doi:10.25143/prom-rsu_2021-08_dt

RĪGA STRADIŅŠ UNIVERSITY

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Medicinal Plants in the Records of Latvian Folk Medicine and Analysis of Their Practical Applications

Doctoral Thesis for obtaining
a doctoral degree (*Ph.D.*)

Sector – Basic Medical Sciences, including Pharmacy
Sub-Sector – Pharmacognosy

Riga, 2021

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Riga, 2021

Annotation

Medicinal plant knowledge in Europe is rooted in a long history of health traditions. Numerous ethnobotanical studies across Europe have addressed the increasing importance of the traditional use of medicinal plants. Latvia is a country with old folk medicine traditions and an extensive folk knowledge archive. The aim of this thesis was to collect and analyse knowledge about the use of the medicinal plants found in the records of Latvian folk medicine and to search for new ideas regarding the practical applications of these plants. This thesis reveals, for the first time to the international scientific community, the important ethnobotanical information contained in the records of Latvian folk medicine that had not yet been translated into English and are therefore less visible to researchers from all over the world.

This thesis provides a list of the used plant species and plant parts, the dosage forms of herbal medicines, the routes of administration, and the disorders treated with medicinal plants mentioned in the records of Latvian folk medicine and used by indigenous people of Latvia in the end of 19th and the beginning of 20th centuries. In total, the thesis includes information on 211 plant taxa, most of which were utilized for the treatment of digestive and respiratory system disorders. To understand whether the information mentioned in the folklore material is relevant today, it was compared with evidence-based information regarding the uses of the listed plant, including official herbal monographs. During this study, it was concluded that only 59 plant taxa mentioned in the studied records of Latvian folk medicine are included in the official monographs of the European Medicines Agency, and most of the plant indications mentioned in the records have not been described in evidence-based monographs.

After the systematic analysis of folklore materials, additional information on the molecular mechanisms of the anti-inflammatory activities of *Pelargonium sidoid*es DC. and *Prunus padus* L. were investigated to confirm their traditional use for the treatment of inflammatory conditions. The obtained results provide evidence that both plant extracts exhibit pronounced *in vitro* and *ex vivo* anti-inflammatory activities, supporting their use in Latvian ethnomedicine as effective anti-inflammatory agents.

The obtained results are important as they provide ideas for further research related to possibilities regarding the use of plants growing in the territory of Latvia and allow new perspectives to be gained for both national and international ethnobotanical research.

Keywords: folk medicine, ethnomedicine, records of Latvian folk medicine, *Pelargonium sidoides* root extract, proanthocyanidins, *Prunus padus* flower extract, anti-inflammatory activity.

Anotācija

Promocijas darba tēma: **Ārstniecības augi latviešu tautas ārstniecības pierakstos un to praktiskā lietojuma analīze**

Daudzi Eiropas valstīs veiktie etnobotāniskie pētījumi vērš uzmanību uz ārstniecības augu tradicionālās izmantošanas pieaugošo nozīmi. Eiropā zināšanas par ārstniecības augu lietošanu balstās senā vēsturē un tradīcijās. Arī Latvija ir valsts ar gadsimtos krātām tautas medicīnas tradīcijām un plašu šo zināšanu arhīvu.

Promocijas darba mērķis bija apkopot un analizēt latviešu tautas ārstniecības pierakstos pieejamās zināšanas par ārstniecības augu lietošanu un meklēt jaunas idejas šo zināšanu praktiskam lietojumam mūsdienās. Promocijas darbs pirmo reizi starptautiskajai zinātniskajai sabiedrībai atklāj latviešu tautas ārstniecības pierakstos iekļauto informāciju par ārstēšanos ar augiem, kas līdz šim nebija tulkota angļu valodā, tāpēc nebija pieejama citu valstu pētniekiem.

Pētījums ietver sarakstu ar Latvijas teritorijā 19. gs. beigās un 20. gs. pirmajā pusē tautas medicīnā izmantotiem augiem, to sugām, ārstniecībā lietotām šo augu daļām un pagatavojumiem no tām, to ievadīšanas veidu un slimībām vai to simptomiem, kas ārstēti ar šiem līdzekļiem. Darbs satur informāciju par 211 augu ģintīm, no kurām lielākā daļa tika izmantotas gremošanas un elpošanas sistēmas traucējumu ārstēšanai. Lai saprastu, vai folkloras materiālos norādītā informācija mūsdienās ir aktuāla, tradicionālais augu lietojums tika salīdzināts ar informāciju, balstītu uz pierādījumiem. Pētījumā secināts, ka 59 augu drogām ir pieejamas oficiālas Eiropas Zāļu aģentūras datubāzē publicētas monogrāfijas, bet lielākajai daļai latviešu tautas ārstniecības pierakstos minēto indikāciju nav zinātniska pierādījuma.

Pēc sistemātiskas folkloras materiālu analīzes turpmākam pētījumam tika izvēlēti *Pelargonium sidoides* DC. un *Prunus padus* L., lai apstiprinātu šo augu drogu tradicionālo lietojumu iekaisuma stāvokļu ārstēšanai. Šim nolūkam pētījuma gaitā tika noskaidroti to pretiekaisuma aktivitātes molekulārie mehānismi. Iegūtie rezultāti sniedz pierādījumu, ka abu augu drogu ekstraktiem *in vitro* un *ex vivo* ir izteikta pretiekaisuma aktivitāte, tā apstiprinot to izmantošanas lietderību latviešu tautas medicīnā par efektīvu līdzekli iekaisuma procesu mazināšanai.

Promocijas darba rezultātu nozīmīgums: tie var rosināt jaunas idejas turpmākiem ar Latvijas teritorijā augošu augu izmantošanas iespējām saistītiem pētījumiem un var pavērt jaunas perspektīvas gan nacionāliem, gan starptautiskiem etnobotāniskiem pētījumiem.

Atslēgvārdi: tautas medicīna, etnomedicīna, latviešu tautas ārstniecības pieraksti, *Pelargonium sidoides* sakņu ekstrakts, proantocianidīni, *Prunus padus* ziedu ekstrakts, pretiekaisuma aktivitāte.

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Abbreviations

ALF	Archives of Latvian Folklore
ANOVA	analysis of variance
BMDMs	bone marrow-derived macrophages
cDNA	complementary deoxyribonucleic acid
COX-2	cyclooxygenase 2
DAD	diode-array detection
DPPH	2,2-diphenyl-1-picrylhydrazyl
EC_{50}	half maximal effective concentration
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
ESCOP	European Scientific Cooperative on Phytotherapy
ESI	electrospray ionization
EU	European Union
FBS	fetal bovine serum
GAE	gallic acid equivalent
GC-MS	gas chromatography-mass spectrometry
GPI	glucose-6-phosphate isomerase
HBSS	Hank's buffered saline solution
HMPC	Committee on Herbal Medicinal Products
HPLC	high-performance liquid chromatography
IC_{50}	half maximal inhibitory concentration
IFN-γ	interferon gamma
IL-10	interleukin 10
IL−1β	interleukin-1 beta
IL-4	interleukin-4
IL-6	interleukin-6
iNOS	inducible nitric oxide synthase
LC-MS	liquid chromatography-mass spectrometry
LC-MS-IT-TOF	liquid chromatography mass spectrometry–ion trap–time of flight
LDH	lactate dehydrogenase
LPS	lipopolysaccharide
M1	classically activated (pro-inflammatory) macrophages
M2	alternatively activated (anti-inflammatory) macrophages
M-CSF	monocyte-colony stimulating factor

MPP	methylene chloride fraction of *P. padus*
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NO	nitric oxide
NSAIDs	nonsteroidal anti-inflammatory drugs
P. padus	*Prunus padus*
P. sidoides	*Pelargonium sidoides*
PACN	proanthocyanidins from *Pelargonium sidoides* root extract
PBMC	peripheral blood mononuclear cells
PPFE	*Prunus padus* flower extract
PSRE	*Pelargonium sidoides* root extract
RT	retention time
SEM	standard error of the mean
SD	standard deviation
TIC	total ion chromatogram
TNF-α	tumor necrosis factor alpha
w/v	weight by volume

Introduction

Currently, ethnobotanical studies on the traditional use of wild and cultivated plants have become increasingly popular and have contributed to bioeconomics and green knowledge. Despite many studies conducted all over the world and in two neighbouring Baltic states, Estonia and Lithuania, Latvia has made a very small contribution to European ethnomedicine (Pardo-de-Santayana et al., 2015). Thus, Latvian knowledge must be analysed and brought to a wider audience.

Ethnobotanical research aims to document traditional knowledge regarding medicinal plants and to provide assessments of their uses. In such research, lists of species and plant families used are complemented with information about the cultural importance of these species, e.g., the frequency or citation of their use. The data obtained are relevant for cross-cultural comparisons wherein data of one ethnic group, culture, or region are compared to those of another. Further information from ethnobotanical studies can initiate new experimental investigations regarding the traditional use of specific plants (Heinrich et al., 2009).

The information found in the records of Latvian folk medicine is based on the knowledge of traditional folk medicine passed from generation to generation. During the 19th century, across Europe, including in the territory of Latvia, folklore research became increasingly popular. The folklore materials collected during the 19th and 20th centuries are an integral part of Latvia's cultural heritage (Ciglis et al., 2016). The records of Latvian folk medicine can be found in collections held by the Archives of Latvian Folklore (ALF). The ALF is the largest centre of archived vernacular knowledge in Latvia. Information gathered on medicinal plant species is dispersed among almost all ALF collections. The second source containing records about medicinal plants comprises four published volumes of “Latvian Folk Beliefs” (Šmits, 1940, 1941). The information found in these sources is not systematized or analysed from a botanical or pharmacological point of view, and this information is not available for international comparison. To understand whether the information mentioned in the folklore materials is relevant today, this information must be compared with evidence-based information on the uses of the relevant plants, including scientific articles and herbal monographs.

There are more than 500 000 species of land plants in the world, but the mechanisms of the actions of their constituents, including the molecular mechanisms of anti-inflammatory activities, are not yet fully understood (Corlett, 2016; Vogl et al., 2013). The ability of plant extracts to reduce inflammation has been indicated by various means in experimental models. Since inflammation enhances the release of specific mediators, inhibition of the production of

these mediators can be used to investigate the anti-inflammatory effects of plants that are widely used in folk medicine for this purpose (Martins et al., 2017).

Aim of the study

This study aims to collect and analyse knowledge about the use of medicinal plants found in the records of Latvian folk medicine, and to search for new ideas for practical applications of these plants.

Objectives of the study

1. To select records of Latvian folklore materials containing information about plant usage for human medicine and to systematically review these usages by analysing and summarizing information on the most common diseases and symptoms indicated in the records.
2. To compare the uses of plants mentioned in the records of Latvian folk medicine with current knowledge regarding their uses in modern evidence-based medicine.
3. To investigate and confirm the activities of the plants that were mentioned in the records of Latvian folk medicine as being used to reduce the inflammatory process.

Hypothesis of the study

1. Most (> 50%) plant indications mentioned in the records of Latvian folk medicine have been described in evidence-based studies and are still relevant today.
2. Folklore research is sources of ideas for discovering new substances/medications and therapy options.

Scientific novelty of the study

Within the framework of this research, records of Latvian folk medicine were identified and systematically reviewed to clarify whether the folklore materials refer to significantly diverse plants for treating different disorders and to determine the most medicinally valuable species in the territory of Latvia during the 19th and 20th centuries:

1. This is the first systematic ethnobotanical study conducted in Latvia that provides a list of medicinal plants, including their health benefits and applications. Each plant mentioned in the records of Latvian folk medicine has been identified according to its scientific plant name in Latin; the plant uses have been grouped

into medicinal use categories; and the plant parts used, the dosage forms of the herbal medicines and the routes of administration have been analysed.

2. Information regarding which plants were the most important in the treatment of different disorders and which were the most common disorders treated with plants in the territory of Latvia during the 19th and 20th centuries is now published and available at the international level for comparisons with other ethnobotanical studies.
3. The plant species mentioned in the records of Latvian folk medicine, as well as their uses, could be useful for future research on herbal medicine inspired by the research of folklore data.
   3.1. The anti-inflammatory activities of *Pelargonium sidoides* DC. root extract (PSRE) and proanthocyanidins in PSRE (PACN) were investigated in bacterial lipopolysaccharide (LPS)-mediated inflammation *in vitro* and justified for their practical application.
   3.2. An investigation of the chemical composition and pharmacological activities of the *Prunus padus* L. flower extract (PPFE) demonstrated a promising result for its potential use in reducing inflammatory conditions.

1 Literature

1.1 Traditional herbal medicine and ethnobotanical studies

The application of plants in traditional medicine has a long history, and the use of plants as medicine is as old as human civilization. Historically, medicinal plants have gained considerable recognition for the prevention and treatment of different sicknesses. Numerous biologically active components have been isolated from plants known in traditional medicine. It is estimated that out of the 122 plant-derived drugs, 80% have been developed on the basis of ethnomedical information (Fabricant and Farnsworth, 2001). Examples of well-known drugs originated from herbal medicine are digoxin from *Digitalis* L. spp., quinine and quinidine from *Cinchona* L. spp., vincristrine and vinblastine from *Catharanthus roseus* (L.) G.Don, morphine and codeine from *Papaver somniferum* L., and atropine from *Atropa bella-donna* L. (Rates, 2001; Vickers et al., 2001). Between 10% and 25% of the drugs prescribed worldwide contain one or more natural bioactive compounds (Cameron et al., 2005; Pan et al., 2013). Interestingly, over the last 30 years, up to 50% of all cancer drugs are either directly or indirectly derived from natural products (Cragg et al., 2012).

The core idea of ethnobotany is to investigate human-plant relationships by recording and preserving local community knowledge (McClatchey et al., 2009). Based on ethnobotanical studies, new bioactive compounds or new plant uses are continually being identified or rediscovered (Atanasov et al., 2015). In the last 10 years, the number of research articles containing the word *ethnopharmacology* was 2038 (data obtained from PubMed, December 2020), which is 5 times greater than the number for the previous decade (Figure 1.1). This finding reflects an increasing tendency to study and document medicinal plants used in traditional medicine.

Figure 1.1. **Growing numbers of citations per year found by searching for the term *ethnopharmacology* in Pub Med database**

Made with PubMed by Year: http://esperr.github.io/pubmed-by-year

Just a few studies have analysed medicinal plant use in the northern Europe; for example, analysis from Lithuania, Estonia, Poland, Belarus, and Russia has been published recently (Kujawska et al., 2017; Pranskuniene et al., 2018; Sak et al., 2014; Shikov et al., 2017; Sõukand et al., 2013). The knowledge of utilization of plants in the Baltic States and especially in Latvian folk medicine is incomplete. Up to now, Latvian ethnologists and folklorists have focused on healing traditions that include the use of plants (Ančevska, 2018), but no one has analysed the plants mentioned in the records of Latvian folk medicine in terms of botanical nomenclature, pharmacognosy and pharmacological activity.

1.2 Records of Latvian folk medicine

During the 19th century, across Europe and including in the territory of Latvia, folklore research became increasingly popular (Ciglis et al., 2016). The targeted collecting of vernacular knowledge in Latvia started in the middle of the 19th century, however, during the latter part of the 19th century, the collection of folklore had become a national movement with active societal involvement (Viksna, 2017). Until 1945, the collection of records continued in a relatively large area. In the Latvian folklore, there are several genres, such as fairy tales, legends, records of folk beliefs, magic practices, folk songs, and records of folk medicine. Latvian folk beliefs are short statements about the order in the nature and human life. Beliefs help to predict the course of future events, to identify actions that help to achieve a positive outcome, and describe prohibitions that help to prevent failure. Folk beliefs also include the translation and divination of dreams and weather predictions (Treija, 2020). Most evidence describing the healing tradition in the territory of Latvia during the 19th and 20th centuries are found among folk beliefs. The folklore researcher P. Šmits has made a significant contribution

to the study of Latvian folklore and folk traditions. He compiled "Latvian Folk Beliefs" (1940–1941) that is one of the most valuable sources containing information about plant use in Latvian folk healing traditions. Although the information on plants in this book is compiled under the title "Latvian Folk Beliefs", the units that describe the use of plants scientifically correctly should be called records of folk medicine. In the Archives of Latvian Folklore (ALF), these units are also registered under the folk medicine genre. Therefore, this term "records of Latvian folk medicine" is used in the dissertation.

Ethnobotanical knowledge found in the records of Latvian folk medicine, can be used to identify what were the most medicinally valuable species in the territory of Latvia during the 19th and 20th centuries and show their importance in local people healthcare. Folklore materials provide information about the health status of the ancient population living in the territory of Latvia and folk healing methods. These records include valuable information that was transmitted orally from generation to generation on what plants were used at that time. Knowledge of the chemical composition of plants might not have been available to local people, but they knew medicinal plants that were useful to treat many kinds of diseases and health conditions. Nowadays, it is important to preserve our folk traditions, including healing traditions to save them as a traditional Latvian heritage, to analyse ethnomedicinal knowledge transfer from generation to generation, to compare information about the use of plants, illnesses and diseases treated by the indigenous people with neighboring or other countries, and this information could be useful in herbal medicine research and development.

1.3 Medicine and folk healing tradition between the 19th and 20th centuries

In the late 19th century, the majority of Latvians lived in the countryside and were engaged in agriculture. Latvians positioned themselves as farmers, and they saw the countryside as the main place where the stability of the nation and its traditions are preserved (Ciglis et al., 2016; Locmele, 2011). In the territory of Latvia, the first doctors possessing a university education started to work in the countryside only in the 19th century. Before then, doctors worked in towns, but most of them were available only to rich people. Scientific medicine searched rational explanations for health problems, but folk healing was still continuing in the countryside (Ančevska, 2018). Folk healing knowledge that was passed from one generation to another by oral tradition was used by ordinary people (Vīksna, 1993). In addition, the ordinary people had to overcome their own illnesses, using their knowledge of folk healing and natural remedies, including plants. Later, by the time of the foundation of the Republic of Latvia, a wide network of drugstores had formed in towns and in the countryside. For instance, 521 drugstores were functioning in Latvia in 1939 (Vīksna, 1993).

1.4 Official pharmacopoeias used in the territory of Latvia in the 19th and 20th centuries

The first and oldest pharmacy in the territory of Latvia opened in 1357 (Lauze et al., 2018). For centuries, pharmacopoeias – official documents with obligatory instructions about the preparation, storage, quality of medicines, the toxic doses of substances, and of those having a strong effect, have been used in pharmacies. The first collection of that kind of instruction in Europe was published in 1498 in Florence (Otter, 2000). From the 18th century and until 1918, Latvia along with two other Baltic states, Estonia and Lithuania, was a part of the tsarist Russian empire. Russian and German pharmacopoeias and manuals were used in pharmaceutical practice at the time (Kondratas et al., 2015). Russia was among the first countries to compile a pharmacopoeia, and the first Russian pharmacopoeia the Pharmacopoeia Rossica was published in 1778 (Shikov et al., 2014). In 1886, this pharmacopoeia was translated into Russian and published as an official document (Otter, 2000). The second (1871), third (1880), and fourth (1891) editions of the Russian Pharmacopoeia were used in the territory of Latvia during the 19th century (Kondratas et al., 2015).

In the 1920ies, pharmacists consulted the 6th edition of the Russian Pharmacopoeia (1910), which was very outdated and did not reflect the rapid progress and innovations of 20th century pharmacy. In 1940, Latvian pharmacists compiled and released the Latvian Pharmacopoeia based on actual knowledge of that time. The compilation of the Latvian Pharmacopoeia took 17 years. The articles in the Latvian Pharmacopoeia were based on the German, Russian, British, U.S., Belgian, Swiss, Italian, Romanian, Spanish, and Swedish pharmacopoeias and comprised 658 monographs (Kondratas et al., 2015). The Latvian Pharmacopoeia was an important source of information compiled by Latvian researchers and pharmacists. Both the Russian and Latvian pharmacopoeias did not mention the use of herbal medicine, just methods for making herbal preparations.

1.5 Folk and evidence-based medicine from a modern perspective

Since the end of the 20th century, the popularity of traditional medicine has grown rapidly. In addition, during recent years there has been a growing interest in alternative treatments. There are several reasons for this, for example, conventional medicine does not always lead to the desired result; misuse and/or abuse of synthetic medicines often cause side effects, but folk medicine practitioners recommend “natural” products as safe; many people worldwide do not have adequate and regular access to treatment with western medicine methods and tools. However, the use of these traditional products is not always allowed by the national authorities that are responsible for testing the effectiveness and safety of medicines. In order to

obtain such permission, new studies should be performed to approve their effects (Wachtel-Galor and Benzie, 2011).

In many cases, the use of herbal medicines in scientific evidence-based medicine is based on folk and traditional medicine, and the use of herbal medicines is not always scientifically proven with laboratory testing or clinical trials (European Medicines Agency, 2017). Folk medicine is an empirical method of treatment with a long history. In folk medicine, knowledge has been transmitted from generation to generation. Folk medicine eliminates the symptoms of the disease without finding out the true cause. Folk medicine varies from nation to nation and is especially popular in rural areas. Until the 19th century, folk medicine was the most important method of treatment (Petrovska, 2012). As modern medicine developed and became more accessible, the importance of folk medicine decreased. At the same time, scientific interest in folk medicine is growing, as ethnopharmacology is considered an essential tool for obtaining new biologically active substances. The list of herbal drugs used in human medicine is shorter than the known number of plant species. Approximately 350,000 known plant species have been identified worldwide (Shikov et al., 2014). Interestingly, only a small part of the therapeutic effects of plants have been scientifically studied, so in most cases the use of plants is based on tradition and *long-standing use*. Folk medicine is the foundation of modern evidence-based medicine.

1.6 Anti-inflammatory drugs and anti-inflammatory activity of plants

Plants possessing anti-inflammatory effects have been used by humans since ancient times. Inflammation is the response to the body's tissue damage or pathogenic irritants. The inflammatory process is involved in a variety of pathological conditions including arthritis, atherosclerosis, metabolic syndrome, sepsis, allergies, autoimmune, and oncological diseases. Nowadays, most of these diseases are either poorly treated or inadequately prevented (Vogl et al., 2013; Waltenberger et al., 2016). Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most widely used drug groups in the world. NSAIDs have anti-inflammatory, antipyretic, and analgesic properties. NSAIDs have a rapid therapeutic effect, but they can also have significant side effects on the digestive system (dyspepsia, heartburn, nausea, gastritis, gastric ulcer), kidney (acute or chronic renal failure) and cardiovascular system (cardiovascular events) (Harirforoosh et al., 2013; Ungprasert et al., 2015). For this reason, new research concerning oral anti-inflammatory drugs with less side effects is needed. Topical NSAIDs have also been widely used to relieve the pain of inflammatory skin conditions. However, no evidence supports the long-term use of topical NSAIDs (Lin et al., 2004). Various plant species with anti-inflammatory activities have been documented in the scientific literature. However, the

compounds responsible for the anti-inflammatory activity have not yet been fully explained. Medicinal plants are used instead of NSAIDs as the use of non-steroidal anti-inflammatory drugs is associated with several of the above-mentioned side effects. Therefore, the search for medicinal plants as a source of anti-inflammatory drugs keeps increasing (Shah et al., 2011). Alkaloids, terpenes, and phenolic compounds such as saponins, lignans, coumarins, tannins, and especially flavonoids are substances of plant origin which proved to have an anti-inflammatory activity (Nunes et al., 2020). Flavonoids represent a group of natural substances characterized by a C6–C3–C6 backbone structure, being found in fruits, seeds, flowers, roots, stems, bark, tea, and wine (Panche et al., 2016). Flavonoids can be divided into several subclasses such as flavanones, flavones, flavanols, flavonols, isoflavones, and anthocyanidins (Figure 1.2). Among the different flavonols, myricetin, kaempferol, and quercetin are the most representative (Serafini et al., 2010). Flavonoids have been shown to possess a wide variety of beneficial effects on human health including antimicrobial, antioxidant, coronary heart disease prevention, hepatoprotective, anticancer, antiviral, anti-inflammatory and immunomodulatory activities (Kumar and Pandey, 2013; Owona et al., 2020). Certain flavonoids act by modulating the induced nitric oxide synthase (NOS) enzyme and cells involved in inflammation, inhibiting the production of pro-inflammatory cytokines, and modulating the activity of arachidonic acid pathways, such as cyclooxygenase, lipoxygenase, and phospholipase A2 (Nunes et al., 2020).

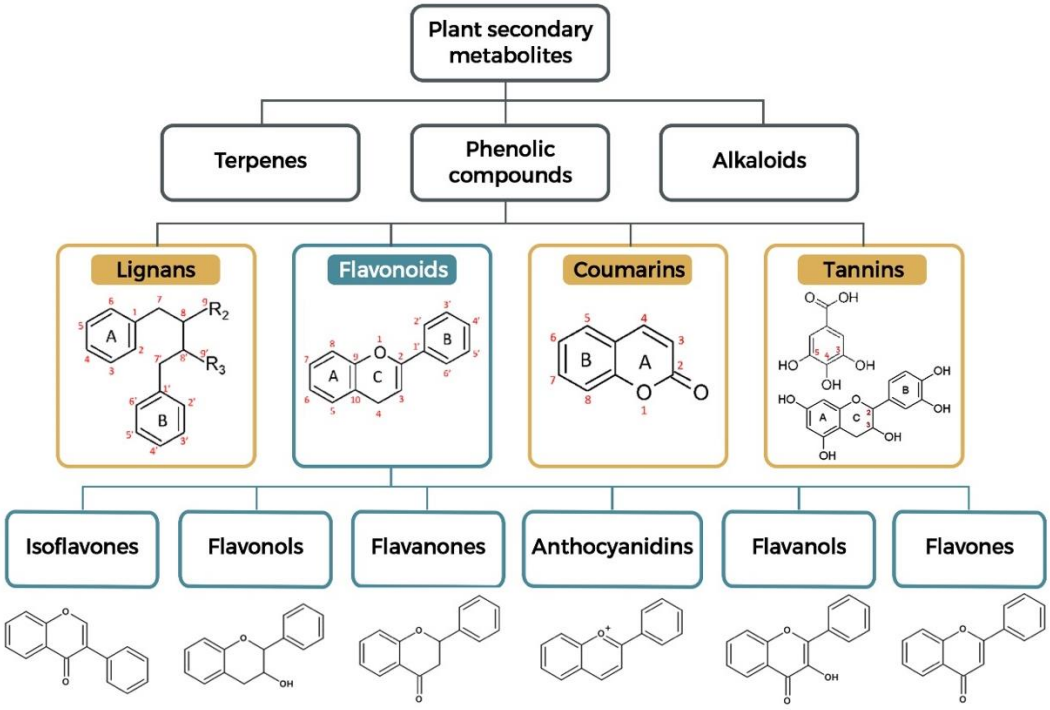


Figure 1.2 **Plant secondary metabolites with anti-inflammatory activity, and basic structure of flavonoids and their subclasses**

Modern technologies allow analysing the identity and pharmacological effects of substances found in plants and obtain evidence-based proof for their traditional use. More studies are needed in order to validate certain plant usage in traditional medicine. Due to the production of secondary metabolites, medicinal plants play an important role in the development of new and potent drugs. In addition, information gathered from folk materials can be further analysed and initiate new ideas for the potential usage of plants in medicine, cosmetics, or as food supplements.

1.7 The role of macrophages and blood leukocytes in inflammation processes

The inflammatory response in various organs or organ systems of the body can be caused by both infectious and noninfectious agents and can be either acute or chronic. As a result, inflammation can cause tissue damage or disease (Chen et al., 2017). Macrophages and leukocytes are immune cells that play a key role against infection. In addition, macrophages are involved in most stages of inflammation, including healing (Butterfield et al., 2006; Oishi and Manabe, 2018). Activated macrophages can be polarized into a pro-inflammatory M1 phenotype and alternative anti-inflammatory M2 phenotype (Mills, 2012). M1 macrophages are involved in initiating and sustaining inflammation, while M2 macrophages are associated with the resolution of chronic inflammation and promote wound healing (Tang et al., 2018). Usually, leukocytes are the first responders to be involved in the immune response and have a higher microbicidal activity; where macrophages are involved later on (Prame Kumar et al., 2018).

The release of inflammatory factors is in the first line of defense of the innate immune system against many common microorganisms and are essential for the control of common bacterial infections. The next step leading to a prolonged and enhanced inflammatory reaction is the induction of inflammatory genes to produce new mediators (Janeway et al., 2001). Activated macrophages and leukocytes are the main source of such pro-inflammatory cytokines as tumor necrosis factor (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) (Duque and Descoteaux, 2014). Bacterial infection also triggers inducible NO synthase (iNOS) to produce reactive nitrogen species stress on various pathogens, including bacteria (Xue et al., 2018).

1.8 Plant species from records of Latvian folk medicine selected for experimental analysis

1.8.1 Pelargonium sidoides

Pelargonium is the second largest genus (after *Geranium*) in the family *Geraniaceae* Juss. *Pelargonium sidoides* DC. is a perennial plant with dark maroon-red to black and linear to spathulate petals, with green sepals having white margins, and fleshy, bright red tubers or rhizomes (Brendler and van Wyk, 2008). *P. sidoides* is a medicinal plant native to South Africa (POWO, 2019). The underground parts of the plant have been widely used in South Africa by local communities as a traditional medicine for curing various ailments, including diarrhoea, colic, gastritis, tuberculosis, cough, hepatic disorders, menstrual complaints and gonorrhoea (Moyo and Van Staden, 2014). In the late 1890s, due to its use in the treatment of tuberculosis, *P. sidoides* was introduced in Europe (Moyo and Van Staden, 2014). *Pelargonium* spp. were among the widely used cultivated species throughout Europe (Pardo-de-Santayana et al., 2015). Two most mentioned species of the genus *Pelargonium* in other ethnomedicinal studies were *P. odoratissimum* (L.) L'Her (flowers and leaves were used internally for reduction of cough and inflammation) and *P. grandiflorum* Willd. (was used internally together with milk and honey in the treatment of pneumonia) (Kujawska et al., 2017; Pranskuniene et al., 2019).

Initially, *P. sidoides* was traditionally used mainly for gastrointestinal disorders, but later its indications were extended to respiratory tract infections. Today, an aequous ethanolic root extract from *P. sidoides* has been formulated into phytopharmaceutical, namely, EPs® 7630 (exclusively contained in Umckaloabo® (in Latvia known as Umckalor®), marketed by Spitzner Arzneimittel, Ettlingen, Germany) (Kolodziej, 2011). It is approved as a traditional herbal medicine with *long-standing use* for the treatment of acute and chronic infections of the upper respiratory tract of viral and bacterial origin (bronchitis, sinusitis, tonsillitis, angina and rhinopharyngitis) (EMA, 2018). The drug is also listed in the monograph of the European Pharmacopoeia, 10th edition (latin name: *Pelargonii radix*; english name: Pelargonium root).

P. sidoides root extracts have been reported to possess antibacterial, antiviral, and immunomodulatory activities. The antibacterial and antiviral effects are attributed to gallic acid and other phenolic compounds, while the immunomodulatory activity is considered due to a combination of phenolic compounds and numerous coumarins (Brendler and van Wyk, 2008; Moyo and van Staden, 2014). According to PubMed database, there are only a few research articles concerning anti-inflammatory effects of *P. sidoides* root extracts. More investigation on the molecular mechanism of anti-inflammatory effect of traditionally used root extracts from *P. sidoides* should be carried out.

It is already known that proanthocyanidins (PACNs) are the major phenolic compounds in *P. sidoides* roots. The chemical nature of PACNs in crude extracts varies depending on the plant species used. *P. sidoides* is one of the richest PACN-containing plants and a significant part of the activities of crude extracts might be assigned to PACNs. Study of Savickiene et al. (2018) has shown that isolated PACNs exhibited higher antioxidant capacity and antibacterial action than *P. sidoides* root extract (PSRE). This suggests that isolated PACNs might also have more pronounced anti-inflammatory activity compared to the whole extract. To test this hypothesis - whether PACNs could indeed have a better anti-inflammatory effect - a part of this dissertation was conducted to investigate PACN activity in bacterial lipopolysaccharide (LPS)-mediated inflammation, including measurement of the secretion of inflammatory cytokines and other mediators, inflammatory gene expression and viability of macrophages and blood leukocytes.

1.8.2 Prunus padus

Prunus padus L., known as bird cherry, is a small to medium-sized tree or bush (2–14 m) in the family *Rosaceae* Juss., which is native to Europe, Asia and Morocco (POWO, 2019). *P. padus* blooms in early spring with white flowers in racemes approximately 10 cm long, which have a pleasant smell, and develops an edible stone fruit, black in color when ripe (Uusitalo, 2004). The species belongs to the subgenus *Padus* in the genus *Prunus* L. (Nestby, 2020). *Prunus* has been among the most frequently mentioned genera that has been used for making recreational tea in eastern and central Europe (Sõukand et al., 2013). Estonian folkloristic data on medicinal plant use revealed that *P. padus* was one of the most frequently utilized plants for medicinal purposes at the end of the 19th and during the 20th century (Sõukand and Kalle, 2011).

The results of studies of extracts from different plant parts of *P. padus* have been summarized in a recent review (Telichowska et al., 2020). This review presents and characterizes the phytochemical content of the investigated extracts and reports their antioxidant, anti-inflammatory, antibacterial, and antidiabetic activities.

P. padus was selected for detailed analysis because it was among the top ten most popular plant species mentioned in the records of Latvian folk medicine and due to the lack of studies related to the pharmacological activities of *P. padus* flowers. In addition, compared to the other most frequently mentioned species from the records of Latvian folk medicine, *P. padus* is less well studied, and in Europe, it is used based on knowledge of ethnobotanical information. Only fruits of *P. padus* are used as medicine and included in the Russian

Pharmacopoeia (Shikov et al., 2017). The infusion of *P. padus* fruits are used for their astringent properties (1:20 w/v; 100 ml once a day).

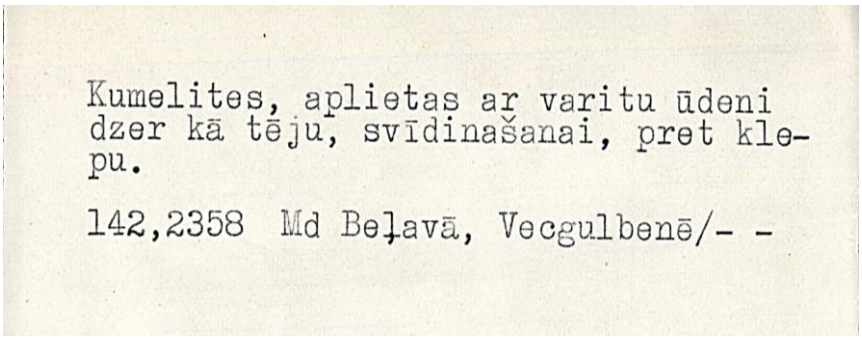
Although all parts of plants, such as the bark, leaves, flowers, and fruits, are used in Europe based on ethnobotanical knowledge, flowers are of interest for experimental analysis because little research has been done on them, especially on the molecular mechanisms of their anti-inflammatory action. To date, several studies have been performed on the phytochemical composition of *P. padus* flower extracts with chromatographic methods (GC-MS, LC-MS). Due to the characteristic scent of flowers, two previous studies have been dedicated to the determination of volatile compounds, and the other two referred to the qualitative and quantitative characterization of phenolic compounds. The anti-inflammatory activity of a chloroform extract of *P. padus* flowers has been investigated and reported so far (Magiera et al., 2019).

2 Materials and methods

2.1 Folklore material research and analysis

2.1.1 Data collection and botanical identification

Data on plants and their uses were collected from the records of Latvian folk medicine, the Archives of Latvian Folklore (ALF) of the Institute of Literature, Folklore and Art at the University of Latvia. In total, more than 40000 records were reviewed to select those containing information about plant usage in human medicine. A part of the records was obtained from the collections digitized by the Digital Archives of Latvian Folklore (http://en.lfk.lv). Information gathered on ethnomedicinal plants was dispersed among almost all manuscript collections analysed. To make it usable for research purposes, a card index was created and systematized according to ailments. Each card contains the text extracted from the original manuscript, as well as a geographical location (e.g. district, city or town). An exemplar file card with information on the name and uses of a plant is shown in Figure 2.1. Some records were collected from the four published volumes titled "Latvian Folk Beliefs" (Šmits, 1940–1941) compiled by the folklore researcher P. Šmits. The collection of folk beliefs published in his compilation contains 36790 records in 1626 alphabetic chapters, it is digitized and published (http://valoda.ailab.lv/folklora/ticejumi/).


Kumelites, aplietas ar varitu ūdeni dzer kā tēju, svīdinašanai, pret klepu.

142,2358 Md Beļavā, Vecgulbenē/- -

Figure 2.1 **An exemplar file card from the Archives of Latvian Folklore with information on the name and uses of a plant from the village Beļava, Vecgulbene**

Translation of the record of Latvian folk medicine: Pour boiling water over the chamomile and drink as tea to induce sweating and to treat cough.

For each plant identified in the records of Latvian folk medicine, taxonomic ranks of family, genus, and species were recorded. The Latvian plant name, mentioned in the records was compared with plant names published in the Latvian plant name dictionary (Ēdelmane and Ozola, 2003). This book contains the most complete register of local plant names collected in Latvia and published in the last century, with references to the sources used. Local plant names in official Latvian language were provided according to the Encyclopedia Nature of Latvia

(https://www.latvijasdaba.lv). Plant names were updated to the currently accepted scientific plant names in Latin following The Plant List website (www.theplantlist.org) and family names were arranged using the Angiosperm Phylogeny Group 4 classification (www.mobot.org/MOBOT/research/APweb/).

2.1.2 Medicinal use categories

The use reports were attributed to 17 medicinal use categories following the The International Classification of Primary Care (ICPC; www.who.int/ classifications/icd/adaptations/icpc2/en/) accepted by the World Health Organization (WHO) and used for disease classification. Following the ICPC, plant uses were grouped as follows: 1) General and unspecified; 2) Blood, blood-forming organs, lymphatics and spleen; 3) Digestive; 4) Eye; 5) Ear; 6) Circulatory; 7) Musculoskeletal; 8) Neurological; 9) Psychological; 10) Respiratory; 11) Skin; 12) Endocrine, metabolic and nutritional; 13) Urology; 14) Pregnancy, childbirth and family planning; 15) Female genital system and breast; 16) Male genital system; and 17) Unspecified medicinal disorders, the last of which was used when it was not possible to code the disease. The number of citations was indicated for each use of medicinal plant.

2.1.3 Plant parts used, preparations and routes of administration

Plant parts, preparations and routes of administration were described for each plant mentioned in the records of Latvian folk medicine. When there was no mention of plant parts, preparations or administration, the category “unspecified” was used.

2.1.4 Folkloristic data comparison with official herbal monographs

Folkloristic data presented in the study were compared with the Russian Pharmacopoeia (1891), which was official pharmacopoeia used in the territory of Latvia during the 19th century. This fourth edition of Russian Pharmacopoeia contains 808 monographs, including 171 individual plant monographs. The second pharmacopoeia used for data comparison was Latvian Pharmacopoeia (1940).

In order to identify species from the folklore material that were medicinally valuable in the territory of Latvia during the 19th and 20th centuries and are still valuable nowadays, comparison of medicinal plant usage was made with the European Union (EU) herbal monographs by the Committee on Herbal Medicinal Products (HMPC) and published by the European Medicines Agency (EMA) (www.ema.europa.eu). These monographs include

information not only from scientific databases but also from the European Scientific Cooperative on Phytotherapy (ESCOP) monographs, WHO herbal monographs, and also monographs from the Pharmacopoeia of China and India that are from outside the EU (EMA, 2014). EU herbal monographs deal with the assessment of clinical safety and efficacy and applies to the both well-established and traditional herbal medicinal products. "*Well-established use*" means that at least one controlled clinical study or alternatively a well-documented clinical experience with sufficient supportive pharmacological data is needed to substantiate efficacy for a *well-established use*. "*Long-standing use*" means that herbal medicine is with plausible efficacy or pharmacological effects, and acceptable level of safety (EMA, 2006).

Medicinal plants from the records of Latvian folk medicine were also compared to the handbook for practice on a scientific basis consisting of 212 herbal drugs used in the central Europe and including monographs of herbal drugs from the German Commission E, the ESCOP and the WHO (Wichtl, 2004).

2.2 Plant material and extract preparation

2.2.1 *Pelargonium sidoides* DC. root extract and proanthocyanidins isolated from root extract

The *P. sidoides* root extract (PSRE) was purchased from Frutarom Switzerland Ltd. Rutiwisstrasse 7 CH-8820 Wadenswil (batch no. 0410100). To obtain proanthocyanidins (PACN) from PSRE, 4 g of PSRE was dissolved in 200 ml of 50% methanol, the solution was centrifuged at 2000 x g for 20 min and filtered through 0.45 µm nylon filters. The solution was purified by gel adsorption over Sephadex LH-20. The proanthocyanidins were released from the gel with 70% aqueous acetone (500 ml) and concentrated under vacuum at 35 °C. Afterwards, the aqueous aliquot was freeze-dried.

PSRE and PCANs water solutions for *in vitro* experiments were prepared at concentration of 6 mg/ml, vortexed for 5–10 min, and filtered using sterile syringe filters (Filtropur S, 0.2 µm, Sarstedt).

2.2.2 *Prunus padus* L. flower extract

The fully opened inflorescences of *P. padus* were collected from Salaspils municipality, Latvia, from April to May 2019 (GPS coordinates: 56°53.7157'N, 24°29.5992'E). A voucher specimen was deposited at the Department of Dosage Form Technology, Riga Stradins University, Latvia, under code PI-019/02. Flowers were air-dried in the shade at room temperature. The extract was prepared based on the method described by Todd et al. (2015)

with a slight modification. Briefly, approximately 130 g of dry *P. padus* flowers were homogenized using a mortar and pestle. One hundred grams of obtained powder was macerated with 1000 ml of 70% ethanol solution in water at room temperature in the dark for 7 days. Afterwards, the extract was filtered through a celite pad to remove all insoluble material. From the filtrate, a 600 ml aliquot was taken and concentrated with a rotary evaporator until the volume was approximately 200 ml, which was followed by lyophilization. The dry mass of the extract was 25.51 g, and the yield was 42.5 mass % for the dried material. The powder was labelled and stored in a refrigerator at –20 °C prior to further analysis.

PPFE water solution for *in vitro* experiments were prepared at concentration of 5 mg/ml, vortexed for 2–3 min, and filtered using sterile syringe filters (Filtropur S, 0.2 µm, Sarstedt).

For chromatographic analysis fifty grams of fresh *P. padus* flowers were macerated in 800 ml of diethyl ether. After 20 days, 50 ml of extract was poured into a 250 ml three-necked round bottom flask equipped with an outlet and a room temperature water bath. An argon stream was bubbled through the solution for 2 h. Bubbling was continued until the solvent volume was approximately 1 ml. The extract was prepared based on the method described by Radulović et al. (2009) with a slight modification.

2.3 Cell culture

2.3.1 Isolation of bone marrow-derived macrophages and treatment with extracts

For bone marrow-derived macrophages (BMDM) isolation male C57BL6/J inbred mice (18–20 weeks old, Envigo, Netherlands) were used. The experimental procedures were carried out in accordance with the guidelines of the European Community (2010/63/EU), local laws and policies and were approved by the Latvian Animal Protection Ethical Committee, Food and Veterinary Service, Riga, Latvia. Mice were euthanized by decapitation, and bone marrow cells were extracted from femur bones and differentiated for 7 days in RPMI 1640 with Glutamax (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS, Merck KGaA, Darmstadt, Germany), 1% antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin) and 10 ng/ml M-CSF (monocyte-colony stimulating factor, PeproTech, London, UK).

The Petri dish containing BMDMs was washed with HBSS (Hank's buffered saline solution, Merck KGaA, Darmstadt, Germany) twice. The cells were detached with 0.5% trypsin (Merck KGaA, Darmstadt, Germany) and placed in medium supplemented with 10% FBS, 1% antibiotics, and the cell suspension was centrifuged at 300 x g at room temperature

for 5 min. The cells were resuspended in medium supplemented with 10% FBS and 1% antibiotic and seeded in 12- or 96-well plates.

After 1h incubation in 37 °C incubator, cells were stimulated with: a) PSRE and PACN at 100 µg/ml and LPS (lipopolysaccharide, Merck KGaA, Darmstadt, Germany) 10 ng/ml with murine IFN-γ (interferon gamma, PeproTech Inc., Rocky Hill, NJ, USA) 100 U/ml for pro-inflammatory gene expression and macrophage polarization to M1 (pro-inflammatory) phenotype for 2 h and 24 h, respectively. The cells were cultured at 11 x 10^5 cells/ml (12 well plate); b) PPFE at the concentrations of 250 µg/ml and 500 µg/ml, and LPS 10 ng/ml with murine IFN-γ 100 U/ml for macrophage polarization to M1 phenotype and IL-4 (interleukin-4, Invitrogen, Paisley, UK) 10 ng/ml for macrophage polarization to M2 (anti-inflammatory) phenotype for 24 h. The cells were cultured at 30 x 10^4 cells/ml (12 well plate).

2.3.2 Human peripheral blood mononuclear cells

Human peripheral blood mononuclear cells (PBMCs) were purchased from ATCC (ATCC® PCS-800-011™, Manassas, VA, USA). The cells were cultured at 3.3 x 10^6 cells/ml (12 well plate) in RPMI medium supplemented with 10% FBS, 1% antibiotics. After 1 h incubation in 37 °C incubator, cells were stimulated with 1 µg/ml LPS in the presence of 100 µg/ml PSRE or PACN, for 6 h.

2.4 *Ex vivo* and *in vitro* methods

2.4.1 Analysis of cell viability by lactate dehydrogenase release, alamarblue and MTT assay

PBMCs' viability was assessed by measuring lactate dehydrogenase (LDH) release in cell culture media after 6 h treatment with PSRE and PACN (100 µg/ml each), and LPS (1 µg/ml). LDH activity was measured using a method based on the reduction of a tetrazolium salt (yellow) to formazan (red) (Buttery et al., 1976). The absorbance of kinetic parameters was determined spectrophotometrically at 503 nm on Hidex Sense microplate reader (Hidex, Turku, Finland).

In addition, PBMCs viability after 24 h incubation with different concentrations of PSRE and PACN was determined with alamarBlue®, (Bio-Rad Laboratories, Hercules, CA, USA) following the manufacturer's instructions. Briefly, the ready-to-use solution was added to each well in a 1:10 ratio and incubated for 2 h in the dark at 37°C; then, fluorescence (Ex 544 nm/Em 590 nm) and optical density (570 and 600 nm) were determined using Hidex Sense microplate reader.

BMDM viability after 24 incubation with different concentrations of PSRE, PACN and PPFE was determined using MTT assay. After incubation with extract, cells were incubated with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, TCI Europe) solution (1 mg/ml) at 37°C for 1–2 h. Thereafter the medium was aspirated and isopropranol was added to each well to dissolve the formazan crystals formed during the incubation period. The absorbance was determined spectrophotometrically at 570 nm using a reference wavelength of 650 nm on Hidex Sense microplate reader.

2.4.2 Annexin V staining assay

The viable cells were analysed on a BD FACSMelody™ (BD Biosciences, San Jose, CA, USA) flow cytometer using Annexin V-APC (allophycocyanin) (BioLegend, San Diego, CA, USA) staining. For analysis of BMDM apoptosis, treated cells were stained with fluorescent annexin V antibody and, afterwards, the proportion of apoptotic (Annexin V positive) cells was evaluated.

2.4.3 Bone marrow-derived macrophage polarisation to M1 phenotype and analysis by flow cytometry

BMDMs were incubated with extracts and LPS+IFN-γ for 24 h. The cells were washed twice with HBSS and harvested with 0.5% trypsin. Then DMEM-high glucose (Merck KGaA, Darmstadt, Germany) or RPMI medium with 10% FBS, 1% antibiotic was added and cell suspension was centrifuged at 300 x g for 5min. Then cells were incubated with specific conjugated antibody mixtures (in concentration 1:100 in cell wash buffer) for 30 min on ice in the dark. The mixture contained following monoclonal antibodies purchased from BioLegend (SanDiego, CA, USA): FITC-conjugated anti-mouse F4/80, phycoerythrin (PE)-conjugated anti-mouse CD86 and biotin-conjugated anti-mouse CD80. Then cells were washed and stained with streptavidin-APC-Cy7. After 30min, cells were washed and stained for evaluation for apoptosis (see method above). After staining, the expression of markers was analysed by flow cytometry.

2.4.4 Bone marrow-derived macrophage polarisation to M2 phenotype and analysis by flow cytometry

Cells were stimulated with extracts and IL-4 10 ng/ml for macrophage polarization to M2 (anti-inflammatory) phenotype for 24 h. The following monoclonal antibodies purchased from BioLegend (SanDiego, CA, USA) were used for flow cytometry analysis:

FITC-conjugated anti-mouse F4/80, PE-conjugated anti-mouse CD206, PE/Cy7-conjugated anti-mouse CD301.

2.4.5 mRNA isolation and quantitative PCR analysis

The method was performed for PSRE and PACN extracts. Total RNA from cells was isolated using PureLink^{TM} RNA Mini Kit (Invitrogen, Carlsbad, CA , USA) according to the manufacturer's protocol. The first-strand cDNA synthesis was carried out using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems^{TM}, Foster City, CA, USA) following the manufacturer's instructions. The quantitative PCR analysis of gene expression was performed by mixing SYBR Green Master Mix (Applied Biosystems^{TM}, Foster City, CA, USA), synthesized cDNA, forward and reverse primers specific for interleukin-1β (IL-1β), interleukin 10 (IL-10), inducible nitric oxide synthase (iNOS), tumor necrosis factor-α (TNF-α), cyclooxygenase 2 (COX-2) and running the reactions on a Mic Real-Time PCR instrument (Bio Molecular Systems, Upper Coomera, Australia). The relative expression levels for each gene were calculated with the ΔΔCt method and normalized to the expression of glucose-6-phosphate isomerase (GPI) gene.

2.4.6 Detection of cytokine release

Medium collected after 6 h treatment with PSRE and PACN extracts (100 μg/ml) and LPS (1 μg/ml) was assayed for cytokine (interleukin-6 (IL-6)) release using human IL-6 ELISA (enzyme-linked immunosorbent assay) kit (Sabbiotech, College Park, MD, USA) by following the manufacturer's protocol.

Medium collected after 6 h treatment with PPFE extract (250 μg/ml and 500 μg/ml), LPS (10 ng/ml) and IFN-γ (100 U/ml) was assayed for cytokine IL-6 release using IL-6 mouse ELISA kit (Invitrogen, Frederick, MD, USA) by following the manufacturer's protocol.

2.5 Qualitative and quantitative analysis

2.5.1 Gas chromatography-mass spectroscopy (GC-MS) of the diethyl ether extract of *Prunus padus*

Fifty grams of fresh *P. padus* flowers were extracted using 800 ml of diethyl ether. After 20 days, 50 ml of extract was poured into a 250 ml three-necked round bottom flask equipped with an outlet and a room temperature water bath. An argon stream was bubbled through the solution for 2 h. Bubbling was continued until the solvent volume was approximately 1 ml. GC-MS analysis was performed based on the procedure described by Radulović et al. (2009)

with slight modifications. Agilent 6890N GC system with a mass selective detector 5975C was applied for analysis. A J&W Scientific HP-5MS column (30 m × 0.25 mm × 0.25 μm) was used for the analysis with helium carrier gas. The GC oven was held at 80 °C for 2 min and then ramped up to 260 °C at 10 °C/min, where it was held for 20 min. A 1 μl injection (split 1:50) was used.

2.5.2 Liquid chromatography mass spectrometry (LC-MS) of *Prunus padus* ethanolic extract and lyophilized sample

The flower extract of *P. padus* was prepared as described previously (3.2.2). LC-MS analysis was performed on the ethanolic extract and lyophilized sample dissolved in ethanol at a concentration of 1 mg/ml.

LC-MS analysis of the ethanolic extract and lyophilized sample dissolved in ethanol at a concentration of 1 mg/ml was performed by the modified procedure described earlier (Mihajlovic et al., 2015). Experiments were conducted on a Shimadzu LC/MS-IT-TOF system (Agilent Technologies, Santa Clara, CA, USA). Separation was carried out with an Acquity BEH C18 column (150 × 2.1 mm I.D.; particle size 1.7 μm) using gradient elution with mobile phase A (0.1% formic acid) and mobile phase B (acetonitrile). Gradient elution was performed under the following conditions: from 2% to 98% B at a flow rate of 0.4 ml/min from 0–20 min. The sample chamber in the autosampler was maintained at 10 °C, while the column was maintained at 40 °C. The injection volume was 1 μl.

A hybrid ion trap/time-of-flight instrument equipped with an ESI source was used for the identification of the flavonoid components. The optimized MS conditions were as follows: nebulizing gas (N_2), 1.5 L/min; drying gas (N_2) pressure, 0.1 MPa; CDL temperature, 200 °C; block heater temperature, 200 °C; ion accumulation time, 100 ms; and detector voltage, 1.8 kV. For fragmentation analysis of flavonoid samples, mass spectrometric analyses were carried out by full-scan MS with a mass range of 120–1000 Da, while the MS2 data were acquired in the range of m/z 100–800. The software automatically selected the precursor ions for MS2 analysis according to criterion settings (such as the ion intensity). Argon was used as the collision gas, and the collision energy was set at 50% for MS2.

2.6 Determination of the total phenolic content

The total phenolic content in PPFE was determined using the Folin–Ciocalteu colorimetric method described by Kähkönen et al. (1999) with slight modifications. In brief, 20 μl of extract was added to a 96-well plate and mixed with 100 μl of 10% Folin-Ciocalteu (Merck KGaA, Darmstadt, Germany) reagent followed by the addition of 80 μl 7.5% sodium

carbonate (Na_2CO_3, Merck KGaA, Darmstadt, Germany) solution. After incubation at room temperature for 30 min in the dark with slight shaking, the absorbance at 765 nm was measured on a Hidex Sense microplate reader. Gallic acid (Merck KGaA, Darmstadt, Germany) was used as a standard for the calibration curve. Total phenolic content was expressed as mg of gallic acid equivalent (GAE) per g of lyophilized extract. All measurements were performed in triplicate.

2.7 DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay

DPPH was used to assess the free radical scavenging property of PPFE. The DPPH radical scavenging activity was measured according to Brand-Williams et al. (1995) with some modifications. For the assay, 20 µl of *P. padus* extract diluted in 70% ethanol was mixed with 180 µl of DPPH (Merck KGaA, Darmstadt, Germany) in methanol (40 µg/ml) in the wells of a 96-well plate. The plate was kept in the dark at room temperature for 15 min. Decreases in the absorbance at 517 nm were measured using a Hidex Sense microplate reader. Ascorbic acid solutions in the concentration range of 0-800 µg/ml were used as a standard, and ethanol was used as a control. The extract was tested over a range of concentrations to establish the EC_{50} (the concentration that reduced the DPPH absorbance by 50%). The radical scavenging activity was calculated using the following formula:

DPPH radical scavenging activity % = $[(A_0 – A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

2.8 Collagenase inhibition activity

Skin anti-aging potential of PPFE was evaluated in a collagenase inhibitory activity assay. The *in vitro* collagenase inhibition assay was performed using a Collagenase Activity Assay Kit (Abcam, Cambridge, UK) by following the manufacturer's instructions. The assay measures collagenase activity using a synthetic peptide (FALGPA) that mimics the structure of collagen. Aliquots of the extract were prepared in water at final concentrations of 250 and 500 µg/ml. The diluted samples (2 µl) were mixed with 10 µl of collagenase (0.35 U/ml), and 88 µL of assay buffer. A positive control was prepared with the enzyme, assay buffer and 2 µl of water. The reaction was started by adding 60 µl of assay buffer and 40 µl of collagenase substrate mixture to each well. The absorbance was measured immediately at 345 nm on a Hidex Sense microplate reader at 37 °C for 5–15 min.

2.9 Data and statistical analysis

The quantitative results are expressed as the mean ± standard error of the mean (SEM) or as the mean ± standard deviation (SD) of three independent experiments and were analysed using GraphPad Prism (GraphPad, Inc., La Jolla, USA) computer software. Statistical analyses were conducted using one-way ANOVA followed by Dunnett's or Tukey post hoc tests. An unpaired t-test was used for the MTT assay. The EC_{50} value was calculated to evaluate the DPPH free radical scavenging activity of the PPFE. Values of $P < 0.05$ were considered to be significant.

3 Results

3.1 Analysis of plant species and their uses described in the records of Latvian folk medicine

3.1.1 Medicinally useful plants from records of Latvian folk medicine

Over 1900 records containing information about medicinal plant usage in the territory of Latvia were found in the folklore materials (Figure 3.1). In total, 211 genera belonging to 71 families were mentioned (see Supplement). The four plant families with the largest number of taxa were *Asteraceae* – 27 taxa (12%), *Rosaceae* – 17 taxa (8%), *Lamiaceae* – 13 taxa (6%), and *Apiaceae* – 10 taxa (5%). The number of medicinal plant species in each plant family is shown in Figure 3.2.

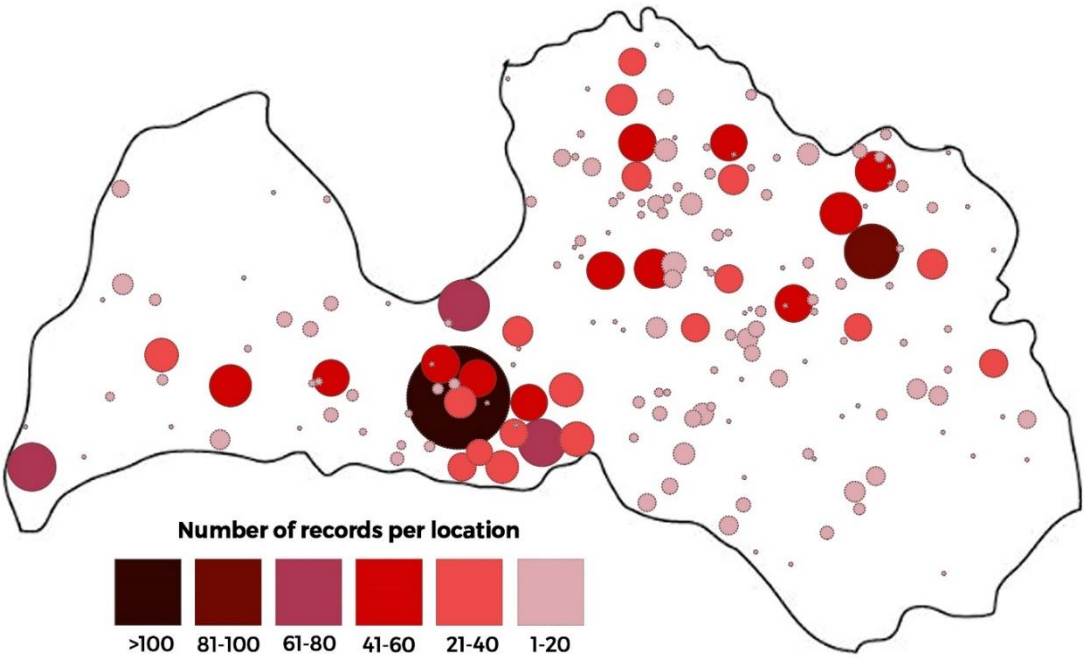


Figure 3.1 **The geographic locations from which the records of Latvian folk medicine were collected**

Each location where the records have been collected is marked by a circle, and the color and size of the circle represents the number of records collected in each location. The top ten locations with the highest number of records are Garoza (319), Vecgulbene (92), Rīga (80), Nīca (71), Skaistkalne (70), Saldus (55), Beļava (54), Alsviķi (50), Kosa (48) and Ozolnieki (46).

Several native plant taxa were mentioned more than 40 times: yarrow (*Achillea millefolium* L.), chamomile (*Matricaria chamomilla* L.), onion (*Allium cepa* L.), wormwood (*Artemisia absinthium* L.), greater plantain/ribwort plantain (*Plantago major* L./*Plantago lanceolata* L.), birch (*Betula* sp. L.), oak (*Quercus robur* L.), bird cherry (*Prunus padus* L.), and juniper (*Juniperus communis* L.). The most frequently mentioned foreign taxa were tobacco (*Nicotiana* L.) and aloe (*Aloe* sp. L.). Plant taxa that were reported more than 20 times in the

folklore materials are shown in Figure 3.3. However, at the same time, a large number of taxa (135) were mentioned in only 1–2 records.

3.1.2 Medicinal use categories

In total, 1976 cases were reported for disease prevention or health improvement that fit into one of the 17 medicinal use categories. Most cases were mentioned for the treatment of symptoms related to digestive system disorders. A significant number of use reports related to treating diseases and symptoms were also mentioned for respiratory system disorders, skin disorders, general and unspecified disorders and musculoskeletal system disorders. All other categories were mentioned in fewer than 100 cases, especially endocrine, blood/blood-forming organs/lymphatics/spleen, and male genital system disorders, which were mentioned relatively rarely (in fewer than 20 cases) (Table 3.1).

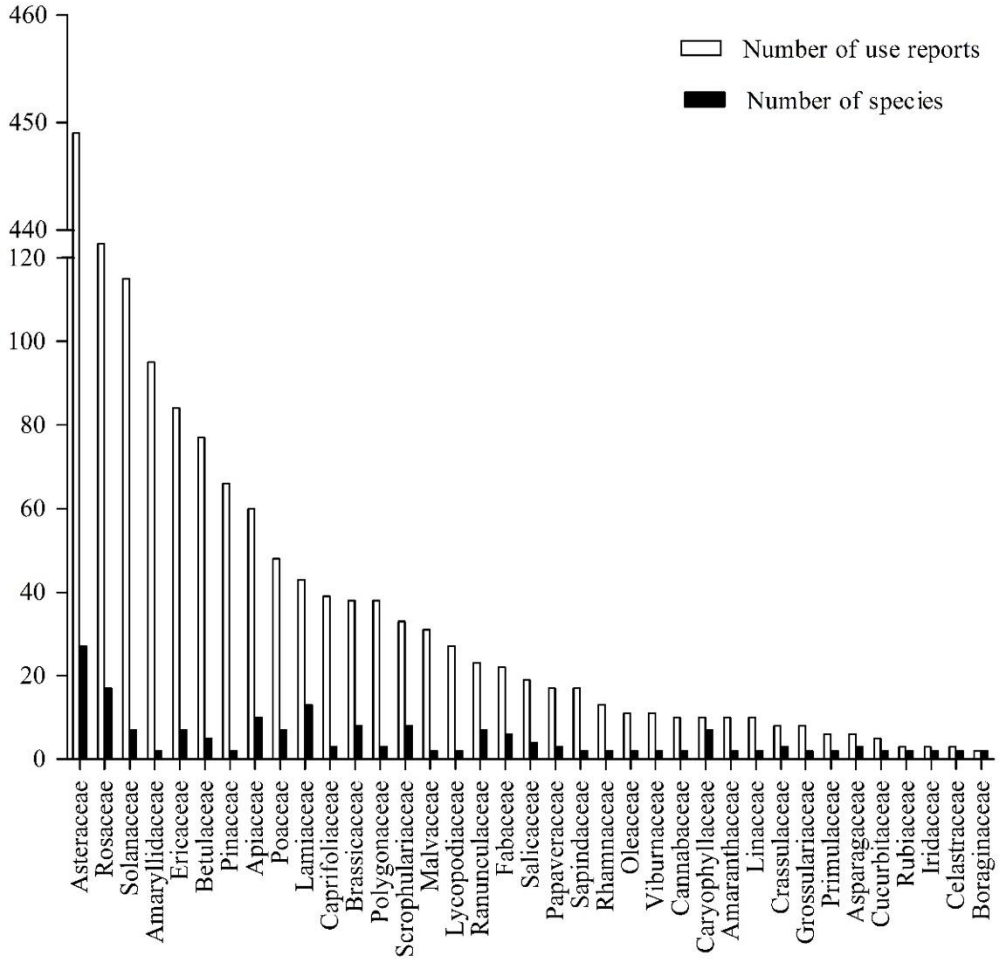


Figure 3.2 **Number of use reports (white bars) and number of medicinal plant species (black bars) in each plant family that contains more than two plant species**

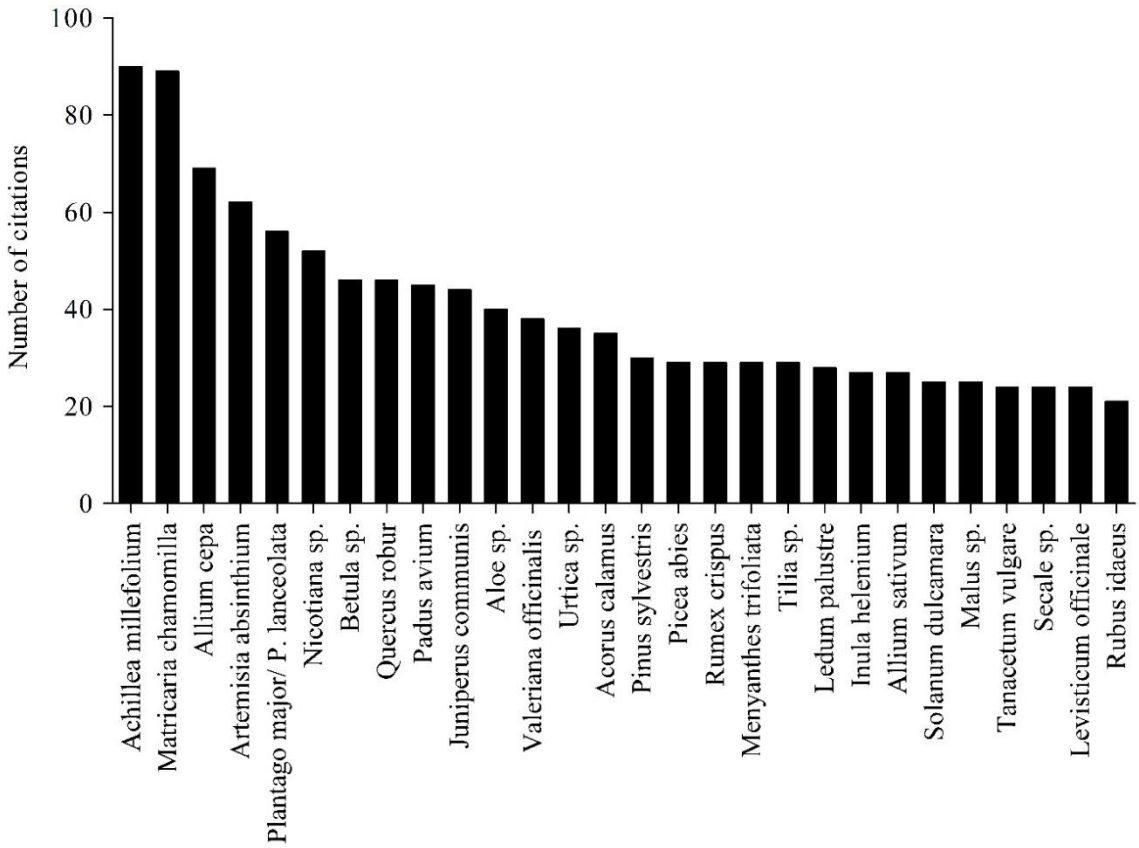


Figure 3.3 **Plant taxa that were reported more than 20 times in records of Latvian folk medicine**

3.1.3 Disorders treated/medicinal effects

More than 120 plant taxa were mentioned as having uses relevant for the category digestive system disorders. Three of 24 disorders were dominant: generalized abdominal pain, toothache, and diarrhoea. *Artemisia absinthium* L., *Secale* sp. L. and *Betula* sp. L. were the species most often described as medicinal treatments for generalized abdominal pain. *A. absinthium* is long known for the traditional use for temporary loss of appetite and for mild dyspeptic/gastrointestinal disorders (EMA, 2017). According to the information found in the records of Latvian folk medicine, tea or decoction was the most commonly used form of herbal medicine. Green shoot tea of *Secale* sp. and a tea or tincture made of buds or bark of *Betula* sp. were also commonly used for pain reduction. For toothache, powder made from *Nicotiana tabacum* L. leaves, fresh bark of *Prunus padus* L. and fresh twigs of *Daphne mezereum* L. were used, and all were applied directly to the tooth's surface. Among more than 30 taxa, mainly *Quercus robur* L., *Rumex crispus* L. and *Vaccinium myrtillus* L. were described as treating diarrhoea. Diarrhoea was treated with a tea or decoction of the bark of pedunculate oak, with a tea or decoction of curled dock and with fruits of bilberry.

Table 3.1

Numbers of use reports and number of medicinal plant taxa mentioned in records of Latvian folk medicine in each of 17 medicinal use categories

Medicinal use category	No. of use reports	No. of medicinal plant taxa
Digestive system disorders	479	121
Respiratory system disorders	414	97
Skin disorders	359	88
General and unspecified disorders	201	72
Musculoskeletal system disorders	116	51
Unspecified medicinal disorders	111	73
Neurological disorders	73	50
Psychological disorders	45	21
Ear disorders	40	15
Urinary system disorders	29	18
Circulatory system disorders	24	18
Eye disorders	23	13
Disorders related to pregnancy, childbirth, family planning	21	16
Female genital system and breast disorders	21	18
Endocrine, metabolic and nutritional disorders	12	10
Blood, blood forming organs, lymphatics, spleen disorders	5	6
Male genital system disorders	3	3
Total	**1976**	

In the category of respiratory system disorders, the most frequently mentioned conditions were cough and chest pain due to cough. Treatment of cough was clearly dominated by three taxa: *Achillea millefolium* L., *Matricaria chamomilla* L. and *Solanum dulcamara* L., which were used in the form of tea. In total, 50 taxa were mentioned to reduce cough. However, half of the taxa were mentioned only once. For the treatment of chest pain, *Aloe* sp. L., *Tilia* sp. L. and *Juniperus communis* L. were used, and the route of administration was as a tea. The category of skin disorders contained the largest number of treated disorders. In this category, cuts and wounds, boils, athlete's foot, and scabies were dominant. More than 80 taxa were mentioned. To treat cuts and wounds, the most frequently used taxa were *Plantago* sp. L. (used mainly as a fresh material made from leaves), *A. millefolium* (used mainly as a juice made from aerial parts) and *Aloe* sp. (used mainly as a fresh material made from leaves). The most common taxa used to treat boils were *Allium cepa* L., *Allium sativum* L., *A. millefolium,* and *Plantago* sp. Bulbs of both onion and garlic were applied to the affected area in a baked or fresh form. Aerial parts of yarrow and leaves of plantain were used as fresh material. Athlete's foot was treated with fresh leaves of *Alnus* Mill., *Malus* Mill., *Plantago* sp. L. and *Achillea millefolium* L. The present study found ethnopharmacological uses of *R. crispus* and

J. communis against scabies. Both species were used externally: curled dock leaves were used as a fresh grated material, and juniper was whisked into a sauna or bath. The category of general and unspecified disorders includes infectious diseases, together with the symptoms of fever and chills. A large number of plant taxa (more than 70) were described in this category, and three diseases dominated: fever, swelling, and tuberculosis. Teas or decoctions of *Tilia* sp. L. flowers; tea from *Rubus idaeus* L. stems, leaves or fruits; and fresh leaves of *Plantago* sp. used externally were mentioned as good for reducing fevers. A tea of *Levisticum officinale* W.D.J. Koch was used to treat swelling, as was a tea/decoction made from fruits of *J. communis*. Additionally, fresh leaves of *Plantago* sp. were used for the same purpose. To treat tuberculosis, the most frequently used taxa were *Pinus sylvestris* L. (used mainly as a tea made from new shoots), *Aloe* sp. (used mainly as a decoction or fresh material made from leaves) and *A. millefolium* (used mainly as a tea made from aerial plant parts). Fifty-one plant taxa were mentioned as being treatments for ailments in the category of musculoskeletal system disorders. Three of 9 disorders were mentioned the most: rheumatism, cramps and bone pain. The leaves, flowers or fruits of *Vaccinium vitis-idaea* L. were used as a tea against rheumatic pains. For external application, a bath or steam infused with *J. communis* was frequently used. Additionally, *Urtica sp.* L., *Acorus calamus* L., and *P. sylvestris* were administered externally and *Aesculus hippocastanum* L. was administered internally for the same purpose. Tea made of *Valeriana officinalis* L. root was the most commonly used remedy for cramps. Bone pain was treated both internally and externally, mainly with *V. vitis-idaea*, *A. calamus* and *A. hippocastanum*.

Thirteen plant taxa were described as having activity for more than 8 medicinal use categories. The plant taxa that had the highest medicinal value were *M. chamomilla*, which was mentioned in 13 use categories, and *Betula* sp., which was mentioned in 11 use categories. These taxa were followed by *Allium cepa* L., *Nicotiana* sp. L., *Valeriana officinalis* L., *Urtica* sp. L., *Artemisia absinthium* L., *Acorus calamus* L., *Prunus padus* L., *Pinus sylvestris* L., *Viola* sp. L., *Levisticum officinale* W.D.J. Koch, and *Verbascum thapsus* L. Almost all these plants, except chamomile and wormwood, belong to different plant families.

3.1.4 Plant parts used

According to the information collected from the records of Latvian folk medicine, almost all plant parts were used as herbal medicine. The leaf (16%) was the most commonly used part for medicinal purposes, closely followed by flowers (14%). The third most commonly used part was the root (9%). The bark (5%) and other aerial parts (5%) were used almost as often as the fruit (6%), showing how important these parts were for preparing medicine.

However, a large number of records (29%) did not contain information about a specific plant part used. Relatively few uses were mentioned for seeds, buds, and stems.

3.1.5 Forms of herbal medicine and routes of administration

Thirty percent of all herbal medicines mentioned were used as a tea. Tea was in the first place in eleven use categories and it was used for both external and internal purposes. Tea was the most common mode of use, followed by fresh plant material (17%), mostly as a topical application and a decoction (11%). Tea, decoction and raw plant material or at least two of them were among the first three of the most commonly used medicines in all use categories. A juice (5%) and a tincture made with alcohol (4%) were also commonly used herbal remedies, and routes of administration were both internal and external. Additionally, making baths was quite common (3%). In 19% of cases, the mode of use was not specified. Water was the solvent most commonly used to extract medicinal compounds from plant material. In some cases, other solvents and methods were used to prepare medicines, including milk, cream, beer, fat, oil, honey, vinegar, or urine. Many records reported the use of medicinal plants as fresh materials by applying them directly to the affected skin or wound, especially squeezed or crushed leaves, roots, or fruits. According to the data presented, both internal and external routes of administration were widespread (51% and 37%, respectively). However, oral administration was the most common method of using medicine. In this study, 41% of plant taxa were reported to be consumed only internally, and 13% only externally. Of those taxa reported to be consumed only internally, most cited were *Vaccinium myrtillus* L. (18 times), *Anthemis tinctoria* L. (15 times), *Helichrysum arenarium* (L.) Moench (12 times), but those to be used only externally were *Daphne mezereum* L. (12 times), *Pelargonium* L'Hér. (10 times), and *Ranunculus acris* L. (8 times).

3.1.6 Comparison of plant taxa mentioned in records of Latvian folk medicine with official herbal monographs

Forty-seven (22%) plant taxa in the records of Latvian folk medicine were mentioned in the fourth edition of the Russian Pharmacopoeia. More than 3/4 of taxa were not included in the respective pharmacopoeia. The Latvian Pharmacopoeia that was published later contains 37 (17%) taxa from the records of Latvian folk medicine that were also mentioned in the Russian Pharmacopoeia (Table 3.2). The Latvian Pharmacopoeia included five taxa that were reported in the records of folk medicine but not included in the 4th edition of Russian Pharmacopoeia. These taxa were *Convallaria majalis* L., *Petroselinum* Hill., *Potentilla erecta* (L.) Raeusch., *Primula veris* L., and *Tanacetum vulgare* L. (Table 3.2.).

Only 59 out of 211 plant taxa mentioned in this study are included in the official monographs of the EMA (see Supplement). Two taxa are currently being evaluated by HMPC; this is the case of oak bark and tormentil rhizome. From these 59 taxa 78% of their uses recorded in the folklore materials match with the indications provided in monographs. Most of the taxa are currently used as traditional herbal medicines. Only few of them are reported as herbal medicinal products with a *well-established use*. Monographs of *well-established use* correspond to these taxa: *Aloe* spp. L., *Frangula alnus* Mill., *Linum usitatissimum* L., *Senna* spp. Mill. – all used for the treatment of constipation; *Hypericum perforatum* L. – for mild to moderate depressive episodes and mild depressive disorders; *Aesculus hippocastanum* L. – for treatment of chronic venous insufficiency; *Salix* spp. – for low back pain; *Zingiber officinale* Roscoe – for the prevention of nausea and vomiting in motion sickness; *Valeriana officinalis* L. – for nervous tension and sleep disorders. The first top ten taxa most cited in the records of Latvian folk medicine but not included in EU herbal monographs are *Allium cepa* L., *Nicotiana* sp. L., *Prunus padus* L., *Acorus calamus* L., *Pinus sylvestris* L., *Picea abies* L., *Rumex crispus* L., *Ledum palustre* L., *Inula helenium* L., *Malus* Mill.

Sixty-five species from the records of Latvian folk medicine are described in the book Herbal Drugs and Phytopharmaceuticals (Wichtl, 2004), 18 plant taxa match at the genus level, and 6 plant taxa belong to other species from the same genus (see Supplement). Monographs on *A. calamus* and *I. helenium* are included in this book. The rhizome of *A. calamus* is used primarily for improving stomach function and increase appetite, and as a carminative. The rhizome of *I. helenium* is used as expectorant (Wichtl, 2004).

Table 3.2

Plant taxa of the records of Latvian folk medicine included in the Russian Pharmacopoeia (*) (4th edition) and Latvian Pharmacopoeia (#)

Aerial parts of	*Artemisia absinthium* L. (*, #)
	Cannabis sativa L. (*, #)
	Centaurium erythraea Rafn (*, #)
	Cicuta virosa L. (*)
	Origanum vulgare L. (*)
	Thymus serpyllum L. (*, #)
	Viola tricolor L. (*, #)
Bark of	*Frangula alnus* Mill. (*, #)
	Quercus robur L. (*, #)
Buds of	*Pinus sylvestris* L. (*)

Table 3.2 continued

Flowers of	*Achillea millefolium* L. (*, #)
	Arnica montana L. (*, #)
	Convallaria majalis L. (#)
	Malva sylvestris L. (*)
	Matricaria chamomilla L. (*, #)
	Sambucus nigra L. (*, #)
	Tanacetum vulgare L. (#)
	Tilia cordata Mill., *Tilia platyphyllos* Scop. (*, #)
	Verbascum thapsus L. (*, #)
Fruits of	*Carum carvi* L. (*, #)
	Laurus nobilis L. (*)
	Petroselinum Hill. (#)
	Pimpinella anisum L. (*, #)
Hops of	*Humulus lupulus* L. (*, #)
Leaves of	*Aloe* L. (*, #)
	Arctostaphylos uva-ursi (L.) Spreng. (*, #)
	Atropa belladonna L. (*, #)
	Datura stramonium L. (*, #)
	Hyoscyamus niger L. (*, #)
	Melissa officinalis L. (*)
	Mentha×piperita L. (*, #)
	Menyanthes trifoliata L. (*, #)
	Nicotiana tabacum L. (*)
	Senna alexandrina Mill. (*, #)
	Tussilago farfara L. (*, #)
Rhizomes of	*Acorus calamus* L. (*, #)
	Dryopteris filix-mas (L.) Schott (*)
	Potentilla erecta (L.) Raeusch. (#)
	Zingiber officinale Roscoe (*, #)
Roots and aerial parts with roots of	*Taraxacum campylodes* G. E. Haglund (*, #)
Roots of	*Albuca bracteata* (Thunb.) J.C.Manning & Goldblatt (*, #)
	Carapichea ipecacuanha (Brot.) L. Andersson (*, #)
	Primula veris L. (#)
	Rheum officinale Baill., *Rheum palmatum* L. (*, #)
	Valeriana officinalis L. (*, #)
Seed cones of	*Juniperus communis* L. (*, #)
Seeds of	*Brassica nigra* (L.) K.Koch (*, #)
	Linum usitatissimum L. (*, #)
	Papaver somniferum L. (*)
Spores of	*Lycopodium* L. (*, #)
Thallus of	*Cetraria islandica* (L.) Ach. (*)
Tuberous roots	*Aconitum napellus* L. (*, #)

3.2 The effect of *Pelargonium sidoides* DC. root extract and proanthocyanidins from *Pelargonium sidoides* root extract on inflammatory responses to bacterial lipopolysaccharide

3.2.1 The effect of *Pelargonium sidoides* root extract and proanthocyanidin fraction on lipopolysaccharide-induced secretion of inflammatory mediator interleukin-6

Bacterial invasion can cause an infiltration of leukocytes that mediate inflammation and disturb osteoblast-osteoclast balance via release of IL-6 (Chang and Yang, 2000). Thus, antiinflammatory properties of PSRE and PACN on LPS-induced release of IL-6 from PBMCs was examined. One hundred µg/ml of PSRE and PACN significantly decreased LPS-induced secretion of IL-6 from PBMCs to 67% and 85% of the level caused by LPS stimulation, respectively (Figure 3.5). Note that neither PSRE nor PACN were toxic to the cells at the concentrations applied as revealed by metabolic viability analysis (Figure 3.4).

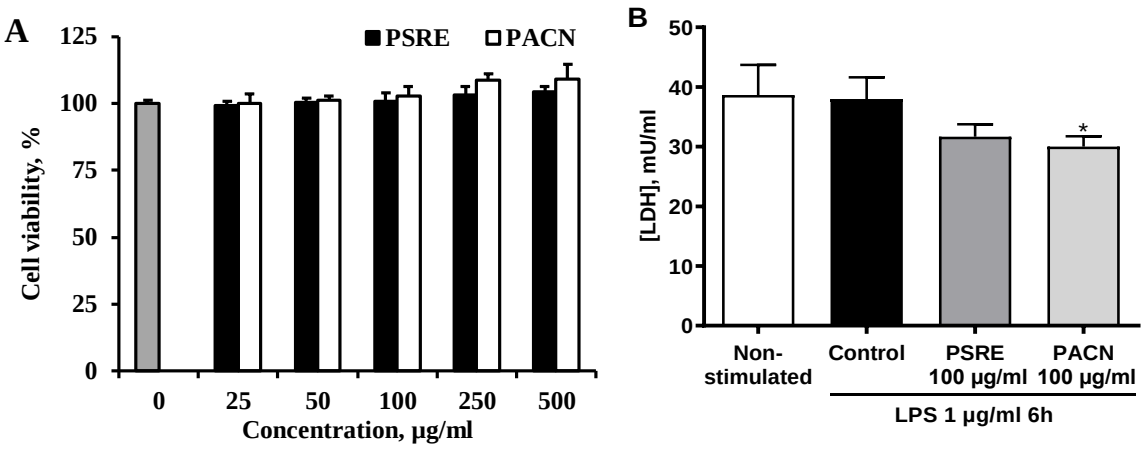


Figure 3.4 **Effects of *Pelargonium sidoides* root extract (PSRE) and proanthocyanidins from PSRE (PACN) on human peripheral blood mononuclear cell viability**

(**A**) Human peripheral blood mononuclear cell viability after 24 h incubation with PSRE and PACN evaluated by Alamar Blue assay. (**B**) Effects of PSRE and PACN on blood mononuclear cell membrane damage measured by lactate dehydrogenase (LDH) assay. The LDH release was tested in media after 6 h treatment with extracts (100 µg/ml) and LPS (1 µg/ml). Values are represented as the mean ± SD of (A) 6 parallels or (B) 3 independent measurements in 3 parallels. Differences between the measurements were tested using one way ANOVA followed by Tukey's multiple comparison test.
*$P < 0.05$ vs. LPS control.

3.2.2 The effect of *Pelargonium sidoides* root extract and proanthocyanidin fraction on lipopolysaccharide-induced expression of inflammation-related genes

The capacity of PSRE and PACN to modulate expression of inflammatory genes IL-1β, TNF-α and iNOS in primary murine bone marrow-derived macrophages and human mononuclear leukocytes under treatment of LPS was examined. Stimulation of macrophages in the presence of interferon-γ (IFN-γ) induced an increase in transcription of all the three genes investigated (Figure 3.7).

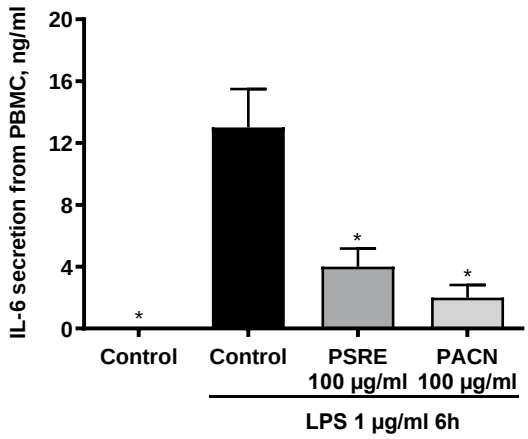


Figure 3.5 **The effect of *Pelargonium sidoides* root extract (PSRE) and proanthocyanidins from PSRE (PACN) on interleukin-6 (IL-6) secretion from peripheral blood mononuclear cells after LPS treatment**

Concentration of IL-6 secreted by PBMCs was estimated by ELISA. Effects of PSRE and PACN extracts (100 µg/ml) on LPS-stimulated (6 h) cells. Data are represented as the mean ± SEM of 3 independent measurement in 3 parallels. Differences between the measurements were tested using one-way ANOVA followed by Tukey's multiple comparison test.
*$P < 0.05$ vs. LPS control.

Both preparations at a dose of 100 µg/ml were not toxic (Figure 3.6) and significantly suppressed the mRNA transcription of IL-1β and iNOS (Figure 3.7 A and B). The level of the IL-1β mRNA decreased by 78% of the initial level with LPS after treatment with PSRE, and by 89% − after treatment with PACN. For iNOS, the decrease in mRNA level after PSRE and PACN treatment was 53% and 64%, respectively. However, the incubation with both substances did not affect LPS plus IFN-γ-induced TNF-α gene expression (Figure 3.7 C).

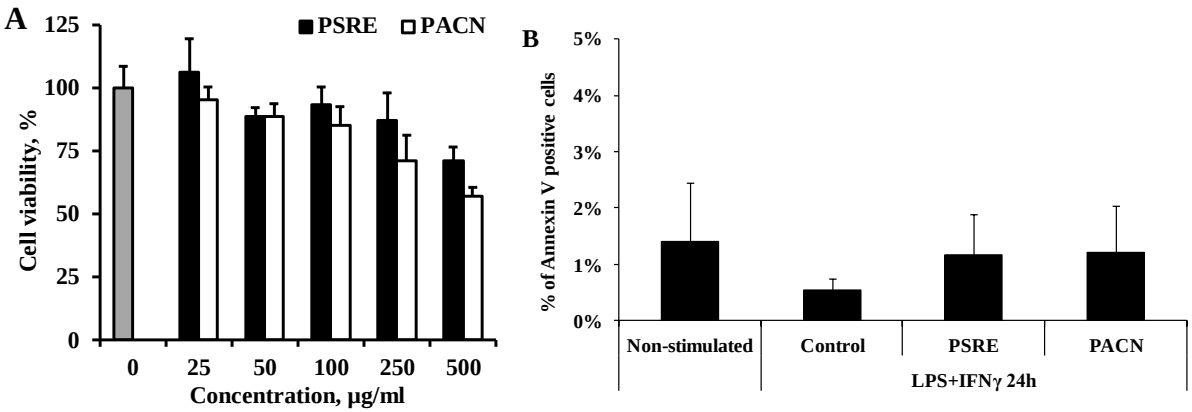


Figure 3.6 **Effects of *Pelargonium sidoides* root extract (PSRE) and proanthocyanidins from PSRE (PACN) on bone-marrow derived macrophage (BMDM) cell viability**

(**A**) BMDM viability after 24 h incubation with PSRE and PACN assessed by MTT assay. (**B**) Detection of apoptosis by staining BMDM for Annexin V. BMDM were stained for Annexin V after 24 h of incubation with PSRE and PACN at 100 µg/ml, and LPS+IFN-γ (10 ng/ml/100 U/ml). Apoptotic cells (Annexin V positive) were detected by flow cytometry. Values are presented as mean ± SD of (A) 6 parallels or (B) 3 independent measurements in 3 parallels.

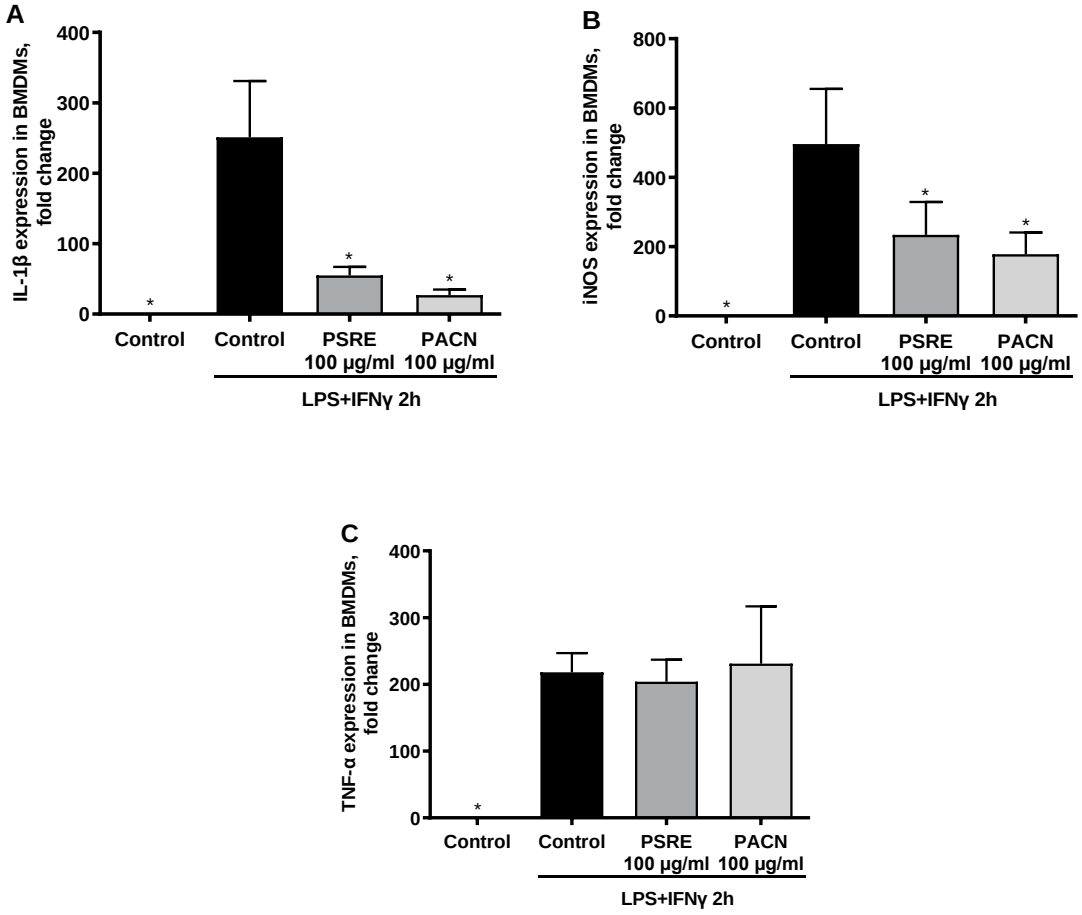


Figure 3.7 **The effect of *Pelargonium sidoides* root extract (PSRE) and proanthocyanidins from PSRE (PACN) on pro-inflammatory gene expression in bone marrow-derived macrophages after LPS and IFN- γ stimulation**

The mRNA level of (**A**) IL-1β, (**B**) iNOS and (**C**) TNF-α was determined by qPCR analysis and normalised against β-actin mRNA. Data are represented as mean ± SEM of 3 independent measurement in 3 parallels. Differences between the measurements were tested using one-way ANOVA followed by Tukey's multiple comparison test.
*$P < 0.05$ vs. LPS + IFN-γ control.

Six hour treatment with LPS caused significant increase in cyclooxigenase-2 (COX-2), TNF-α and IL-1β gene transcription in human PBMCs (Figure 3.8). PSRE and PACN at a concentration of 100 µg/ml significantly suppressed mRNA transcription of COX-2 and IL-1β (Figure 3.8 A and C). When LPS was together with PSRE, COX-2 and IL-1β mRNA levels dropped by 50% and 56%, respectively. For PACN, mRNA synthesis for these cytokines was suppressed by 63% and 76%. Similarly to BMDMs case, neither PSRE, nor PACN significantly affected TNF-α gene expression (Figure 3.8 B).

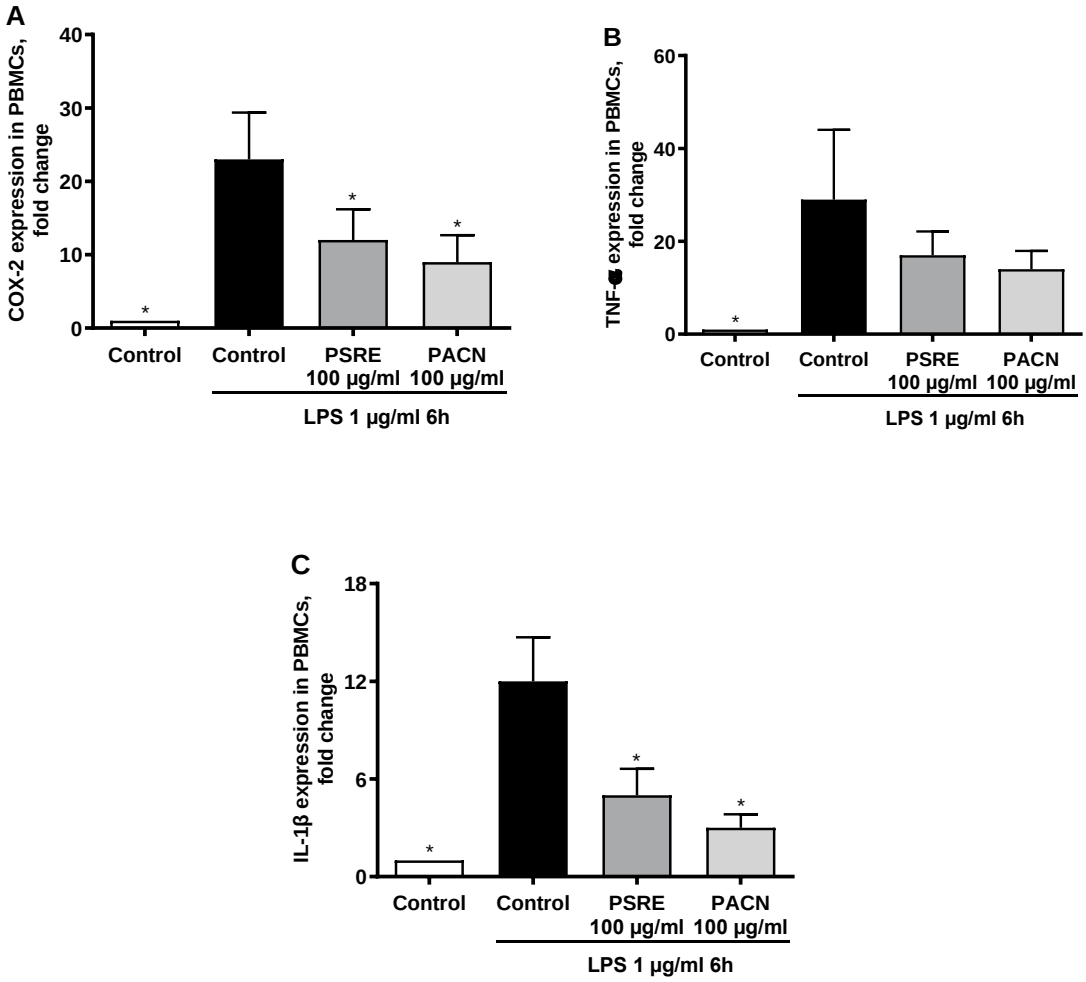


Figure 3.8 **The effect of *Pelargonium sidoides* root extract (PSRE) and proanthocyanidins from PSRE (PACN) on pro-inflammatory gene expression in peripheral blood mononuclear cells after LPS stimulation**

The mRNA level of (**A**) COX-2, (**B**) TNF-α and (**C**) IL-1β was determined by qPCR analysis and normalised against β-actin mRNA. Data are represented as mean ± SEM of 3 independent measurement in 3 parallels. Differences between the measurements were tested using one-way ANOVA followed by Tukey's multiple comparison test.
*$P < 0.05$ vs. LPS control.

3.2.3 The effect of *Pelargonium sidoides* root extract and proanthocyanidin fraction on lipopolysaccharide-induced macrophage conversion to M1 phenotype

Flow cytometry analysis revealed that in response to LPS and IFN-γ, the amount of M1-polarised macrophages increased 9.3 times compared to the untreated control (Figure 3.9). Both PSRE and PACN at a concentration of 100 µg/ml were effective in reducing the level of CD80 and CD86-positive cells. The population of cells with the exposed markers after treatment with PSRE was by 58% lower, and after treatment with PACN by 71% lower than after LPS and IFN-γ stimulation without the treatments. The results indicate that both substances were potent in preventing macrophage conversion to pro-inflammatory M1 phenotype under exposure to LPS and IFN-γ treatment.

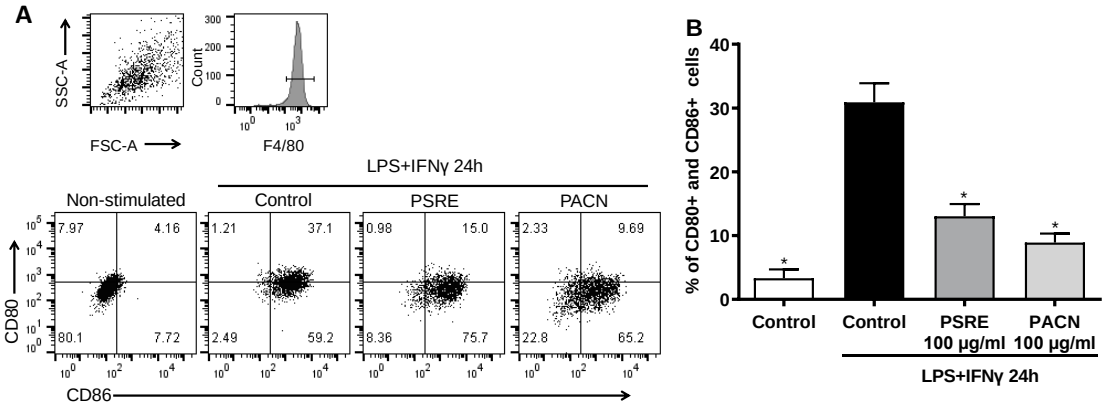


Figure 3.9 **Expression of pro-inflammatory cell surface markers CD80 and CD86 analysed by flow cytometry 24 h after treating LPS + IFN-γ-activated BMDMs with PSRE and PACN**

(**A**) Characteristic mouse macrophage marker F4/80-positive cells were gated for double CD80 and CD86 analysis as a measure of M1 macrophage phenotype (top right small quadrant). Representative plots of a total of three independent experiments in three replicates are presented in the bottom. (**B**) Data are represented as mean ± SEM of three independent measurements in three parallels. Differences between the measurements were tested using a one-way ANOVA by Tukey's multiple comparison test. *$P < 0.05$ vs. LPS + IFN-γ control.

3.3 Chemical composition of *Prunus padus* L. flower extract and the effect on inflammatory responses to bacterial lipopolysaccharide

3.3.1 Gas chromatography-mass spectrometry analysis of the diethyl ether extract of *Prunus padus*

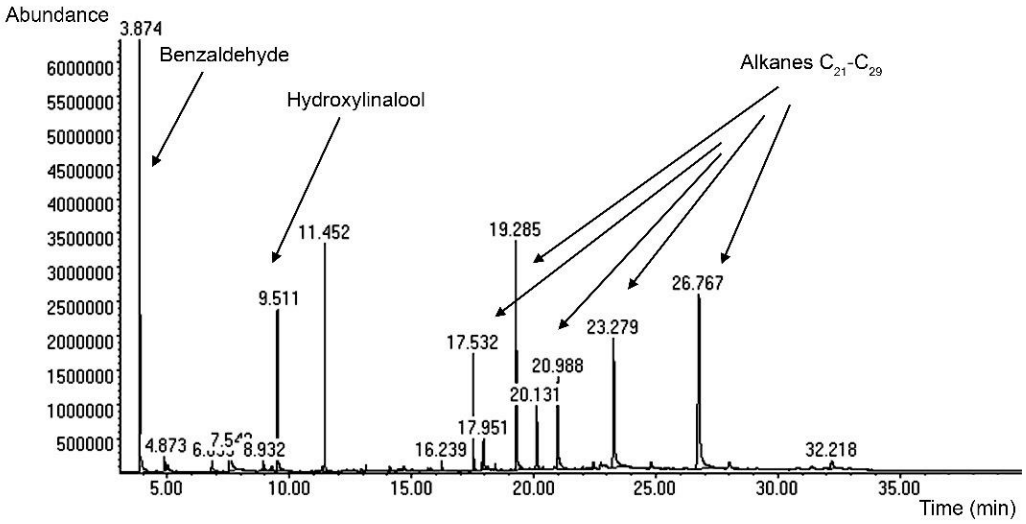


Figure 3.10 **Total ion current chromatogram of the diethyl ether extract of *Prunus padus* flowers**

The analysed sample of the diethyl ether extract of *P. padus* flowers was characterized by high amounts of benzaldehyde, 8-hydroxylinalool and saturated C21-C29 alkanes (Figure 3.10). A detailed list of the phytochemical components detected by GC-MS in *P. padus* is shown in Table 3.3.

Table 3.3

Volatile compounds detected in diethyl ether extract of *Prunus padus* flowers

RT, min	Compound	TIC, %
3.8	Benzaldehyde	17.2
4.9	Benzyl alcohol	1.3
6.8	Benzoic acid	0.9
7.5	Benzofuran, 2,3-dihydro	3.4
8.9	2-Methoxy-4-vinylphenol	0.9
9.5	8-Hydroxylinalool	7.7
11.4	BHT(stabilizator from diethylether)	7.8
16.3	Hexadecanoic acid	0.7
17.5	Heneicosane	4.4
17.9	9,12,15-Octadecatrienoic acid	2.1
19.3	Tricosane	9.9
20.1	DEHA (plasticizer)	3.0
20.9	Pentacosane	6.1
23.3	Heptacosane	10.7
26.7	Nonacosane	22.6

* Abbreviations: RT, retention time; TIC, % of total ion current. Compound identification was performed using the NIST/EPA/NIH Mass Spectral Library, Vers.2.0d.

3.3.2 Liquid chromatography-mass spectrometry analysis of the 70% ethanolic extract of *Prunus padus*

Both the ethanolic extracts of *P. padus* flowers and ethanol solutions of lyophilized samples were analysed under optimized LC-MS conditions to obtain the DAD, MS and MS/MS data. The total ion chromatograms (TIC) of both extracts are shown in Figures 3.11 and 3.12.

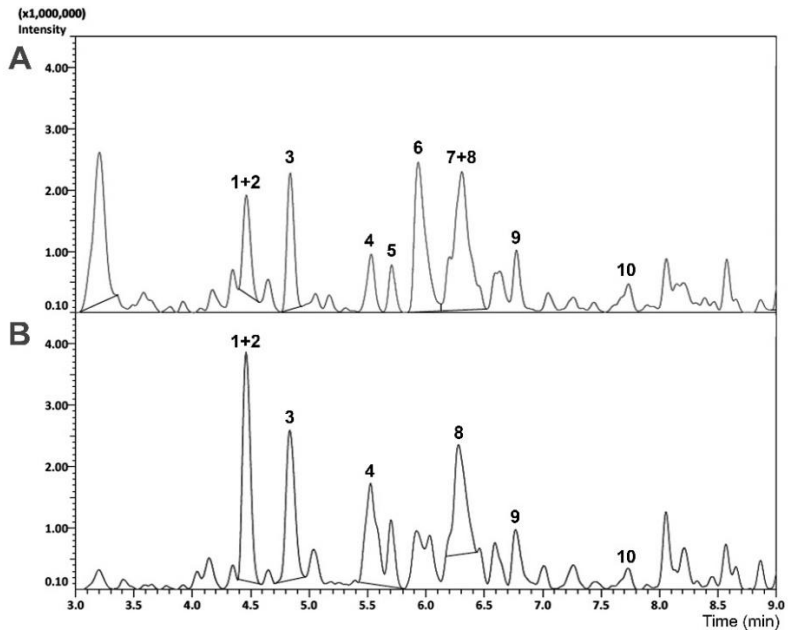


Figure 3.11 **Comparison of positive (A) and negative (B) ion electrospray ionization mass chromatograms of ethanolic extract of *Prunus padus* flowers:**

1 – vanilloside, **2** – zinolol, **3** – chlorogenic acid, **4** – isomer of p-coumaroylquinic acid, **5** – quercetin diglucoside, **6** – quercetin diglycoside, **7** – rutin, **8** – quercetin diglycoside, **9** – cynarine, **10** – dicaffeoylcoumaroyl spermidine

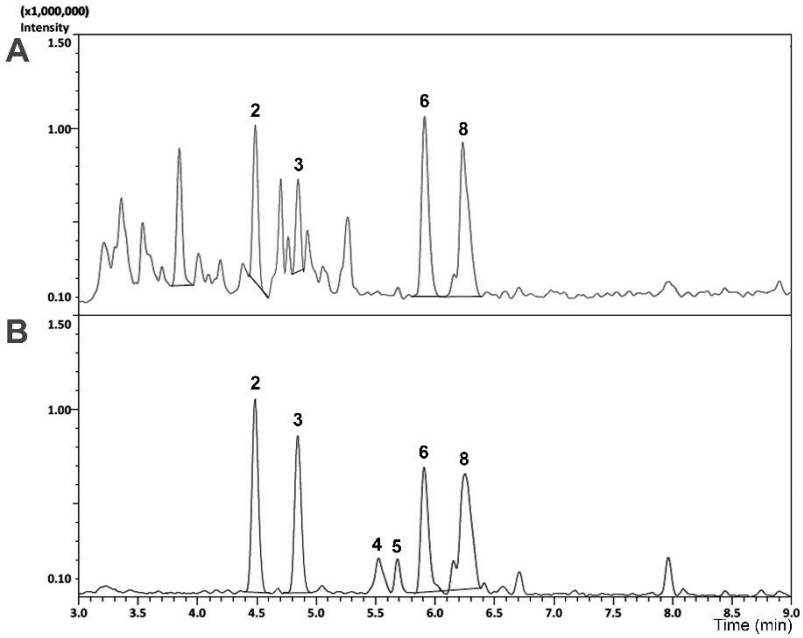


Figure 3.12 **Comparison of positive (A) and negative (B) ion electrospray ionization mass chromatograms of lyophilized sample of *Prunus padus* flowers dissolved in ethanol (1 mg/ml)**

2 – zinolol, **3** – chlorogenic acid, **4** – isomer of p-coumaroylquinic acid, **5** – quercetin diglucoside, **6** – quercetin diglycoside, **8** – quercetin diglycoside

Mass spectrometric identification of individual compounds was confirmed by comparison of the retention time and the MS and MS/MS spectra with available standards. If no standards were available, a tentative identification was made using the PubChem

database and published literature. The high-resolution mass spectrometry characteristics and identification data for the 10 major peaks in the MS chromatograms of PPFE are shown in Table 3.4.

The main compounds identified in PPFE were flavonoids and phenolic acids (quinic acid derivatives and chlorogenic acid). The characteristic peak of quercetin aglycone was observed in at least four mass spectra of the identified compounds and corresponded to quercetin diglycoside **6** and **8**, quercetin diglucoside **5** and rutin **7**. In addition, two quinic acid derivatives, coumaroyl quinic acid **4** and dicaffeoylquinic acid (cynarine) **9,** were detected in the analysed samples.

Two nitrogen-containing compounds, the polyamine derivative (di-caffeoyl coumaroyl spermidine) **10** and the hydroxyhydroquinone glycoside (zinolol) **2**, were found in the initial ethanolic extract. LC-MS analysis of the lyophilized sample after reconstitution in ethanol showed only traces of these compounds.

Table 3.4

Phytocomponents identified in the ethanolic extracts of *Prunus padus* flowers

	RT (min)	DAD spectrum, λ_{max} (nm)	MS (m/z)				Molecular formula	MW	Compound identity	Identification (Lit)
			$[M + H]^+$	MS2, fragments	$[M - H]^-$	MS2, fragments				
1	4.46	258	315.11	-	313.09	-	$C_{14}H_{18}O_8$	314	Vanilloside	https://pubchem.ncbi.nlm.nih.gov/ compound/Vanilloside
2	4.47	240	332.13	163.06	-	-	$C_{14}H_{21}NO_8$	331	Zinolol	(Ammar et al., 2008)
3	4.84	321	355.10	-	353.08		$C_{16}H_{18}O_9$	354	Chlorogenic acid	a
4	5.52	280	339.11	-	337.09	191.05	$C_{16}H_{18}O_8$	338	Isomer of p-Coumaroylquinic acid	(Marchelak et al., 2017)
5	5.71	253	627.14	303.05	625.14	-	$C_{27}H_{30}O_{17}$	626	Quercetin diglucoside (isomer)	(Mikulic-Petkovsek et al., 2016)
6	5.93	245	597.14	465.10, 303.05	595.13	-	$C_{26}H_{28}O_{16}$	596	Quercetin diglycoside (quercetin-3-vicianoside)	(Olszewska and Kwapisz, 2011; Slimestad et al., 2005; Song et al., 2012)
7	6.21	256	611.15	465.10, 303.05	609.14	301.04	$C_{27}H_{30}O_{16}$	610	Rutin	a
8	6.25	257	581.14	465.10, 303.05, 287.05	579.14	463.09	$C_{26}H_{28}O_{15}$	580	Quercetin diglycoside	b
9	6.76	328	517.13	499.12, 319.08	515.12	-	$C_{25}H_{24}O_{12}$	516	Cynarine	(Simirgiotis et al., 2015)
10	7.72	248	616.26	454.23	614.25	-	$C_{34}H_{37}N_3O_8$	615	Di-caffeoyl coumaroyl spermidine	(Hanhineva et al., 2008; Mihajlovic et al., 2015)

* a – identified by comparing with the standard compound
* b – a large group of isomeric compounds corresponding to quercetin conjugates with two carbohydrate residues

The comparison of the HPLC-UV (254 nm) chromatograms of both the ethanolic extract and the ethanolic solution of the lyophilized sample of *P. padus* flowers is shown in Figure 3.13. The chromatographic profiles of both analysed samples (before and after lyophilization) show three to four intense peaks in the retention time interval from 3 to 9 min (Table 3.5), constituting 65–75% of the total peak area.

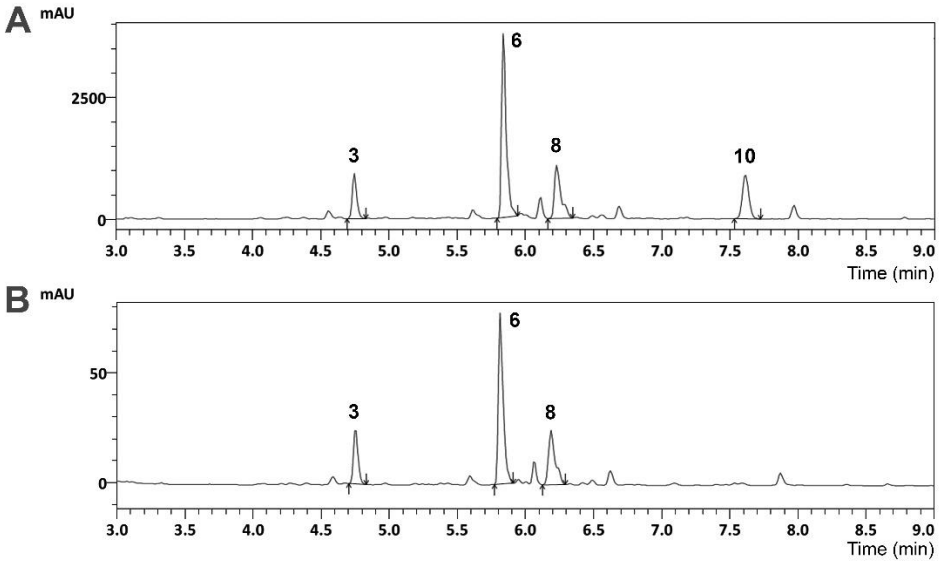


Figure 3.13 **UV (λ = 254 nm) chromatograms of *Prunus padus* flowers**

(**A**) Ethanolic extract. (**B**) Lyophilized sample dissolved in ethanol (1 mg/ml): **3** – chlorogenic acid, **6** – quercetin diglycoside, **8** – quercetin diglycoside, **10** – dicaffeoylcoumaroyl spermidine

Table 3.5

Comparison of the UV profiles of the ethanolic extract and the ethanolic solution of the lyophilized sample of *Prunus padus* flowers

RT, min	Content, % of the total peak area (UV)		Tentative identification	Identification reference in Table 3.4
	Ethanolic extract	Ethanol solution of lyophilized sample		
4.74	8.9	12.4	Chlorogenic acid	3
5.83	38.2	36.9	Quercetin diglycoside	6
6.22	15.2	16.7	Quercetin diglycoside	8
7.61	11.8	-	N’,N”-dicaffeoyl,N’”-coumaroyl spermidine	10

The main components in the HPLC-UV plots were identified as chlorogenic acid **3**, the quercetin diglycosides **6** and **8**, and N’,N”-dicaffeoyl,N’”-coumaroyl spermidine **10**. The latter was not detected in samples of the lyophilized powder after dissolution in ethanol.

3.3.3 Total content of the phenolic compounds and the DPPH free radical scavenging activity of the *Prunus padus* flower extract

The capacity of PPFE to scavenge the stable DPPH radical and the total phenolic content are shown in Table 3.6. The EC_{50} value of PPFE was approximately 11-fold higher than that of L-ascorbic acid. The total phenolic content of PPFE was 85.19 mg GAE/g.

Table 3.6

Total phenolic content and DPPH free radical scavenging activity of *Prunus padus* flower extract

Sample	Total phenolic content (mg GAE/g lyophilized extract)[a]	EC_{50} value of DPPH radical scavenging activity (mg/ml)[b]
PPFE	85.19 ± 3.26	0.55 ± 0.10
L-ascorbic acid	-	0.05 ± 0.00006

* Data are represented as the mean ± SD of three independent measurements made in parallel.
* [a]Total phenolic content is expressed as the gallic acid equivalents per gram (mg GAE/g) of lyophilized extract.
* The [b]EC_{50} (mg/ml) value corresponds to the concentration that reduced the DPPH absorbance by 50%.

3.3.4 The effect of *Prunus padus* flower extract on collagenase activity

The results of the collagenase inhibition assay are shown in Figure 3.14. PPFE exhibited the ability to inhibit collagenase activity in a dose-dependent manner. PPFE showed the most significant inhibitory effect on collagenase activity at concentrations of 250 µg/ml and 500 µg/ml and reduced the enzyme activity by 20% at the highest concentration tested.

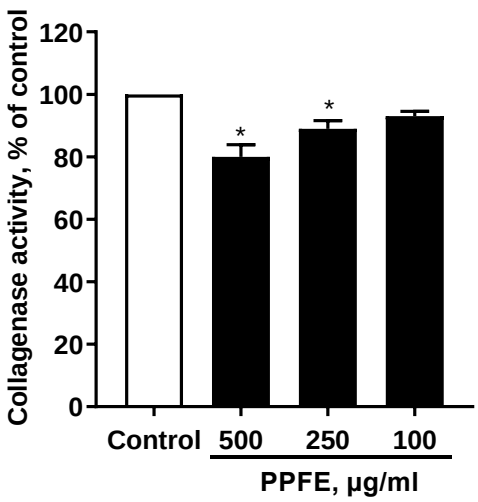


Figure 3.14 **Inhibitory effects of *Prunus padus* flower extract (PPFE) on collagenase activity**

The results are expressed as the percentage of the control/vehicle measurement. Five hundred and 250 represent the concentrations (µg/ml). Data are represented as the mean ± SEM of three independent measurements in two parallels. Differences between the measurements were tested using one-way ANOVA followed by Dunnett's test. *Significantly different from the control ($p < 0.05$).

3.3.5 The effect of *Prunus padus* flower extract on LPS-induced secretion of inflammatory mediator interleukin-6

Since the inflammatory mediator IL-6 is a strong pro-inflammatory cytokine that appears to play a crucial role in the immune system in inducing an inflammatory response (Hodge et al., 2005), its secretion in BMDM cell media was analysed using ELISA. The data regarding IL-6 secretion demonstrate that PPFE suppresses LPS/IFN-γ-induced IL-6 release from BMDMs. PPFE at a concentration of 500 µg/ml significantly decreased the secretion of IL-6 by 35%, and PPFE at a concentration of 250 µg/ml inhibited secretion by 25% compared with that by the LPS/IFN-γ-pretreated control BMDMs (Figure 3.15). According to the MTT assay results, PPFE was not toxic to BMDMs when applied for 24 h at concentrations ranging from 50 to 750 µg/ml (Figure 3.16).

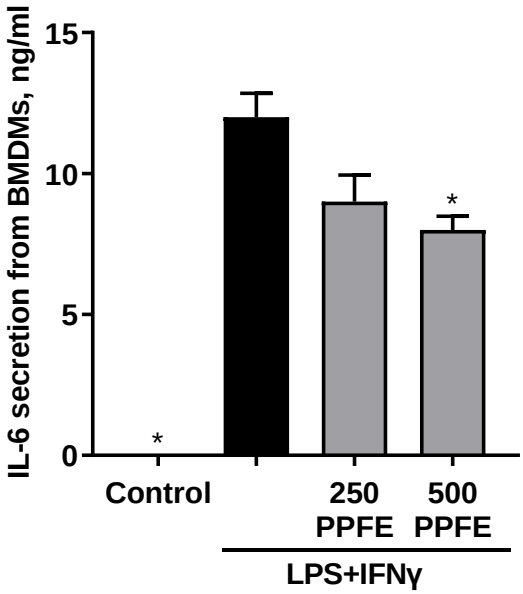


Figure 3.15 **The concentration of IL-6 secreted by BMDMs in cell media was estimated by ELISA**

Effects of PPFE (250 µg/ml and 500 µg/ml) on LPS- and IFN-γ-stimulated (6 h) cells. Five hundred and 250 represent the concentrations (µg/ml). Data are represented as the mean ± SEM of three independent measurements in three parallel experiments. Differences between the measurements were tested using one-way ANOVA followed by Dunnett's test.
*Significantly different from the LPS control ($p < 0.05$).

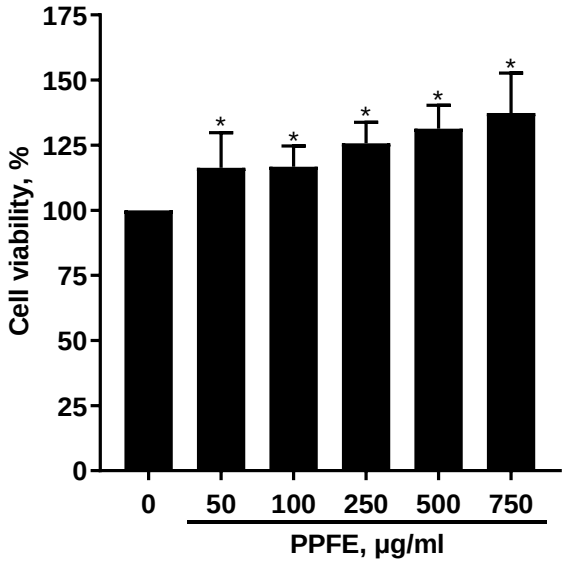


Figure 3.16 **Effects of *Prunus padus* flower extract (PPFE) on bone marrow-derived macrophage viability measured by MTT assay**

Cell viability was tested after 24 h incubation. Values are represented as the mean ± SD of three independent measurements in twelve parallels.
* - significantly different from the untreated control (unpaired t-test, $p<0.05$).

3.3.6 The effect of *Prunus padus* flower extract on LPS-induced macrophage conversion to M1 and M2 phenotypes

In response to LPS/IFN-γ-driven polarization, the number of M1-polarized macrophages increased 8.2-fold compared to that in the untreated control (Figure 3.17 B). The percentage of CD80+ and CD86+ double-positive cells treated with LPS/IFNγ was 32%. PPFE at both concentrations (250 µg/ml and 500 µg/ml) reduced the population of M1 macrophages after 24 h to 28% and 23%, respectively.

The percentage of CD206+ and CD301+ double-positive cells stimulated with IL-4 was 21%. Treatment with 250 µg/ml and 500 µg/ml PPFE increased the macrophage population after 24 h to 23% and 27%, respectively (Figure 3.17 C). The results of the study indicate that PPFE had an effect on both M1 and M2 macrophage polarization.

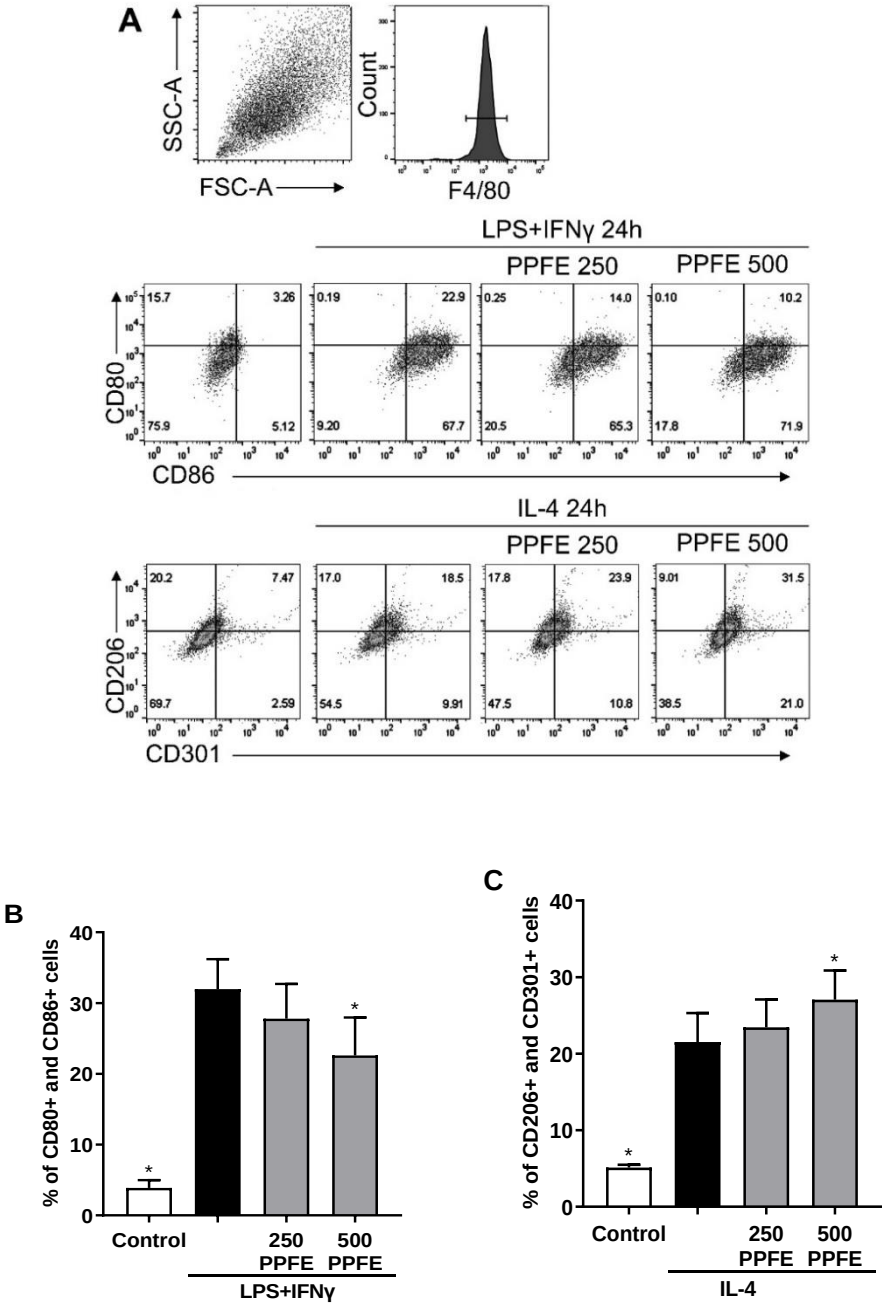


Figure 3.17 **Flow cytometry analysis of BMDM polarization toward the M1 and M2 phenotypes**

(**A**) Upper right quadrant: F4/80-positive cells were gated for double CD80 and CD86 analysis (M1 macrophage phenotype) and for double CD206 and CD301 analysis (M2 macrophage phenotype). The dot plot representation of a total of three independent experiments with three replicates is shown at the bottom of the figure.
(**B**) Expression of the pro-inflammatory cell surface markers CD80 and CD86 were analysed by flow cytometry 24 h after treating BMDMs with extract (250 µg/ml and 500 µg/ml) and LPS/IFN-γ. (**C**) Expression of the anti-inflammatory cell surface markers CD206 and CD301 were analysed by flow cytometry 24 h after treating BMDMs with the extract and IL-4. Five hundred and 250 represent the concentrations (µg/ml). Data are represented as the mean ± SEM of three independent measurements in three parallel experiments. Differences between the measurements were tested using one-way ANOVA followed by Dunnett's test.
*Significantly different from the LPS or IL4 control ($p < 0.05$).

4 Discussion

In the present thesis, the uses of various medicinal plants that were important in Latvian traditional medicine during the 19th and 20th centuries are analysed, and new ideas for practical applications of these plants are sought, providing evidence that this knowledge is still relevant for research purposes today. This is the first comprehensive overview of Latvian folk herbal traditions from the 19th and 20th centuries. A significant number of records of Latvian folk medicine regarding the use of plants in the treatment of various ailments are listed and systematically reviewed. Folkloristic data were compared with the Russian Pharmacopoeia (1891), which was the official pharmacopoeia used in the territory of Latvia during the 19th century, and were also compared with the first Latvian Pharmacopoeia (1940), which was compiled by Latvian researchers and pharmacists. The medicinal plants with herbal monographs published by the European Medicines Agency and those included in the book Herbal Drugs and Phytopharmaceuticals (Wichtl, 2004) are analysed and are shown in the data table. Inspired by the knowledge found in the records of Latvian folk medicine, the anti-inflammatory effects of *Pelargonium sidoides* DC., and *Prunus padus* L. Are further investigated.

4.1 Differences between the traditional and modern uses of medicinal plants

Treatments with medicinal plants have been known to healers since ancient times. In India and Egypt, phytotherapy was used several thousand years ago. The inhabitants of ancient Greece, China, and America used traditional medicinal plants to treat different ailments (Petrovska, 2012). Local people in the territory of Latvia also used medicinal plants. This is evidenced by both oral and written records, including records of Latvian folk medicine. Latvians continue to use plants extensively, although the life habits of Latvians have changed. Today, herbal drugs and natural substances are more often purchased at the point of sale rather than intentionally collected in forests, meadows, or gardens. In modern medicine, plant extracts are widely used as tablets and capsules. For instance, the most frequently used plant taxa in the records of Latvian folk medicine, yarrow, chamomile, and onion, are currently subjected to extract preparations to obtain tablets and capsules (www.zva.gov.lv; registri.pvd.gov.lv). In folk medicine, these plants are used in other kinds of preparations, such as decoctions, teas, juices, and tinctures. To ensure that all manufactured plant extracts are of the same quality and purity, standardization is required (Wachtel-Galor and Benzie, 2011). According to the annual report of the State Agency of Medicines, called “Statistics on medicines consumption 2018”, the following plant species were included in the best-selling medicines: hawthorn

(*Crataegus* spp.), peppermint (*Mentha x piperita* L.), valerian (*Valeriana officinalis* L.), motherwort (*Leonurus cardiaca* L.), ginkgo (*Ginkgo biloba* L.), yellow gentian (*Gentiana lutea* L.), common verbena (*Verbena officinalis* L.), cowslip (*Primula veris* L.), curly dock (*Rumex crispus* L.), and black elder (*Sambucus nigra* L.). In the records of Latvian folk medicine, all of the abovementioned plants, except ginkgo and gentian, were mentioned. The nomenclature of the most frequently mentioned plants in the records of Latvian folk medicine differs from the data published by the State Agency of Medicines. With the decline in the individual tradition of collecting herbal crude drugs in modern society, there is a lack of knowledge about many plant species from Latvian flora. This could suggest that the previous generations used plants more as medicine than the current generations do. In general, the use of medicine has increased due to the availability of a wide range of synthetic drugs, and the use of herbal medicinal products is increasing every year, while in the past, treatment with natural products was the main method and practically the only method (Sõukand et al., 2017).

The climatic conditions and geographic location of Latvia's territory combine north-south and east-west types of vegetation, which results in rich plant diversity. In total, 1800 species of vascular plants are found in the flora of Latvia (Priedītis, 2014). A considerable number of plant taxa (211) were mentioned in the records of Latvian folk medicine. The most dominant plant species across Europe are *Asteraceae* and *Rosaceae* (Sõukand et al., 2013). The plant taxa mentioned in the records also mainly belong to these two families. Additionally, plants from the *Lamiaceae* family are widespread in Europe. *Lamiaceae* is the third most common family recorded in the records of Latvian folk medicine. However, the plants of this family are mostly distributed in countries of the Mediterranean. Most plant species studied are found in the previously mentioned families (see Supplement). Currently, the most predominant families representing the flora of Latvia are the *Asteraceae*, *Rosaceae*, *Fabaceae* and *Brassicaceae* ((Priedītis, 2014); https://www.latvijasdaba.lv). The main plant families of Latvia's flora have not changed throughout the centuries. Local plants that were mentioned in the records of Latvian folk medicine probably were used not only because they were accessible in a certain region but also due to bioactive compounds that provide various physiological effects. Although most plants mentioned in the records belong to *Asteraceae* and *Rosaceae* families, the 13 plants that were described as having activity for more than 8 medicinal use categories were from a range of different families (*Betulaceae*, *Amaryllidaceae*, *Solanaceae*, *Caprifoliaceae*, *Urticaceae*, *Acoraceae*, *Pinaceae*, *Violaceae*, *Apiaceae* and *Scrophulariaceae*), showing that plants from these families have beneficial properties for the treatment of many different diseases (see Supplement).

Trees and shrubs were mentioned relatively often in the studied records of Latvian folk medicine. This could be explained by Latvia's location in a forest zone. The most frequently mentioned plant species that included trees and shrubs were birch, oak, bird cherry, and juniper, as well as pine, spruce, linden, and alder. These previously mentioned perennial plants, as well as yarrow and wormwood, which were mentioned more than 40 times in the records, are apophytes. Apophytes are native plants that have left their natural habitats and passed spontaneously to artificial sites (Sõukand and Kalle, 2011). In turn, yarrow, onion, and chamomile, also among the most frequently mentioned plants, are anthropophytes. Anthropophytes are found in man-made soils and cannot survive without certain conditions sustained by human activities (Sõukand and Kalle, 2011).

Two most mentioned disease categories in other ethnomedicinal studies are respiratory system disorders and digestive system disorders (Phumthum et al., 2018; Sõukand et al., 2017). A large number of records of Latvian folk medicine mentioned the treatment of symptoms related to respiratory system disorders, allowing for the mapping of useful medicinal plant species. These plant species were used to treat mostly upper respiratory tract ailments that equally affected both children and adults. Only slightly described in the records of Latvian folk medicine were nervous system disorders, oncological diseases, and cardiovascular system disorders. They have become relevant health problems in modern societies with increased life expectancy (Phumthum et al., 2018; Sak et al., 2014). In the records of Latvian folk medicine, these three health problems were rarely mentioned, and it could be explained by the limited knowledge of their treatment and insufficient diagnostic capabilities. In the folklore materials, only one taxon, cranberry (*Oxycoccus* Hill.), was mentioned for the treatment of cancer. In recent preclinical *in vitro* and *in vivo* studies, cranberry extracts have shown the ability to inhibit the growth of different human cancer cell lines (Seeram et al., 2006). This is the first ethnobotanical study on Latvian folk herbal traditions, in which diseases have been reported according to the ICPC categorisation, allowing to compare these data with other ethnomedicinal studies.

Many plant species mentioned in the studied records of Latvian folk medicine were used for multiple purposes. This could be explained by the fact that birch, chamomile, valerian, and other wild plants were growing nearby, were easily accessible and were known to possess anti-inflammatory properties. Inflammation and pain have long been well-known symptoms of many diseases, and they are often associated with each other (Maroon et al., 2010). This likely explains why, in the records of Latvian folk medicine, a large number of plant taxa (90) were used for pain reduction.

In addition to wild plant taxa, foreign plant taxa were also used for medicinal purposes in the territory of Latvia. *Aloe* sp. L. are native to the Arabian Peninsula, and also widespread in the Canary Islands, the Cape Verde Islands, Madeira and Spain (Grace et al., 2015). Aloe is a frequently used indoor house plant that is effective in the treatment of respiratory and skin disorders. *Tilia* sp. L., *Juniperus communis* L., *Pinus sylvestris* L., *Achillea millefolium* L. from Latvia's flora were used for the same purpose as aloe. The number of times aloe was mentioned suggested that this plant grew in almost every household in Latvia in the beginning of the 20th century. Along with aloe, *Albuca bracteata* (Thunb.) J. C. Manning & Goldblatt and *Pelargonium* L'Hér. Were used for cough and earache, respectively. Additionally, in Lithuanian ethnobotanical studies, pelargonium and aloe were mentioned; pelargonium was used for respiratory tract disorders and aloe for indigestion and appetite loss (Pranskuniene et al., 2018). *Aloe*, *Pelargonium* and *A. bracteata* were foreign plant taxa that in the territory of Latvia were used throughout the year, as well as in winter, as fresh material to treat different ailments.

Analysis of the records of Latvian folk medicine demonstrated that, in some cases, plants were used to improve health without a specific disease indication (i.e., beneficial for health). Some examples include *Angelica sylvestris* L., *Helianthus tuberosus* L., *Lepidium sativum* L., *Thymus serpyllum* L. and *Rumex* L (see Supplement). Although the records of Latvian folk medicine do not report the medicinal use category of these species, in many countries of Europe and worldwide they are mentioned in folk medicine for treating various disorders. In folk medicine, *T. serpyllum* has been used as a stomachic, carminative, expectorant, for bladder and kidney disorders; externally for rheumatic pain and sprains (Wichtl, 2004). *H. tuberosus*, known as the Jerusalem artichoke, has been used for the treatment of diabetes and rheumatism (Johansson et al., 2015). Angelica root has been occasionally used as an expectorant, as a diuretic, an emmenagogue, and for nervous insomnia (Wichtl, 2004). Many species belonging in the *Rumex* genus have been used in folk medicine because of their gentle laxative effect and also for the treatment of skin diseases (Vasas et al., 2015). *L. sativum* has been used mainly for treating airway disorders, such as asthma, bronchitis, and cough (Rehman et al., 2012).

For the treatment of yellow fever (jaundice), plants with yellow flowers were used, for example, yellow chamomile (*Anthemis tinctoria* L.), yellow everlasting (*Helichrysum arenarium* (L.) Moench), and marigold (*Calendula officinalis* L.). Due to this disease, the yellow colour of flowers was associated with the yellow colouring of skin and mucous membranes. Interestingly, the indication attributed to calendula and sandy everlasting is

described in the scientific literature. *C. officinalis* ethanolic and aqueous extracts of dried and ground flowers and leaves exhibited hepatoprotective and renoprotective properties against aflatoxin-induced liver injury in rats in a dose-dependent manner (Hamzawy et al., 2013). Lyophilised water extracts from the inflorescences of sandy everlasting were more effective in rat liver than well-known hepatoprotectant silibinin at the tested concentrations (Czinner et al., 1999; Czinnera et al., 2001). A common tradition in herbal folk medicine in the territory of Latvia was to use treatments with attributes similar to the ailments.

In Northern and Southern Europe, people recognized the anti-diarrhoeal effects of *Quercus robur* L., *Rumex crispus* L. and *Vaccinium myrtillus* L. (Pranskuniene et al., 2018; Šarić-Kundalić et al., 2011; Shikov et al., 2014). Although local people had no or poor knowledge about the chemical composition of medicinal plants, the astringent properties and the treatment effects were observed, and this information was passed on to subsequent generations. In the case of tannin-containing botanicals with a hard to very hard consistency, such as roots and bark of oak, decoction is an especially suitable method of medicine preparation that was mentioned in the records and is also used today (Sõukand et al., 2017). Additionally, due to a wide range of herbal preparations described in the records, it is evident that people had some knowledge of what type of solvents should be used, methods of preparation, and how medicines should be applied to obtain the best results.

In other studies conducted in Europe, leaves and flowers were the most common plant parts used (Fortini et al., 2016; Menković et al., 2011; Pranskuniene et al., 2018). According to the records of Latvian folk medicine, almost all plant parts were used as herbal medicine. However, leaves, followed by flowers, represented the most commonly used parts. The more frequent use of leaves and flowers could be explained by the fact that these plant parts are abundantly available in this geoclimatic region (Pranskuniene et al., 2018). It is more difficult to collect and prepare roots, fruits, or bark because they are mostly hard and require additional preparation that can also be time consuming. In some tropical areas with pronounced dry seasons, roots may be the only source of biologically active plant parts because roots remain in the soil, are easily available, and are not exposed to intense sunlight (Phumthum et al., 2018). The incorrect harvesting of plant material or harvesting in large quantities might damage or destroy plants. The use of leaves may not cause harmful effects on plant growth compared with the collection of roots, bulbs, whole plants or stems. For instance, leaves are the renewable parts of plants, with their removal resulting in less damage to the plant.

According to the information provided in the records of Latvian folk medicine and in studies of both neighbouring countries, Lithuania and Estonia, the oral administration of herbal medicines was the most common route of administration (Pranskuniene et al., 2018; Sõukand

and Kalle, 2011). In addition, during the plant growing season, the application of fresh and unprocessed herbal drugs is not time consuming but is effective, especially when applied topically to the skin. Many records mentioned that plant leaves, such as plantain, yarrow, aloe, and apple tree leaves were applied directly to fresh wounds or insect or animal bites, as they had properties that reduced inflammation and healed wounds. The preparation of a decoction requires a longer boiling time compared with tea, so this method was used for parts that were harder or denser, such as bark and fruits. Active substances from the bark, roots and fruits cannot be extracted in a short period of time (Liu, 2008). Tinctures made of fresh birch buds, valerian roots, and wormwood stems were very convenient for use in case of respiratory and digestive system disorders because tinctures have a long shelf life. To improve the taste of herbal medicines that were used for treating respiratory and digestive system disorders, honey or sugar was added. Several studies have also mentioned natural sweeteners to improve taste and facilitate the intake of medicines (Phumthum et al., 2018; Pranskuniene et al., 2018; Tariq et al., 2015). Externally applied honey with fresh plant parts such as garlic and onion bulb, or tobacco leaves was used to treat even deep lesions such as abscesses. In the records of Latvian folk medicine, milk, cream, beer, fat, oil, vinegar and even urine were mentioned as solvents instead of water. As mentioned in the records of Latvian folk medicine, bathing in boiled plant water was commonly used to reduce pain, treat skin diseases, or to calm the nervous system for babies, young children and adults.

Daphne mezereum L. Bark, *Pelargonium* L'Hér. Leaves, *Ranunculus acris* aerial parts are reported only for external use and it could be explained by the fact that these taxa contain toxic compounds and may not be safe for oral intake. The bark of *D. Mezereum* is known as a toxic drug due to the presence of diterpene esters (Görick and Melzig, 2013). Hepatotoxicity of *Pelargonium sidoides* root has been reported (Teschke et al., 2012) however, studies on the toxicity of *Pelargonium* species leaves are not assesed. The *Ranunculus* species have potential toxicity caused by the ranunculin and its enzymatic degradation products: protoanemonin and anemonin (Neag et al., 2018). The records of Latvian folk medicine mentioned many other plant taxa, such as *Tussilago farfara* L., *Ledum palustre* L., *Nicotiana* sp. L. And *Acorus calamus* L., which are nowadays known as toxic substance-containing plants (Dampc and Luczkiewicz, 2013; Dobravalskyte et al., 2013; Olas and Bryś, 2018).

In the records of Latvian folk medicine, fresh and raw plant material was most often used to treat digestive, respiratory, skin and neurological disorders. The frequent use of raw material for digestive system disorders could be explained by the fact that toothache was one of the most common diseases treated in this category. For respiratory system disorders, raw

plant material was commonly used. For the voice loss, fresh slices of garden radish were placed under the foot, grated turnip or horseradish were applied to the neck. Garden radish, turnip and horseradish belong to *Brassicaceae* family and are known to be rich in pungent sulfur- containing compounds that stimulate blood flow, especially to the skin (Duke, 2000). This could explain why these taxa were used to reduce the symptoms of the common cold. Only few fresh herbal drugs, such as spruce seeds and yarrow leaves were used internally for reduction of cough. Traditionally, raw materials applied externally have been used for skin disorders. Cranberry was the only taxon mentioned in the records of Latvian folk medicine for treating skin disorders (sunburn) and used orally. In the category of neurological diseases, external application of raw material was mentioned for treating headache. The analgesic effect probably was not achieved by the active substances present in the medicinal plant, but cold application itself alleviated headache. Other studies indicate that cold applications do provide some symptomatic relief of headache (Diamond and Freitag, 1986; Zanchin et al., 2001). As medicinal plants from the records of Latvian folk medicine were used for different purposes, the methods involved in preparation of them were very diverse.

Taking into consideration the ethnobotanical knowledge recorded in studies of other Baltic states, such as Estonia and Lithuania, and comparing medicinal plant use, certain differences are noticed. The plant taxa mentioned most in Estonian studies were similar to those described in the records of Latvian folk medicine: *Plantago* sp. L., *Allium cepa* L., *Matricaria* sp. L., *Achillea millefolium* L., *Juniperus communis* L., *Betula* sp. L., and *Prunus padus* L. (Sõukand and Kalle, 2011). Other plants, such as *Chelidonium majus* L., *Secale cereale* L., *Potentilla anserina* L., *Tussilago farfara* L., *Valeriana officinalis* L., *Solanum dulcamara* L., *Thymus serpyllum* L. and *Pinus sylvestris* L. were also mentioned in the records but are not found among the first top ten plants. Compared to the Lithuanian study, there is one commonly used taxon, *Matricaria chamomilla* L. Others such as *Rubus idaeus* L., *Calendula officinalis* L., and *Tilia cordata* Mill. were rarely mentioned in records of Latvian folk medicine (Pranskuniene et al., 2018). With regard to botanical genera, the dominant taxa in Europe included *Mentha* L., *Tilia* L., *Thymus* L., *Origanum* L., *Rubus* L. and *Matricaria* L. (Łuczaj et al., 2012; Sõukand et al., 2013).

4.2 Future perspectives for medicinal plants from the records of Latvian folk medicine

Fifty-nine plant taxa reported in this study are important for medical practice nowadays, and they are used as traditional herbal medicines according to the EU monographs (see Supplement). However, top ten plant taxa the most frequently cited in the records of Latvian folk medicine and not presented in both: Herbal Drugs and Phytopharmaceuticals (Wichtl,

2004) and herbal monographs by the EMA are interesting for future analysis (Figure 4.1). More ethnobotanical, phytochemical, and pharmacological studies are needed in order to promote and facilitate the therapeutic approval of these species as traditional herbal medicines.

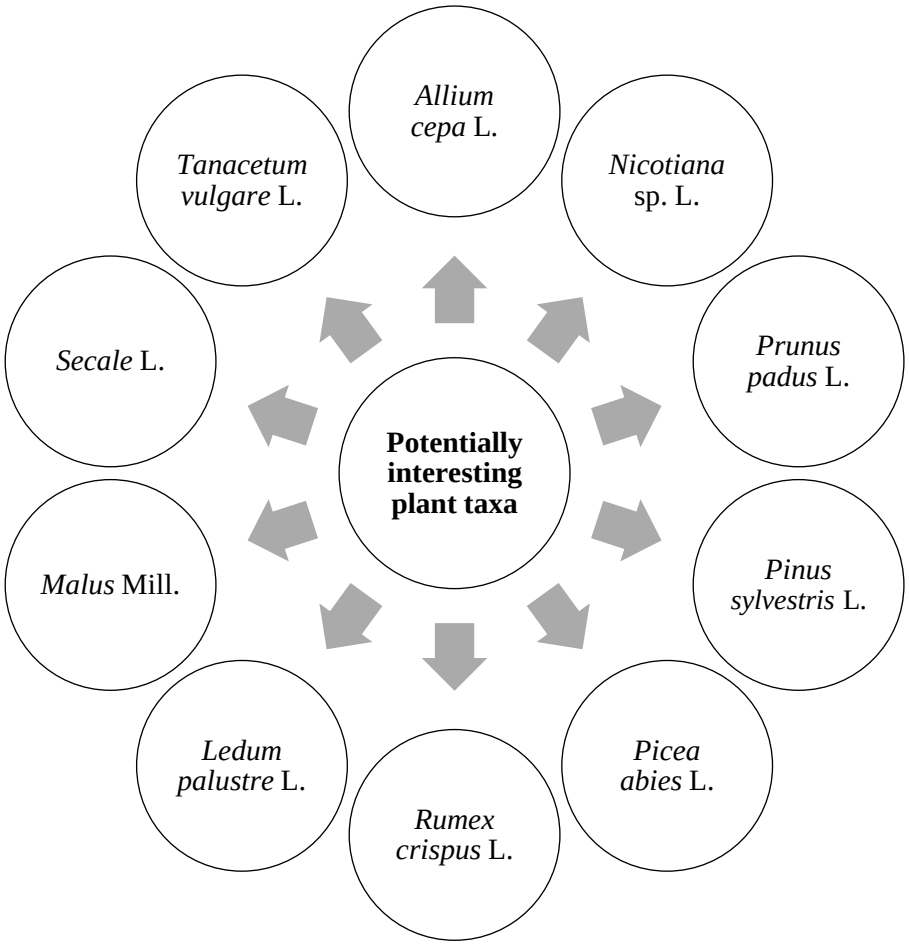


Figure 4.1 **Potentially interesting plant taxa for future studies**

Top ten plant taxa the most frequently cited in the records of Latvian folk medicine and not presented in both: herbal monographs by the EMA and Herbal Drugs and Phytopharmaceuticals (Wichtl, 2004).

Four plant taxa that are mentioned in the records of Latvian folk medicine and Herbal Drugs and Phytopharmaceuticals (Wichtl, 2004) are not presented in herbal monographs by the EMA at this moment (see Table 4.1). In both the records of Latvian folk medicine and monographs from Herbal Drugs and Phytopharmaceuticals the use of these species is identical. The efficacy and safety of these plant taxa are well supported by pharmacological studies according to Wichtl (2004).

Table 4.1

Plant taxa and their uses that are mentioned in the both the records of Latvian folk medicine and Herbal Drugs and Phytopharmaceuticals by Wichtl (2004) but are not presented in EMA herbal monographs

Scientific plant name	Part of plant used	Therapeutic uses
Apium graveolens L.	Seeds	As an adjuvant therapy in the treatment of rheumatic conditions
Euphrasia rostkoviana Hayne	Herb	For treating eye diseases (for inflammatory or catarrhal conjunctivitis)
Inula helenium L.	Root	As an expectorant in case of bronchitis
Petroselinum crispum (Mill.) Fuss	Root	As a diuretic

4.3 Analysis of *Pelargonium*

4.3.1 Differences between the traditional and modern use of *Pelargonium*

In Latvian folk medicine, *Pelargonium* is colloquially called the “ear flower”. This is not without reason because, in Latvia, the leaves of this plant have traditionally been used to treat earaches. Evidence of this can be found in the records of Latvian folk medicine, in which seven out of ten mentions of *Pelargonium* were dedicated to reducing earaches. While in the studied Latvian folk medicine records, the *Pelargonium* leaves were the most commonly mentioned part of the plant used for medicinal purposes, the use of *Pelargonium* roots, especially the roots of *P. sidoides* DC., is more commonly recognized worldwide to treat coughs, sore throats, and other respiratory ailments (Kolodziej, 2011; Saraswathi et al., 2011). Another widely used *Pelargonium* species, which is cultivated so that essential oils can be obtained from its leaves, is *P. graveolens* L’Hér. The most important *Pelargonium* species explored to date include *P. graveolens*, *P. sidoides*, *P. reniforme* Curtis, and *P. radula* (Cav.) L’Hér. In the studied records of Latvian folk medicine, specific species were not mentioned. From the data collected, it can be concluded that the local people in the territory of Latvia recognized the most effective use of *Pelargonium* mainly in external applications. Using the leaves of *Pelargonium* for medicinal purposes was a sustainable way of using the plant. The use of other plant parts, such as roots, may be unsustainable and may contribute to the destruction of the plant. However, environmental factors may influence the traditional uses of plants. For example, it may be more difficult to obtain enough leaves to make medicine in very dry regions, such as in South Africa, so, in those regions, roots would be preferred (Phumthum et al., 2018).

Various phytochemical compounds can be found in the different parts of a plant, and these compounds can possess diverse bioactive properties. There are differences and similarities in chemical composition among the aerial and underground parts of *Pelargonium* plants. The presence of significant amounts of phenolic compounds in the leaves and roots of

P. reniforme has been reported (Adewusi and Afolayan, 2009). The occurrence of coumarin sulphates, coumarin glycosides, and proanthocyanidins was confined to *P. sidoides*. In addition, *P. sidoides* comprises a variety of phenolic and polyphenolic compounds and is rich in flavonoids and hydrolysable tannins (Saraswathi et al., 2011). In PSRE, which was investigated in this study, six phenolic compounds were identified and quantified: catechin, epicatechin, epigallocatechin, epigallocatechin gallate, gallic acid, and quercetin (Savickiene et al., 2018). In addition, the phenolic compounds identified in the PACN fraction were prodelphinidin oligomers, from dimers to hexamers (Savickiene et al., 2018). The essential oil of *P. graveolens* contains citronellol, citronellyl formate, citronellyl acetate, and geraniol. When applied topically, the essential oil may have antibacterial and antifungal properties (Fekri et al., 2019; Saraswathi et al., 2011). Experimental studies have shown that the characteristic components of the essential oil of *P. graveolens*, citronellol and geraniol, exhibit anti-inflammatory properties, supporting their common use and demonstrating their therapeutic potential for treating inflammation-associated disorders (Su et al., 2010). Currently, there is no evidence supporting the idea that *P. graveolens* was the species used for medicinal purposes in the territory of Latvia during the 19th and 20th centuries. In Lithuanian ethnobotanical studies, the aerial parts of *Pelargonium odoratissimum* (L.) L'Hér. Were mentioned for the treatment of respiratory tract disorders (Pranskuniene et al., 2019, 2018).

4.3.2 Anti-inflammatory activity of PSRE and PACN

In the experimental part of this study, the anti-inflammatory properties of PSRE and PSRE-derived PACN were investigated. A previous study by Savickiene et al. 2018 reported that PACN possessed stronger antioxidant and antibacterial properties than those of PSRE. Evaluation of pro-inflammatory cytokine secretion and gene expression revealed that PSRE and PACN suppress at least three different inflammatory processes: cytokine secretion (IL-6 from bone marrow-derived macrophages), inflammatory gene expression (IL-1β, iNOS and COX-2) and macrophage conversion to pro-inflammatory M1 phenotype (Figure 4.2). Similar anti-inflammatory activity of PSRE together with *Coptis chinensis* Franch. Root extract was recently shown in LPS-stimulated RAW 264.7 cells (Park et al., 2018). The extract combination significantly decreased the levels of iNOS, PGE2, TNF-α, IL-1β, and IL-6 in RAW 264.7 macrophages, and the results were also confirmed *in vivo* in a paw oedema rat model. Although the study reported lower levels of TNF-α secretion from LPS-stimulated RAW 264.7 cells, in our study, we did not observe significant changes in TNF-α gene expression in both LPS-stimulated leukocytes and LPS/IFN-*γ*-stimulated macrophages after

PSRE and PACN treatment. Although observed anti-inflammatory properties of PACN and PSRE were of comparative levels, PACN had a stronger efficiency in preventing mediator release. Stronger anti-inflammatory activity of PACN might be due to greater amounts of prodelphinidins found in the roots of *P. sidoides*. These compounds possess higher antioxidant capacity and share certain important structural peculiarities, namely, hydroxyl groups in B ring (especially in C4' position and catechol group), hydroxyl groups in the A ring at the C5 and C7 positions (Ambriz-Pérez et al., 2016).

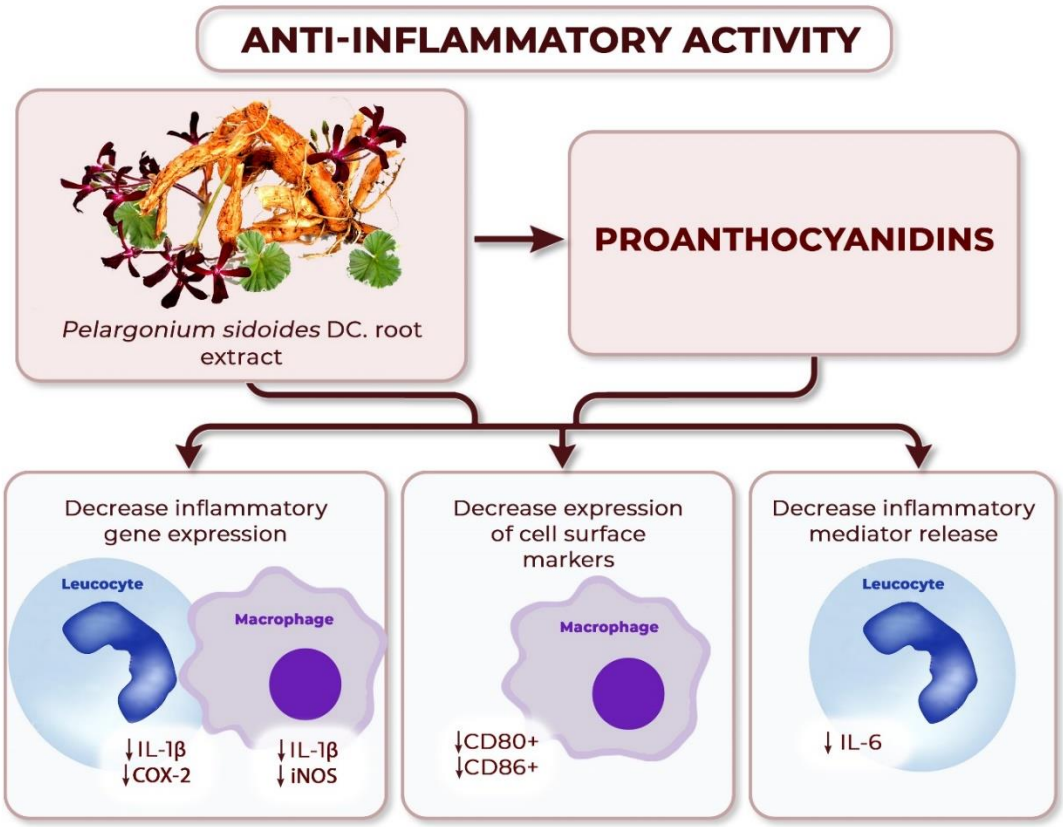


Figure 4.2 **Anti-inflammatory activity of *Pelargonium sidoides* root extract (PSRE) and proanthocyanidins from PSRE in bone marrow-derived macrophages and human peripheral blood mononuclear cells**

P. sidoides root extracts and the proantocyanidins obtained from these extracts are useful not only in the treatment of coughs and colds but also due to their anti-inflammatory and antibacterial effects, which may help reduce infection and inflammation-related diseases such as periodontitis (Jekabsone et al., 2019). To avoid the negative impacts of antibiotics and synthetic antiseptics, natural, biologically active compounds from *P. sidoides* can be used as alternative prevention and treatment options. The studied records of Latvian folk medicine also mentioned the use of *Pelargonium* to reduce toothaches. In summary, the findings of this study

confirm the traditional use of *Pelargonium* for the effective treatment of inflammatory conditions.

4.4 Analysis of *Prunus padus*

4.4.1 Differences between the traditional and modern use of *Prunus padus*

In European ethnobotanical studies, the use of the fruits and flowers of *P. padus* has been commonly reported (Kujawska et al., 2017; Pranskuniene et al., 2019; Sõukand et al., 2017). However, in the records of Latvian folk medicine, the bark was the most frequently mentioned part of the plant. The use of *P. padus* bark was mentioned ten times more often than the use of fruits and eight times more often than the use of flowers. The symptoms and uses mentioned in other studies are the same as those mentioned in the Latvian records: the fruits were used to treat diarrhoea and the flowers were used for joint and rheumatic pain and for treating erysipelas (Kujawska et al., 2017; Pranskuniene et al., 2019; Sõukand et al., 2017). In addition, the use of *P. padus* was also reported in the records of Latvian folk medicine for treating disorders such as headaches, toothaches, pain in the ears, neck and stomach, coughs, bruises, and swelling.

P. padus is still a common wild plant in Latvia and in Baltic states in general. Estonian ethnobotanical research on medicinal plants revealed that *P. padus* was among the top ten most frequently used taxa from the 19th to 20th centuries. Moreover, according to the records of Latvian folk medicine, *P. padus* was frequently used for multiple disorders comprising 8 medicinal use categories; however, currently, it is almost forgotten and is not available for purchase as tea, food supplements or medicine.

4.4.2 Anti-inflammatory activity of PPFE

The major volatile constituents in *P. padus* flower extracts reported in other studies were benzaldehyde, 2-phenylethanol, and (Z)-8-hydroxylinalool (Radulović et al., 2009; Surburg et al., 1990). The results obtained in the present study are in line with the chemical composition of *P. padus* flower extracts described in the literature. The benzaldehyde content detected in the present study is similar to that reported by (Radulović et al., 2009), whereas we did not detect 2-phenylethanol, which represented 15% of the composition of the extract in the previously published study. Similarly, a decreased content of 8-hydroxylinalool compared to that in previously published data (Radulović et al., 2009) was found (7.7% and 30.4%, respectively). The total content of alkanes found in the present study (26.4%) is comparable to

that found in the published data (39.4%). Several factors, such as genetic variability, climatic conditions and environmental impact, could contribute to the differences in the phytochemical composition of *P. padus* flowers.

The flowers of *P. padus* have been reported to have significant and dose-dependent *in vitro* antioxidant activity that correlates with the polyphenol content (Olszewska and Kwapisz, 2011). High levels of quercetin diglycosides and chlorogenic acid in *P. padus* flower extracts have also been observed (Olszewska and Kwapisz, 2011). In the previously mentioned study conducted in Poland, the ratio between the content of chlorogenic acid and quercetin diglycosides was reported to be similar to that in the present study; however, according to the present study, the content of quercetin diglycosides was higher than that of chlorogenic acid. Quercetin has been reported as a long-lasting anti-inflammatory agent that possesses strong anti-inflammatory activity (Li et al., 2016). The molecular mechanism of the anti-inflammatory effect of quercetin on LPS-induced gene and protein expression of inflammatory mediators and cytokines in macrophages has been reported, including its role in reducing proinflammatory cytokine IL-6 release in the cell media (Endale et al., 2013; Y. Yang et al., 2012). Chlorogenic acid along with quercetin could be the main compounds responsible for the anti-inflammatory effect of PPFE (Figure 4.3).

Another compound detected in *P. padus* ethanol extract was a spermidine derivative, di-caffeoyl-coumaroyl spermidine, which is present uniquely in the stamen and pistil of flowering plants (Hanhineva et al., 2008; Z. Yang et al., 2012). Spermidine derivatives have been tentatively identified in extracts of the *Prunus spinosa* L. flower (Marchelak et al., 2017). In the current study, the presence of spermidine derivatives in *P. padus* flowers was tentatively identified for the first time. However, spermidine derivative was not detected after lyophilization of ethanolic extract. Therefore, the spermidine derivative was not involved in the effects induced by PPFE.

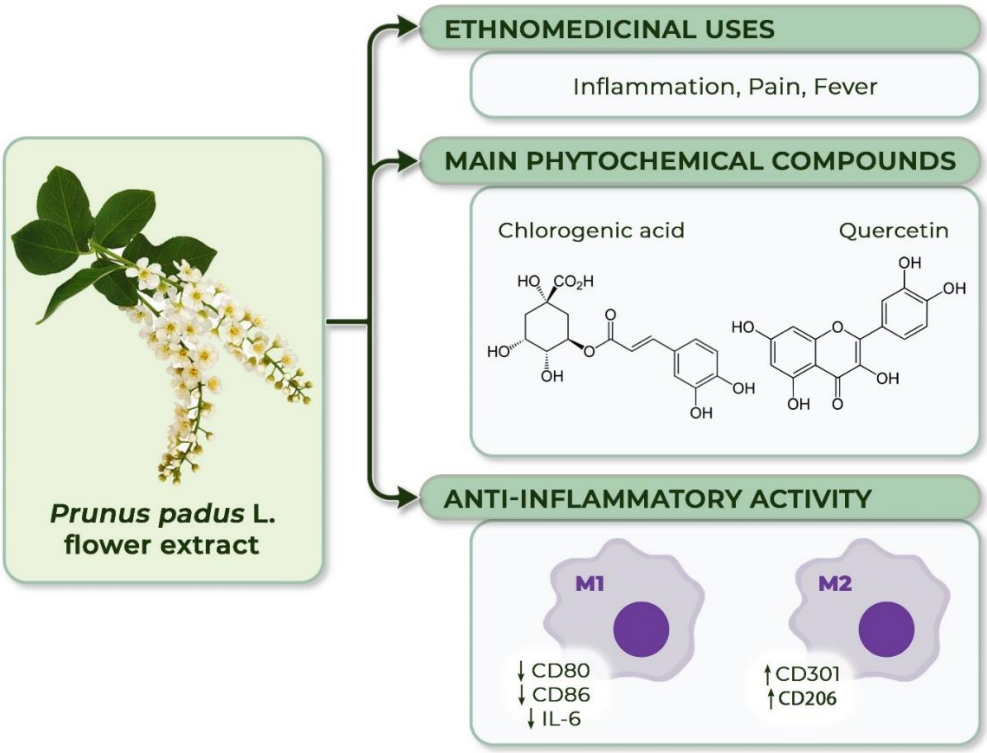


Figure 4.3 **Anti-inflammatory activity of *Prunus padus* flower extract in classically activated (M1) and alternatively activated (M2) bone marrow-derived macrophages**

The previous literature data referring to intracellular enzyme inhibition by *P. padus* are limited to studies on bark. *P. padus* bark extract at a dose of 350 µg/ml, inhibited elastase and tyrosinase activity for 36% and 38%, respectively (Hwang et al., 2014). Although the polyphenol concentration in *P. padus* bark extract was eight times higher than that in PPFE, in comparison with other known antioxidant-containing plants, such as thistle, slippery elm bark, and pine needles, *P. padus* bark possessed moderate elastase and mild tyrosinase inhibitory effects (Hwang et al., 2014). Quercetin, as one of the core components in the *P. padus* flower extract, is known to result in significant collagenase inhibition (Shin et al., 2019; Sin and Kim, 2005). These previous studies and findings from the present investigation suggest that quercetin detected in PPFE may play a significant role in protection against collagen degradation. However, the concentration of PPFE needed to induce anti-collagenase activity seems to be very high.

A recent study of the anti-inflammatory effects of *P. padus* flowers showed that the lipophilic triterpenes (corosolic, ursolic and oleanolic acids) from the flowers exhibited a relatively high inhibitory activity towards proinflammatory enzymes, such as lipoxygenase (IC_{50}: 12.8 µg/U) and hyaluronidase (IC_{50}: 22.0 µg/U), compared to the positive controls. The extracts were 5.8-fold less active than indomethacin and dexamethasone (Magiera et al., 2019).

In an experimental model similar to the experimental model set up in the present study, the anti- inflammatory activity of the *P. padus* stem extract in LPS- and IFN-γ-stimulated murine peritoneal macrophages was shown (Choi et al., 2012). The study demonstrated that the methylene chloride fraction of *P. padus* (MPP) stem extract has significant inhibitory effects on proinflammatory mediators, including nitric oxide (NO), inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), via the downregulation of NF-kB translocation to the nucleus (Choi et al., 2012). Based on previous studies, the authors speculated that compounds such as isorhamnetin, astragalin, quercetin, and chlorogenic acid, which were also detected in present study, might be responsible for the anti-inflammatory action of MPP (Choi et al., 2012). In the present study, primary BMDMs were chosen as the cellular model because they are a better model than immortalized cell lines and represent a homogenous population of cells that can be activated to analyse the inhibition of proinflammatory cytokine activity. Based on the results of present study, two inflammatory processes were suppressed by PPFE: cytokine secretion (IL-6 from BMDMs) and macrophage polarization toward the proinflammatory M1 phenotype characterized by the presentation of the surface markers CD80 and CD86. In addition, PPFE exhibited promising anti-inflammatory effects on M2 macrophages, which are involved in tissue healing in the late stages of inflammation (Figure 4.3). In summary, these findings suggest the potential use of PPFE as a source of natural anti-inflammatory agents.

Conslusions

1. This ethnobotanical study provides the first comprehensive overview of Latvian folk herbal traditions from the 19th and 20th centuries. More than 200 different plant taxa were identified as being used in traditional medicine in the territory of Latvia.
2. One hundred fifty-two taxa mentioned in the studied records of Latvian folk medicine are still not included in EU herbal monographs, which provide scientific information on the safety and efficacy of the use of various herbs. The plant species and their uses described in the studied records of Latvian folk medicine could be potentially useful for future research on herbal medicine.
3. Both *Pelargonium sidoides* root extract (PSRE) and proanthocyanidins obtained from PSRE (PACN) exhibit equally pronounced *in vitro* anti-inflammatory activities by suppressing at least three different inflammatory processes: cytokine secretion, inflammatory gene expression and macrophage conversion to the pro-inflammatory M1 phenotype.
4. The ethanol extract of *Prunus padus* L. flowers (PPFE) is a rich source of bioactive compounds such as quercetin, chlorogenic acid, and other phenolic compounds that possess considerable anti-inflammatory properties, supporting its use in ethnomedicine for reducing inflammatory processes.

Approbation of the study – publications and thesis

Doctoral thesis is based on following SCI publications:

1. **Sile, I**., Romane, E., Reinsone, S., Maurina, B., Tirzite, D., Dambrova, M. 2020. Medicinal plants and their uses recorded in the Archives of Latvian Folklore from the 19th century. *J Ethnopharmacol*. 249, 112378.
2. **Sile, I**., Romane, E., Reinsone, S., Maurina, B., Tirzite, D., Dambrova, M. 2020. Data on medicinal plants in the records of Latvian folk medicine from the 19th century. *Data Brief*. 28, 105024.
3. **Sile, I**., Videja, M., Makrecka-Kuka, M., Tirzite, D., Pajuste, K., Shubin, K., Krizhanovska, V., Grinberga, S., Pugovics, O., Dambrova, M. 2021. Chemical composition of *Prunus padus* L. flower extract and its anti-inflammatory activities in primary bone marrow-derived macrophages. *J Ethnopharmacol*. 268, 113678.
4. Jekabsone, A., **Sile, I**., Cochis, A., Makrecka-Kuka, M., Laucaityte, G., Makarova, E., Rimondini, L., Bernotiene, R., Raudone, L., Vedlugaite, E., Baniene, R., Smalinskiene, A., Savickiene, N., Dambrova, M. 2019. Investigation of Antibacterial and Antiinflammatory Activities of Proanthocyanidins from *Pelargonium sidoides* DC Root Extract. *Nutrients*. 11, 2829.

Publications in Latvian peer-reviewed scientific journals:

1. **Sile, I**., Reinsone, S., Romane, E., Dambrova, M. 2017. Medicinal Plants in Latvian Folk Beliefs [in Latvian]. *RSU Collection of Scientific Papers*. 233−240.

Results are reported in the following international conferences:

1. **Sile, I**., Makarova, E., Makrecka-Kuka, M., Videja, M., Savickiene, N., Dambrova, M. 2019. Comparison of antiinflammatory activities of *Pelargonium sidoides* root extract and isolated proanthocyanidins. *FEBS3+ Conference of Latvian, Lithuanian and Estonian Biochemical Societies*, Riga, Latvia, June 17−19, 2019, Book of Abstracts, P.91.
2. **Sile, I**., Romane, E., Reinsone, S., Tirzite, D., Dambrova, M. 2019. The use of ethnomedicinal plants in the Latvian-populated territory. *RSU International Conference on Medical and Health Care Sciences: Knowledge for Use in Practice*, Riga, Latvia, April 1−3, 2019. Book of Abstracts, P.397.
3. **Sile, I**., Romane, E., Shubin, K., Grinberga, S., Makarova, E., Dambrova, M. 2018. Analysis of traditional medicinal use in Latvia and chemical composition of flower and fruit extracts of bird cherry. *18th World Congress of Basic and Clinical Pharmacology*, Kyoto, Japan, July 1−6, 2018.
4. **Sile, I**., Romane, E., Shubin, K., Grinberga, S., Dambrova, M. 2017. Analysis of chemical composition and traditional medicinal use in Latvia of bird cherry flowers *Padus avium*. *2nd International Conference in Pharmacology: from Cellular Processes to Drug Targets (ICP2017RIGA)*, Riga, Latvia, October 19−20, 2017. Book of Abstracts: Intrinsic Activity. 5 (Suppl. 1):A2.33, doi:10.25006/IA.5.S2-A2.33.

Results are reported in following local conferences:

1. **Sīle, I**., Romāne, E., Šubins, K., Grīnberga, S., Makarova, E., Dambrova, M. 2018. Parastās ievas drogu ķīmiskā sastāva analīze, antiradikālā aktivitāte un ārstnieciskais pielietojums latviešu tautas ticējumos, *IV Pasaules latviešu zinātnieku kongress*, Sekc. “Medicīna un veselības zinātnes” [Rīga, Latvija, 18.–20. jūnijs, 2018].

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Acknowledgements

I would like to express my sincerest gratitude to my scientific supervisor, Prof. Maija Dambrova, for her guidance, knowledge and patience. I am grateful to have her support and helpful suggestions during all stages of this research. I have learned so many useful knowledge in science and everyday life from her. I would also like to thank my supervisor Dr. pharm. Edita Romāne for her support and valuable advice during my PhD study.

I am very thankful to Elīna Makarova, she was my first teacher in the research laboratory. I would like to thank her for her technical advices and kind support. Thanks to Marina Makrecka-Kūka who introduced me to flow cytometry. She was always willing to help and maintain a positive attitude no matter what. I would like to thank Melita Vidējā and Kārlis Vilks for help and assistance. They never refused to help or give advice. Thanks to Dace Tirzīte, who introduced me to DPPH and Folin-Ciocalteu assay. She always read the manuscripts carefully and with great interest. Special thanks to Solveiga Grīnberga and Osvalds Pugovičs for chromatography analysis, Kirill Shubin and Kārlis Pajuste for preparation of extracts. Thanks to Edijs Vāvers who introduced me to GraphPad Prism. He always was interested in how I was doing with my research and has always been very supportive. Thanks to Ludmila Jackeviča who was a person working with cell culture. I would like to thank Līga Zvejniece, Helēna Cīrule, Lāsma Ļauberte for their advice and help to carry out the experiment.

Special thanks to Sanita Reinsone who introcuced me to the Archives of Latvian Folklore and provided useful knowledge about the records of Latvian folk medicine. Thanks to Baiba Mauriņa, who helped to find the old Russian Pharmacopoeia (1891) which stored in P. Stradins Museum for History of Medicine.

Many thanks to Riga Stradins University which is my *Alma Mater* and supported this thesis with doctoral studies grants. Thanks to Department of Dosage Form Technology for financial support to visit laboratory at the Department of Food Chemistry, Technology and Biotechnology, Gdansk University of Technology.

This thesis would not have been possible without the excellent conditions provided by the Latvian Institute of Organic Synthesis and the financial support of the research project PELARGODONT funded by a grant (No. S-M-ERA.NET-17-2) from the Research Council of Lithuania and the State Education Development Agency of Latvia, and support of the European Regional Development Fund project “Innovative solutions for growing technologies and applications of spring medicinal and aromatic plants” (Nr. 1.1.1.1/18/A/043).

Lastly, I would like to thank my family for their understanding and encouragement over four years.

Supplement

List of plants identified in the records of Latvian folk medicine to either species or genus level

Note: Ext. – external use; O. ad. – oral administration; Unsp – unspecified.

* Assessment by the European Medicines Agency's Committee on Herbal Medicinal Products. Status type - C: Ongoing call for scientific data; D: Draft under discussion; F: Assessment finalised; P: Draft published.

** Herbal drugs listed in: M. Wichtl, Herbal Drugs and Phytopharmaceuticals: A Handbook for Practice on a Scientific Basis, Third ed., CRC press, Stuttgart, 2004.

The use of plant not identified in the records of Latvian folk medicine.

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
Acoraceae	*Acorus calamus* L.	*smaržīgā kalme*	Leaves, rhizome	Bath, decoction, decoction with honey, raw material, tea, tincture	Ext., O. ad.	Digestive	Diarrhoea	3		× (rhizome)
							Dysentery	3		
							Generalized abdominal pain	3		
							Hernia	2		
							Stomach function disorders	2		
							Teething problem	1		
							Toothache	3		
						Endocrine, metabolic and nutritional	Loss of appetite	2		
						General and unspecified	Fever	1		
						Musculoskeletal	Bone pain	2		
							Rheumatism	6		
						Neurological	Headache	1		
						Psychological	Feeling anxious/ nervous/ tense	1		
							Tobacco abuse	1		
						Respiratory	Chest pain	1		
							Respiratory complaint	2		
						Skin	Hair loss	1		
Amaranthaceae	*Atriplex* L.	*balodene*	Aerial parts	Raw material	Ext.	Skin	Athlete's foot	1		
	Beta vulgaris L.	*parastā biete*	Leaves, roots	Juice, raw material	Ext., O. ad.	Digestive	Stomach function disorders	1		
						General and unspecified	Fever	1		
							Tuberculosis	2		
						Neurological	Epilepsy	1		
							Headache	1		
						Skin	Abscess	1		
							Cuts and wounds	2		
Amaryllidaceae	*Allium cepa* L.	*dārza sīpols*	Bulb	Baked, bath, decoction, juice, raw material,	Ext., O. ad.	Blood, blood forming organs, lymphatics, spleen	Spleen problems	1		

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				steamed material, tea, tincture, with vinegar		Circulatory	Cardiovascular problems	1		
						Digestive	Dysentery	1		
							Toothache	7		
							Worms	1		
						Ear	Earache	6		
						Eye	Eye problems	1		
						General and unspecified	Bleeding	1		
							Fever	1		
							Bacterial infection	1		
						Respiratory	Cough	5		
							Diphtheria	1		
							Runny nose	2		
							Sore throat	2		
							Voice loss	1		
						Skin	Snake bite	5		
							Abscess	18		
							Burns	2		
							Ulcer	1		
							Hair loss	2		
							Insect bite	2		
							Lichen	1		
							Athlete's foot	1		
							Warts	3		
						Urology	Cystitis	1		
	Allium sativum L.	*ķiploks*	Bulb	Decoction, juice, raw material, with milk	Ext., O. ad.	Digestive	Hernia	1	F: EMA/HMPC/7685/2013 Cough and cold Atherosclerosis#	× (bulb powder)
							Toothache	4		
							Worms	2		
						Ear	Earache	4		
						General and unspecified	Bacterial infection	1		
						Musculoskeletal	Cramps	1		
						Respiratory	Laryngo-tracheo-bronchitis	1		
						Skin	Snake bite	1		
							Abscess	5		
							Ulcer	1		
							Splinter	1		
							Insect bite	1		
							Cuts and wounds	1		
							Rash	1		
							Lichen	1		
							Warts	1		
Apiaceae	*Angelica sylvestris* L.	*meža zirdzene*	Unsp	Unsp	Unsp	Unsp	Unsp	1		× (root of *A. Sylvestris*, *A. archangelica*)

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	Apium graveolens L.	*selerija*	Unsp	Decoction	O. ad.	Musculoskeletal	Rheumatism	1		× (fruit)
	Carum carvi L.	*parastā ķimene*	Fruits	Baked, decoction, tea	Ext., O. ad.	Digestive	Diarrhoea	1	F: EMA/HMPC/715092/2013 Bloating and flatulence#	× (fruit)
							Heartburn	2		
						Ear	Earache	1		
						Endocrine, metabolic and nutritional	Loss of appetite	1		
						General and unspecified	Fever	1		
						Respiratory	Respiratory complaint	2		
	Cicuta virosa L.	*indīgais velnarutks*	Roots	Raw material, tea	Ext., O. ad.	Male genital system	Male sexual function complaint	1		
						Skin	Lichen	1		
							Cuts and wounds	1		
	Daucus L.	*burkāns*	Aerial parts, roots	Decoction, juice, raw material, tea	O. ad.	Digestive	Jaundice	2		
							Worms	1		
						General and unspecified	Tuberculosis	2		
						Respiratory	Chest pain	1		
	Levisticum officinale W.D.J. Koch	*ārstniecības lupstājs*	Flowers, herb, leaves	Bath, compresses, decoction, raw material, tea, with urine	Ext., O. ad.	Blood, blood forming organs, lymphatics, spleen	Blood problems	1	F: EMA/HMPC/524621/2011 (root) Adjuvant in minor urinary complaints#	× (root)
						Circulatory	Cardiovascular problems	1		
						Digestive	Sharp, throbbing abdominal pain	1		
							Toothache	1		
						Female genital system and breast	Excessive menstrual bleeding	1		
						General and unspecified	Swelling	11		
						Musculoskeletal	Bone pain	1		
							Rheumatism	1		
						Respiratory	Respiratory complaint	2		
							Sore throat	2		
						Skin	Erysipelas	1		
							Scabies	1		
							Snake bite	1		
	Petroselinum Hill	*pētersīlis*	Aerial parts, roots	Decoction, tea, with wine	Ext., O. ad.	Digestive	Diarrhoea	1		× (fruit, root of *P. crispum*)

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						General and unspecified	Swelling	1		
							Fever	1		
						Pregnancy, childbirth, family planning	Induced abortion	1		
						Psychological	Learning problems	1		
						Skin	Cuts and wounds	1		
						Urology	Kidney problems	1		
							Urinary retention	1		
							Urination problems	2		
	Pimpinella anisum L.	*parastais aniss*	Fruits	Drops	O. ad.	Respiratory	Chest pain	1	F: EMA/HMPC/321184/2012 Cough and cold Bloating and flatulence#	× (fruit)
							Cough	1		
							Acute upper respiratory infection	1		
	Pimpinella L.	*noraga*	Roots	Decoction, tea	O. ad.	Circulatory	Cardiovascular problems	1		× (root of *P. major*)
						Digestive	Sharp, throbbing abdominal pain	1		
	Sium latifolium L.	*platlapu cemere*	Unsp	Unsp	O. ad.	Digestive	Generalized abdominal pain	1		
Apocynaceae	*Vinca minor* L.	*mazā kapmirte*	Unsp	Unsp	Unsp	Unsp	Unsp	1		
Asparagaceae	*Albuca bracteata* (Thunb.) J.C.Manning & Goldblatt	*neīstais jūras sīpols*	Bulb, leaves	Decoction, juice, raw material, with cream	Ext., O. ad.	Neurological	Headache	1		
						Respiratory	Cough	3		
	Convallaria majalis L.	*parastā kreimene*	Flowers	Tincture	O. ad.	Digestive	Sharp, throbbing abdominal pain	1		
						Psychological	Feeling anxious/ nervous/ tense	1		
	Polygonatum odoratum (Mill.) Druce	*ārstniecības mugurene*	Unsp	Unsp	Unsp	Musculoskeletal	Bone pain	1		
Asphodelaceae	*Aloe* sp. L.	*alveja*	Leaves	Boiled material, decoction, juice, raw material, tincture, with honey, with milk, with oil	Ext., O. ad.	Circulatory	Heart pain	1	F: EMA/HMPC/625788/2015 Constipation	× (dried juice of the leaves of *A. barbadensis*, *A. capensis*)
						Digestive	Constipation	1		
							Hernia	1		
							Sharp, throbbing abdominal pain	1		
							Vomiting	1		
						General and unspecified	Tuberculosis	6		

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						Respiratory	Chest pain	11		
							Cough	5		
							Respiratory complaint	1		
							Shortness of breath	1		
						Skin	Abscess	2		
							Burns	1		
							Cuts and wounds	8		
							Scar	1		
Asteraceae	*Achillea millefolium* L.	*parastais pelašķis*	Aerial parts, flowers, roots	Decoction, juice, raw material, tea, tincture	Ext., Inhalation, O. ad.	Digestive	Diarrhoea	1	F: EMA/HMPC/143949/2010 (flower) F: EMA/HMPC/290284/2009 (herb) Bloating and flatulence Small superficial wounds Spasm associated with menstrual periods# Loss of appetite#	× (aerial part)
							Flatulence	1		
							Generalized abdominal pain	4		
							Hernia	1		
							Toothache	1		
						Ear	Earache	1		
						General and unspecified	Bleeding	21		
							Tuberculosis	4		
						Musculoskeletal	Cramps	1		
						Psychological	Feeling anxious/ nervous/ tense	1		
						Respiratory	Cough	35		
							Runny nose	1		
							Sore throat	1		
						Skin	Athlete's foot	3		
							Boils	4		
							Cuts and wounds	8		
							Dry skin	1		
							Scabies	1		
	Anthemis tinctoria L.	*dzeltenā ilzīte*	Flowers	Decoction, tea, tincture	O. ad.	Digestive	Jaundice	15		
	Arctium lappa L.	*lielais dižadzis*	Leaves, roots	Decoction, juice, raw material	Ext., O. ad.	Digestive	Dysentery	1	F: EMA/HMPC/246763/2009 (root) Seborrhoeic skin conditions Loss of appetite# Adjuvant in minor urinary complaints#	× (root)
							Toothache	1		
						General and unspecified	Swelling	1		
						Skin	Boils	1		
							Cuts and wounds	1		
							Hair loss	1		
	Arnica montana L.	*kalnu arnika*	Flowers		Ext., O. ad.	Digestive	Hernia	3		× (flower)

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				Decoction, juice, tea, tincture		Skin	Cuts and wounds	1	F: EMA/HMPC/198793/2012 Bruises, sprains, localised muscular pain	
	Artemisia abrotanum L.	*dievkociņš*	Leaves	Decoction, raw material, tea, with urine	Ext., O. ad.	Digestive	Generalized abdominal pain	1		
							Sharp, throbbing abdominal pain	2		
							Toothache	2		
						Respiratory	Chest pain	1		
							Respiratory problems	1		
						Skin	Lice	1		
							Lichen	2		
	Artemisia absinthium L.	*vērmele*	Flowers	Decoction, raw material, tea, tincture	Ext., O. ad.	Digestive	Stomach function disorders	2	F: EMA/HMPC/751490/2016 (herb) Loss of appetite Mild dyspeptic/ gastrointestinal disorders	× (aerial part)
							Diarrhoea	2		
							Dysentery	3		
							Generalized abdominal pain	22		
							Heartburn	1		
							Hernia	2		
							Jaundice	1		
							Nausea	2		
							Toothache	2		
							Worms	2		
						Endocrine, metabolic and nutritional	Loss of appetite	2		
						Female genital system and breast	Menstrual problems	1		
						General and unspecified	Bacterial infection	2		
							Fever	3		
							Swelling	1		
						Musculoskeletal	Cramps	1		
						Neurological	Headache	1		
						Respiratory	Chest pain	2		
							Cough	4		
							Respiratory problems	2		
						Skin	Boils	1		
							Cuts and wounds	2		
							Erysipelas	1		

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	Artemisia vulgaris L.	*parastā vībotne*	Aerial parts, roots	Decoction, tea, with milk	Ext., O. ad.	Digestive	Generalized abdominal pain	1		× (aerial part)
						Female genital system and breast	Absent menstruation	2		
							Excessive menstrual bleeding	1		
						Neurological	Epilepsy	3		
							Headache	2		
						Respiratory	Chest pain	1		
							Respiratory problems	1		
						Skin	Snake bite	1		
	Calendula officinalis L.	*ārstniecības kliņģerīte*	Flowers	Tea	O. ad.	Digestive	Diarrhoea	1	F: EMA/HMPC/437450/2017 Skin inflammation and minor wounds# Minor inflammations in the mouth or the throat#	× (flower)
							Jaundice	1		
	Carduus L.	*dzelksnis*	Roots	Tea	O. ad.	Respiratory	Chest pain	2		
	Cyanus segetum Hill	*parastā rudzupuķe*	Aerial part, flowers, straw	Bath, decoction, tea	Ext., Fumigation, O. ad.	Digestive	Diarrhoea	1		
							Sharp, throbbing abdominal pain	1		
						Eye	Eye pain	1		
						General and unspecified	Fever	2		
							Tuberculosis	2		
						Psychological	Sleep disturbance	2		
						Respiratory	Chest pain	1		
							Cough	3		
	Cichorium intybus L.	*parastais cigoriņš*	Aerial parts, roots	Tea	O. ad.	Digestive	Nausea	1	F: EMA/HMPC/121816/2010 (root) Mild digestive disorders (abdominal fullness, flatulence, slow digestion) Loss of appetite#	
						Respiratory	Respiratory problems	1		
	Cirsium arvense (L.) Scop.	*tīruma usne*	Unsp	Tea	O. ad.	Circulatory	Cardiovascular problems	1		

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	Cirsium vulgare (Savi) Ten.	*asais dadzis*	Inflores-cence, roots, seeds	Ashes, raw material, tea	Ext., O. ad.	Digestive	Sharp, throbbing abdominal pain	2		
							Toothache	1		
						Respiratory	Cough	1		
						Skin	Herpes simplex	1		
	Dahlia Cav.	*dālija*	Roots	Tea	O. ad.	Digestive	Jaundice	1		
						Neurological	Epilepsy	1		
							Headache	1		
	Gnaphalium uliginosum L.	*dumbrāja zaķpēdiņa*	Aerial parts	Powder, tea	Ext., O. ad.	Respiratory	Cough	1		
						Skin	Lichen	2		
	Helianthus annuus L.	*vasaras saulgrieze*	Seeds	Oil	Ext.	Skin	Dry skin	1		
	Helianthus tuberosus L.	*topinambūrs*	Unsp	Unsp	Unsp	Unsp	Unsp	1		
	Helichrysum arenarium (L.) Moench	*dzeltenā salmene*	Flowers	Decoction, tea	O. ad.	Digestive	Colic	1	F: EMA/HMPC/41 108/2015 Digestive disorders with a feeling of fullness and bloating	× (flower)
							Jaundice	9		
						Musculoskeletal	Cramps	1		
							Musculoskeletal problems	1		
	Inula helenium L.	*helēniju ālante*	Flowers, roots	Decoction, tea, tincture	Ext., O. ad.	Digestive	Generalized abdominal pain	4		× (rhizome)
							Hernia	10		
							Sharp, throbbing abdominal pain	2		
						General and unspecified	Frostbite	2		
						Neurological	Headache	1		
						Respiratory	Chest pain	2		
							Respiratory problems	2		
						Skin	Bruise/ contusion	1		
							Skin problems	1		
	Leucanthemum vulgare (Vaill.) Lam.	*parastā pīpene*	Flowers, leaves	Decoction, raw material, tea	Ext., O. ad.	General and unspecified	Fever	1		
						Musculoskeletal	Cramps	1		
						Skin	Cuts and wounds	4		
							Freckles	1		

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	Matricaria chamomilla L.	*ārstniecības kumelīte*	Flowers, leaves	Bath, compresses, decoction, raw material, tea	Ext., Inhalation, O. ad.	Digestive	Constipation	2	F: EMA/HMPC/55 843/2011 (flower) Minor inflammation of the skin (sunburn), superficial wounds and small boils (furuncles) Minor gastro-intestinal complaints such as bloating and minor spasms Cough and cold Minor ulcers and inflammations of the mouth and throat Irritations of skin and mucosae in the anal and genital region	× (flower)
							Diarrhoea	1		
							Generalized abdominal pain	4		
							Toothache	2		
						Ear	Earache	7		
						Endocrine, metabolic and nutritional	Feeding problem of infant/child	1		
						Eye	Eye pain	8		
						Female genital system and breast	Induced abortion	1		
							Female genital candidiasis	1		
						General and unspecified	Fever	3		
							Swelling	1		
						Musculoskeletal	Rheumatism	1		
						Neurological	Headache	6		
						Pregnancy, childbirth, family planning	Complicated labour/delivery livebirth	2		
							Post-partum problems	3		
						Psychological	Sleep disturbance	1		
							Feeling anxious/ nervous/ tense	1		
						Respiratory	Chest pain	1		
							Cough	20		
							Voice loss	2		
							Acute upper respiratory infection	5		
							Sore throat	5		
							Pneumonia	1		
							Runny nose	3		
						Skin	Boils	1		
							Cuts and wounds	3		
							Vernix	1		
						Urology	Cystitis	1		
							Kidney problems	1		
	Scorzonera humilis L.	*zemā raudupe*	Roots	Tincture	O. ad.	General and unspecified	Fever	1		
	Tagetes L.	*samtene*	Unsp	Tea	O. ad.	Neurological	Headache	1		

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	Tanacetum vulgare L.	*parastais biškrēsliņš*	Flowers, fruits, roots	Decoction (with milk), raw material, tea (with milk), tincture	Ext., O. ad.	Digestive	Generalized abdominal pain	2		
							Hernia	1		
							Worms	16		
						General and unspecified	Fever	2		
						Musculoskeletal	Pain in joint	1		
						Neurological	Headache	1		
						Respiratory	Chest pain	2		
							Cough	2		
						Skin	Lichen	1		
	Taraxacum campylodes G.E.Haglund	*ārstniecības pienene*	Flowers, flying seeds, leaves, roots	Decoction, juice, raw material, tea	Ext., O. ad.	Digestive	Generalized abdominal pain	2	F: EMA/HMPC/579636/2008 (leaf) Adjuvant in minor urinary complaints# F: EMA/HMPC/212895/2008 (root with herb) Mild digestive disorders (abdominal fullness, flatulence, slow digestion) Loss of appetite# Adjuvant in minor urinary complaints#	× (root with aerial part)
							Hernia	3		
						Respiratory	Runny nose	1		
						Skin	Blisters	1		
							Cuts and wounds	1		
							Lichen	2		
							Warts	2		
	Tripleurospermum inodorum (L.) Sch.Bip.	*nesmaržīgā suņkumelite*	Flowers	Decoction	O. ad.	Respiratory	Sore throat	1		
	Tussilago farfara L.	*parastā mālīpe*	Leaves	Bath, raw material, tea	Ext., O. ad., Smoking	Digestive	Generalized abdominal pain	1		× (leaf)
							Hernia	2		
						General and unspecified	Swelling	4		
						Musculoskeletal	Bone pain	1		
						Neurological	Headache	2		
						Respiratory	Cough	3		
						Skin	Erysipelas	1		
Balsaminaceae	*Impatiens noli-tangere* L.	*meža sprigane*	Unsp	Powder	Ext.	Skin	Lichen	1		
Betulaceae	*Alnus glutinosa* (L.) Gaertn.	*melnalksnis*	Bark, buds, leaves	Decoction	O. ad.	Digestive	Diarrhoea	1		
							Generalized abdominal pain	1		
						Respiratory	Sore throat	1		
	Alnus incana (L.) Moench	*baltalksnis*	Unsp	Tea	O. ad.	General and unspecified	Fever	1		

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	Alnus Mill.	*alksnis*	Bark, fruits, leaves, twigs	Bath, decoction, powder, raw material, tea	Ext., O. ad.	Digestive	Diarrhoea	2		
						Ear	Earache	1		
						Musculoskeletal	Cramps	1		
						Neurological	Headache	1		
						Respiratory	Sore throat	1		
						Skin	Animal bite	1		
							Athlete's foot	9		
	Betula L.	*bērzs*	Bark, buds, flowers, leaves, wood, twigs	Bath, decoction, raw material, steamed material, tea, tincture, with beer, wood tar	Ext., O. ad.	Circulatory	Cardiovascular problems	2	F: EMA/HMPC/573241/2014 (leaf) Adjuvant in minor urinary complaints	× (leaf)
							Swollen ankles/oedema	1		
						Digestive	Diarrhoea	2		
							Stomach cleansing	1		
							Dysentery	2		
							Generalized abdominal pain	6		
							Sharp, throbbing pain	2		
							Toothache	1		
						Ear	Earache	2		
						Endocrine, metabolic and nutritional	Loss of appetite	1		
						General and unspecified	Bleeding	1		
							Fever	1		
							Tuberculosis	2		
						Musculoskeletal	Bone pain	2		
							Rheumatism	2		
						Neurological	Epilepsy	2		
						Pregnancy, childbirth, family planning	Complicated labour/delivery livebirth	2		
						Respiratory	Chest pain	3		
							Cough	3		
							Diphtheria	1		
						Skin	Athlete's foot	1		
							Burns	1		
							Cuts and wounds	1		
							Hair loss	3		
							Localized swelling/lump	1		
							Warts	2		
						Urology	Kidney problems	2		
		āra bērzs			Ext., O. ad.	Digestive	Diarrhoea	1		

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	Betula pendula Roth		Buds, leaves, twigs	Raw material, steamed material, tincture			Generalized abdominal pain	1		
						General and unspecified	Swelling	1		
						Musculoskeletal	Bone pain	1		
						Skin	Abscess	1		
	Corylus avellana L.	*parastā lazda*	Buds, flowers	Tea	O. ad.	General and unspecified	Tuberculosis	1		
						Pregnancy, childbirth, family planning	Female infertility	1		
Boraginaceae	*Pulmonaria obscura* Dumort.	*ārstniecības lakacis*	Unsp	Tea	O. ad.	Respiratory	Respiratory problems	1		× (aerial part)
	Symphytum L.	*tauksakne*	Roots	Raw material	Ext.	Skin	Abscess	1	F: EMA/HMPC/572846/2009 Sprains and bruises#	× (root of *S. officinale*)
Brassicaceae	*Armoracia rusticana* P.Gaertn., B.Mey. et Scherb.	*mārrutks*	Leaves, roots	Decoction, raw material, tea	Ext., O. ad.	Digestive	Toothache	2		
						Female genital system and breast	Absent menstruation	1		
						General and unspecified	Tuberculosis	1		
						Musculoskeletal	Rheumatism	1		
						Respiratory	Chest pain	1		
							Sore throat	1		
	Brassica napus L.	*kālis*	Seeds	Unsp	O. ad.	Pregnancy, childbirth, family planning	Complicated labour/delivery livebirth	1		
	Brassica oleracea L.	*dārza kāposts*	Leaves, seeds	Decoction, raw material	Ext., O. ad.	Neurological	Headache	2		
						Pregnancy, childbirth, family planning	Female infertility	1		
						Skin	Cuts and wounds	1		
	Brassica rapa L.	*rācenis*	Leaves, seeds, tuber	Decoction, dew, raw material, tincture	Ext., O. ad.	Digestive	Diarrhoea	1		
						Eye	Eye problems	1		
						General and unspecified	Chickenpox	1		
							Measles	3		
						Respiratory	Sore throat	1		
						Skin	Scabies	1		
	Capsella bursa-pastoris (L.) Medik.	*ganu plikstiņš*	Unsp	Unsp	Unsp	Pregnancy, childbirth, family planning	Female infertility	1	F: EMA/HMPC/262766/2010 (herb) Heavy menstrual bleeding#	× (aerial part)
	Lepidium sativum L.	*dārza cietķērsa*	Unsp	Unsp	Unsp	Unsp	Unsp	1		
	Raphanus raphanistrum	*rutks*	Roots	Juice, raw material	Ext., O. ad.	Digestive	Hernia	1		

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
	subsp. *sativus* (L.) Domin						Worms	1		
						General and unspecified	Tuberculosis	1		
						Musculoskeletal	Bone pain	1		
						Neurological	Headache	2		
						Respiratory	Chest pain	1		
							Cough	4		
							Voice loss	1		
	Sinapis L.	*sinepe*	Unsp	Tea, tincture, with fat	Ext.	Digestive	Toothache	2		
						Skin	Boils	1		
							Cuts and wounds	1		
Campanulaceae	*Campanula trachelium* L.	*strēļu pulkstenīte*	Unsp	Unsp	Unsp	Unsp	Unsp	1		
Cannabaceae	*Cannabis sativa* L.	*sējas kaņepe*	Aerial parts, leaves, stem	Juice, raw material	Ext., Inhalation, Smoking	Digestive	Toothache	2		
						General and unspecified	Toxic effect non-medicinal substance	1		
						Skin	Burns	1		
							Cuts and wounds	1		
	Humulus lupulus L.	*parastais apinis*	Flowers, roots, stem	Ashes, bath, decoction, oil, tea	Ext., Inhalation, O. ad.	Digestive	Toothache	2	F: EMA/HMPC/682384/2013 (strobiles)	× (strobile, grains)
						Neurological	Headache	1		
							Walking difficulties in children	1		
						Skin	Hair loss	1	Mental stress and sleep disorders	
							Herpes simplex	1		
							Scabies	1		
Caprifoliaceae	*Knautia arvensis* (L.) Coult.	*tīruma pēterene*	Unsp	Unsp	O. ad.	Musculoskeletal	Fracture	1		
	Succisa pratensis Moench	*pļavas vilkmēle*	Unsp	Unsp	Unsp	Digestive	Toothache	1		
	Valeriana officinalis L.	*ārstniecības baldriāns*	Flowers, roots	Bath, decoction, juice, raw material, tea, tincture	Ext., Fumigation, O. ad.	Circulatory	Cardiovascular problems	3	F: EMA/HMPC/150848/2015, Corr. (root) Sleep disorders and temporary insomnia Mental stress and mood disorders	× (root)
						Digestive	Diarrhoea	1		
							Generalized abdominal pain	3		
							Dental problem	1		
							Toothache	1		
						Musculoskeletal	Cramps	6		
							Rheumatism	2		
						Neurological	Epilepsy	2		
							Headache	3		
						Pregnancy, childbirth, family planning	Complicated labour/delivery livebirth	2		
						Psychological	Feeling anxious/ nervous/ tense	7		

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
							Sleep disturbance	3		
						Respiratory	Chest pain	1		
						Skin	Lichen	1		
						Urology	Kidney problems	1		
Caryophyllaceae	*Agrostemma githago* L.	*lauka kokalis*	Unsp	Tea	O. ad.	General and unspecified	Measles	1		
							Scarlet fever	1		
	Dianthus deltoides L.	*dzirkstelīte*	Unsp	Tea	O. ad.	Digestive	Dysentery	1		
							Sharp, throbbing abdominal pain	1		
	Herniaria L.	*trūkumzālīte*	Unsp	Unsp	Unsp	Digestive	Sharp, throbbing abdominal pain	1	D: EMA/HMPC/1887935/2018 (herb) Urinary tract and genital disorders#	× (aerial part of *H. glabra*)
	Silene L.	*plaukšķene*	Unsp	Decoction	O. ad.	Digestive	Generalized abdominal pain	1		
	Stellaria L.	*virza*	Aerial parts	Raw material	Ext.	Neurological	Headache	1		
						Skin	Athlete's foot	1		
	Stellaria media (L.) Vill.	*parastā virza*	Aerial parts	Steamed material	Ext.	Respiratory	Respiratory problems	1		
	Viscaria vulgaris Bernh.	*lipīgā sveķene*	Flowers	Tincture	O. ad.	Digestive	Sharp, throbbing abdominal pain	1		
Celastraceae	*Euonymus* L.	*segliņš*	Leaves	Tea	O. ad.	Respiratory	Chest pain	1		
	Parnassia palustris L.	*purva atālene*	Unsp	Decoction	O. ad.	Circulatory	Cardiovascular problems	1		
						Musculoskeletal	Cramps	1		
Crassulaceae	*Sempervivum globiferum* L.	*atvašu saulrietenis*	Unsp	Juice	Ext.	Ear	Earache	2		
	Sedum L.	*laimiņš*	Aerial parts	Raw material	Ext.	Musculoskeletal	Rheumatism	1		
	Sedum acre L.	*kodīgais laimiņš*	Aerial parts	Bath, tea	Ext., O. ad.	General and unspecified	Fever	1		
						Neurological	Epilepsy	1		
	Sedum maximum (L.) Hoffm.	*lielais laimiņš*	Aerial parts	Raw material	O. ad.	Psychological	Sleep disturbance	2		
	Sempervivum tectorum L.	*jumtu saulrietenis*	Bulb	Juice	Ext.	Ear	Earache	1		
Cucurbitaceae	*Cucumis sativus* L.	*gurķis*	Fruits, seeds	Juice, pickled, raw material	Ext., O. ad.	Digestive	Jaundice	1		
							Worms	1		
						Eye	Eye pain	1		
						Respiratory	Sore throat	1		

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
	Cucurbita L.	*ķirbis*	Fruits	Raw material	O. ad.	Urology	Kidney problems	1	F: EMA/HMPC/136024/2010 (seed) Lower urinary tract symptoms related to benign prostatic hyperplasia or related to an overactive bladder#	× (seed of *C. pepo*)
Cupressaceae	*Juniperus communis* L.	*Zviedrijas kadiķis*	Aerial parts, fruits, roots	Bath, decoction, juice, raw material, tea	Body steaming, Ext., O. ad., Sauna whisk	Digestive	Bad breath	1	F: EMA/HMPC/441929/2008 (berry) Adjuvant in minor urinary complaints Digestive disorders (dyspepsia, flatulence)	× (wood, berry)
							Generalized abdominal pain	1		
							Hypersalivation	1		
							Sharp, throbbing abdominal pain	1		
						General and unspecified	Bacterial infection	1		
							Fever	1		
							Swelling	9		
							Tuberculosis	1		
						Musculoskeletal	Rheumatism	8		
						Neurological	Headache	1		
						Respiratory	Chest pain	4		
							Cough	2		
							Respiratory problems	2		
						Skin	Scabies	4		
						Urology	Bladder problems	1		
							Kidney problems	2		
Cyperaceae	*Carex* L.	*grīslis*	Unsp	Unsp	O. ad.	Digestive	Generalized abdominal pain	1		
Droseraceae	*Drosera rotundifolia* L.	*apaļlapu rasene*	Leaves	Dew	Ext.	Eye	Eye problems	2		× (aerial part)
						Skin	Dry skin	1		
Dryopteridaceae	*Dryopteris filix-mas* (L.) Schott	*melnā ozolpaparde*	Leaves, roots	Unsp	Ext.	Ear	Earache	1		
						Musculoskeletal	Rheumatism	1		
						Skin	Snake bite	1		
Equisetaceae	*Equisetum* L.	*kosa*	Aerial part	Decoction, tea	O. ad.	Blood, blood forming organs, lymphatics, spleen	Blood problems	1	F: EMA/HMPC/278091/2015 Adjuvant in minor urinary complaints Superficial wounds#	× (aerial part of *E. arvense*)
						Respiratory	Chest pain	1		
						Urology	Cystitis	2		
	Equisetum hyemale L.	*ziemzaļā kosa*	Aerial part	Decoction, tea	O. ad.	Digestive	Digestive problems	1		

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
						Respiratory	Acute upper respiratory infection	1		
	Equisetum sylvaticum L.	*meža kosa*	Aerial part	Tea	O. ad.	Urology	Kidney problems	1		
Ericaceae	*Arctostaphylos uva-ursi* (L.) Spreng.	*parastā miltene*	Leaves	Tea	O. ad.	Circulatory	Swollen ankles/oedema	1	F: EMA/HMPC/750269/2016 Lower urinary tract infections (burning sensation during urination and/or frequent urination in women)	× (leaf)
						Musculoskeletal	Rheumatism	1		
						Psychological	Feeling anxious/ nervous/ tense	1		
						Urology	Cystitis	2		
	Calluna vulgaris (L.) Hull	*sila virsis*	Aerial parts, flowers	Bath, tea	Ext., O. ad.	Digestive	Generalized abdominal pain	1		
						Musculoskeletal	Rheumatism	1		
						Respiratory	Respiratory problems	1		
	Chimaphila umbellata (L.) Nutt.	*čemuru palēks*	Unsp	Decoction, tincture	O. ad.	Digestive	Hernia	2		
	Ledum palustre L.	*purva vaivariņš*	Flowers, leaves	Bath, decoction, tea	Ext., O. ad.	Digestive	Bad breath	1		
						General and unspecified	Swelling	1		
							Tuberculosis	2		
						Musculoskeletal	Rheumatism	3		
						Psychological	Acute alcohol abuse	2		
						Respiratory	Chest pain	3		
							Cough	11		
							Shortness of breath	1		
						Skin	Cuts and wounds	1		
							Dandruff	1		
							Rash on head	1		
							Snake bite	1		
	Oxycoccus Hill	*dzērvene*	Fruits	Decoction, juice, raw material, tea	Ext., O. ad.	Circulatory	Cardiovascular problems	1		
						Digestive	Toothache	1		
						Ear	Earache	1		
						General and unspecified	Cencer	1		
							Fever	2		
						Respiratory	Pneumonia	2		
						Skin	Sunburn	1		
	Vaccinium myrtillus L.	*mellene*	Fruits, leaves	Jam, juice, raw material, tea	O. ad.	Digestive	Diarrhoea	9	F: EMA/HMPC/37	× (leaf, dried fruit)
							Dysentery	1		

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
							Generalized abdominal pain	3	5808/2014 (fresh fruit) Discomfort and heaviness of legs related to minor venous circulatory disturbances# Cutaneous capillary fragility# F: EMA/HMPC/678995/2013 (dried fruit) Diarrhoea Inflammations of the oral mucosa#	
							Stomach function disorder	2		
							Toothache	1		
						Respiratory	Chest pain	1		
							Cough	1		
	Vaccinium vitis-idaea L.	*brūklene*	Flowers, fruits, leaves	Bath, decoction, jam, tea	Ext., O. ad.	Blood, blood forming organs, lymphatics, spleen	Promote formation of blood	1		
						Circulatory	Cold feet	1		
						Digestive	Sharp, throbbing abdominal pain	1		
						General and unspecified	Fever	1		
						Musculoskeletal	Bone pain	3		
							Rheumatism	10		
						Respiratory	Chest pain	1		
							Runny nose	1		
Euphorbiaceae	*Euphorbia helioscopia* L.	*saules dievkrēsliņš*	Unsp	Decoction	O. ad.	Urology	Urinary retention	2		
Fabaceae	*Lotus* L.	*vanagnadziņš*	Unsp	Unsp	Unsp	Digestive	Generalized abdominal pain	1		
	Pisum L.	*zirnis*	Seeds	Decoction, soaked	Ext., O. ad.	Digestive	Teething problem	1		
						Endocrine, metabolic and nutritional	Loss of appetite	1		
	Senna Mill.	*senna*	Leaves	Tea	O. ad.	Digestive	Constipation	1	F: EMEA/HMPC/51869/2006 Corrigendum Constipation	× (leaf, pods of *S. angustifolia*)
	Trifolium arvense L.	*tīruma āboliņš*	Flowers	Tea	O. ad.	Respiratory	Respiratory problems	1		
	Trifolium aureum Pollich	*dzeltenais āboliņš*	Flowers, leaves	Decoction, tea	O. ad.	Digestive	Generalized abdominal pain	1		
						Neurological	Epilepsy	1		
						Respiratory	Voice loss	1		
	Trifolium L.	*āboliņš*	Flowers	Bath, decoction	Ext., O. ad.	Digestive	Dental problem	1		

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
						Female genital system and breast	Absent menstruation	1		
						Respiratory	Cough	1		
						Urology	Kidney problems	1		
	Trifolium pratense L.	*pļavas āboliņš*	Flowers	Decoction, tea	O. ad.	General and unspecified	Tuberculosis	1		
						Respiratory	Sore throat	1		
	Trifolium repens L.	*ložņu āboliņš*	Flowers	Bath, decoction, tea	Ext., O. ad.	Digestive	Teething problem	1		
						Female genital system and breast	Female genital candidiasis	1		
						Respiratory	Sore throat	2		
	Trifolium spadiceum L.	*brūnais āboliņš*	Flowers	Tea	O. ad.	Respiratory	Cough	1		
	Vicia faba L.	*lauka pupa*	Flowers, stem	Juice, tea	Ext., O. ad.	Female genital system and breast	Absent menstruation	1		
						Skin	Scar	1		
	Vicia L.	*vīķis*	Unsp	Tea	O. ad.	Digestive	Generalized abdominal pain	1		
Fagaceae	*Quercus robur* L.	*parastais ozols*	Bark, buds, fruits, leaves, seeds	Bath, compresses, decoction, powder, raw material, roasted material, steamed material, tea	Ext., O. ad., Smoking	Circulatory	Stroke	1	C: EMA/HMPC/3203/2009 (bark) Diarrhoea Inflammation of the oral mucosa or skin	× (bark)
						Digestive	Diarrhoea	16		
							Dysentery	6		
							Generalized abdominal pain	5		
							Hernia	3		
							Toothache	7		
						Female genital system and breast	Absent menstruation	1		
							Excessive menstrual bleeding	1		
						Neurological	Headache	2		
						Pregnancy, childbirth, family planning	Complicated labour/delivery livebirth	1		
						Respiratory	Cough	1		
							Respiratory problems	2		
							Sore throat	2		
						Skin	Cuts and wounds	2		
Gentianaceae	*Centaurium erythraea* Rafn	*čemuru augstiņš*	Unsp	Tea	O. ad.	Digestive	Generalized abdominal pain	1	F: EMA/HMPC/277493/2015 (herb) Mild dyspeptic/ gastrointestinal disorders	× (aerial part)

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
									Loss of appetite#	
Geraniaceae	*Pelargonium* L'Hér.	*pelargonija*	Leaves, roots	Raw material, tea	Ext.	Digestive	Sharp, throbbing abdominal pain	1	F: EMA/HMPC/444244/2015 (root) Cough and cold#	
							Toothache	2		
						Ear	Earache	7		
Grossulariaceae	*Ribes nigrum* L.	*parastā upene*	Bark, buds, twigs	Tea	O. ad.	General and unspecified	Swelling	1	F: EMA/HMPC/745353/2016 (leaf) Articular pain Adjuvant in minor urinary complaints#	× (leaf)
						Musculoskeletal	Rheumatism	1		
						Respiratory	Chest pain	1		
							Cough	1		
							Respiratory problems	1		
							Acute upper respiratory infection	2		
	Ribes rubrum L.	*sarkanā jāņoga*	Fruits	Juice	O. ad.	General and unspecified	Tuberculosis	1		
Hypericaceae	*Hypericum* L.	*asinszāle*	Aerial part	Tea, tincture	O. ad.	Digestive	Dysentery	1	P: EMA/HMPC/101304/2008 Depressive disorders# P: EMEA/HMPC/745582/2009 Mental exhaustion# Skin inflammation and minor wounds# Gastrointestinal discomfort	× (aerial part)
							Generalized abdominal pain	1		
						Respiratory	Cough	1		
	Hypericum perforatum L.	*divšķautņu asinszāle*	Aerial part	Unsp	Unsp	Digestive	Dysentery	1		
Iridaceae	*Gladiolus imbricatus* L.	*jumstiņu gladiola*	Bulb	Raw material	Ext., O. ad.	Digestive	Generalized abdominal pain	1		
							Toothache	1		
	Iris L.	*skalbe*	Fruits	Tincture	Ext.	Unsp	Unsp	1		× (rhizome)
Lauraceae	*Laurus* L.	*laurs*	Bark, fruits, leaves	Decoction with beer, powder, tea	O. ad.	Female genital system and breast	Absent menstruation	2		
							Excessive menstrual bleeding	1		
						General and unspecified	Fever	1		
							Scarlet fever	1		
							Measles	1		
						Pregnancy, childbirth, family planning	Induced abortion	1		
Lamiaceae	*Glechoma hederacea* L.	*efejlapu sētložņa*	Flowers	Bath, raw material, tea	Ext., O. ad.	Digestive	Toothache	1		
						General and unspecified	Scarlet fever	1		
							Swelling	1		
	Lamium album L.		Unsp		O. ad.		Menstrual pain	2		

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
		baltā panātre		Decoction, juice, tea		Female genital system and breast				× (aerial part, flower)
							Absent menstruation	1		
							Excessive menstrual bleeding	1		
	Leonurus cardiaca L.	*sirds mātere*	Unsp	Tea	O. ad.	Pregnancy, childbirth, family planning	Post-partum problems	2	F: EMA/HMPC/127428/2010 (herb) Nervous tension# Nervous cardiac complaints (palpitations)#	× (aerial part)
	Melissa officinalis L.	*ārstniecības melisa*	Unsp	Tea	O. ad.	Digestive	Generalized abdominal pain	1	F: EMA/HMPC/196745/2012 (leaf) Gastrointestinal disorders (bloating and flatulence) Mental stress and sleep disorders#	× (leaf)
	Mentha aquatica L.	*ūdensmētra*	Aerial parts	Tea	O. ad.	Digestive	Diarrhoea	1		
	Mentha L.	*mētra*	Aerial parts	Tincture	O. ad.	Digestive	Generalized abdominal pain	1		
	Mentha spicata L.	*krūzmētra*	Aerial parts	Decoction, tea	Ext., O. ad.	General and unspecified	Fever	1		× (leaf)
						Skin	Hair loss	1		
	Mentha x piperita L.	*piparmētra*	Leaves, roots	Raw material, tea	Ext., O. ad.	Digestive	Diarrhoea	1	F: EMEA/HMPC/193909/2007 (leaf) Gastrointestinal disorders (dyspepsia and flatulence)	× (leaf)
							Generalized abdominal pain	1		
							Toothache	2		
						General and unspecified	Fever	2		
						Male genital system	Male sexual function problems	1		
						Neurological	Headache	1		
						Respiratory	Chest pain	1		
							Acute upper respiratory infection	2		
	Nepeta cataria L.	*kaķumētra*	Unsp	Tea	O. ad.	Pregnancy, childbirth, family planning	Lactation problems	1		
	Origanum L.	*raudene*	Unsp	With beer	O. ad.	Female genital system and breast	Absent menstruation	1		

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
	Prunella vulgaris L.	*parastā brūngalvīte*	Unsp	Tea	O. ad.	Digestive	Diarrhoea	1		
						Respiratory	Sore throat	1		
	Salvia pratensis L.	*pļavas salvija*	Unsp	Unsp	Unsp	General and unspecified	Fever	1		× (leaf of *S. officinalis*)
						Psychological	Acute alcohol abuse	2		
							Feeling anxious/ nervous/ tense	1		
	Satureja hortensis L.	*pupumētra*	Unsp	Tea	O. ad.	General and unspecified	Swelling	1		
	Thymus L.	*mārsils*	Aerial parts	Decoction, tea	Ext., O. ad.	Neurological	Headache	1	F: EMA/HMPC/342332/2013 Expectorant in cough associated with cold	
						Respiratory	Cough	1		
							Chest pain	2		
						Skin	Bruise/ contusion	1		
	Thymus pulegioides L.	*lielais mārsils*	Aerial parts	Tea	O. ad.	Digestive	Generalized abdominal pain	1		
							Stomach function disorder	1		
	Thymus serpyllum L.	*mazais mārsils*	Aerial parts	Unsp	Unsp	Unsp	Unsp	1		× (aerial part)
Linaceae	*Linum catharticum* L.	*pļavas liniņš*	Flowers	Tincture	Ext.	Skin	Abscess	2		
							Bruise/ contusion	2		
	Linum usitatissimum L.	*sējas lins*	Seeds	Decoction, tea, with pork fat	Ext., O. ad.	Digestive	Dysentery	1	F: EMA/HMPC/377675/2014 Constipation# Gastrointestinal discomfort#	× (seed)
						General and unspecified	Measles	1		
						Respiratory	Chest pain	1		
							Sore throat	2		
						Skin	Hair loss	1		
Lobariaceae	*Lobaria pulmonaria* (L.) Hoffm.	*parastais plaukšķērpis*	Thallus	Decoction, tea	O. ad.	General and unspecified	Tuberculosis	1		
						Respiratory	Respiratory problems	2		
Lycopodiaceae	*Huperzia selago* (L.) Bernh. ex Schrank & Mart.	*apdzira*	Aerial parts, spores	Decoction, decoction with beer, tea	Ext., O. ad.	Digestive	Hernia	2		
							Induces vomiting	7		
						General and unspecified	Leprosy	1		
						Skin	Dandruff	2		
							Hair loss	1		
							Lice	3		
							Scabies	2		
	Lycopodium L.	*staipeknis*	Spores	Powder	Ext.	General and unspecified	Bleeding	2		× (aerial part)

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
						Skin	Cuts and wounds	3		
							Diaper rash	2		
							Athlete's foot	1		
	Lycopodium clavatum L.	*vālīšu staipeknis*	Spores	Powder	Ext.	Skin	Burns	1		
Malvaceae	*Malva* L.	*malva*	Flowers	Tea	O. ad.	Unsp	Unsp	1	F: EMA/HMPC/749511/2016 Oral or pharyngeal irritation and associated dry cough# Gastrointestinal discomfort#	× (flower, leaf of *M. sylvestris*)
	Tilia sp. L.	*liepa*	Bark, flowers, leaves, sapwood	Bath, compresses, decoction, tea	Ext., O. ad.	General and unspecified	Fever	6	F: EMA/HMPC/337066/2011 (flower) Common cold Mental stress	× (flower of *T. cordata*)
							Tuberculosis	1		
						Psychological	Nightmares	1		
						Respiratory	Acute upper respiratory infection	4		
							Chest pain	6		
							Cough	4		
							Respiratory problems	6		
						Skin	Burns	2		
Menyanthaceae	*Menyanthes trifoliata* L.	*trejlapu puplaksis*	Aerial parts, leaves, roots	Bath, decoction, juice, tea	Ext., O. ad.	Circulatory	Swollen ankles/oedema	2	D: EMA/HMPC/187996/2018 (leaf) Gastrointestinal disorders Loss of appetite#	× (leaf)
						Digestive	Diarrhoea	1		
							Generalized abdominal pain	4		
							Hernia	2		
						General and unspecified	Fever	1		
							Swelling	2		
							Tuberculosis	3		
						Neurological	Headache	1		
						Respiratory	Chest pain	1		
							Cough	4		
							Respiratory problems	4		
						Skin	Cuts and wounds	1		
							Athlete's foot	1		
Myrtaceae	*Myrtus* L.	*mirte*	Leaves	Decoction, tea, with milk	Ext., O. ad.	Digestive	Generalized abdominal pain	1		
							Hernia	1		
							Toothache	1		
						Musculoskeletal	Cramps	2		
						Neurological	Epilepsy	1		

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
							Hair loss	1		
Nymphaeaceae	*Nuphar lutea* (L.) Sm	*dzeltenā lēpe*	Leaves	Bath, raw material	Ext.	Neurological	Headache	3		
						Skin	Vernix	4		
Oleaceae	*Fraxinus excelsior* L.	*parastais osis*	Flowers, leaves, twigs	Ashes, tea	O. ad.	Neurological	Headache	2	F: EMA/HMPC/239271/2011 (leaf) Adjuvant in minor urinary complaints Articular pain#	
						Urology	Kidney problems	1		
	Syringa L.	*ceriņš*	Flowers, twigs	Steamed material, tea	Ext., O. ad.	Digestive	Diarrhoea	1		
							Teething problem	1		
						General and unspecified	Measles	1		
							Scarlet fever	1		
						Neurological	Headache	1		
						Respiratory	Cough	3		
Orchidaceae	*Dactylorhiza* Neck. Ex Nevski	*dzegužpirkstīte*	Roots	Tincture	O. ad.	Pregnancy, childbirth, family planning	Female contraception	1		
							Induced abortion	1		
							Labour pain	1		
	Dactylorhiza incarnata (L.) Soó	*stāvlampu dzegužpirkstīte*	Roots	Unsp	O. ad.	Pregnancy, childbirth, family planning	Female infertility	1		
	Dactylorhiza maculata (L.) Soó	*plankumainā dzegužpirkstīte*	Roots	Raw material, tincture	Ext., O. ad.	Digestive	Generalized abdominal pain	3		
							Toothache	2		
Oxalidaceae	*Oxalis* L.	*zaķskābene*	Leaves	Juice	Ext.	Digestive	Toothache	1		
Paeoniaceae	*Paeonia* L.	*peonija*	Flowers	Tea	O. ad.	Digestive	Diarrhoea	1		× (flower of *P. officinalis*
						Musculoskeletal	Cramps	1		
						Respiratory	Chest pain	1		
Papaveraceae	*Chelidonium majus* L.	*lielā strutene*	Aerial parts	Juice, tea	Ext., O. ad.	Digestive	Diarrhoea	1		× (aerial part)
						Eye	Eye problems	1		
						Skin	Lichen	2		
							Warts	2		
	Fumaria officinalis L.	*ārstniecības matuzāle*	Unsp	Decoction, tea	Ext.	Skin	Dandruff	2	F: EMA/HMPC/574766/2010 (herb) Increase bile flow for the relief of symptoms of indigestion (sensation of fullness, flatulence, slow digestion)#	× (aerial part)
							Hair loss	1		
	Papaver L.	*magone*	Seeds	Decoction with milk,	O. ad.	Digestive	Dysentery	1		× (flower of *P. rhoes*)

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
				raw material, tea, tea with milk		General and unspecified	Measles	1		
						Psychological	Sleep disturbance	6		
Parmeliaceae	*Cetraria islandica* (L.) Ach.	*Islandes ķērpis*	Thallus	Decoction	O. ad.	General and unspecified	Tuberculosis	1	F: EMA/HMPC/678891/2013 Oral or pharyngeal irritation and associated dry cough Loss of appetite#	× (thallus)
						Respiratory	Respiratory problems	1		
Pinaceae	*Picea abies* (L.) H.Karst.	*parastā egle*	Bark, buds, cones, leaves, resin, seeds, shoots	Bath, compresses, decoction, raw material, tea, with milk, with vinegar	Ext., O. ad.	Digestive	Dysentery	1		
						General and unspecified	Bleeding	2		
							Tuberculosis	2		
						Musculoskeletal	Rheumatism	4		
						Neurological	Headache	3		
						Respiratory	Chest pain	2		
							Cough	1		
							Respiratory problems	2		
							Sore throat	6		
							Runny nose	1		
						Skin	Abscess	3		
							Cuts and wounds	5		
							Scabies	1		
							Athlete's foot	1		
	Pinus sylvestris L.	*parastā priede*	Bark, buds, leaves, resin, seeds, shoots, twigs, wood	Bath, compresses, decoction, fermented material, juice, powder, raw material, tea	Ext., Inhalation, O. ad.	Digestive	Diarrhoea	1		
							Generalized abdominal pain	1		
						Endocrine, metabolic and nutritional	Loss of appetite	1		
						General and unspecified	Tuberculosis	6		
						Musculoskeletal	Rheumatism	6		
						Neurological	Headache	1		
						Respiratory	Acute upper respiratory infection	3		
							Chest pain	1		
							Cough	1		
							Respiratory problems	4		
							Sore throat	3		
						Skin	Cuts and wounds	1		
							Freckles	1		
							Warts	1		
						Urology	Kidney problems	1		
Plantaginaceae	*Plantago* L.	*ceļteka*		Decoction, juice, raw	Ext., O. ad.	Circulatory	Heart pain	1	F: EMA/HMPC/43	× (aerial part, leaf)

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
			Inflores-cence, leaves	material, tea, tincture		Digestive	Generalized abdominal pain	3	7858/2010 (leaf) Oral or pharyngeal irritations and associated dry cough	
							Toothache	2		
						General and unspecified	Bleeding	7		
							Fever	4		
							Swelling	6		
						Neurological	Dizziness	1		
							Headache	1		
						Respiratory	Acute upper respiratory infection	1		
							Respiratory problems	1		
						Skin	Abscess	3		
							Athlete's foot	5		
							Cuts and wounds	7		
							Lichen	1		
	Plantago lanceolata L.	*šaurlapu ceļteka*	Leaves	Raw material	Ext.	General and unspecified	Swelling	1		
						Skin	Abscess	1		
							Cuts and wounds	2		
	Plantago major L.	*lielā ceļteka*	Inflores-cence, leaves	Decoction, raw material	Ext., O. ad.	Digestive	Diarrhoea	1		
						General and unspecified	Fever	4		
						Skin	Swelling	2		
							Cuts and wounds	4		
							Localized swelling/lump	1		
Poaceae	*Agropyron* Gaertn.	*vārpata*	Roots	Decoction, powder, tea	O. ad.	Digestive	Sharp, throbbing abdominal pain	1	F: EMA/HMPC/563408/2010 (rhizome) Adjuvant in minor urinary complaints#	× (rhizome of *A. repens*)
						Musculoskeletal	Cramps	2		
						Urology	Incontinence urine	1		
	Alopecurus L.	*lapsaste*	Unsp	Tea	O. ad.	Digestive	Dysentery	1		
	Avena L.	*auza*	Fruits, straw	Bath, decoction, tea	Ext., Inhalation, O. ad., Smoking	Digestive	Jaundice	1	F: EMEA/HMPC/368600/2007 (fruit) Skin inflammation and minor wounds# F: EMEA/HMPC/202966/2007 (herb) Mental stress and sleep disorders#	× (aerial part of *A. sativa*)
							Toothache	1		
						Musculoskeletal	Rheumatism	1		
						Neurological	Walking difficulties in children	1		
						Respiratory	Chest pain	1		
							Cough	9		
							Acute upper respiratory infection	2		
							Voice loss	1		

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
	Hordeum L.	*miezis*	Green shoots	Decoction	O. ad.	Digestive	Jaundice	1		
	Phalaris arundinacea L.	*parastais miežubrālis*	Leaves	Tea	O. ad.	Digestive	Generalized abdominal pain	1		
	Secale L.	*rudzi*	Flowers, green shoots, spike, straw	Bath, decoction, tea, tincture, with alkaline solution, with beer, with milk	Ext., O. ad.	Digestive	Generalized abdominal pain	7		
						Musculoskeletal	Rheumatism	1		
						Pregnancy, childbirth, family planning	Labour pain	2		
						Respiratory	Chest pain	1		
							Cough	6		
							Runny nose	1		
							Respiratory problems	3		
						Skin	Corn/callosity	1		
							Lichen	1		
	Triticum L.	*kviesis*	Bran	Bath	Ext.	Urology	Kidney problems	1		
Polygalaceae	*Polygala amarella* Crantz	*rūgtā ziepenīte*	Unsp	Bath, tea	Ext., O. ad.	Psychological	Sleep disturbance	1		
						Respiratory	Respiratory problems	1		
						Skin	Freckles	1		
							Sunburn	1		
Polygonaceae	*Polygonum* L.	*sūrene*	Unsp	Raw material, tea	Ext., O. ad.	Skin	Fleas	2		
	Polygonum aviculare L.	*maura sūrene*	Unsp	Bath, tea	Ext.	Musculoskeletal	Musculoskeletal problems	1		× (aerial part)
						Urology	Kidney problems	2		
	Rheum rhaponticum L.	*dārza rabarbers*	Leaf stalks	Jam, powder, with water	O. ad.	Respiratory	Cough	1		× (root of *R. palmatum*)
						Digestive	Diarrhoea	1		
							Generalized abdominal pain	2		
	Rumex acetosella L.	*mazā skābene*	Leaves	Juice	Ext.	General and unspecified	Bleeding	1		
	Rumex crispus L.	*cirtainā skābene*	Flowers, leaves, roots	Decoction, raw material, tea, with cream	Ext., O. ad.	Digestive	Diarrhoea	11		
							Generalized abdominal pain	4		
						General and unspecified	Swelling	1		
						Skin	Erysipelas	1		
							Scabies	10		
	Rumex L.	*skābene*	Unsp	Unsp	Unsp	Unsp	Unsp	1		

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
Polytrichaceae	*Polytrichum commune* Hedw.	*parastais dzegužlins*	Tea	Unsp	Unsp	Unsp	Unsp	1		
Primulaceae	*Primula farinosa* L.	*bezdelīgactiņa*	Aerial parts	Tea	O. ad.	Digestive	Generalized abdominal pain	1		
						Respiratory	Respiratory problems	1		
	Primula veris L.	*gaiļbiksīte*	Flowers	Tea	O. ad.	Respiratory	Acute upper respiratory infection	1	F: EMA/HMPC/136582/2012 Expectorant in cough associated with cold	× (flower, root)
							Chest pain	1		
							Respiratory problems	2		
Ranunculaceae	*Aconitum napellus* L.	*zilā kurpīte*	Roots	Raw material, tea	Ext.	Digestive	Sharp, throbbing abdominal pain	1		
							Toothache	3		
						Skin	Lichen	1		
	Actaea spicata L.	*vārpainā krauklene*	Aerial parts	Unsp	Ext.	Psychological	Feeling anxious/ nervous/ tense	1		
	Anemone L.	*vizbulis*	Roots	Tea	O. ad.	Respiratory	Chest pain	1		
	Anemone ranunculoides L.	*dzeltenais vizbulis*	Unsp	Tea	O. ad.	General and unspecified	Tuberculosis	1		
	Consolida regalis Gray	*tīruma zilausis*	Unsp	Tea	O. ad.	Digestive	Sharp, throbbing abdominal pain	2		× (flower)
	Ranunculus acris L.	*kodīgā gundega*	Flowers, leaves	Juice, raw material	Ext.	Digestive	Toothache	2		
						Skin	Freckles	1		
							Scabies	1		
							Sunburn	1		
							Ulcer	1		
							Warts	2		
	Thalictrum L.	*saulkrēsliņš*	Unsp	Juice, tea	Ext., O. ad.	Digestive	Generalized abdominal pain	1		
							Worms	1		
						Skin	Dandruff	1		
							Rash on head	1		
	Trollius europaeus L.	*Eiropas saulpurene*	Flowers	Decoction	O. ad.	Respiratory	Cough	1		
Resedaceae	*Reseda* L.	*rezēda*	Unsp	Unsp	Unsp	Skin	Rash	1		
Rhamnaceae	*Frangula alnus* Mill.	*trauslais krūklis*	Bark, fruits	Decoction, raw material, tea	Ext., O. ad.	Digestive	Constipation	2	F: EMEA/HMPC/76307/2006 Corrigendum (bark) Constipation	× (bark)
							Flatulence	1		
							Generalized abdominal pain	1		
						Musculoskeletal	Rheumatism	1		
						Respiratory	Cough	3		
							Runny nose	1		

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
						Skin	Scabies	3		
	Rhamnus L.	*pabērzs*	Bark	Powder	O. ad.	Respiratory	Cough	3		× (fruit of *R. chatarticus*)
Rosaceae	*Alchemilla* L.	*rasaskrēsliņš*	Aerial parts, roots	Tea	O. ad.	Respiratory	Cough	4		× (aerial part of *A. vulgaris*)
	Prunus avium (L.) L.	*saldais ķirsis*	Bark, flowers, twigs	Decoction, tea	O. ad.	Female genital system and breast	Excessive menstrual bleeding	2		
						Neurological	Headache	1		
						Respiratory	Cough	1		
	Comarum palustre L.	*purva vārnkāja*	Unsp	Raw material, tea	Ext., O. ad.	General and unspecified	Sweating problem	1		
						Musculoskeletal	Bone pain	2		
							Sprain/strain of joint	1		
						Neurological	Headache	1		
						Respiratory	Cough	1		
	Crataegus L.	*vilkābele*	Unsp	Tea	Unsp	Skin	Animal bite	1	F: EMA/HMPC/159075/2014 (leaf and flower) Nervous cardiac complaints (palpitations, perceived exstra heart beat due to mild anxiety)# Mental stress and sleep disorders#	× (leaf with flower, fruit)
	Crataegus laevigata (Poir.) DC.	*divirbuļu vilkābele*	Bark	Tea	O. ad.	Respiratory	Cough	1		
	Filipendula ulmaria (L.) Maxim.	*parastā vīgrieze*	Flowers, roots	Bath, decoction, juice, powder, raw material, tea	Ext., O. ad.	Digestive	Diarrhoea	2	F: EMA/HMPC/434894/2010 (flower) Common cold Articular pain	× (flower)
							Generalized abdominal pain	3		
							Toothache	2		
						Eye	Eye problems	1		
						Musculoskeletal	Bone pain	1		
						Psychological	Feeling anxious/ nervous/ tense	1		
						Respiratory	Cough	1		
						Skin	Erysipelas	1		
							Rabies	4		
							Snake bite	2		
	Fragaria vesca L.	*meža zemene*	Flowers, fruits	Decoction, raw material, tea	Ext., O. ad.	Neurological	Headache	1	F: EMA/HMPC/432278/2015 (leaf)	× (leaf)
						Respiratory	Chest pain	1		
							Cough	2		

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
						Skin	Warts	1	Adjuvant in minor urinary complaints# Diarrhoea#	
	Malus Mill.	*ābele*	Flowers, fruit bark, fruits, leaves, wood	Wood tar, juice, raw material, tea	Ext., O. ad.	Digestive	Generalized abdominal pain	1		
						Eye	Eye pain	1		
						Neurological	Headache	1		
						Musculoskeletal	Rheumatism	1		
						Respiratory	Respiratory problems	4		
						Skin	Athlete's foot	8		
							Warts	8		
	Padus avium Mill.	*parastā ieva*	Bark, flowers, fruits, leaves, twigs	Decoction, raw material, steamed material, tea, tincture	Ext., O. ad.	Circulatory	Localized swelling/lump	1		
						Digestive	Constipation	1		
							Diarrhoea	4		
							Generalized abdominal pain	1		
							Toothache	9		
						Ear	Ear problems	2		
						General and unspecified	Fever	1		
							Swelling	5		
						Musculoskeletal	Bone pain	1		
						Neurological	Headache	4		
						Respiratory	Cough	1		
							Sore throat	1		
						Skin	Bruise/ contusion	2		
							Dry skin	1		
							Erysipelas	10		
							Freckles	1		
	Potentilla L.	*retējs*	Roots	Tea, tincture	Ext., O. ad.	Digestive	Dysentery	1	C: EMA/HMPC/5513/2010 (rhizome) Diarrhoea Inflammations of the oral mucosa	
							Generalized abdominal pain	5		
							Hernia	1		
							Sharp, throbbing abdominal pain	1		
							Toothache	1		

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
						Endocrine, metabolic and nutritional	Overeating	1		
						Respiratory	Respiratory problems	2		
	Potentilla anserina L.	*maura retējs*	Unsp	Tea	O. ad.	Digestive	Sharp, throbbing abdominal pain	1		× (aerial part)
	Potentilla erecta (L.) Raeusch.	*stāvais retējs*	Unsp	Unsp	Unsp	Unsp	Unsp	1	C: EMA/HMPC/5513/2010 (rhizome) Diarrhoea# Inflammations of the oral mucosa#	× (root)
	Prunus L.	*plūme*	Flowers, resin	Raw material	O. ad.	Neurological	Headache	1		× (flower of *P. spinosa*)
						Respiratory	Acute upper respiratory infection	1		
	Pyrus L.	*bumbiere*	Flowers	Unsp	Unsp	Neurological	Headache	1		
	Rosa L.	*roze*	Leaves	Decoction, raw material, tea	Ext., O. ad.	Circulatory	Cardiovascular problems	1	F: EMA/HMPC/137299/2013 (flower) Skin inflammation Inflammations of the oral and pharyngeal mucosa#	× (pseudo-fruit, "seeds")
						Eye	Eye pain	1		
						General and unspecified	Fever	1		
						Skin	Erysipelas	1		
	Rubus caesius L.	*zilganā kazene*	Bark, roots	Decoction, tea, with cream	Ext., O. ad.	Digestive	Diarrhoea	1		× (leaf)
						Female genital system and breast	Menstrual problems	2		
						Respiratory	Cough	2		
						Skin	Scabies	1		
	Rubus chamaemorus L.	*lācene*	Unsp	Tea	O. ad.	Respiratory	Cough	2		
	Rubus idaeus L.	*meža avene*	Flowers, fruits, leaves, stem, stem with leaves and fruits	Jam, juice, tea	Ext., O. ad., Smoking	General and unspecified	Fever	5	F: EMA/HMPC/44211/2012 (leaf) Minor inflammations in the mouth or the throat Spasms associated with menstrual periods# Diarrhoea#	× (leaf)
							Sweating problem	1		
						Neurological	Headache	1		
						Respiratory	Chest pain	2		
							Cough	5		
							Runny nose	1		
							Acute upper respiratory infection	7		
	Rubus saxatilis L.	*klinšu kaulene*	Stem	Decoction	O. ad.	Musculoskeletal	Back pain	1		
	Sorbus aucuparia L.	*parastais pīlādzis*	Bark, buds, flowers,	Jam, raw material, tea	Ext., O. ad.	Digestive	Constipation	2		
							Toothache	1		

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
			fruits, wood			Endocrine, metabolic and nutritional	Loss of appetite	1		
						General and unspecified	Swelling	1		
						Musculoskeletal	Rheumatism	1		
						Neurological	Headache	1		
						Psychological	Feeling anxious/ nervous/ tense	1		
						Respiratory	Cough	2		
Rubiaceae	*Galium odoratum* (L.) Scop.	*smaržīgā madara*	Unsp	Tea, tincture	O. ad.	Digestive	Diarrhoea	1		× (aerial part of *G. verum*)
							Generalized abdominal pain	1		
	Carapichea ipecacuanha (Brot.) L.Andersson	*ipekakuāna*	Roots	Unsp	O. ad.	Digestive	Diarrhoea	1		× (root)
Salicaceae	*Populus* L.	*apse*	Bark, buds, shoots, stem	Ashes, decoction, tea, tincture	Ext., O. ad.	Circulatory	Heart pain	1		
						Digestive	Generalized abdominal pain	2		
							Toothache	1		
						General and unspecified	Fever	1		
							Tuberculosis	1		
						Musculoskeletal	Back pain	1		
						Respiratory	Respiratory problems	2		
	Salix caprea L.	*blīgzna*	Bark, twigs	Raw material, tea	Ext., O. ad.	Digestive	Toothache	1		
						Skin	Bruise/ contusion	1		
	Salix L.	*kārkls*	Bark, leaves, sapwood	Bath, decoction, raw material, steamed material, tea	Ext., O. ad.	Digestive	Diarrhoea	1	F: EMA/HMPC/80 630/2016 – Corr (bark) Articular pain Fever associated with common cold# Headache#	× (bark)
							Generalized abdominal pain	1		
						Eye	Eye pain	1		
						Musculoskeletal	Bone pain	1		
							Musculoskeletal problems	1		
						Respiratory	Chest pain	1		
						Skin	Sunburn	1		
	Salix viminalis L.	*klūdziņu kārkls*	Unsp	Tea	O. ad.	Respiratory	Chest pain	1		
Sapindaceae	*Acer* L.	*kļava*	Leaves, wood	Charcoal, raw material	Ext.	Neurological	Headache	2		
						Skin	Warts	1		
	Aesculus hippocastanum L.	*parastā zirgkastaņa*	Flowers, seeds	Baked, decoction, tea, tincture	Ext., O. ad., Smoking	Digestive	Hernia	1	F: EMEA/HMPC/2 25319/2008 (seed) Chronic venous insufficiency#	× (bark, leaf, seed)
							Toothache	1		
						Female genital system and breast	Female genital candidiasis	1		
							Bone pain	2		

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
						Musculoskeletal system disorders	Rheumatism	6	Venous circulatory disturbances# Bruises of oedema and haematoma# F: EMA/HMPC/354156/2011 (bark) Venous circulatory disturbances# Itching and burning associated with haemorrhoids#	
						Respiratory	Chest pain	1		
							Cough	1		
							Runny nose	1		
Saxifragaceae	*Chrysosplenium alternifolium* L.	*pamišlapu pakrēslīte*	Unsp	With milk	O. ad.	Digestive	Generalized abdominal pain	1		
Scrophulariaceae	*Euphrasia rostkoviana* Hayne	*rostkova žibulītis*	Unsp	Unsp	Unsp	Eye	Eye problems	1		× (aerial part)
	Gratiola officinalis L.	*ārstniecības rūgtene*	Flowers	Tea	O. ad.	Digestive	Colic	1		
	Linaria Mill.	*vīrcele*	Unsp	Tea	O. ad.	Psychological	Nightmares	1		
	Linaria vulgaris Mill.	*parastā vīrcele*	Unsp	Unsp	Ext.	Skin	Lichen	1		
	Pedicularis L.	*jāņeglīte*	Aerial parts	Raw material	Ext.	Skin	Lice	1		
	Verbascum thapsus L.	*deviņvīruspēks*	Flowers, leaves	Decoction, powder, tea, tincture	Ext., O. ad.	Digestive	Generalized abdominal pain	3	F: EMA/HMPC/611537/2016 (flower) Sore throat associated with dry cough and cold	× (flower of *V. densiflorum*)
							Hernia	5		
						Female genital system and breast	Menstrual problems	1		
						General and unspecified	Fever	2		
						Male genital system	Male genital problems	1		
						Musculoskeletal	Rheumatism	1		
						Respiratory	Cough	4		
							Throat problems	2		
						Skin	Dandruff	1		
							Erysipelas	1		
							Hair loss	1		
						Urology	Incontinence urine	2		
	Veronica beccabunga L.	*avota veronika*	Leaves	Juice, raw material	Ext., O. ad.	Digestive	Hernia	1		
						General and unspecified	Swelling	1		
	Veronica officinalis L.	*zemteka*	Unsp	Unsp	Unsp	Musculoskeletal system disorders	Bone pain	1		× (aerial part)

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
	Veronica L.	*veronika*	Unsp	Tea	O. ad.	Digestive	Generalized abdominal pain	1		
Solanaceae	*Atropa belladonna* L.	*melnā velnoga*	Unsp	Unsp	Unsp	Neurological	Headache	1		
	Datura stramonium L.	*parastais velnābols*	Seeds	Raw material, seeds	O. ad.	Digestive	Sharp, throbbing abdominal pain	5		
						Neurological	Headache	1		
	Hyoscyamus niger L.	*driģene*	Leaves, seeds	Raw material	Ext.	Digestive	Caries	1		
							Toothache	4		
							Sharp, throbbing abdominal pain	2		
						Ear	Ear problems	1		
						General and unspecified	Fever	1		
						Musculoskeletal	Bone pain	1		
						Neurological	Headache	1		
						Pregnancy, childbirth, family planning	Complicated labour/delivery livebirth	1		
						Skin	Cuts and wounds	1		
							Rabies	2		
	Nicotiana L.	*tabaka*	Leaves	Ashes, decoction, powder, raw material, tincture	Ext., O. ad., Smoking	Blood, blood forming organs, lymphatics, spleen	Spleen problems	1		
						Digestive	Generalized abdominal pain	2		
							Toothache	15		
							Worms	2		
						Ear	Earache	1		
						Eye	Eye pain	2		
						General and unspecified	Swelling	1		
						Neurological	Headache	1		
						Psychological	Chronic alcohol abuse	2		
						Respiratory	Sore throat	1		
							Runny nose	1		
						Skin	Athlete's foot	2		
							Boils	3		
							Corn/callosity	1		
							Cuts and wounds	4		
							Dandruff	1		
							Erysipelas	1		
							Lice	2		
							Lichen	6		

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
							Scabies	2		
							Snake bite	1		
	Solanum dulcamara L.	*bebrukārkliņš*	Fruits, stem	Decoction, raw material, tea	Ext., O. ad., Smoking	Digestive	Hernia	1	F: EMA/HMPC/734361/2011 (stem) Recurrent eczema#	
							Tooth extraction	2		
							Toothache	2		
						Female genital system and breast	Menstrual problems	1		
						Psychological	Nightmares	1		
						Respiratory	Acute upper respiratory infection	1		
							Cough	17		
	Solanum americanum Mill.	*melnā naktene*	Fruits	Bath, with milk	Ext., O. ad.	Psychological	Sleep disturbance	3		
	Solanum tuberosum L.	*kartupelis*	Flowers, leaves, tuber	Potato water, raw material, tea	Ext., O. ad.	Endocrine, metabolic and nutritional	Loss of appetite	3		
						General and unspecified	Fever	1		
							Frostbite	1		
						Neurological	Headache	2		
						Skin	Athlete's foot	1		
							Burns	1		
							Lice	1		
							Lichen	1		
							Warts	2		
Thymelaeaceae	*Daphne mezereum* L.	*parastā zalktene*	Fruits, twigs	Bath, raw material	Ext.	Digestive	Tooth extraction	3		
							Toothache	8		
						Skin	Itchy skin	1		
Urticaceae	*Urtica* L.	*nātre*	Aerial parts, roots, stem	Bath, decoction, juice, raw material, tea, tincture	Ext., O. ad., Sauna whisk, Smoking	Digestive	Generalized abdominal pain	1	F: EMA/HMPC/508015/2007 (leaf) Articular pain Adjuvant in minor urinary complaints F: EMEA/HMPC/170261/2006 (herb) Articular pain Seborrhoeic skin conditions Adjuvant in minor urinary complaints F: EMA/HMPC/461160/2008 (root) Lower urinary tract symptoms related to	× (aerial part/leaf, fruit, root of *U. dioica*)
							Sharp, throbbing abdominal pain	1		
							Hernia	1		
							Toothache	2		
						Female genital system and breast	Female genital candidiasis	1		
						General and unspecified	Swelling	1		
						Musculoskeletal	Rheumatism	5		
						Respiratory	Chest pain	2		
							Cough	3		
							Respiratory problems	1		
						Skin	Burns	1		
							Itchy skin	1		
	Urtica dioica L.	*lielā nātre*	Roots	Decoction	O. ad.	General and unspecified	Swelling	1		

Family	Scientific plant name	Local plant name	Part used	Herbal preparation	Administration	Disorder	Therapeutic uses	No. of citations	Assessment by the EMA*	Wichtl (2004)**
	Urtica urens L.	*sīkā nātre*	Aerial parts, roots	Decoction, juice, raw material	Ext., O. ad., Sauna whisk	Blood, blood forming organs, lymphatics, spleen	Blood problems	1	benign prostatic hyperplasia	
						Musculoskeletal	Bone pain	2		
							Leg pain	1		
							Rheumatism	2		
						Neurological	Headache	2		
						Respiratory	Cough	1		
						Skin	Abscess	2		
							Dandruff	1		
							Itchy skin	1		
							Warts	1		
						Urology	Urinary retention	1		
Verbenaceae	*Verbena officinalis* L.	*ārstniecības verbēna*	Unsp	Raw material	Ext.	Skin	Cuts and wounds	1		× (aerial part)
Viburnaceae	*Sambucus nigra* L.	*melnais plūškoks*	Flowers, fruits, leaves, twigs with leaves	Decoction, tea	Ext., O. ad.	General and unspecified	Fever	4	F: EMA/HMPC/61 1512/2016 (flower) Cough and cold	× (flower, fruit)
							Tuberculosis	1		
						Musculoskeletal	Rheumatism	1		
						Respiratory	Cough	1		
							Chest pain	1		
							Acute upper respiratory infection	1		
						Skin	Cuts and wounds	1		
	Viburnum opulus L.	*parastā irbene*	Fruits	Decoction	O. ad.	General and unspecified	Swelling	1		× (bark of *V. prunifolium*)
Violaceae	*Viola* L.	*vijolīte*	Roots	Powder, tea, with milk	Ext., O. ad.	Circulatory	Cardiovascular problems	1	F: EMA/HMPC/13 1734/2009 (herb with flower) Seborrhoeic skin conditions#	× (flowering aerial parts of *V. tricolor*)
						Digestive	Colic	1		
							Diarrhoea	1		
							Generalized abdominal pain	1		
						Female genital system and breast	Excessive menstrual bleeding	1		
						Musculoskeletal	Cramps	1		
						Neurological	Headache	1		
						Pregnancy, childbirth, family planning	Complicated labour/delivery livebirth	1		
						Respiratory	Chest pain	1		
							Cough	3		
							Respiratory complaint	1		
							Acute upper respiratory infection	1		
	Viola arvensis Murray	*tīruma vijolīte*	Unsp	Tea	O. ad.	Respiratory	Sore throat	1		
	Viola tricolor L.	*trejkrāsu vijolīte*	Unsp	Decoction, tea	O. ad.	Musculoskeletal	Cramps	1		
						Respiratory	Cough	1		
						Skin	Skin problems	1		
Zingiberaceae	*Zingiber officinale* Roscoe	*ingvers*	Rhizome	Tea	O. ad.	Digestive	Generalized abdominal pain	1	F: EMA/HMPC/74 9154/2010 Motion sickness# Gastrointestinal disorders (bloating and flatulence)	× (rhizome)