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SIGNIFICANCE AND EFFICACY OF TRITERPENE SAPONIN HERBAL DRUGS WITH EXPECTORANT ACTION IN COUGH THERAPY

SUMMARY

Medicinal plants, about 10,000 species, are used in medicine to treat diseases or preserve human health because they are rich in the content of alkaloids, glycosides, resin, essential oils, etc. Phytopreparations are very important in the treatment of productive cough, both in self-medication and on the recommendation of health professionals. Medicinal preparations containing saponosides stimulate the glands to secrete bronchial secretions through a gastropulmonary reflex mechanism, which results in the production of rarer, watery mucus in the bronchial glands. They act on thick mucus as surfactants, reduce its viscosity and facilitate expectoration. Among triterpene saponosides used as expectorants, ivy leaf extract has proven therapeutic efficacy based on *in vitro* and *in vivo* studies. For other saponin expectorants (primrose root and flower, liquorice root (radix dulcis = sweet root), senega root, grindelia herb, common polypody rhizome), only the traditional application has been documented, which is confirmed by many studies. Due to the increased application, as well as the necessary confirmations of efficacy, there is a need for future research to analyze in more detail the pharmacological effects of triterpene saponins of expectorant action and their effect on the human organism.

Keywords: HMP-herbal medicinal products, cough, ivy, primrose, liquorice and senega

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INTRODUCTION

Herbal medicines (HMP - *herbal medicinal products*) are medicinal preparations that contain active substances of plant origin, they are also called phytopreparations (Applequist, 2006; Mauseth, 2016; Petrović, 2016; Heinrich *et al.*, 2018; Petrović *et al.*, 2021). They are obtained from plant organs or products that are formed in their parts (fatty oils, essential oils, resins). Medicinal properties of plants are defined by one or more pharmacologically active substances (API-*active pharmaceutical ingredient),* a group of ingredients with similar or different chemical structure (Mauseth, 2016; Heinrich *et al.*, 2018; Watson, 2019; Popović *et al.*, 2021).

Medicinal properties can be derived from secondary metabolites, a combination of different compounds (alkaloids, tannins, phenols), which are stored only in certain parts of the plant or in the whole plant (Heinrich *et al.*, 2018; Popović *et al.*, 2018; Šarčević Todosijević *et al.*, 2019). The compounds are specific and depend on the family, genus and species of plant, some are constantly present in plants, while some occur in response to the presence of microorganisms or physical injury (Šarčević Todosijević *et al.*, 2019). Medicinal substances are extracted from herbal drugs by various pharmaceutical-technological processes and are part of traditional, but also modern medicine (Applequist, 2006; Watson, 2019).

The compounds found in plants can have a physiological effect on the organism, and for that reason their efficiency is determined by long-term tests, and the acquired knowledge is used in the production of medicinal preparations. Each phytopreparation has a certain pharmacological effect and a procedure for application (Heinrich *et al.*, 2018; Šarčević Todosijević *et al.*, 2018; 2019; Popović *et al.*, 2019). The effect of medicinal products containing medicinal herbs depends on the content of active pharmaceutical ingredients and combinations of medicinal herbs contained in these preparations. Compared to synthetic drugs, herbal medicines take longer to exert their effects and have weaker therapeutic effects (Petrović & Vukomanović, 2018; Kruttschnitt *et al.*, 2020; Dročić *et al*., 2020; Đorđević *et al.*, 2020).

Assessing their effectiveness is therefore difficult. However, compounds isolated from plants are very often used for the production of modern, synthetic medicinal preparations (Heinrich *et al*., 2018; Petrović & Vukomanović, 2018).

In recent years, there has been a trend of returning to traditional organic plant production and the growing popularity of their use in treatment (Popović *et al*., 2018; Stevanović *et al.*, 2019; Vojnov *et al*., 2020; Ikanović & Popović, 2020; Stevanović *et al*., 2021; Popović *et al*., 2021; Lemenkova, 2021; Kwee & Maw, 2021; Petrović *et al*., 2021). Medicinal herbs can be used at home conditions in the form of teas, macerates, tinctures, powders or juices. In the pharmaceutical industry, herbal extracts are mainly tableted or made into syrups, powders, lozenges, effervescent tablets or solutions (Applequist, 2006; Watson, 2019; Petrović *et al*., 2021).

Herbal medicines are registered on the basis of clinical confirmation of efficacy, on the basis of experimental data or data obtained from the literature. In the absence of clinical research, many years of experience in traditional medicine are accepted, provided that no side effects are recorded (Petrović *et al.*, 2016).

In Europe, the trade in herbal preparations is regulated by a European directive that regulates the quality, safety and efficacy of herbal preparations, and confirmed by the Committee on Herbal Medicinal Products (HMPC) European Medicines Agency (EMA) (CPMP/QWP/2819/00).

Preparations containing herbal components must have a standard quality, which means their physical, chemical and microbiological stability, as well as a standardized composition with a high degree of purity (HSTPD, 2019; STPAC 1996; GST 2002).

Herbal drugs are rarely fresh, so dried parts of plants or whole plants are most often used to extract medicinal substances, from which various preparations are obtained by processing. Extraction of API from plants is usually done by extraction, distillation, squeezing, purification or fermentation..Powdered herbal drugs, extracts, essential oils, fatty oils, squeezed juices or processed exudates are used as semi-finished products for the production of herbal medicines. Essential oils are obtained by squeezing, distillation or extraction (Applequist, 2006; HSTPDM, 2009; CPMP/ICH/367/96).

The method of extraction of medicinal substances from plants consists of three phases (Applequist, 2006; Petrović, 2016):

1) Analysis of extracts by mass spectrometry to identify components and their relative amounts.

2) Examination of extracts in order to determine their effect on cells and their significance for a certain disease.

3) Optimization of extracts to improve their effects.

Newer sophisticated pharmaceutical-technological separation operations yield more efficient extracts with higher concentrations of active ingredients.

In recent years, the use of medicinal herbs has been on the rise as a support to official medicine and as an adjunct to therapy, and it is often used in the initial stages of some diseases. Therefore, there is an interest and need to better examine the chemical composition of plants, isolate certain compounds and analyze their effect on the human organism (Popović *et al.*, 2021; CPMP/ICH/367/96).

According to some data, over 220 pharmaceutical companies in the world, half of which are in the USA, are working to discover API from plants, define their biological role, test biosynthetic pathways and find new biotechnologies, to confirm the effectiveness of traditional drugs and medicines. For many medicines of traditional medicine, these studies have confirmed the effectiveness, and for some new indications have been discovered or new unknown compounds have been isolated (Reynolts, 1991).

In this study, a review of triterpenoid saponin herbal expectorant drugs used in cough therapy is considered, and they are considered in HMPC/EMA.

MATERIAL AND METHODS

According to the pharmacological action, Medicinal plants are divided into two groups: medicinal plants with a mild effect (mint, chamomile, linden, pomegranate, etc.) and medicinal plants with strong effects, which produce very strong poisons such as morphine, heroin, atropine, etc., whose use and handling prescribed by the law on poisonous plants. According to the mode of action on the human body, they are most often classified into several groups: a) plants that affect the work of the heart and blood vessels; b) herbs that stimulate the nervous system; c) herbs that have a calming effect on the nervous system; d) herbs for pain relief; e) plants that regulate the work of the digestive system; f) herbs that facilitate expectoration; g) herbs that accelerate wound healing. The data about plant material and its chemical analysis were described in the previous study of (Šarčević Todosijević *et al.,* 2019). All research in this study was done "at the table" based on results of numerous studies.

RESULTS AND DISCUSSION

Medicinal plants. The greatest success in agriculture will be to achieve the desired increase in production by reducing the negative environmental conditions. This can only be achieved by implementing sustainable methods and sustainable solutions in agriculture. The fact that the agricultural activities and practices are compatible with the environment and being permanent is great importance in terms of contributing to the sustainability of the ecology (Markoski *et al.*, 2018; Tugrul, 2019, Ikanović *et al.,* 2020; Markoski *et al.,* 2021). This family also includes many medicinal species. Geographical position, climatic conditions have enabled the rich biodiversity on the mountain Bjelasica in Montenegro (Balijagić *et al.,* 2021) and Serbia (Popovic *et al.,* 2021). Growing of medicinal and aromatic plants has a long tradition in agro-ecological the conditions of Europe and lead the origin from the Mediterranean region. Herbal medicine entered Western medical history around the time of Hippocrates, and herbs were used from the 5th century BCE on, to alleviate the manifestations of a particular disease. Herbs have proven potent immunomodulatory and antiviral activities. Medicinal plants have gained great popularity for beneficial applications in animals and humans. Herbs have proven potent immuno-modulatory and antiviral activities (Popović *et al*., 2021). Areas under medicinal and aromatic plants in Serbia show in Table 1.

Table 1. Areas under medicinal and aromatic plants in Serbia, 2004-2019

Parameter	Average (000 ha)	Minimum (000 ha)	Maximum (000 ha)	CV* (%)
Serbia	1.80	1.10	2.20	39.70

Source: Unstad comtrade, database 2021; * Data processing of authors

According to the data from the Serbian Chamber of Commerce, in 2012, there was 1,337 hectares under grown plants (1,419 hectares in 2011). If we add to it a portion of the areas 960 under spice plants that are listed as vegetables and a portion of the areas on which it is produced for foreign customers, it makes a total of approximately 20,000 hectares. According to the areas of growing, the Republic of Serbia can be divided into two production regions: the lowlands (Vojvodina) and the highlands (Central Serbia). In the former region, the most commonly grown ones are: chamomile, peppermint, marigold, lemon balm, coriander, lavender, white and black mustard, valerian, fennel, parsley, basil, cumin, dill, tarragon, marshmallow, celery, thyme, sage and some other less included species. In the hilly and mountainous part of our country, there are plantations of the following: marigold, lemon balm, arnica, lavender, gentian, sage, St. John's wort and others (Filipović & Popović, 2014).

Glycyrrhiza glabra - Licorice or licorice root is a medicinal plant that acts on the respiratory and digestive organs, strengthens the body's defense capabilities and improves mood. Licorice has health effects including immunomodulatory, antimicrobial, antioxidative, anti-inflammatory, antidiabetic, hepatoprotextive, antiviral, anti-infective, and radical-scavenging activities.

Hedera helix - Ivy (Fig. 1a) is a plant that belongs to a type of wild vine, originating from Europe and Asia with a moderate climate. Ivy contains a chemical substance called hederin, which has a beneficial effect on the respiratory tract and relaxation of muscle mass, it has a good effect in the treatment of cellulite, calluses, burns, ulcers and some rheumatic conditions. Ivy leaf can help with polyps in the nose, problems with the spleen, inflammation of the urinary tract etc.

Primulae - Primrose (Fig. 1b) is a perennial plant which grows throughout Europe up to an altitude of 2000 m. It blooms from March to May. Medicinal parts of the plant are *radix*, flowers and leaves. The flowers are collected in May, i.e. when the plant is in bloom while the root is collected in autumn. It has a healing effect, on expectoration, thanks primarily to triterpene saponins, and with nervousness and insomnia, in gout and migraines. It has a diuretic and antirheumatic effect.

Polygalae radix - senega (Fig.2) is a species of flowering plant in the milkwort family, *Polygalaceae*. It is native to North America, where it is distributed in southern Canada and the central and eastern United States. Senega root was marketed as a treatment for pneumonia (Popović *et al.*, 2021).

a. b.https://regiuplant.ro/en/produse/ciubotica-cucului-primula-afficinalis/ c.https://stock.adobe.com/search?k=grindelia d.

Figure 1. *Hederae helicis folium (a); Primulae officinalis* (L.) *(b); Grindelia (c); Polypodium vulgare (d)*

Cough. Cough is a protective and defensive act of the respiratory system that aims to expel mucus, foreign particles and various irritants that are inhaled or formed at the site of inflammation of the mucous membranes of the larynx, trachea and large bronchi. Cough is a protective function of the body that occurs during expiration in order to remove the contents from the airways, but it can also be an indicator of the disease.

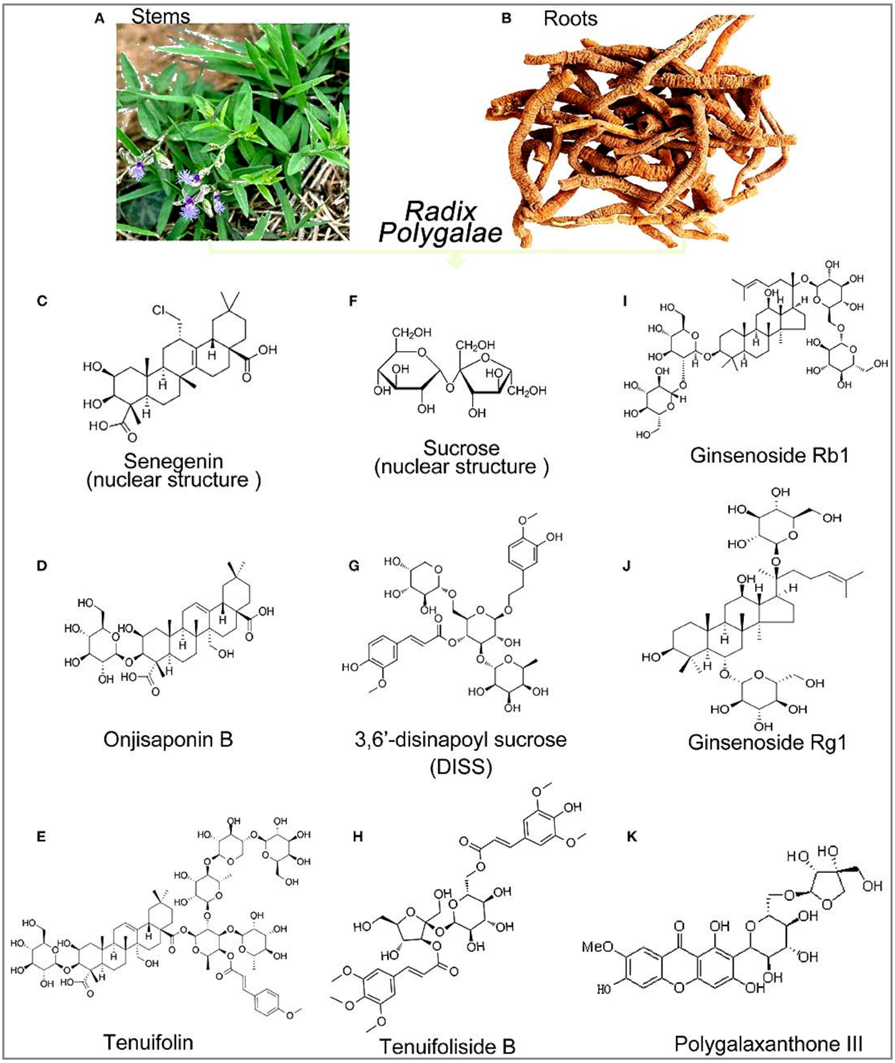

Figure 2. *Polygalae* (*Popović et al.*, 2021)

According to the duration, cough is divided into acute (lasts less than 2 weeks), subacute (lasts 2-8 weeks) and chronic cough (lasts longer than 8 weeks), and according to the pathological classification into productive and dry (irritating) cough (Kardos *et al*., 2019; Canning *et al*., 2014). The most common causes of acute cough are upper respiratory tract diseases (viral infections), allergic rhinoconjunctivitis, pulmonary embolism, pneumothorax, as well as heartdiseases with lung congestion (Kardos *et al.*, 2019).

The most common diseases of the respiratory system - chronic obstructive pulmonary disease (COPD) and bronchial asthma, are often the causes of acute as well as chronic cough (Petrović & Vukomanović, 2019).

Subacute cough is a consequence of viral infections, but also acute bacterial sinusitis. The category of subacute cough, according to the new classification, includes viral infections caused by adenoviruses, as well as infections caused by the bacteria *Bordetella pertussis* and *Mycoplasma pneumonia*. In the etiology of chronic cough, there are diseases of the upper respiratory tract (eg. rhinitis, pharyngitis, laryngitis), non-obstructive chronic bronchitis, non-asthmatic eosinophilic bronchitis, lung cancer, etc. Other causes of chronic cough, which are not related to the respiratory system, are gastroesophageal reflux or the use of some drugs (ACE inhibitors) (Kardos *et al.*, 2019), as well as exposure to various chemical and mechanical irritants (cigarette smoke, air pollution, moisture, allergens, etc.).

The treatment of patients who have a cough depends on the etiological factors that caused it, as well as the pathogenetic basis. For these reasons, antitussives are used for irritable, nonproductive cough, while expectorants are used for productive cough (Heinrich *et al.*, 2018; Petrović & Vukomanović, 2018).

Untimely and inadequate cough therapy can lead to multiple health complications, from cardiovascular and neurological to psychosocial and dermatological, which negatively affect and reduce the quality of life.

Plant expectorants. Expectorants are medicinal preparations that act on the formation, density and flow of bronchial secretions. These preparations usually contain an extract of one plant or a combination of several plant species. Their use facilitates the removal of bronchial mucus from the airways. Expectorants can be mucolytics, secretolytics or secretomotorics (Franova *et al.*, 2006; Wagner *et al.*, 2015; Petrović & Vukomanović, 2018; Anheyer *et al.*, 2018; Filipović *et al.*, 2021).

Mucolytics change the properties of bronchial mucus, they break it down chemically, softening and reducing its viscosity. By including mucolytics in therapy, the water content in the bronchial mucus increases, which affects the increased productivity of cough.

Secretolytic preparations stimulate the mucous membrane of the stomach and respiratory tract via the cranial nerve X (*N. vagus*). The secretion of bronchial glands increases, mucus is increased, which acts to reduce its surface tension and less formation of deposits on the walls of the bronchi. The bronchial mucus is then less thick and sticky, so its flow is accelerated and the cilia make it easier to clean the airways and cough. Natural secretolytics are preparations based on mint, primrose, ipecac, ivy, liquorice, senega, thyme, eucalyptus, anise, pine and others (Filipović *et al.*, 2021). These are herbal drugs that act on the mucous membrane of the stomach or duodenum, triggering afferent mechanisms, which causes reflex stimulation and increased secretion. They can cause vomiting if taken in higher doses because they stimulate the vomiting center.

Secretomotorics encourage the removal of mucus with better mobility of cilia, and essential oils are used for that purpose. These include essential oils of: anise, fennel, thyme, eucalyptus, white pine, juniper, larch.

Herbal cough preparations generally contain one component, but can be made from combinations of different plants (ivy, primrose, marshmallow, plantago, mint, chamomile, thyme) (Applequist, 2006; Franova *et al*., 2006; Wagner *et al.*, 2015; Mauseth, 2016; Heinrich *et al.*, 2018; Anheyer *et al.*, 2018).

Saponin expectorants. Saponosides are a type of compounds that belong to glycosides, whose structure consists of sugar (glycone) and non-sugar component (aglycone). Under the influence of hydrolytic enzymes, glycosides decompose into carbohydrates and essential oils with a pungent odor and taste. According to the aglycone component, sapogenol (or sapogenin), steroid, terpene and triterpenesaponosides are distinguished. Steroid saponosides have a hormone-like structure and are important as precursors for the synthesis of steroid hormones, while triterpenesaponosides can be toxic compounds with a weaker or stronger hemolysis ability. Saponosides are secondary metabolites of plants, which synthesize them as phytoalexins, in order to protect themselves from bacterial and fungal infections. They are dissolved in the cells of underground organs, in fruits, seeds, in some plants they are present in all parts of the plant, and in the cuticle of plants they build a waxy protective film. Due to the characteristic structure of molecules with a highly hydrophilic and lipophilic part, saponosides reduce the surface tension of heterogeneous systems, they are soluble in water, in which they build colloidal solutions. Rubbing saponin drugs in water creates foam, due to the rich foam that is formed when their solutions are mixed, they were called saponins in the 19th century (lat. *sapo*= soap). Due to their ability to act as surface active agents (SAA), they have been used in householdsfor making detergents. More recently, their properties have been used for emulsification and spraying, while their SAA property allows them to penetrate cells more easily (Applequist, 2006; Wagner *et al.*, 2015; Heinrich *et al.*, 2018).

They are safe for oral use, while parenteral use is not recommended due to their hemolytic activity. Inhalation is also contraindicated, because inhaling saponin powder triggers the sneezing reflex and can damage the airways. Saponosides damage cell membranes, leading to local irritation, and are cytotoxic at higher doses.

Most saponoside-based drugs are triterpene glycosides containing a polar glycoside (sugar) moiety and a non-polar (aglycoside) moiety, sapogenin. Sapogenins are mainly composed of pentacyclic compounds, while the glycosidic part consists of monosaccharides (D-glucose, D-galactose, L-rhamnose, L-arabinose, D-xylose, L-fucose) and carboxylic acids (D-glucuronic acid and D-galacturonic acid). Minor changes in the structure of molecules can affecton large differences in the activity of these compounds. They can have different pharmacological properties, because the properties of saponosides depend on their chemical structure (Applequist, 2006; Wagner *et al.*, 2015; Heinrich *et al.*, 2018).

In recent years, the effects of triterpenesaponosides have been studied because there is growing evidence of their cytoprotective and anti-inflammatory role, as well as antitumor activity because they can induce apoptosis of tumor cells and reduce their invasiveness. However, evidences of their effectiveness were not followed by adequate explanations of the mechanism of their action (Heinrich *et al.*, 2018; Petrović & Vukomanović, 2018). Saponosides react with cell membranes and their action depends on membrane structure, saponin side chains, and the nature of aglycones. When given in concentrations above 100 µm, they affect the formation of pores on the membranes, which increases the permeability (Mauseth, 2016; Heinrich *et al.*, 2018). When applied in lower concentrations, below 10 µm, they are important for physiological processes because they affect on intracellular signaling pathways. Despite a large number of studies, it is still not possible to predict the action of these compounds based on their structure. The mechanism of the expectorant action of drugs with saponosides has not yet been fully elucidated. One plant can contain several different saponosides. Some of them can be toxic (sapotoxins), and some can significantly reduce the symptoms of the disease and enable faster recovery.

Clinical studies have confirmed the effectiveness of the use of ivy herb extracts and fixed combinations of thyme herb and primrose root as expectorants in productive cough, while for a number of other herbal drugs and their preparations used in cough therapy, only traditional use has been documented (Bauer, 1973; Büechi *et al.*, 2005; Kemmerich *et al.*, 2006; Kemmerich, 2007).

Table 2. Triterpene saponosides with expectorant properties according to HMPC/EMA

Number	Species	Plant parts
1.	*Hederae helicis folium*	Ivy leaf
2.	*Primulae radix*	Primrose root
3.	*Primulae flos*	Primrose flower
4.	*Liquiritiae radix*	Sweet root
5.	*Polygalae radix*	Senega root
6.	*Grindeliae herba*	Grindelia herb
7.	*Polypodii rhizoma*	Common polypody rhizome

Herbal drugs with expectorant action belonging to triterpene saponosides, and documented in HMPC/EMA monographs are shown in Table 2, (EMA/HMPC/325716/2017;EMA/HMPC/325715/2017;EMA/HMPC/136582/2012; EMA/HMPC/571119/2010; EMA/HMPC/748220/2011).

From many herbal saponin drugs with expectorant action, only ivy leaf extract is approved by the EMA for regular therapy, because its therapeutic efficacy has been confirmed on clinical studies in several *in vitro* and *in vivo* studies (Wagner *et al.*, 2015; Šarčević Todosijević *et al.*, 2018).

For the primrose root and flower, as well as liquorice root, senega root, grindelia herb (Fig. 1c) and common polypody rhizome, only the traditional application has been documented, and they are treated as additions to therapy. In HMPC/EMA monographs of traditional saponin expectorants, is stated that they are not used for children (liquorice root and grindelia herb are contraindicated even in adolescents under 18 years), as well as during pregnancy and lactation (ESCOP 2003, EMA/HMPC/571119/2010, EMEA/HMPC/600668/2007, Šarčević Todosijević *et al.*, 2018).

The use of grindelia (gum weed) herb (*Grindeliae herba*, *Grindelia robusta* Nutt., Asteraceae) is contraindicated for people who are hypersensitive to plants from the Asteraceae family (EMA/HMPC/748220/2011). In addition, common polypody rhizome (*Polypodii rhizoma*, *Polypodium vulgare* L. (Fig. 1d), *Polypodiaceae*) has a mild laxative effect, and is contraindicated for people with peptic ulcer (EMEA/HMPC/600668/2007).

Ivy. Ivy (*Hedera helix* L., Araliaceae) is a perennial evergreen climbing plant. It reaches a length of up to 50 meters, has leathery leaves, with green-white flower crowns collected in racemose inflorescences, the fruit is a red berry (Mauseth, 2016; Šarčević Todosijević *et al.*, 2018). Ivy is a poisonous plant, whose healing properties come from saponosides and flavonoid heterosides that have spasmolytic activity (Heinrich *et al.*, 2018).

Dried leaves (*Hederae helicis folium*) are used in therapy. They contain: triterpene saponosides 5 - 8%, hederasaponoside B and C, sesquiterpenes, flavonoids (rutin), phenolcarboxylic acids, polyacetylenes (falcarinol), phytosterols, etc. (Heinrich *et al.*, 2018; Šarčević Todosijević *et al.*, 2018).

Ivy leaf extract is very effective in diluting thick bronchial secretions and facilitates airway cleansing, so it is one of the most important and most commonly used saponin drugs with expectorant action (mucolytic). It is indicated for the treatment of uncomplicated acute bronchitis and cough therapy, which has been confirmed in *in vitro* and *in vivo* studies where its spasmolytic, bronchodilator and expectorant effects have been proven (Holzinger, 2011; Zeil *et al.*, 2014; Song *et al.*, 2015; Barnes *et al.*, 2020). The above pharmacological effects of ivy are due to saponoside, α-hederin, which is formed from hederacoside C in the body, and is considered the most important API of ivy leaves. The secretolytic effect of α-hederin is achieved through β_2-adrenergic receptors, because it increases the amount of surfactant in type II pneumocytes in the alveoli, which results in dilution of bronchial mucus and facilitation of expectoration (EMA/HMPC/325716/2017). On the other hand, the enhanced response of β_2- adrenergic receptors in the bronchi is responsible for the bronchospasmolytic and secretolytic properties of ivy leaf extract. Has been shown that α- hederin inhibit the internalization of β_2-adrenergic receptors and affect the regulation of β_2-adrenergic receptors (Büechi *et al*., 2005; EMA/HMPC/325716/2017). In addition to its effect on the respiratory system, ivy leaf extract acts as a keratolytic and rubefacient of the skin, has antioxidant and antiviral effects, and thanks to polyacetylene (falcarinol) it also has antibacterial, antifungal, analgesic and sedative effects (Holzinger, 2011; Song *et al.,* 2015; Heinrich *et al.,* 2018; Kardos *et al.*, 2019). Unlike spasmolytic, bronchodilator and expectorant effects, these pharmacological effects have no proven clinical efficacy.

Ivy leaf should not be used in the form of herbal tea, but dry, liquid and semi-solid extracts of ivy leaf should be used in liquid and solid dosage pharmaceutical forms for *per os* application, for a maximum of 7 days. To achieve the effectiveness of the preparation as an expectorant, the preparation must contain 25 mg of saponoside in one tablet or 700 mg of saponoside in 100 ml of syrup.30% ethanol is used to prepare the extract, and the daily dose of the raw drug is 0.3-0.8 grams. Cough syrup is made from a standardized dry extract of ivy leaves.

Frequent use of ivy-based preparations can cause allergic reactions (urticaria, rash, dyspnea, anaphylactic reaction) and which is mainly due to the presence of polyacetylene falcarino (Bauer, 1973; Holzinger, 2011; EMA/HMPC/325715/2017).

Primrose. *Primrose (Primula officinalis*, *Primula veris* L., Primulaceae) is a perennial herbaceous plant with yellow flowers, the fruit is a cylindrical cocoon, the leaves are ovate, rounded at the top, wrinkled, with hairs on the reverse. *Primula veris* is widespread on the edges of mountain forests, and *Primula elatior* is characteristic of wet meadows and mountain pastures (Mauseth, 2016; EMA/HMPC/748220/2011).

In the treatment of cough, dried rhizome and root (*Primulae radix etrhizoma*) are used. They have a characteristic faint odor and a spicy and unpleasant taste. The plant blooms in early spring, so the rhizome and root are removed after the plant blooms. The rhizome is elongated, dark in color, and the roots are thin, brittle, light pink (*P. elatior*) or white (*P. veris*), about 2 mm thick (EMA/HMPC/748220/2011; Mauseth, 2016).

The drug is rich in flavonoids and saponosides, it contains 5-10% of triterpene saponosides (primula acid), aglycone protoprimulagenin A (13β, 16α, 28β-trihydroxy-oleanolicacid), protoprimula-saponin A, flavonoids (gosipetin) and others. Protoprimulagenin A (pentaglycoside) *Primula elatior* contains four sugar components: glucose, galactose, rhamnose and glucuronic acid. Protoprimula-saponin A contains protoprimulagenin A and saccharose (xylose). The most important saponin aglycones from *Primula veris* are anagaligenin, priverogenin B and priverogenin-B 22-acetate. Primula acid A is the most important saponoside of primrose, which is found in the root and flower of primrose (*Primulae flos*) (EMA/HMPC/104095/2012; EMA/HMPC/136582/2012; Heinrich *et al.*, 2018).

In addition to these components, primrose root also contains phenolic glycosides (primverin and primulaverin), which are present in both species in variable amounts (up to 2.3%) and change during drying, giving a characteristic primrose odor. There are saponosides and flavonoids in the flower, and only saponosides in the root. Flavonoids have a proven beneficial effect on blood vessels, because they reduce their fragility and permeability, and in addition, it is important that they have a vasodilatory, anti-inflammatory and antibacterial effect. The concentration of saponosides in the flower of primrose is slightly lower than in the root. Fresh root has the most saponosides, and in the dried one the concentration of medicinal ingredients decreases by prolonging the standing time. Characteristic sugars in underground organs are: primoverose, glucose and volemitol. Underground organs do not contain quinoid compounds such as primin, which cause allergic reactions as in the aboveground parts of species of the genus *Primula.* All organs of primrose contain a lot of ascorbic acid, mostly leaves and flowers. The leaf also contains β-carotene (EMA/HMPC/104095/2012; EMA/HMPC/136582/2012; Heinrich *et al.*, 2018).

Primrose extract is mostly used due to its secretolytic and secretomotor action. It achieves expectorant effect by irritating the gastric mucosa, which also has an effect on the increased secretion of bronchial glands. Saponins, due to their ability to reduce the surface tension of bronchial mucus, spread from the pharynx to the surrounding mucosa and dilute the content that is present in the respiratory tract. This facilitates the expulsion of excess mucus, the cilia of the bronchial epithelium become more mobile, so the primrose extract is also a secretomotor. Since they show their effect by irritating the gastric mucosa, caution is necessary. In patients with gastritis or gastric ulcer, the use primrose preparations is not recommended. In addition to expectorant action, preparations containing primrose extract exhibit antibacterial, antiviral, antifungal and anti-inflammatory effects (Franova *et al.*, 2006; EMA/HMPC/104095/2012; EMA/HMPC/ 136582/2012).

Primrose extract is prepared in the form of a decoction or liquid extract. The extract of primrose flower is sometimes combined with other plants (eg mullein) in the cough preparations, and cough syrup is made from the roots.

According to the guidelines of the German Commission E, (LGCM, 2021) the daily dose of primrose flower extract is 2 - 4 grams of drug, and 2.5 - 7.5 grams of flower tincture, while the daily dose of primrose root extract is 0.5 - 1.5 grams of drug and 1.5 - 3 grams of root tincture.

The European Medicines Agency recommends as a lower limit 0.125 ‰ concentration of ethanol in the blood after a single oral dose. There is also no time limit for the use of primrose. High doses of primrose extract cause nausea, abdominal colic, vomiting, diarrhea (EMA/HMPC/104095/2012.; EMA/HMPC/136582/2012).

Liquorice. Liquorice (*Glycyrrhiza glabra* L., syn. *Liquiritia officinalis*, Fabaceae) is a plant with an upright stem and purple flowers, blooms in July and August. It has a branched root, the leaves are odd feathered, elliptical in shape, the fruit is a flat pod of brown color. It grows in dry sandy places or moderately moist meadows in southeastern Europe and the eastern and central Mediterranean (EMA/HMPC/748220/2011; Mauseth, 2016). Liquorice is one of the oldest medicinal plants used for thousands of years in Indian, Chinese and European medicine.

Dried peeled or unpeeled parts of the roots (*Liquiritiae radix*) of cultivated plants are used as drugs. When dried, peeled and cut, the root is yellow, has a fibrous fracture, is dusted due to the starch present, with a visible cambium line. It has a sweet taste and a pleasant smell. The unpeeled root is light brown in color and has a smooth bark wrinkled lengthwise. After crushing the dried drug, a yellow powder with small starch grains, parts of the trachea with reticulate and dotted thickenings of the walls, bundles of fibers surrounded by calcium oxalate crystals and parts of the parenchyma were obtained (Mauseth, 2016; Heinrich *et al.,* 2018).

Liquorice root (*radix dulcis* = sweet root) contains a large number of chemical compounds, among which the most important are: amino acids, flavonoids, phytoestrogens, coumarins, vitamins, minerals, carbohydrates, fats, pentacyclictriterpenes and many others. It contains saponin glycoside glycyrrhizin (root 6 - 13%, extract 20 - 25%) and triterpene glycyrrhizinic acid. Glycyrrhizin is a mixture of the potassium and calcium salts of glycyrrhizinic acid. Hydrolysis of glycosides produces aglyconeglycyrrhetinic acid and two molecules of glucuronic acid. Glycyrrhetinic acid has a pronounced hemolytic effect. Liquorice root contains triterpene saponins, flavonoids (formononetin, glabren, glabridin, liquiritigenin, isoliquiritigenin), coumarins and sterols. In addition to these, it contains flavonoid glycosides (liquiritin (flavanone), isoliquiritin (chalcone), liquirithoside, ramnoliquiritin, isoflavones, isoflavanones), coumarin derivatives (herniarin, umbeliferon) and asparagine. From the flavonoid glycoside liquiritoside, glucose, rhamnose and dihydroxy-flavanon are formed by hydrolysis. Fresh root contains fatty and mineral substances, bitter ingredients, saccharose and a little mannitol. In addition to active pharmaceutical ingredients, liquorice root contains 25-30% starch, up to 10% glucose and saccharose (EMA/HMPC/571119/2010; Heinrich *et al.,* 2018).

Flavonoids and pentacyclictriterpenes have the greatest pharmacological activity. It is assumed that glycyrrhizin is responsible for the expectorant effect of liquorice extract. Saponosides locally stimulate the mucous membrane, increase salivation due to the action on the salivary glands, as well as on the bronchial glands, which affects the dilution of the mucus. Animal studies have shown central antitussive activity, which is attributed to glycyrrhizinic acid.

Also, in preclinical studies, its secretolytic action has been indicated, it has a very successful effect on the dissolution and elimination of bronchial secretion, and it is assumed that by increasing bronchial secretion, mucus is reflexively triggered and that the reflex begins in the stomach (EMA/HMPC/571119/2010).

The recommended daily dose in the treatment of cough is 5 - 15 grams of dried liquorice root (Franova *et al.,* 2006; EMA/HMPC/571119/2010).

In addition to expectorant action, liquorice root acts as a taste corrector, mild laxative, antioxidant, immunostimulant, hepatoprotective, anticoagulant and anti-inflammatory agent. Liquorice root is a very good inhibitor of COX_2 i 5-

lipoxygenase, which affects the reduced synthesis of eicosanoids and leukotrienes. It would be a great replacement for NSAIDs (nonsteroidal anti-inflammatory drugs) if there were no negative effects on the gastric mucosa. It is fifty times sweeter than saccharose, so it is used in the food industry. Liquorice ingredients are very successfully used in dermatological preparations for the treatment of hyperpigmentation.

Glycyrrhetinic acid is often an ingredient in topical preparations for the treatment of hyperpigmentation due to its strong effect on skin whitening, but it is also used in the treatment of atopic eczema, seborrhea, burns due to its anti-inflammatory properties.

Many studies indicate that long-term use of liquorice-based preparations worsens hypertension, edema, hypokalemia, and myoglobinuria occurs less frequently (due to increased concentration of myoglobin in the blood). Glycyrrhizin has a pseudoaldosterone effect because glycyrrhizinic acid blocks mineralocorticoid receptors, thereby increasing the levels of cortisol in the kidneys, which then binds to mineralocorticoid receptors.

Glycyrrhizin is metabolized in the body to glycyrrhizinic acid, which inhibits the enzyme 11-β hydroxysteroid dehydrogenase, which converts cortisol to cortisone. Higher doses of glycyrrhetinic acid affect sodium reabsorption, and due to sodium and water retention, they affect high blood pressure and hypokalemia (Franova *et al.,* 2006; EMA/HMPC/571119/2010).

Senega. *Senega* (*Polygala senega* L., Poygalaceae) is a perennial plant that grows in North America and reaches a height of about 30 cm [2].

Dried parts of the roots are used as drugs (*Polygalae radix*). The root has a characteristic unpleasant odor (due to the content of methyl salicylate), soapy taste (Heinrich *et al.*, 2018).

The chemical composition of senega root consists of saponosides 5-10%, a mixture of triterpene glycosides, senegasaponins A - D, bisdesmoside type, senegins (polygalic acid), aglycone presenegin, methyl salicylate 0.1 - 0.3%, phenolic acids, lipids, oligosaccharides, etc. (Heinrich *et al.,* 2018; ESCOP, 2003).

Senega root has a secretolytic effect because it reduces the viscosity of bronchial mucus. Senegin and polygalic acid, as well as all saponins, have an irritating effect on the gastric mucosa and reflexively increase the secretion of bronchial mucus. It is used in the treatment of productive cough in the form of encapsulated dry extract, decoction or in the form of syrup. Daily doses are 1.5-3 g of the drug, or 2.5-7.5 g of tincture (ESCOP, 2003; Wagner *et al.,* 2015; Franova *et al.*, 2018).

In addition to its expectorant action, like all other triterpene saponin expectorants, senega root is also a galactagogue (affects the increased secretion of milk in breastfeeding mothers), so it is contraindicated in lactation. It can also cause gastritis, diarrhea and emesis due to irritation of the gastric mucosa (ESCOP, 2003).

Prospective Use of Triterpene Saponin Herbal Drugs with Expectorant Action in Veterinary Medicine. In veterinary medicine, as in human medicine, there is increasing popularity of herbal drugs. However, veterinary practitioners still heavily rely on modern medicines as there is not enough scientific evidence on the benefits of herbal drugs. In recent years a few studies have provided evidence of the benefits of the usage of herbals with expectorant actions in animals (Van den Hoven, 2003; Song *et al.*, 2015). South Korean study provided evidence of expectorant and antitussive effects of *Hedera helix* (HH) and *Rhizoma coptidis (RC)* extracts mixture in mice and guinea pigs. It is found that optimal expectorant effect is dose-dependent when the mixture is prepared in a 3:1 ratio of HH and RC respectively. The benefits of the utility of *Primula* extract on the lung function in horses is studied in an Australian study (Song *et al.*, 2015). Herbal tablets Bronchipret (film-coated tablets of dried extract of *Thymus vulgaris* (160 mg/tabl) and dried *Primula veris* root extract (60 mg/tabl.)) that were used in this study had some effect on improving lung function on horses with heaves (recurrent airway obstruction syndrome), although authors report limitations of the study by using doses originaly adjusted for humans (Van den Hoven, 2003).

Ginger and rosemary oil can potentially be used in treating diseases caused by bacteria, *Escherichia coli, Staphylococcus aureus and Salmonella spp.* Imamović *et al.* (2021). Needless to say that this is a very promising field in veterinary as in human medicine as easiness of usage, quality of ingredients and lack of serious adverse drug effects makes herbal remedies superior to synthetic medicines.

CONCLUSIONS

Most herbal expectorants based on saponosides are triterpene glycosides, so their importance in cough therapy is dominant. In recent years, their use in the treatment of cough and as an adjunct to the treatment of respiratory infections has been increasing. Therefore, education is necessary about the rational use, as well as all interactions, side effects that may occur. In addition, there is a need to conduct more detailed analysis of the pharmacological effects of triterpene saponosides with expectorant action in order to their wider application.

ABBREVIATION

ACE inhibitors = angiotensin-converting enzyme inhibitors; SAA = surface-active agents; API = *active pharmaceutical ingredient;* EMA = European Medicines Agency; HMPC = Committee for Herbal Medicinal Products; HMP = herbal medicinal products, COPD = chronic obstructive pulmonary disease.

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REFERENCES

Anheyer, D., Cramer,H., Lauche, R., Saha, F. J. & Dobos, G. (2018). *Herbal Medicine in Children With Respiratory Tract Infection: Systematic Review and Meta-Analysis. AcadPediatr*. Jan-Feb;18(1):8-19.

Applequist W. (2006). The identification of medicinal plants, Missouri, St. *Louis: Missouri Botanical Garden Press.* Book.

Balijagić, J., Arslanović, S. & Mustajbašić, D. (2021). Medicinal plants fam. Asteraceae from Bjelasica Mountain used in folk and scientific medicine. *Agriculture and Forestry*, 67(1): 271-281.

Bauer, L. (1973). Die Feinstruktur der menschlichen Bronchial schleim hautnach Behandlungmit Ozothin. *Klinische Wochenschrift.* 51(9):450-53.

Barnes, L.A., Leach, M., Anheyer, D., Brown, D., Carè, J., Lauche, R., Medina, D. N., Pinder, T. A., Bugarcic, A. & Steel, A. (2020). The effects of *Hedera helix* on viral respiratory infections in humans: A rapid review. *Adv Integr Med.* Dec. 7(4): 222-226.

Büechi, S., Vögelin, R., von Eiff, M. M., Ramos, M. & Melzer, J. (2005). Open trial to assess aspects of safety and efficacy of a combined herbal cough syrup with ivy and thyme. *Forsch Komplementarmed Klass Naturheilkd.* Dec. 12(6):328-32.

Canning, B. J., Chang, A. B., Bolser, D. C., Smith, J. A., Mazzone, S. B. & Garvey, Mc. (2014). Anatomy and neurophysiology of cough: CHEST Guideline and Expert Panel report. Chest. 146(6): 1633-48.

CPMP/ICH/367/96. Specifications, *Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances* (CPMP/ICH/367/96).

Dročić, M., Šarčević Todosijević, Lj., Petrović, B.,Vukomanović, P., Đorđević, S., Đorđević, N., Popović, V. & Živanović, Lj. (2020). The possibility of application of plants in the prevention and treatment of diabetes, in X*XV Symposium on Biotechnology*. Čačak, Serbia, Thematic Proceedings 1, p.105-110.

Đorđević, S., Šarčević Todosijević, Lj. Popović, V., Perić, M., Živanović, Lj., Đorđević, N. & Stevanović, A. (2020). Healty safe food – *Risk of carcinogenic substances. XXIV Internat. Eco-Conference@ 2019, XI Safe Food,* 23-25.09.2020. Novi Sad, 315-322.

European Medicinal Agency, EMA/HMPC/325716/2017. Final: *European Union herbal monograph on Hedera helix L., folium, London*, 21 November 2017.

European Medicines Agency, EMA/HMPC/325715/2017. Final: *Assessment report on Hedera helix L., folium, London*, 21 November 2017.

European Medicinal Agency, EMA/HMPC/104095/2012. Final: *Community herbal monograph on Primulaveris L. and/or Primulaelatior (L.) Hill, radix, London*, 19 September 2012.

European Medicinal Agency, EMA/HMPC/136582/2012. Final: C*ommunity herbal monograph on Primulaveris L. and/or Primulaelatior (L.) Hill, flos,* London, 19 September 2012.

European Medicinal Agency, EMA/HMPC/571119/2010. *Final: Community herbal monograph on Glycyrrhizaglabra L. and/or Glycyrrhiza inflata Bat. and/or Glycyrrhiza uralensis Fisch., radix;* London, 22 May 2012

ESCOP Monographs. 2nd ed. Exeter, The European Scientific Cooperative on Phytotherapy. Stuttgart: *Georg ThiemeVerlag*; New York: Thieme New York, 2003.

European Medicinal Agency. Doc. Ref.: EMEA/HMPC/600668/2007. *Final: Community herbal monograph on Polypodium vulgare L., rhizoma*, London, 6 November 2008.

European Medicinal Agency. EMA/HMPC/748220/2011. *Final: Community herbal monograph on Grindelia robusta Nutt., Grindelia squarrosa (Pursh) Dunal,* *Grindelia humilis Hook.* E*t Arn., Grindelia camporum Greene, h*erba, London, 20.10.2012.

Filipović, V. & Popović, V. (2014). State of the Production and the Collecting of Medicinal Plants in the Republic of Serbia. Inter. Scie. Conf. „*Sustainable agriculture and rural development in terms of Republic of Serbia strategic goals realization within the Danube region – rural development and (un) limited resources*“. Ed.: Cvijanović D., Subić, J., Vasile, A.J. Institute of Agricultural Economics, Belgrade, Serbia, 5-6/6/2014. *Economics of agriculture, Thematic proceedings*, 957 – 973.

Filipović, V., Ugrenović, V., Maksimović, Z., Popović, V., Paunović, D., Šarčević-Todosijević, Lj. & Popović, S. (2020). Influence of phytohormones on vegetative propagation of different forms of pannonian thyme (*Thymus pannonicus* All.). *Selekcija i semenarstvo*, 26(2): 39-52. DOI:10.5937/SelSem1901001M

Franova, S., Nosalova, G. & Mokry, J. (2006). *Phytotherapy of cough, Advances in Phytomedicine.* 2:111-31.

Guideline on Quality of Herbal Medicinal Products/Traditional Herbal Medicinal Products. *CPMP/QWP/2819/00, rev 1.*

Guideline on Stability Testing, *Stability of Existing Active Substances and Related Finished Products* (CPMP/QWP/122/02, rev 1 corr).

Heinrich, M., Barnes, J. Prieto-Garcia, S. & Gibbons, E. (2018). Williamson, Fundamentals of Pharmacognosy and Phytotherapy, 3rd *Ed Elsevier,* New York, USA.

HSTPDM (2009). Handbook of Stability Testing in Pharmaceutical Development, Regulations, Methodologies and Best Practices, *Kim Huynh-Ba Pharmalytik Newark*, Delaware.

Holzinger, F. & Chenot, J.F. (2011). Systematic review of clinical trials assessing the effectiveness of ivy leaf (*hedera helix*) for acute upper respiratory tract infections. *Evid Based Complement Alternat Med.* 382789.

Ikanović, J., Popović, V., Pavlović, S. (2020). Agroecology and soil protection. Book. Banja Luka, 1-220.

Ikanović, J. & Popović, V. (2020). Organic plant production. *Book.* Bijeljina, B & H, 250.

Imamović, B., Komlen, V., Gavrić, T., Sunulahpašić, A., Lalević, B. & Hamidović, S. (2021). Antimicrobial activity of ginger (*Zingiber officinale*) and rosemary (*Rosmarinus officinalis*) essential oils. *Agriculture and Forestry*, 67 (1): 231-238.

Kardos, P., Dinh, Q. T., Fuchs, K. H., Gillissen, A., Klimek, L., Koehler, M., Sitter, H. & Worth, H. (2019). Leitlinie der Deutschen Gesellschaft für Pneumologie und Beatmungs medizin zur Diagnostik und Therapie von erwachsenen Patienten mit Husten [Guidelines of the German Respiratory Society for Diagnosis and Treatment of Adults Suffering from Acute, Subacute and Chronic Cough]. *Pneumologie*. 73(3):143-180.

Kemmerich, B., Eberhardt, R. & Stammer, H. (2006). Efficacy and tolerability of a fluid extract combination of thyme herb and ivy leaves and matched placebo in adults suffering from acute bronchitis with productive cough. A prospective, double-blind, placebo-controlled clinical trial. *Arzneimittelforschung*. 56(9):652-60.

Kemmerich, B. (2007). Evaluation of efficacy and tolerability of a fixed combination of dry extracts of thyme herb and primrose root in adults suffering from acute bronchitis with productive cough. A prospective, double-blind, placebo-controlled multicentre clinical trial. *Arzneimittelforschung*. 57(9): 607-15.

Kruttschnitt, E., Wegener, T., Zahner, C. & Henzen-Bücking, S. (2020). Assessment of the Efficacy and Safety of Ivy Leaf (*Hedera helix*) Cough Syrup Compared with Acetylcysteine in Adults and Children with Acute Bronchitis. *Evid Based Complement Alternat Med.* May 4; 1910656.

List of German Commission E Monographs. Phytotherapy. *Primrose flower (Primulae flos),* Berlin, Germany, 2021.

Markoski, M., Mitkova, T., Tanaskovik, V. & Spalevic V. (2018). Soil distribution in Strumica river basin and its importance for agricultural production. *Agriculture and Forestry,* 64 (4): 121-128.

Markoski, M., Mitkova, T., Tanaskovik, V., Spalevic, V. & Novicevic, R. (2021). Soil distribution in Crna River basin and its importance for agricultural production. *Agriculture and Forestry*, 67 (1): 125-138

Mauseth, J.D. (2016). Botany, An Introduction to Plant Biology, *6th Edition, Jones & Bartlett Learning, Massachusetts,* USA.

Petrović, B. (2016). Introduction to Pharmaceutical Science, *Medical Sanitary School of Applied Sciences "Visan", Belgrade,* Serbia, 206.

Petrović, B. & Vukomanović, P. (2018). Pharmacology, *Medical Sanitary School of Applied Sciences "Visan"*, Belgrade, Serbia.

Petrović, B., Vukomanović, P.**,** Popović, V., Jovović, Z., Nikolić, M., Šarčević-Todosijević Lj. & Jović S. (2021). Herbal Remedies in the Treatment of Anxiety Disorders. Chapter 7. Ed. Emerald Mila. Book Title: *An Introduction to Medicinal Herbs. NOVA Science publishers,*USA,DOI: https://doi.org/10.52305/TKAL3430, *p.205-236.*

Popovic, V., Maksimovic, L., Vasic, M., Marjanovic-Jeromela, A., Mihailovic, V., lkanovic, J., Stojanovic, D. & Filipovic, V. (2018a). Yield and oil content and total phenol content in seeds of poppy (*Papaver somniferum* L.) in organic cropping system. 59. *Production and Processing of Oilseed Proceeding of the 55th Oil Industry Conference.* Herceg Novi, Montenegro. 85-94.

Popović, V., Marjanović Jeromela, A., Jovovic, Z., Janković, S., Filipović, V., Kolarić Lj., Ugrenović, V. & Šarčević Todosijević, Lj. (2019). Linseed (*Linum usitatissimum* L.) production trends in the World and in Serbia. Ed. Janjev. I. Chapter 5. Book Title: *Serbia: Current Issues and Challenges in the Areas of Natural Resources, Agriculture and Environment. NOVA Science publishers,* USA, p. 123-148. p. 1-383

Popović V., Ikanović J., Rajičić V., Ljubičić N., Kostić M., Radović M., Mačkić K. & Šarčević Todosijević Lj. (2020). Millet–*Panicum miliaceum* L. production trend in the world. Importance of millet in nutrition and for bioenergy. *XXIV International Eco-Conference@ 2020, XI Safe Food, 23-25.9.2020. Novi Sad*, 297-306.

Popović M.V., Šarčević Todosijević Lj., Petrović B., Ignjatov M., Popović B.D., Vukomanović P., Milošević D. & Filipović V. (2021). Economic Justification Application of Medicinal Plants in Cosmetic and Pharmacy for the Drugs Discovery. Chapter 3. *Ed. Emerald M. An Introduction to Medicinal Herbs. NOVA Science publishers,* USA, ISBN: 978-1-68507-147-9, pp. 63-106. https://doi.org/10.52305/TKAL3430

Popović, V., Mihailović, V., Lakić, Ž., Vučković, S., Kolarić ,Lj., Jaćimović, G., Šarčević- Todosijević, Lj. & Đekić, V. (2018). Effects of nutrition on biomass production of Lacy phacelia in organic cropping system. *Green Room Sessions 2018 International GEA (Geo Eco-Eco Agro) Conference,* Podgorica,1-4.11.2018. 53-59.

Reynolts, T. (1991). Tropical rein Forest Conversation Tide to Drug Development. *Journal of the national Cancer Institute,* 9: 1594.

Stevanović, A., Šarčević Todosijević, Lj. & Popović, V. (2018). Application of microbiological fertilizers in the organic production systems. *Sustainable primary agricultural production in Serbia - state, possibilities, limitations and chances,* Faculty of Biofarming, Bačka Topola, p. 13-20

Stevanović, A., Šarčević Todosijević, Lj., Bošković, J., Popović, V. & Živanović, Lj. (2019). Organic production, genetically modified organisms and conservation of biodiversity - leading challenges in environmental protection, *Sustainable primary agricultural production in Serbia - state, possibilities, limitations and chances.* Faculty of Biofarming, Bačka Topola, p. 95-102.

Song, K.J., Shin, Y. J., Lee, K. R., Lee, E. J., Suh, Y. S. & Kim, K. S. (2015). Expectorant and antitussive effect of Hedera helix and Rhizomacoptidis extracts mixture. *Yonsei Med J.*, 56(3):819-24.

Šarčević Todosijević, Lj., Petrović B., Marinković T., Živanović Lj. & Popović V. (2018). The review of medicinal herbal taxons of Magnoliophyta division on the locality of Košutnjak. *XXIII Symposium on Biotechnology,* Čačak, Serbia, 2018, p. 339-345.

Šarčević Todosijević, Lj., Popović, V., Popović, S. & Živanović, Lj. (2019a). The possibility of the use of allelopathic relationships in plant growing. Chapter 4. Ed. Janjev. I. Book Title: *Serbia: Current Issues and Challenges in the Areas of Natural Resources, Agriculture and Environment. NOVA Science publishers, USA*, ISBN: 978-1-53614-897-8, p. 105-122, p. 1-383.

Šarčević Todosijević, Lj., Petrović, B., Vukomanović, P., Živanović, Lj., Garčić, J. & Popović V. **(**2019b). Antimicrobial activity of secondary plant metabolites, *Thematic Proceedings, XXIV Symposium on Biotechnology*, with International Participation, Čačak, Serbia. p. 357-364.

Tugrul, K.M. (2019). Soil Management in Sustainable Agriculture. In book: Soil Management and Plant Nutrition for Sustainable Crop Production, Publisher: *IntechOpen. DOI: 10.5772/intechopen.88319*

Vojnov, B., Šeremešić, S., Ćupina, B., Krstić, Đ., Vujić, S., Živanov, M. & Pavlović, S. (2020). Hot water extractable organic carbon of chernozem under the system of cover crop incorporation and subsequent sowing of spring crops. *Zemljište i biljka*. 69(2): 82-94. DOI 10.5937/ZemBilj2002082V

Van den Hoven, R., Zappe, H., Zitterl-Eglseer, K., Jugl, M. & Franz. C. (2003). Study of the effect of Bronchipret on the lung function of five Austrian saddle horses suffering recurrent airway obstruction (heaves). *Vet. Rec.* 152(18): 555-557.

Watson, D.G. (2019). Pharmaceutical Analysis, Churchill Livingstone, London, UK

Wagner, L., Cramer, H., Klose, P., Lauche, F., Gass, G. & Dobos, J. (2015). Langhorst. Herbal Medicine for Cough: a Systematic Review and Meta-Analysis. *Forsch Komplementmed*. 22(6): 359-368.

Zeil, S., Schwanebeck, U. & Vogelberg, C. (2014). Tolerance and effect of an add-on treatment with a cough medicine containing ivy leaves dry extract on lung function in children with bronchial asthma. *Phytomedicine*. 21(10):1216-1220.