bieb. was used in an *in vivo* model to demonstrate its protective effect against myeloid Graffi tumour in hamsters. Survivability, tumour growth and tumour transplantability were followed. Comparative studies revealed that the intraperitoneal administration of PMS: (i) decreased the tumour transplantability; (ii) inhibited tumour growth in the early stages of tumour progression; (iii) increased the mean survival time; (iv) reduced the percentage mortality. These results suggest that appropriate use of PMS could outline a promising strategy for the treatment of myeloid Graffi tumour [30,31].

Presence of kaempferol-3-*O*-rutinozide (nicotifolin), kaempferol-3-*O*-glucoside (astragalin), quercetin-3-*O*-rutinoside (rutin), quercetin-3-*O*-glucoside (isoquercitrin), quercetin-3-*O*-galactoside (hyperoside), isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, apigenin, luteolin, ferulic and caffeic acids was established in the herb of *Astragalus vesicarius* [32].

Glycyrrhiza glabra L.

G. glabra is a perennial herbaceous plant growing in a moisture place in North Bulgaria. Radix Liquiritiae shows anti-inflammatory, expectorant, demulcent, laxative and diuretic activity. It is taken internally in the treatment of bronchial problems, asthma, peptic ulcer, catarrhal infections of the urinary tract, arthritis and allergic complaints. In addition the plant has been used for mild Addison's disease and other adrenal insufficiencies, such as hypoglycemia [1]. Rajesh and Latha [33] showed that *G. glabra* is a potential antioxidant and attenuates the hepatotoxic effect of CCl_4.

The saponin glycyrrhizin (known also as glycyrrhizic acid) is the main constituent of the roots; in the Bulgarian populations its content varies from 8 to 11%. The plant also contains various sugars, starches, flavonoids, amino acids and essential oil [34]; isoflavonoids (glabridin, hisplaglabridin A, hisplaglabridin B and 4' *O*-methyl glabridin) were reported to provide protection against oxidative stress [33].

Geraniaceae

Geranium macrorrhizum L.

G. macrorrhizum is a perennial herb native from the Balkans, occurring occasionally also in the Carpathian Mountains and in the Alps. It is known as

"Zdravets" which means "healthy" in Bulgarian. The aerial parts as well as the roots have been shown to contain physiologically active principles causing hypotensive and spasmolytic effects. Central depressive action of methanol extracts has also been demonstrated. An essential oil is distilled from leaves and steams with an average yield of 0.1%. In this Zdravets oil oxygenated sesquiterpenes are prevailing with germacron as the major component (50%). The whole plant is rich in tannins with more in the stems than in the green foliage. No alkaloids and cardiological glycosides have been found. The presence of six flavonol aglycones in aerial parts of *G. macrorrhizum* has beem established (kaempferol 3-methyl ether (*isokaempferide*), kae-3,7-dimethyl ether (*kumataketin*), kae-3,4'-dimethyl ether (*ermanin*), quercetin, qu-7,3'-dimethyl ether and qu-3,7,3',4'tetramethyl ether (*retusin*)); two of these, namely ermanin and retusin were said to be present in the roots too.

A methanol extract from leaves possesses strong hypotensive activiy, cardiotonic, capillary anticomplementary and sedative action as well [34-36].

Geranium sanguineum L.

G. sanguineum is also a perennial grassy plant. In Bulgarian folk medicine the root systems are used as astringent and for anti-inflammatory treatments of diarrhea, gastric-enteric catarrh and dysentery. It is applied also in cases of scrofula tuberculosis [35]. A polyphenol complex obtained from the roots of *G. sanguineum* inhibited the reproduction of influenza viruses type A and B *in vitro* and *in ovo* and protected mice from mortality in the experimental influenza infection [38]. The polyphenol complex contains flavonoids (0.172%), tannins (16.15%) and catechins (2.91%). It was found also that the polyphenol complex significantly inhibited the reproduction of influenza virus A/FPV in CPE and also has effect on the process of differentiation and the biosynthesis of antibiotic substances from *Streptomycetes* [39]. Water and ethanol extracts from root systems of *G. sanguineum* are used in cases of gastrointestinal disorders and against infections and inflammatory conditions. The water extract inhibited the replication of herpes simplex virus type 1 and type 2. The ethanol root extract of plant showed a broad spectrum of antimicrobial activities as well as preventive effects against radiation [40,41].

Lamiaceae

Mentha piperita L.

M. piperita is distributed on moist and defended from wind places. It's one of the world's oldest medicinal herbs, and it's used in both Eastern and Western traditions. Ancient Greek, Roman and Egyptian cultures used the herb in cooking and medicine. The rural people from Bulgaria use it against pains in stomach and enteritis. They use it also in liver and gallic trouble, as

carminative drug, as well for elimination the symptoms of intoxication from gastrointestinal origin [7]. The wild *Mentha piperita* isn't used for obtaining the oil since the content of menthol is too low in comparison with that of the cultivated Bulgarian representatives of such plant. The Oleum Menthae is getting through distillation with water vapor of dry aerial part of the plant. The oil is dissolved in 95% ethanol. The main compounds of the oil are menthol (50-80%) and the ketons menthon and piperiton, menthofuran, jasmon and pulegon. The oil also contains terpenoids as α-pinen and β-pinen, α-phellandren, and also ester-connected with menthol or free acetic acid and isovaleric acid. The quality depends mainly upon menthol content in the oil [6]. The herb, the oil and the menthol find wide applications in different cases. Folium Menthae is used as analgesic, anodyne, antibacterial, antiparasitic, antispasmodic, carminative, antiseptic and as refreshing remedy for gastrointestinal deseases and gallic-trouble accompanied with colics, nausea vomiting. The herb has salient cholagogic and choleretic action. Oleum Menthae is used as analgesic, carminative, refreshing and topical anaesthetize remedy.

Mentha spicata (L.) Huds. group

The cultivated Bulgarian representatives of *Mentha spicata* group contain essential oil together with apigenin, apigenin-7-*O*-glucoside, luteolin-7-*O*-glucoside, hesperidin, chlorogenic acid, ursolic and oleanolic acid. The total content of flavonoids is 0.07-0.22%, as the average content (0.15%) and the maximum content (0.22%) of flavonoids is registered in *Mentha spicata ssp. spicata* [42]. Flavonoids in spearmint (*Mentha spicata* L.) are the accompanying biologically active substances, which enhance its antispasmodic, cholagogic, antiseptic, adstingent, diuretic, and antioxidant effect [6,43-45]. Folia *Menthae spicatae,* with the exception of essential oil, also contains diosmetin, apigenin, luteolin, hesperidin, vitamin C, ursolic and oleanolic acid, 5-10% of tannins of the labiathen type, bitter compounds and microelements (Cu, Mg, Sr) [46,47]. The herbal tea inhibited Fe absorption.; therefore, its effect should be considered, especially for children and anemic patients [48]. Luteolin and apigenin enhance the diuretic effect of this drug, while hesperidin, together with vitamin C, has a favourable effect on its P-vitamin activity. The biological effect of phenolic acids is connected with increase the antiseptic effect of polyphenols [7].

Sideritis scardica Griseb.

S. scardica is a perennial herbaceous plant, endemic from Balkans. Aerial parts of plants are used for treatment of cold and chill diseases [1]. Extracts with butanol and with ethyl acetate and the total methanol extracts from *S. scardica* showed a strong radical scavenging activity against $DPPH^{\bullet}$, close

to that of rosmarinic acid. The antioxidant activity of *Sideritis* extracts was attributed to the presence of flavonoids and phenylpropanoid glycosides [49].

Oleaceae

Fraxinus ornus L.

It is a tree up to 20 m in height with a smooth, grey bark. It grows wild in the Mediterranean region and south-cental Europe, northwards to the south Czech Republic and north- eastern Romania. *Fraxinus ornus* bark is used in the traditional medicine for wound healing and treatment of inflammation, arthritis and dysentery. Biological studies on crude extract, many of its constituents and some extractives have revealed significant antimicrobial, antioxidative and photodynamic damage prevention, wound healing, anti-inflammatory, immunomodulatory and antiviral activities providing a support to the ancient claims.

Chemical studies on bark, leaves and flowers of *F. ornus* have shown the presence of many compounds belonging mainly to the groups of hydroxycoumarins, secoiridoid glucosides, phenylethanoids and flavonoids. *F. ornus* is rich in hydroxycoumarins. Esculin, esculetin, fraxin and fraxetin are the main components of the bark. Esculetin (6,7-dihydroxycoumarin) and its diacetate exhibited a marked inhibitory effect on Newcastle diseases replication in call cultures at concentrations of 36 μM and 62 μM respectively [50]. Fraxetin is a stronger antioxidant than esculetin [51]. Esculetin, fraxin and fraxetin are mainly responsible for the antimicrobial properties of *F. ornus* bark extracts [52]. The bark is a source for the industrial production of esculin. The leaves and the flowers contain cichoriin as a main component. Oleoside type secoiridoid glucosides were found in the bark and the leaves. The bark contains ligstroside, insularoside, hydroxyornoside, oleuropein, framoside and hydroxyframoside A and B. Flavonoids are mainly derivatives of quercetin. They occur free or as glycosides. From the bark, six caffeic acid esters were isolate and identified. Caffeic, gallic and *p*-coumaric acids were found in the bark. The presence of ursolic acid in leaves and flowers and fatty acids in the flowers was reported. The bark was found to contain tannins (2%) of pyrocatechol type [53].

Onagraceae

Epilobium hirsutum L.

E. hirsutum is a perennial plant that occurs in moist places up to 1400 m asl in whole country. Water-alcohol extract and four fractions from the polyphenolic mixture of *E. hirsutum* have a significant inhibitory effect on the reproduction of influenza viruses *in vitro*, *in ovo* and *in vivo* ; in addition the extracts showed a broad-spectrum of antimicrobial activity. Main biological

active constituents of the polyphenolic mixture are flavonoids and tannins [35]. Battinelli [54] reported that ethanolic extracts of *E. hirsutum* showed antimicrobial activity in a range of concentrations between 10 and 650 μg/ml of dry extract on Gram-positive and Gram-negative bacteria, yeasts and fungi *in vitro*. The plant is used also in enlarged prostate, prostatitis, cystitis, burning feeling when urinating, and burning feeling after prostate operation. There are data for anti-tumour action of *Epilobium* extract [55].

Ranunculaceae

Paeonia peregrina Mill

P. peregrina is a perennial herbaceous plant that occurs rarely in whole Bulgaria up to 1000 m asl. *Paeonia* radix is used as an anticoagulant, anti-inflammatory, analgesic and sedative agent. It is also frequently used as a remedy for female genital diseases. Chemical investigations on *P. peregrina* roots described the presence of anthocyanidins, proanthocyanidins, in flowers, flavonoid glycosides in leaves, benzoic and gallic acids in leaves and roots, terpenoids, triterpenoids, tannins in roots. The lipids of seeds and alkaloids of roots have been also studied [56]. In the Bulgarian folk medicine the herb is used also for treatment of psycho-neurological diseases (epilepsy) [7].

Rosaceae

Alchemilla vulgaris comp.

In Bulgarian folklore *A. vulgaris*. is known as king's herb. It is distributed in all Bulgarian mountains over 1000 m altitudes. The rural people used it for treatment on inflammation on mouth cavity; hemorrhage from nose, furuncles as well as at gynecological disorders. *A. vulgaris* is valuable remedy for the uterus, helping to regulate the glandular activity of the uterine lining and reduce excessive bleeding. The infusion of plant is used as astringent, antidiarrhetic and inti-inflammatory agent. *A. vulgaris* contains about 10% tannins, flavonol glycosides, leucoanthocyanidins, sugars, resins and vitamin C. In the roots gallo- and ellagitannins predominate [1,7,8].

Rutaceae

Ruta graveolens L.

R. graveolens is native of the Mediterranean region. It is known as medicinal plant containing a diversity of biologically active compounds: alkaloids, coumarins, flavonoids and essential oil. Quinoline alkaloids are the main biologically active compounds in the aerial parts of plants. The total alkaloid mixture of the aerial parts of *R. graveolens* varies from 0.20% to 0.96% between the different Bulgarian populations and has a pronounced

spasmolytic effect while the alkaloid arboretrin has a powerful abortive effect. Four new 2-alkyl-4(1H)-quinolone alkaloids together with 13 known components have been isolated [57] from the aerial parts of *R. graveolens* cultivated in Bulgaria. Rutin is the main flavonoid glycoside in the plant and reduces the permeability and fragility of capillaries. The amount of rutin in the cultivated plants varies from 1% to 2%. The essential oil of *R. graveolens* has anti-inflammatory and antihelmintic effect while the coumarins have a sedative and strongly expressed photodynamic effect [58]. The methanol, petroleum ether, ethyl acetate and water-methanol extracts of *R. graveolens* were found to possess antimicrobial and cytotoxic activities [59].

In Bulgarian folk medicine the aerial parts of plants are used as sedative, soporific and anti-helmintic mean. The plant is applied also as spasmolitic drug in case of menstrual pains, stomach-intestial inflammation [7].

Scrophulariaceae

Verbascum densiflorum Bertol. (*Verbascum thapsiforme* Schrad)

V. thapsiforme is a biennial herbaceous plant, widespread in Bulgaria. *Verbasci* Flos is a traditional herb for treatment of sore throat and cough. Other uses of the flowers are for chills, dry coughs, and phlegm congestion due to the mild expectorant action of the saponins. Both flowers and leaves possess mildly demulcent, expectorant, and astringent properties. Expectorant actions may be due to the plant's saponin content. Verbasci Flos contains water-soluble mucilage polysaccharides, which after hydrolysis yield mainly D-galactose, as well as arabinose, D-glucose, traces of D-xylose, L-rhamnose, D-mannose and L-fucose. Other components: uronic acids, flavonoids (apigenin, luteolin and their 7-*O*-glucosides together with kaempferol and rutin), protocatechuic acid, caffeic acid and caffeic acid derivatives including ferulic acid and verbascoside, iridoid monoterpenes (aucubin, 6-*β*-xylosylaucubin, methylcatalpol, isocatalpol), triterpenes.

The lyophilized infusion from flowers of *V. thapsiforme* (FVI) showed antiviral activity in *in vitro* studies against Fowl plague virus, several influenza A strains, influenza B strain as well as Herpes simplex virus. Influenza viruses titer decreased by 1- 3 log units, while of H. simplex virus by 2.3 log. FVI has shown virucidal activity on H. simplex virus at 300 μg/ml, but did not inactivate influenza viruses. Phytochemical investigations of FVI have shown the presence of flavonoids, iridoids, phenolic acids, saponins, amino acids and free sugars [60-62].

Solanaceae

Atropa bella-donna L.

Belladonna is native from Europe and Asia. Some people say it originated in India. It has been cultivated on almost all continents for centuries. During

that time, it has been used for its many medical properties and its hallucinogenic effects. According to old legends, the plant belongs to the devil that takes care of it as the need arises, and only takes a night off once a year. That date is Walpurgis, when he prepares for the witches' sabbath. The apples of Sodom are held to be related to this plant. The generic name of the plant, Atropa, comes from the Greek word Atropos, one of the Fates who held the shears to cut the thread of human life, a reference to the poisonous nature of Belladonna.

Atropa bella-donna is widely distributed in mountainous parts in Bulgaria and especially in Stara planina, Rhodops, Sredna gora. All parts of the plant may prove to be poisonous even when taken in small quantities. The alkaloid contents of the *Atropa belladonna* can be 0.3-0.75% in the leaves, 0.2-0.6% in the steams and up to 1.3% in the roots; the main active constituents are the tropane alkaloids atropine, L-hyoscyamine, hyoscine (scopolamine), apoatropine and the pyrrolidine alkaloid cuscohygrine. In the leaves are present free volatile bases (pyridine, *N*-metylpyridine, *N*-methylpyrrolidine and nicotine); the plant also contains cumarines (umbelliferone, esculetin, scopolin and its aglycone scopoletin), flavonol glycosides of quercetin and kempferol and tannins [7]. Atropine, hyoscyamine and scopolamine interfere with the acetylcholine transference in the synapses. Acetylcholine is a neurotransmitter of the parasympathetic nervous system. These substances don't prevent the liberation of the neurotransmitter, but merely block the receptor sites. This blocking action prevents specific acetylcholine binding to appropriate receptors. Atropine, hyoscyamine and scopolamine increase parasympathetic activity. They produce Sleep EEG patterns and abolish the awakening effect (ß-waves), decrease heart rate, stimulate salivary glands, etc. Atropine is an acetylcholine antagonist; it reverses it's effects completely. Atropine counteracts the effects of cholinesterase-inhibitors. Nerve gasses, like sarin, and some organic insecticides (alkylphosphates: malathion, parathion, etc) inhibit cholinesterases.

The herb has a narcotic, sedative, anodyne, antispasmotic and myriatic effect [6]. In the beginning of this century the Bulgarian natural healer Ivan Raev has introduced this plant for treatment of Parkinson disease, in view of the fact that *Atropa bella-donna* contains atropine. This method was later called “cura bulgara”.

Today, Belladonna is still a very important plant in the scientific and medical communities because of its chemical constituents.

Datura stramonium L.

The primary biologically active substances in *D. stramonium* are the alkaloids atropine and scopolamine. Atropine has been used in treating Parkinson's disease, peptic ulcers, diarrhea, and bronchial asthma. Scopolamine

is available by prescription primarily for treating motion sickness. In ancient herbal medicine, *D. stramonium* is used internally to treat madness, epilepsy, and melancholia. Externally, it formes the basis of ointments for burns and rheumatism. More recently, preparations from the plant have been used as ingredients in some asthma medicines. With this exception, however, plant is generally considered too toxic for medical applications nowadays. The seeds of *D. stramonium* are used for obtaining L-hyoscyamine [7].

The alkaloid spectrum in roots, leaves and seeds of Bulgarian *D. stramonium* have been investigated by GC-MS. Twenty-nine tropane alkaloids have been detected. Twelve of them are new constituents for the species and the two tropane esters 3-(3'-acetoxytropoyloxy)tropane and 3-(2'-hydroxytropoyloxy)tropane are described for the first time [63]. The plant contains also a flavonoid (rutin) and tannins (4-6%) [6].

Valerianaceae

Valeriana officinalis L.

In Bulgaria *V. officinalis* is widespread from sea level up to 2000 m above sea level, occurring widely in the mountains and foothills and along the Black Sea coast, but rarely in the plains and lowlands. The roots of *V. officinalis* show a powerful sedative action that is due to valepotriates and epoxy-iridoid esters [7]. It has been described that the flavonoid 2S(-)-hesperidin in valeriana has sedative and sleep-enhancing properties whereas 6-methylapigenin was found to have anxiolytic properties and was able to increase the sleep-enhancing properties of hesperidin [64]. The total valepotriate content of Bulgarian *V. officinalis* exceedes 0.6% and is above 1% in some populations. Valepotriates are concentrated in the roots in contrast to flavonoids that are concentrated mainly in the aboveground parts [65].

Zygophyllaceae

Tribulus terrestris L.

The plant has long been quite popular in the folk medicine of the Oriental countries and Bulgaria as a medicinal plant in the treatment of sexual deficiency. The plant is used against various diseases, including cardio vascular, impotence, edema, eye trouble, skin itch. It is also included in many dietary supplements claimed to have biostimulating activity. Recently, antitumoral activity and effects on cardiovascular system have been also found [66]. *T. terrestris* growing in Bulgaria is a source for the industrial production of the original preparation “Tribestan” produced by Sopharma Joint Stock Co., Bulgaria. Tribestan consists of the *n*-butanol extract of the aerial parts of the same plant and is successfully applied for treatment of sexual deficiency. The active components of Tribestan are saponins (furostanol and spirostanol);

flavonoid glycosides, alkaloids and some amides have been reported to occur in this plant [67].

References

1. Ivancheva S. and Stancheva, B. 2000, J. Ethnopharmacol., 69, 165.
2. Sidjimova, B., Berkov, S., Popov, S. and Evstatieva, L. 2003, Pharmazie, 54, 891.
3. Bastos, J., Xu, L., Nanayakkara, N., Burandt, C., Moraes-Cerdeira, R. and McChesney, J. 1996, J. Nat. Prod., 59, 638.
4. Jackson, A. and Martin, J. 1966, J. Chem. Soc., 2061.
5. Nikolova, M. and Gevrenova, R. 2005, Pharm. Biol., 43, 289.
6. Asenov, I. and Nikolov, S. 1988, Pharmacognosy, Science & Art, Sofia.
7. Petkov, V., 1982, Modern Phytotherapy, Science & Art, Sofia.
8. Asenov, I., Gusev, Ch., Kitanov, G., Nikolov, S. and Petkov, T. 1998, Herbal collection, Biler, Sofia.
9. Candan, F., Unlu, M., Tepe, B., Daferera, D., Polissiou, M., Sokmen, A., and Akpulat, HA. 2003, J Ethnopharmacol., 87, 215.
10. Peneva, P., Ivancheva, S., Vitkova, A. and Kozovska V. 1985, Plant Sciences, 22, 50.
11. Kalvatchev, Z., Walder, R. and Garzaro, D. 1997, Biomed. Pharmacother., 51, 176.
12. Leporatti, M.L. and Ivancheva St. 2003, J. Ethnopharmcol., 87, 123.
13. Vitkova, A. 1997, Annuaire de l'Universite de Sofia 'St. Kliment Ohridski", 88, 85.
14. Bocheva, A., Mikhova, B., Taskova R., Mitova, M. and Duddeck, H. 2003, Fitoterapia, 74, 559.
15. Topashka-Ancheva, M., Taskova, R., Handjieva, N., Mikhova, B. and Duddeck H. 2003, Z. Naturforsch, 58c, 833.
16. Mitova, M., Taskova, R., Popov, S., Berger, R., Krings, U. and Handjieva N. 2003, Z. Naturforsch, 58c, 697.
17. Taskova, R., Mitova M., Topashka M., Seizova K. and Duddeck H. 2003, Compt. rend Acad. bulg. Sci., Chimie organique 56, 13.
18. Jalil, S., Taskova, R., Mitova, M., Duddeck, H., Choudhary, M.. and Atta-ur-Rahman, I. 2003, Z. Naturforsch. 58c, 830.
19. Taskova, R., Mitova M., Najdenski H., Tzvetkova I. and Duddeck H. 2002, Fitoterapia 73, 54.
20. Peneva, P., Ivancheva, S. and Terzieva L. 1989, Plant Sci., 26, 25.
21. Ganeva, Y., Chanev, Chr. and Dentchev, T. 2003, Farmacia, 50, 3.
22. Macchioni, F., Perrucci, S., Cecchi, F., Cioni, P.L., Morelli, I., and Pampiglione S. 2004, Medical & Veterinary Entomology, 18, 205.
23. Gross, S., Goodarzi, G., Watabe, M., Bandyopadhyay, S., Pai, S. and Watabe, K. 2002, Nutr. Cancer, 43, 76.
24. Ivancheva, S. and Vitkova, A. 1997, Farmacia, 44, 17.
25. Philipov, S., Evstatieva, L. and Michailova, M. 1995, Phytol. Balcan., 2, 75.
26. Kaneda, Y., Torii, M., Tanaka, T. and Aikawa, M. 1991, Ann. Trop. Med. Parasitol., 85, 417.
27. Ivanovska, N. and Philipov, S., 1996, Int. J. Immunopharmacol. 18, 552.

28. Shamsa, F., Ahamadiani, A. and Khosrokhavar, R. 1999, J. Ethnopharmacol., 64, 161.
29. Genova E., Kitanov G. and Stafanova I. 1995, Pharmacia, 42, 15.
30. Krasteva I., Toshkova R. and Nikolov S. 2004, Phytother Res.,18, 255.
31. Krasteva, I., Nikolov, S. and Pavlova, D. 1999, Farmacia 46, 6.
32. Krasteva, I., Benbassat, N. and Nikolov, S. 2000, Farmacia, XLVII, 20.
33. Rajesh, M. and Latha, M. 2004, Ind. J. Pharmacol., 36, 284.
34. Genova, E., Hristova, J. and Beeva J. 1998, Pharmacia, 45, 10.
35. Ivancheva, S. and Wollenweber E. 1989, Indian Drugs, 27, 167.
36. Ivancheva, S., Manolova, N., Serkedjieva, J., Dimov, V. and Ivanovska, N. 1992, Plant Polyphenols, W. Hemingway and Laks, P. (Eds.), Premium Press, New York and London, 717.
37. Ivanovska, N., Vuleva, V., Stefanova, Z. and Ivancheva, S. 1993, Comptes rendus de l'Academie bulgare des Sciences, 46, 131.
38. Toshkova, R., Nikolova, N., Ivanova, E., Ivancheva, S. and Serkedjieva J. 2004, Pharmazie 59, 150.
39. Naidenova, M., Ivancheva, S. and Serkedjieva J. 1998, Polyphenol Communications, Charbonnier, F., Delacotte, J., Rolando, Ch. (Eds.), Impression Reflex Printing-Publilettrage, France, 519.
40. Serkedjieva, J. and Ivancheva, S. 1999, J. Ethnopharmacol., 64, 59.
41. Velev, G., Ivancheva, S. and Bourzeix, M. 1994, Polyphenols Communications, Brouillard, R., Jay, M., and Scalbert. A. (Eds.), INRA Edition, Paris, 401.
42. Stoeva, T., Ivancheva, I. and Behar, M. 1997, Phytol. Balcan., 3, 89.
43. Tsitsin, NV. 1962, Atlas of Medicinal Plants in USSR, Gos. Izd. Med. Lit., Moscow.
44. Stoyanov, N. and Kitanov, B. 1960, Wilde Nutzpflanzen Bulgariens, Verlag der Bulgarischen Akademie der Wissenschaften, Sofia.
45. Tourova, A. D. and Sapozhnikova, E. N. 1983, Medicinal plants in USSR and their use, Medicina, Moscow.
46. Dorfler, H. P. and Roselt, G. 1989, Heilpflanzen gestern und heute, Verlag der Bulgarischen Akademie der Wissenschaften, Sofia.
47. Gossudarstvennaya Pharmacopeya SSSR 10, 1973, 854.
48. Akdogan, M., Gultekin, F. and Yontem, M. 2004, Toxicol. Ind. Health, 20, 119.
49. Koleva, I.I., Linssen, J.P., van Beek, T.A., Evstatieva, L.N., Kortenska, V. and Handjieva, N. 2003, J. Sci. Food Agric., 83, 809.
50. Galabov, A., Iosifova, T., Vassileva, E. and Kostova, I.1996, Z. Naturforsch., 51c, 558.
51. Marinova, E., Yanishlieva, N. and Kostova, I. 1994, Food Chemistry, 51, 125.
52. Kostova, I, Nikolov, N. and Chipilska, L. 1993, J. Ethnopharmacol., 39, 205.
53. Kostova, I. 2001, Fitoterapia, 72, 471.
54. Battinelli, L., Tita, B., Evandri, MG. and Mazzanti, G. 2001, Farmaco, 56, 345.
55. Voynova, E., Dimitrova, S., Naydenova, E. and Karadjov, P. 1991, Acta Physiol. Pharmacol. Bulg., 17, 50.
56. Todorova, D. and Kostova, I. 1997. Comptes rendus de l'académie bulgare des sciences, 50, 43.
57. Kostova, I., Ivanova, A., Mikhova, B. and Klaiber, I. 1999, Monatshefte für Chemie 130, 703.

58. Vitkova, A. and Philipov, S. 1999, Phytol. Balcan., 5, 53.
59. Ivanova, A., Mikhova, B., Najdenski, H., Tsvetkova, I. and Kostova, I. 2005, Fitoterapia (in press).
60. Slagowska, A., Zgorniak-Nowosielska, I. and Grzybek, J. 1987, Pol. J. Pharmacol. Pharm., 39, 55.
61. Zgorniak-Nowosielska, I., Grzybek, J., Manolova, N., Serkedjieva, J. and Zawilinska, B. 1991, Arch. Immunol. Ther. Exp., 39, 103.
62. Serkedjieva, J. 2000, Phytother. Res., 14, 571.
63. Philipov, S. and Berkov, S. 2002, Z. Naturforsch., 57c, 559.
64. Marder, M., Viola, H., Wasowski, C., Fernandez, S., Medina, J. and Paladini, A. 2003, Pharm. Biochem. Behav., 75, 537.
65. Evstatieva, L., Handjieva, N., Popov, S. and Pashankov, P. 1993, Pl. Syst. Evol., 185, 67.
66. Xu, Y., Chen, H., Liang, H., Gu, Z., Lui, W., Leung, W. and Li, T. 2000, Planta Med., 66, 545.
67. Kostova, I., Dinchev D., Rentsch G., Dimitrov, V. and Ivanova A. 2002, Z. Naturforsch., 57c, 33.