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Rīga Stradiņš University

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

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

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

Riga, 2021

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Abbreviations

ALF	Archives of Latvian Folklore
ANOVA	analysis of variance
BMDMs	bone marrow-derived macrophages
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
FBS	fetal bovine serum
GAE	gallic acid equivalent
GC-MS	gas chromatography-mass spectrometry
HPLC	high-performance liquid chromatography
IC_{50}	half maximal inhibitory concentration
IFN-γ	interferon gamma
IL	interleukin
Inos	inducible nitric oxide synthase
LC-MS	liquid chromatography-mass spectrometry
LDH	lactate dehydrogenase
LPS	lipopolysaccharide
M1	classically activated (pro-inflammatory) macrophages
M2	alternatively activated (anti-inflammatory) macrophages
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
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
TNF-α	tumor necrosis factor alpha

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. 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 Materials and methods

1.1 Folklore material research and analysis

1.1.1 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). Some records were collected from the four published volumes titled "Latvian Folk Beliefs" (Šmits, 1940-1941) compiled by the folklore researcher P. Šmits.

For each plant identified in the records of Latvian folk medicine, taxonomic ranks of family, genus, and species were recorded.

1.1.2 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. The number of citations was indicated for each use of medicinal plant.

1.1.3 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.

1.1.4 Folkloristic data comparison with official herbal monographs

Folkloristic data presented in the study were compared with the Russian Pharmacopoeia (1891) and 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). Medicinal plants from the records of Latvian folk medicine were also compared to the handbook for practice on a scientific basis (Wichtl, 2004).

1.2 Plant material and extract preparation

1.2.1 *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, PSRE was dissolved in 50% methanol (1:50 w/v), the solution was centrifuged at 2000 x g for 20 min

and filtered. The solution was purified by gel adsorption over Sephadex LH-20. The proanthocyanidins were released from the gel with 70% aqueous acetone and concentrated under vacuum at 35 °C. Afterwards, the aqueous aliquot was freeze-dried. *In vitro* experiments were carried out using PSRE and PCAN extracts dissolved in distilled water.

1.2.2 *Prunus padus* L. flower extract

The fully opened inflorescences of *P. padus* were collected. Flowers were air-dried in the shade at room temperature. Dry *P. padus* flowers were homogenized using a mortar and pestle. Obtained powder was macerated with 70% ethanol solution in water (1:10 w/v) at room temperature in the dark for 7 days. Afterwards, the extract was filtered through a celite pad to remove all insoluble material. The filtrate was concentrated with a rotary evaporator, which was followed by lyophilization. *In vitro* experiments with *Prunus padus* L. flower extract (PPFE) were carried out using lyophilized plant material dissolved in distilled water.

1.3 Cell culture

1.3.1 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).

1.3.2 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.

1.4 *Ex vivo* and *in vitro* methods

1.4.1 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).

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.

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 (Hidex, Turku, Finland).

1.4.2 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.

1.4.3 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 staining, the expression of markers was analysed by flow cytometry.

1.4.4 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.

1.4.5 mRNA isolation and quantitative PCR analysis

Total RNA from cells was isolated using PureLink™ 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™, 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™, 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.

1.4.6 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.

1.5 Qualitative and quantitative analysis

1.5.1 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. A 1 µl injection (split 1:50) was used for GC-MS analysis.

1.5.2 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 (1.2.2). LC-MS analysis was performed on the ethanolic extract and lyophilized sample dissolved in ethanol at a concentration of 1 mg/ml. Experiments were

conducted on a Shimadzu LC/MS-IT-TOF system (Agilent Technologies, Santa Clara, CA, USA).

1.6 Determination of the total phenolic content

The total phenolic content in PPFE was determined using the Folin–Ciocalteu colorimetric method. 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 alibration curve. Total phenolic content was expressed as mg of gallic acid equivalent (GAE) per g of lyophilized extract.

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

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%).

1.8 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.

1.9 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.

2 Results

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

2.1.1 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. In total, 211 genera belonging to 71 families were mentioned. 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 2.1.

Plant taxa that were reported more than 20 times in the folklore materials are shown in Figure 2.2. However, at the same time, a large number of taxa (135) were mentioned in only 1–2 records.

2.1.2 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 (Table 2.1).

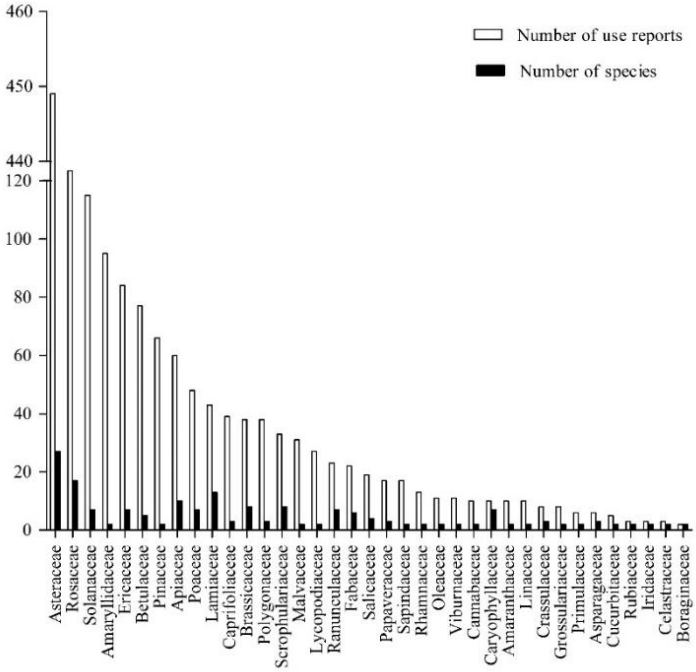

Figure 2.1 **Number of use reports (white bars) and number of medicinal plant species (black bars) in each plant family that contains more than two plant species**

2.1.3 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. 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, 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. described as treating diarrhoea.

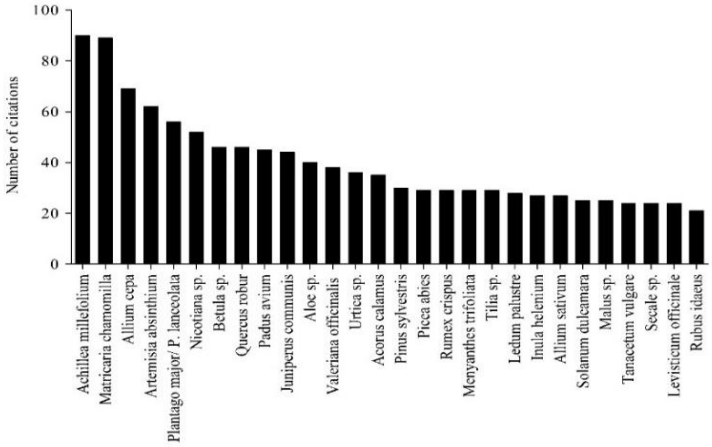

Figure 2.2 **Plant taxa that were reported more than 20 times in records of Latvian folk medicine**

Table 2.1

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

Table 2.1 continued

Medicinal use category	No. of use reports	No. of medicinal plant taxa
General and unspecified disorders	201	72
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. 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. In the category of skin disorders, cuts and wounds, boils, athlete's foot, and scabies were dominant. To treat cuts and wounds, the most frequently used taxa were *Plantago* sp. L., *A. millefolium* and *Aloe* sp. 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. 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., *Aloe* sp. and *A. millefolium*. Three of 9 disorders in the category of musculoskeletal system 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.

2.1.4 Plant parts used

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%). However, a large number of records (29%) did not contain information about a specific plant part used.

2.1.5 Forms of herbal medicine and routes of administration

Thirty percent of all herbal medicines mentioned were used as a tea. Tea 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. 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. According to the data presented, both internal and external routes of administration were widespread (51% and 37%, respectively). In this study, 41% of plant taxa were reported to be consumed only internally, and 13% only externally.

2.1.6 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. 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.

Only 59 out of 211 plant taxa mentioned in this study are included in the official monographs of the EMA. 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*. 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.

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

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

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 2.4). Note that neither PSRE nor PACN were toxic to the cells at the concentrations applied as revealed by metabolic viability analysis (Figure 2.3).

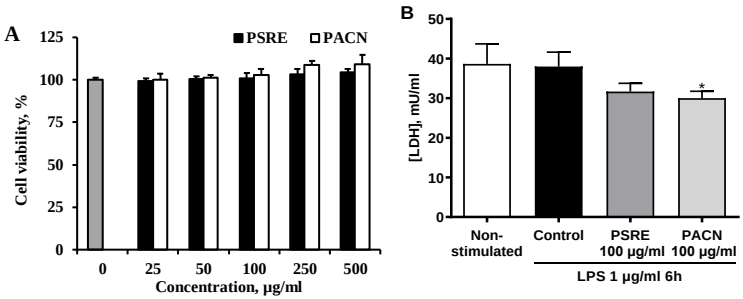

Figure 2.3 **Effects of *Pelargonium sidoides* root extract (PSRE) and proanthocyanidins from PSRE (PACN) on human peripheral blood mononuclear cell viability**

(**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.

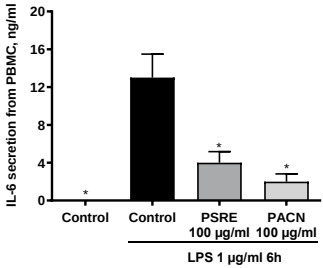

Figure 2.4 **The effect of *Pelargonium sidoides* root extract (PSRE) and proanthocyanidins from PSRE (PACN) on interleukin-6 (IL-6) secretion from peripheral blood mononuclear cells after LPS treatment**

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.

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

Both preparations at a dose of 100 µg/ml were not toxic (Figure 2.5) and significantly suppressed the mRNA transcription of IL-1β and iNOS in primary murine bone marrow-derived macrophages (Figure 2.6 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 2.6 C).

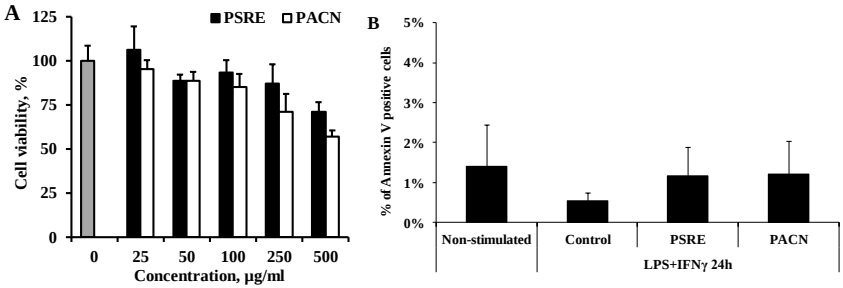

Figure 2.5 **Effects of *Pelargonium sidoides* root extract (PSRE) and proanthocyanidins from PSRE (PACN) on bone-marrow derived macrophage (BMDM) cell viability**

(**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.

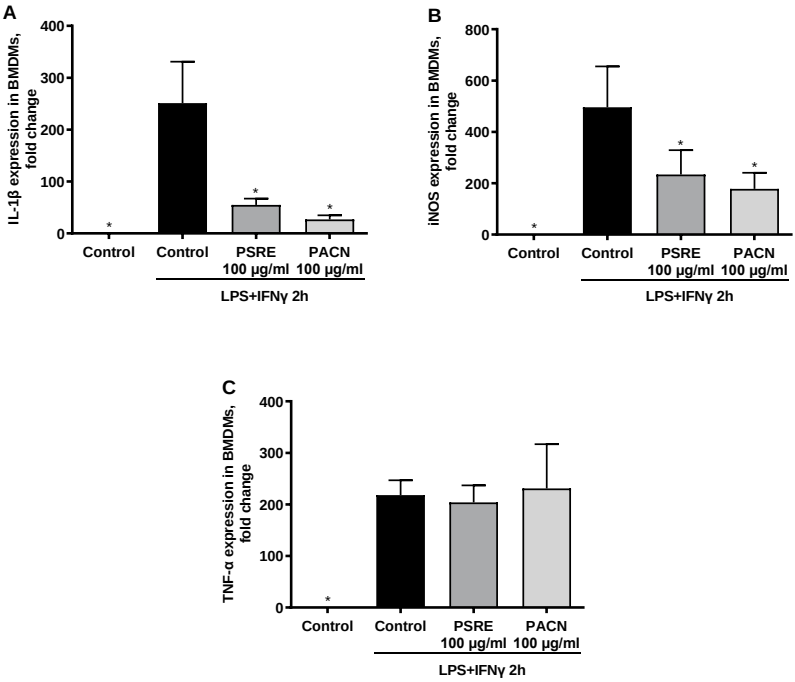

Figure 2.6 **The effect of *Pelargonium sidoides* root extract (PSRE) and proanthocyanidins from PSRE (PACN) on pro-inflammatory gene expression in bone marrow-derived macrophages after LPS and IFN- γ stimulation**

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 2.7). PSRE and PACN at a concentration of 100 µg/ml significantly suppressed mRNA transcription of COX-2 and IL-1β (Figure 2.7 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 2.7 B).

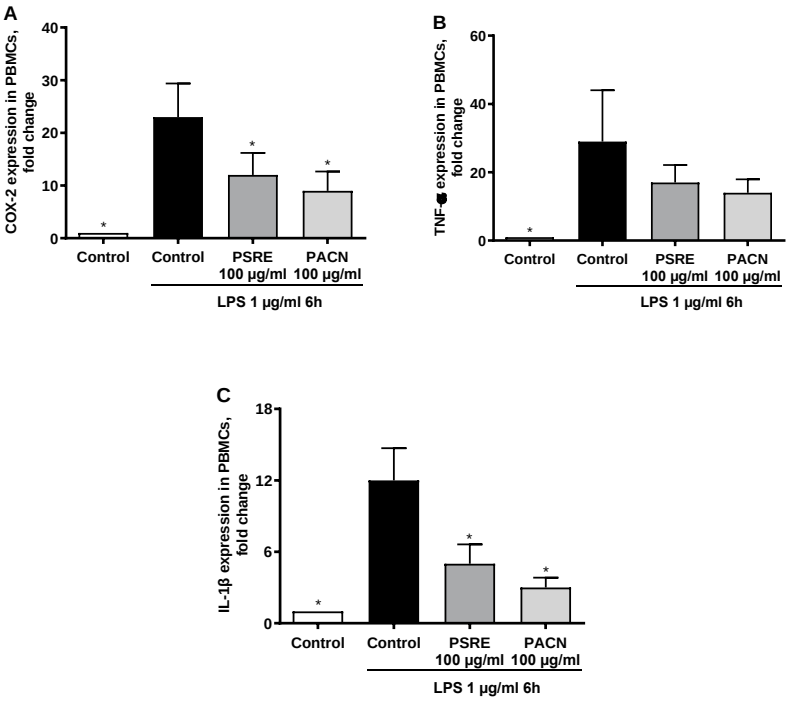

Figure 2.7 **The effect of *Pelargonium sidoides* root extract (PSRE) and proanthocyanidins from PSRE (PACN) on pro-inflammatory gene expression in peripheral blood mononuclear cells after LPS stimulation**

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.

2.2.3 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 2.8). 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.

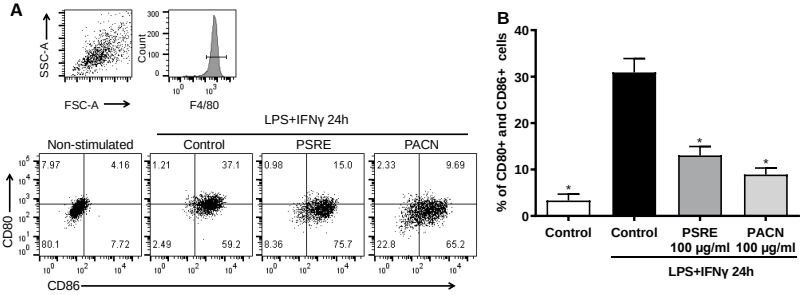

Figure 2.8 **Expression of pro-inflammatory cell surface markers CD80 and CD86 analysed by flow cytometry 24 h after treating LPS + IFN-γ-activated BMDMs with PSRE and PACN**

(**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.

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

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

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. A detailed list of the phytochemical components detected by GC-MS in *P. padus* is shown in Table 2.2.

Table 2.2

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.

2.3.2 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 high-resolution mass spectrometry characteristics and identification data for the 10 major peaks in the MS chromatograms of PPFE are shown in Table 2.3.

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 2.3

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)

Table 2.3 continued

	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				
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 chromatographic profiles of both analysed samples show three to four intense peaks in the retention time interval from 3 to 9 min (Table 2.4), constituting 65–75% of the total peak area.

Table 2.4

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 2.3
	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

2.3.3 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 2.5.

Table 2.5

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%.

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

The results of the collagenase inhibition assay are shown in Figure 2.9 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.

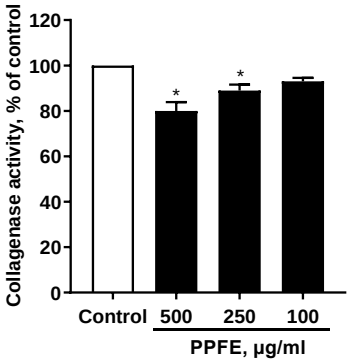

Figure 2.9 **Inhibitory effects of *Prunus padus* flower extract (PPFE) on collagenase activity**

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 ($p < 0.05$).

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

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 2.10). 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 2.11).

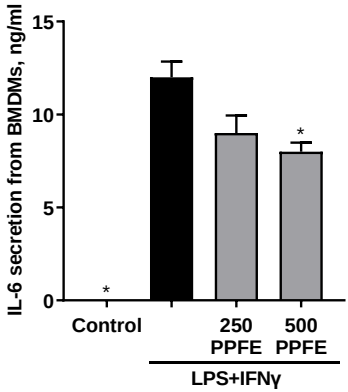

Figure 2.10 **The concentration of IL-6 secreted by BMDMs in cell media was estimated by ELISA**

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 ($p < 0.05$).

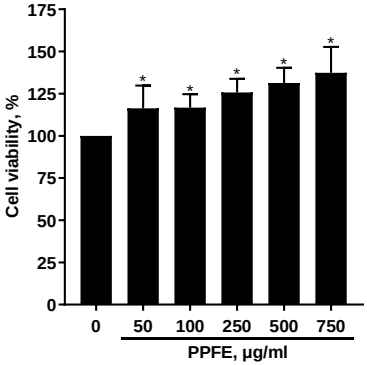

Figure 2.11 **Effects of *Prunus padus* flower extract (PPFE) on bone marrow-derived macrophage viability measured by MTT assay**

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, $p<0.05$).

2.3.6 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 2.12 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 2.12 C). The results of the study indicate that PPFE had an effect on both M1 and M2 macrophage polarization.

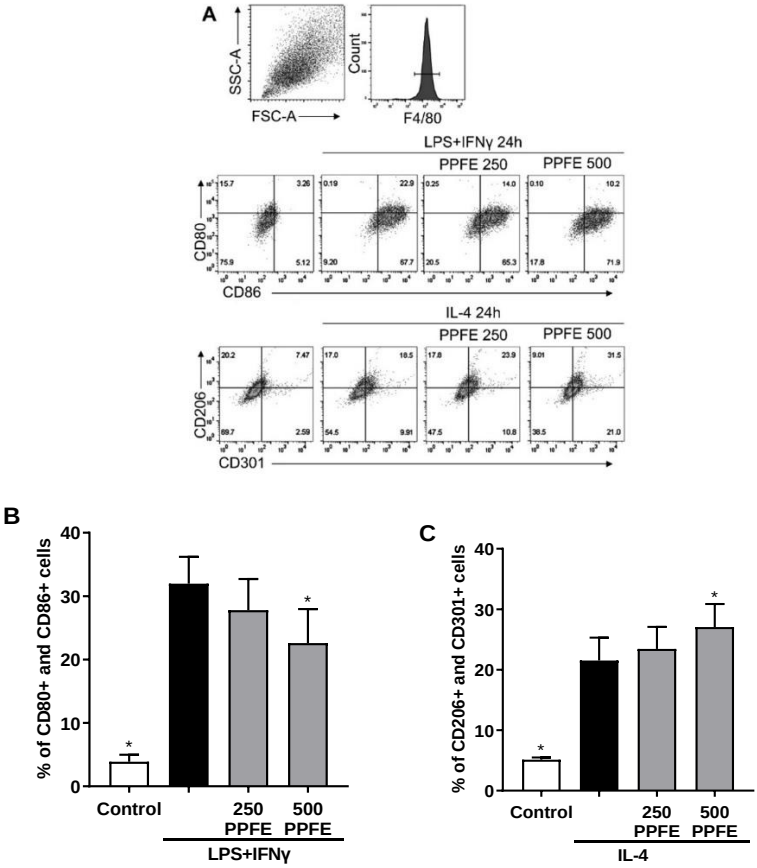

Figure 2.12 **Flow cytometry analysis of BMDM polarization toward the M1 and M2 phenotypes**

(**A**) Upper right quadrant: F4/80-positive cells were gated for double CD80 and CD86 analysis and for double CD206 and CD301 analysis. 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. 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 ($p < 0.05$).

3 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.

3.1 Differences between the traditional and modern uses of medicinal plants

Local people in the territory of Latvia 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.

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).

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. 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.

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.

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 is a frequently used indoor house plant that is effective in the treatment of respiratory and skin disorders. 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.

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. 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.

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).

3.2 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. 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. More ethnobotanical, phytochemical, and pharmacological studies are needed in order to promote and facilitate the therapeutic approval of these species as traditional herbal medicines.

3.3 Analysis of *Pelargonium*

3.3.1 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.

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).

3.3.2 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. 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*.

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.

3.4 Analysis of *Prunus padus*

3.4.1 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.

3.4.2 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 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.

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.

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) (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. In summary, these findings suggest the potential use of PPFE as a source of natural anti-inflammatory agents.

Conclusions

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.

Aprobation 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].
2. **Sīle, I.**, Romāne, E., Šubins, K., Grīnberga, S., Makarova, E., Dambrova, M. 2018. Parastās ievas ziedu un augļu ķīmiskā sastāva analīze un drogu izmantošana latviešu tautas ticējumos, *RSU 2018. gada zinātniskā konference*, Sekc. "Darba un vides veselība, arodslimības, farmācija" [Rīga, Latvija, 22.–23. marts, 2018]: Tēzes, 199. lpp.
3. **Sīle, I**., Reinsone, S., Romāne, E., Dambrova, M. 2017. Ārstniecības augi latviešu tautas ticējumos. *RSU 2017. gada zinātniskā konference*, Sekc. "Darba un vides veselība, arodslimības, farmācija" [Rīga, Latvija, 6.–7. aprīlis, 2017]: Tēzes, 234. lpp.

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