Bearberry (*Arctostaphylos uva-ursi (*L.) Spreng.): chemical content and pharmacological activity.

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ABSTRACT

Although many plants have been known to man for centuries scientifically obtained data about their chemical content and pharmacological activity are often still lacking. Through the ages errors about plants used in different countries officially as medicinal plants have been introduced and when included in complex pharmaceutical formulations may become unsafe. One of these medicinal plants is the bearberry (*Arctostaphylos uva-ursi Spreng.* from the family *Ericaceae)*. The leaves of this plant and in rare cases the stems are used for the treatment of urinary tract diseases. The aim of this review was to examine available data about main medicinal usage of the bearberry leaves as a source of biological active substances from different groups. Another important task is connected with unification of methods for quality control of bearberry leaves. The botanical classification of *Arctostaphylos uva-ursi Spreng.* and its subspecies also require further study. At present, all subspecies of *Arctostaphylos uva-ursi Spreng.* refer only to this species. Therefore analyzing the plant material quantitatively and qualitatively has been difficult.

KEY WORDS: *Arctostaphylos uva-ursi*, biological active substances, medicinal use, ethnomedicine, bearberry

INTRODUCTION

According to The Plant List there are 75 species of the genus *Arctostaphylos* in the world and 113 synonyms (1). In different countries, this plant is known as bearberry (English), busserole (French), Bärentraube (German), oreja de oso (Spanish), uva ursina (Italian).

According to A.L. Takhtajan's "System and Phylogeny of the Flowering Plants" the genus *Arctostaphylos* refers to phylum: Magnoliophyta, Class: Magnoliopsida (Dicotyledons), Subclass: Magnoliidae, Order: Ericales; Fmily: Ericaceae, Subfamily: Arbutoideae. The subfamily includes 5 genera: *Arbutus, Comarostaphylos, Ornithostaphylos, Arctostaphylos* (including *Xylococcus)* and *Arctous*. Several species traditionally included in the

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Arctostaphylos genus are now included in the *Arctous* genus: *Arctostaphylos alpine* is now called *Arctous alpina* (L.) Nied.; *Arctostaphylos rubra* (Rehder & E.H.Wilson) Fernald now is *Arctous rubra* (Rehder & E.H.Wilson) Nakai. (2).

S.K. Czerepanov's "Plantae vasculares Rossicae et Civitatum collimitanearu" registers two species in Russia for the genus *Arctostaphylos* Adans. i.e., *A. caucasica* Lipsch. and *A. uva-ursi* (L.) Spreng. (3).

Arctostaphylos caucasica Lipschitz is the relict of an endemic species in the Greater Caucasus region. The status of this species has not yet been determined, some researches consider this the same as *Arctostaphylos uva-ursi* Spreng., others regard it as a subspecies of *Arctostaphylos uva-ursi* Spreng. subsp. *caucasica* Kvaratzchelia (nomen nudum) = *A. uva-ursi* (L.) Spreng. subsp. *caucasica* (Lipsch) A. Schreter, and other researchers consider it an independent species (4).

The latest update of the APG IV considers the clades in all those taxons to have unique relationship with each other. *Arctostaphylos uva-ursi* Spreng. belongs to the family Ericaceae, clade Asterids, order Ericales, subfamily Arbutoideae, genus *Arctostaphylos* (5).

Bearberry is an evergreen prostrate shrub. Young non-winter stems are green or green-brown color, second-year winter stems are yellow-brown color, third-year stems are olive color and perennial old stems have many-layers of dark-brown, easily crumbling corked tissues. Leaves are alternate, thick, coriaceous, with entire edge and, have a linear-obovate shape, 1.0-2.2 cm in length and 0.5-1.2 cm in width. Leaves have a short petiole, their surface is dark green, lighter below and with observable veins, shiny, and the venation is pinnate. Young leaves are paler, thinner, with hairiness edge. The life span of the leaf is 2 years, by the end of the third year it completely dies off. Flowers are ovate-pitchers, pale pink, 3-5 mm in length, with 5-toothed fused petal. Flowers occur in a drooping apical racemose inflorescence (3-5 flowers per inflorescence). The fruit is a red flattened mealy drupe with 5 seeds (6).

Arctostaphylos caucasica Lipsch. is an evergreen shrub which grows in the mountains of West Transcaucasia and North Caucasia. *A. caucasica* grows in the forest and subalpine belt, in the pine forests, on limestone, and dry gravelly slopes. This species has several features such as large and broad leaves with a mucronulate apex and slightly thickened edge, the first-year stems have fewer leaves. These features should not be used to determine which species it is because they do not belong to generative parts of plant and may be variable. Bearberry with large leaves occurs in the Alps and the Aleutian Islands. Therefore *Arctostaphylos caucasica* may be only a subspecies of *A. uva-ursi* (L.) Spreng. subsp. *caucasica* (Lipsch) A. Schreter and used as this typical species (4).

Ethnomedicinal use

The main therapeutic effects of bearberry leaves and stems are diuretic and antiseptic. Leaves of *Arctostaphylos uva-ursi* and *A. caucasica* also provide antihelmintic, astringent, sedative, hemostatic, tonic, and metabolic effects.

Bearberry raw material has been used to treat the following diseases:

- Kidney and urinary tract, the leaves and diuretic polyherbal drug (1) (includes *Calendula officinalis* flowers, *Mentha piperita* leaves, *Arctostaphylos uva-ursi* leaves, *Anethum graveolens* fruits, and *Eleutherococcus senticosus* rhizomes with roots) and polyherbal drug (2) (*A. uva-ursi* leaves, *Juniperus communis* fruits, and *Glycyrrhiza glabra* roots) are used to treat nephritis, kidney stones, and dysuria in Russia
- Reproductive system, the leaves are used for leukorrhea, metrorrhagia, and involution of the myometrium
- Digestive system, the leaves are used for the treatment of stomach hypofunction, heartburn, and chronic colitis, the fruit for the treatment of gastritis and diarrhea
- Infections, invasion, the leaves are used to treat gonorrhea, and malaria
- Eye diseases, the flowers are used to treat blepharitis, and conjunctivitis

- Mental disorders, the leaves are used to treat neurosis, insomnia, and alcoholism
- Skin and subcutaneous tissue, the flowers are used to treat pemphigus, the leaves are used for diathesis and the treatment of sores
- Metabolic diseases, the leaves are used to treat gout
- Traumatology, the leaves are used to treat purulent wounds
- Musculoskeletal system, the leaves are used for polyarthritis treatment
- Cardiovascular system, the flowers are used to treat heart disease
- Blood and organs of hematopoiesis, the leaves are used to treat anemia
- Endocrine system, the leaves are used for treating diabetes
- Other symptoms and syndromes, ascites, hearts and kidney edema (7).

Chemical content

Scientists from various countries have identified phenologlycosides and flavonoids including antocyanes, hydroxycinnamic acids, saponins, lignans, iridoides, polysaccharides and essential oils in the stems and leaves of the bearberry.

Phenols and phenologlycosides

Keller determined the total amount of hydroquinone in an extract of bearberry leaves after hydrolysis using a cerimetric titration method (8). The author also compared the cerimetric results with using the iodometric method and determined that they varied slightly. In 1960 Keller called into question the validity of using iodometric titration for the quantitative determination of arbutin in bearberry leaves (9).

In 1979 Jahodár and Leifertova, scientists from Czech Republic isolated p-methoxyphenol from bearberry leaves using column chromatography (CC) (10). Sticher *et. al.,* from Switzerland determined in the same year using high-performance liquid chromatography (HPLC) that it was a highly accurate method for the determination of the main components in bearberry leaves. The presence of abutin, hydroquinone and methylarbutin has also been established (11).

Karikas *et. al.,* isolated 3 g of phenologlycosides from air-dried bearberry leaves collected in Spain. These were subjected to column chromatography and arbutin (40 mg), methylarbutin (20 mg) and mixture of previously unknown glycosides were discovered. In the mixture of glycosides the authors identified piceoside (4-acetylphenyl-β-D-glucopyranoside) and piceoside tetraacetate by HPLC and protone nuclear magnetic resonance (^{1}H-NMR) (12).

Linnenbrink & Kraus compared different bearberry leaf samples and an *in vitro* culture of bearberry leaves. In the samples of natural origin, the total amount of arbutin was 6.3-14.6% in fresh raw material. The highest amount of arbutin was found in young leaves. During their growing period, the amount of arbutin decreased. These authors did not find methylarbutin in the samples investigated. In callus, the average amount of arbutin was 1.7% in fresh leaves and 4.1% in fresh stems (13).

Kubo *et. al.,* worked on isolating substances from the leaves of *Arctostaphylos uva-ursi*. The authors isolated arbutin, 1,2,3,6-tetra-O-galloyl-β-D-glucose, 1,2,3,4,6-penta-O-galloyl-β-D-glucose, methylgallate and gallic acid using CC. All the isolated substances were tested for pharmacological activities (14-17).

Parejo *et. al.,* detected the presence of arbutin in bearberry leaves collected from four natural habitats (in the Catalan Pyrenees) during different seasons (spring and autumn) from 1997 and 1998 using HPLC. The authors determined that the amount of arbutin depended on both the habitat and the season. Average amount of arbutin was 6.30-9.16%, the highest amount was detected in the fall (18).

Lamien-Meda *et. al.,* in 2008 used gas chromatography with flame ionization detection (GC-FID) to identify arbutin and hydroquinone in bearberry leaves. The results obtained were comparable with results from HPLC analysis (19).

Alam *et. al.,* in 2010 developed a high performance

thin-layer chromatography (HPTLC) method for the identification of arbutin in bearberry leaves. Separating arbutin in methanol extracts was established using aluminium foil with silica gel 60 F254, a mixture of methanol and chloroform (3:7 v/v) as eluent. The retardation factor was R_f=0.32±0.02; analysis time was 10 minutes, and detection was carried out at a wavelength of 285 nm (20).

Senchenko *et. al.,* developed a method of quantifying arbutin in bearberry leaves using capillary electrophoresis. They used borate buffer (pH 9.0), a quartz capillary with diameter 50 μm and effective length 65 cm. Detection was carried out by spectrophotometry at 254 nm, and the voltage was 20 kV. Using these conditions of analysis, arbutin may be separated from other components (21).

Using a HPLC Kurkin *et. al.,* from Samara (Russia) discovered arbutin in the samples of bearberry leaves, collected in the Mari El Republic. The authors suggested reversed-phase HPLC conditions for its determination: 0.01 M KH_2PO_4 with H_3PO_4 and acetonitrile (9:1) as the mobile phase, and detection at 280 nm. The amount of arbutin in the sample was 10.85-11.16% (22).

Shimizu *et. al.,* from Okayama University (Japan) isolated corilagin from *A. uva-ursi* leaves by CC. This material was investigated in pharmacological tests (23).

Chauhan *et. al.,* used an HPLC to investigate an aqueous extract from bearberry leaves. The authors identified phenologlycoside, arbutin, phenolic acid, gallic acid, flavonoids myricetin and isoquercetin (24). Phenologlycosides from *Arctostaphylos uva-ursi* Spreng. are shown in Figure 1.

Olennikov and Chekhirova investigated phenolic complexes in different parts of *Arctostaphylos uva-ursi* (leaves, stems and roots). The plant material was collected in the Republic of Buryatia (25). An alcohol extract (ethanol 70%) was separated using silica gel, polyamide and Sephadex LH-20 chromatography. Identification of the compounds was carried out using HPLC. The following compounds were found in all parts of the plant (except where indicated):

- simple phenols and their glycosides (arbutin, 6''-galloylarbutin were found in all parts of the plant except the roots; picein 6''-galloylpicein except in the leaves; gallic acid; bergein was found only in the roots;
- galloylglycosides (1,6-di-O-galloylglucose, 3,4,6-tri-O-galloylglucose, 1,2,3,4,6-penta-O-galloylglucose);
- catechins ((+)-catechin, (-)-catechin, (-)-epigallocatechin except in the roots; (-)-epicatechingallate except in the leaves; (+)-galloylcatechingallate except in the roots; (-)-epigallocatechingallate except in the roots; (-)-epigallocatechinmethylgallate, (-)-epigallocatechindigallate except roots);
- tannins (corilagin, chebulagic acid);
- phenylpropanoids (caffeic acid, 5-O-caffeoylquinic acid, 3-O-caffeoylquinic acid, 4-O-caffeoylquinic acid, 1,3-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, cinnamic acid, hydroxycinnamic acid, 2-methoxycinnamic acid, ferulic acid, isoferulic acid, all compounds were found in all parts except in the roots);
- flavonoids (quercetin, quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-arabinofuranoside, quercetin-3-rhamnoside, quercetin-3-rutinoside, quercetin-3-gentiobioside).

The total amount of phenolic complexes in the *A. uva-ursi* from the Republic of Buryatia was 336.78 mg/g in the leaves, 202.23 mg/g in the stems, 15.73 mg/g in the roots. The amount of simple phenols and phenologlycosides in the samples was 97.38 mg/g, 87.7 mg/g and 2.96 mg/g respectively, galloylglycosides 38.54 mg/g, 10.23 mg/g and 0.25 mg/g respectively, catechins 112.04 mg/g, 74.32 mg/g and 12.52 mg/g respectively, tannins 72.58 mg/g, 19.15 mg/g and 0.66 mg/g respectively, phenylpropanoids 5.84 and 4 mg/g in the leaves and stems respectively and in the roots trace amounts of flavonoids – 10.4 and 6.83 mg/g in the leaves and stems respectively and in the roots – trace amount (25). The qualitative content of flavonoids and hydroxycinnamic acids from *Arctostaphylos uva-ursi* (L.) Spreng. are shown in Tables 1 and 2.

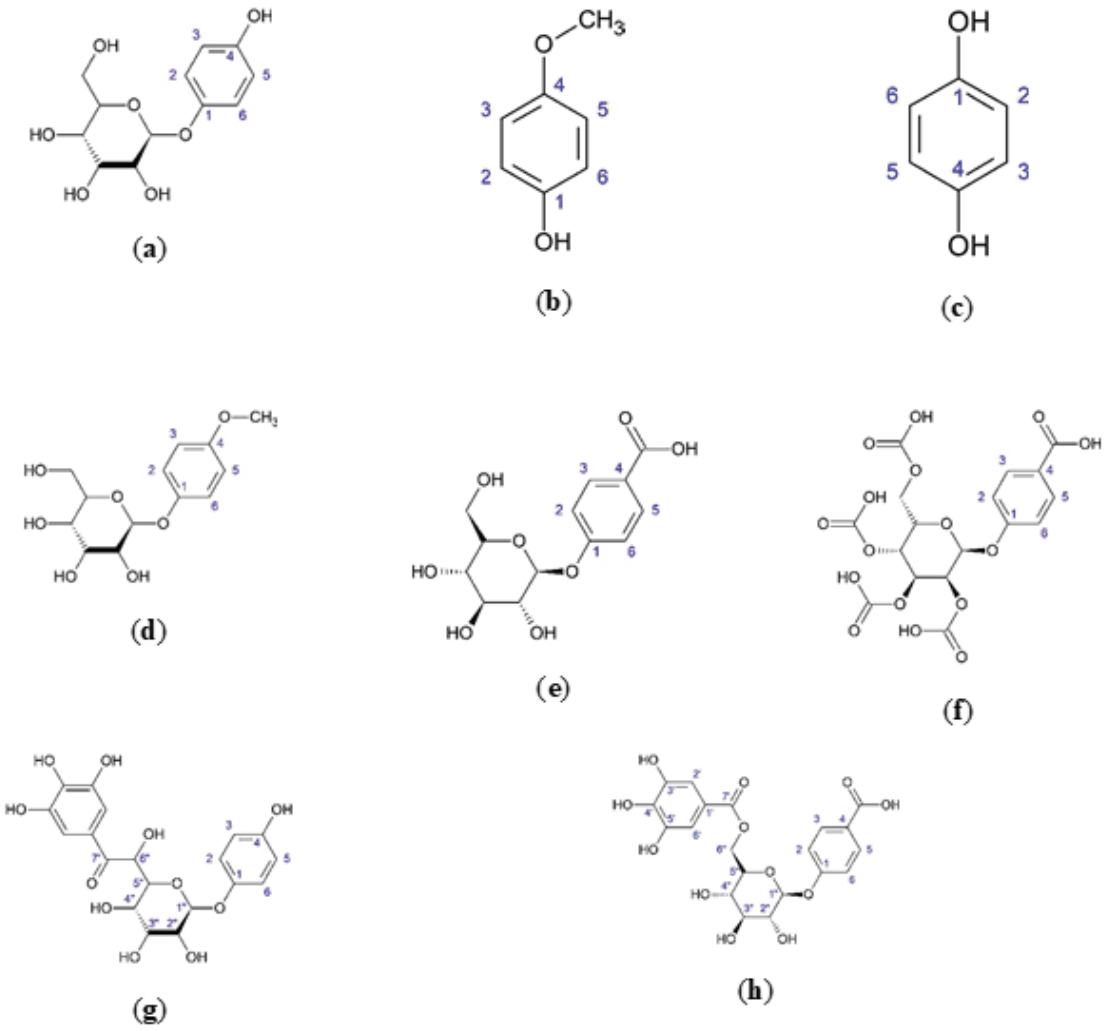
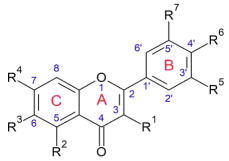


Figure 1 Phenologlycosides from *Arctostaphylos uva-ursi* (L.) Spreng.: (a) arbutin (b) p-methoxyphenol (c) hydrochinon (d) methylarbutin (e) picein (4-acetyl-phenyl-beta-D-glucopyranoside, piceoside), (f) picein tetraacetate, (g) 6"-0-galloylarbutin, (h) 6"-0-galloylpicein.

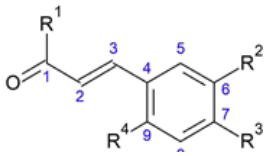
Panusa *et. al.,* identified from a methanol extract of *Arctostaphylos uva-ursi* leaves, 88 phenolic compounds by ultra-high pressure liquid chromatography with photodiode array, coupled with electrospray ionization-quadrupole-time of flight-mass spectrometry (UHPLC-PDA-ESI-TOF/MS) within 5 minutes: phenologlycosides (arbutin, methylarbutin, galloylarbutin), gallic acid and gallotannins (mono-, di-, tri-, tetra and pentagalloylglucoside, galloylshikimic acid), flavonoids (quercetin, quercetingalloylhexoside, quercetin-3-rutinoside, quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-arabinofuranoside, quercetin-3-rhamnoside, myricetin, myricetin hexoside, myricetin pentoside, kaempferol, kaempferol hexoside, kaempferol pentoside). The authors determined the main flavonoid components in the bearberry leaves; quercetin glycosides and quercetin-3-galactoside (hyperoside) had the highest amounts. The qualitative content of tannins from *Arctostaphylos uva-ursi* (L.) Spreng. is shown in Figures 2 and 3. Wähner *et. al.,* also identified antocyanes cyanidin and delphinidin in the bearberry leaves (Figure 4) (27).

Table 1 Flavonoids from Arctostaphylos uva-ursi (L.) Spreng.



No	NAME	TYPE OF FLAVONOID	R^1	R^2	R^3	R^4	R^5	R^6	R^7	GLYCOSIDE
1	Quercetin	Flavonol quercetin	OH	OH		OH		OH	OH	
2	Quercitrin		3-O-α-L-rhamnoside	OH		OH		OH	OH	O-glycoside
3	Isoquercitrin		3-O-β-D-glucopyranoside	OH		OH		OH	OH	O-glycoside
4	Quercitin-3-gentiobioside		3-O-β-D-glucopyranosyl-(1->6)-β-D-glucopyranoside	OH		OH		OH	OH	O-glycoside
5	Hyperoside (quercetin-3-O-galactoside)		3-O-β-D-galactoside	OH		OH		OH	OH	O-glycoside
6	Avicularin (quercetin-3-O-α-L-arabinofuranoside_		3-O-α-L-arabinofuranoside	OH		OH		OH	OH	O-glycoside
7	Rutin (quercetin-3-rutinoside)		3-O-β-D-rutinoside	OH		OH		OH	OH	O-glycoside
8	Kaempferol	Flavonol kaempferol	OH	OH		OH		OH		
9	Kaempferol-pentoside		3-O-pentoside	OH		OH		OH		O-glycoside
10	Kaempferol-hexoside		3-O-hexoside	OH		OH		OH		O-glycoside
11	Myricetin	Flavonol myricetin	OH	OH		OH	OH	OH	OH	
12	Myricetin hexoside		3-O-hexoside	OH		OH	OH	OH	OH	O-glycoside
13	Myricetin pentoside		3-O-pentoside	OH		OH	OH	OH	OH	O-glycoside

Table 2 Hydroxycinnamic acids from *Arctostaphylos uva-ursi* (L.) Spreng.



No	NAME	R^1	R^2	R^3	R^4
1	Ferulic acid (4-hydroxy-3-methoxycinnamic acid)	OH	OCH_3	OH	H
2	Isoferulic acid (3-hydroxy-4-methoxycinnamic acid)	OH	OH	OCH_3	H
3	Cinnamic acid	OH	H	H	H
4	Caffeic acid	OH	OH	OH	H
5	Neo-chlorogenic acid (5-O-caffeoylquinic acid)	5-O-1,3,5-trihydroxycyclohex-1-carboxylic acid	OH	OH	H
6	1,3-dicaffeoylquinic acid	cis-3-O-(1-O-caffeoyl)-quinic acid	OH	OH	H
7	3,5-dicaffeoylquinic acid	cis-3-O-(5-O-caffeoyl)-quinic acid	OH	OH	H
8	2-hydroxycinnamic acid	OH	H	H	OH
9	2-hydroxycinnamic acid	OH	H	H	OCH_3
10	Chlorogenic acid (3-O-caffeoylquinic acid)	trans-3-O-quinic acid	OH	OH	H
11	Cryptochlorogenic acid (4-O-trans-caffeoylquinic acid)	4-O-1,3,5-trihydroxycyclohex-1-carboxylic acid	OH	OH	H

Polysaccharides

Olennikov and Nazarova investigated polysaccharide complexes in bearberry leaves originating in the Republic of Buryatia (28). The authors found water-soluble polysaccharides (WSP) using successive pre-extraction of plant material in a Soxhlet apparatus with chloroform, ethyl acetate and acetone. The raw material was then extracted with water at 20 and 100°C to obtain two fractions WSPSc and WSPSh. These fractions were precipitated with acetone and deproteinized using pronase from *Streptomyces griseus*. Pectinic substances (PS) were isolated after collecting WSP using extraction with a mixture of oxalic acid (0.5%) and ammonium oxalate (0.5%) (1:1). Then the solvent was dialyzed and PS were precipitated using acetone. Hemicellulose components (HC) were isolated after collecting PS using extraction with water and sodium hydroxide 5% and subsequent neutralization by acetic acid. The precipitate of the fraction HC_A was washed by acetic acid 10%, water and ethanol 95%. Then supernatant was dialyzed, concentrated and fraction HC_B was precipitated by acetone. From the 200 g of plant material the authors isolated 0.708 g of WSPSc and 0.512 g of WSPSh, 6.39 g of PS, 2.06 g of HC_A and 1.12 g of HC_B. WSPSc consisted of arabinose, glucose, mannose, rhamnose, galacturonic acid; WSPSh – arabinose, glucose, mannose, rhamnose, xylose, galacturonic acid; PS – arabinose, galactose, glucose, rhamnose, xylose, galacturonic acid; HC_A – arabinose, galactose, glucose, mannose, rhamnose, xylose, galacturonic and glucuronic acids; HC_B – arabinose, galactose, glucose, mannose, rhamnose, xylose, galacturonic and glucuronic acids.

Iridoides

Jahodár *et. al.,* collected *A. uva-ursi* in the Botanical Garden in Brno (Czech Republic) (29). Fresh leaves (200 g) were extracted using 1500 ml of ethanol. The solvent was evaporated under vacuum, the dry residue was dissolved in water, and then liquid-liquid extraction was carried out successively using chloroform and ether. Then the aqueous phase was separated using aluminum oxide and water as eluent. The eluate was concentrated and then separated using silica gel and acetone as mobile phase; 50 mg of iridoide monotropeine was obtained (Figure 5).

Saponins

Takada *et. al.,* (Japan, 2010) used leaves of *Arctostaphylos uva-ursi* as source of ursolic acid for investigation of its anti-inflammatory activity (30).

Caligiani *et. al.,* developed a simple method for analysis of saponins in plant material using gas chromatography with mass-spectrometry (GC-MS) (31). They found 1549 mg/kg of total amount of saponins in bearberry leaves including oleanolic acid (488±42 mg/kg), ursolic acid (1034±43 mg/kg), maslinic acid (11±3 mg/kg) and corosolic acid (16 ± 2 mg/kg) (Figures 6-7).

Essential oils

Radulović *et. al.,* in 2010, investigated essential oils from bearberry leaves (32). They detected 50 substances using gas-liquid chromatography (GLC). The main compounds were α-terpineol (7.8%), linalool (7.3%), hexadecanic acid (4,5%), (E)-gernyl acetone (4.1%), geranyol (3.0%), hexahydrofarnesyl acetone (2.3%), thymol (2.0%), isomenthol (1.9%), 6-methyl-3,5-heptadien-2-one (1.8%), dodecanoic acid (1.8%), (E,E)-2,4-decadienal (1.7%), riesling acetal (1.4%), borneol (1,4%), cis-linalooloxide (furanoid) (1.3%), geranial (1.3%), (E)-β-ionone (1.3%), linoleic acid (1.2%), α-humulene (1.2%), teradecanoic acid (1.2%), terpinen-4-ol (1.0%) (Figure 8). In the essential oil monoterpenes are 35.6% and sesquiterpenes 7.4%.

Trace elements

Afanasyeva and Ayushina collected roots, leaves and stems of *Arctostaphylos uva-ursi* from different habitats in the Republic of Buryatia (33). The authors determined the amount of trace elements using atomic absorption spectroscopy with an AAnalyst 400 AA Spectrometer (Perkin Elmer). The average content of trace elements such as Mn, Fe, Zn, Cu, Cr, Ni, Pb, Co, Cd was measured and their distribution in bearberry established. The richest plant part was the roots, with highest amount of trace elements. The concentrations of Mn, Fe, Zn, Pb, Co, Cd decrease in the following order: roots >

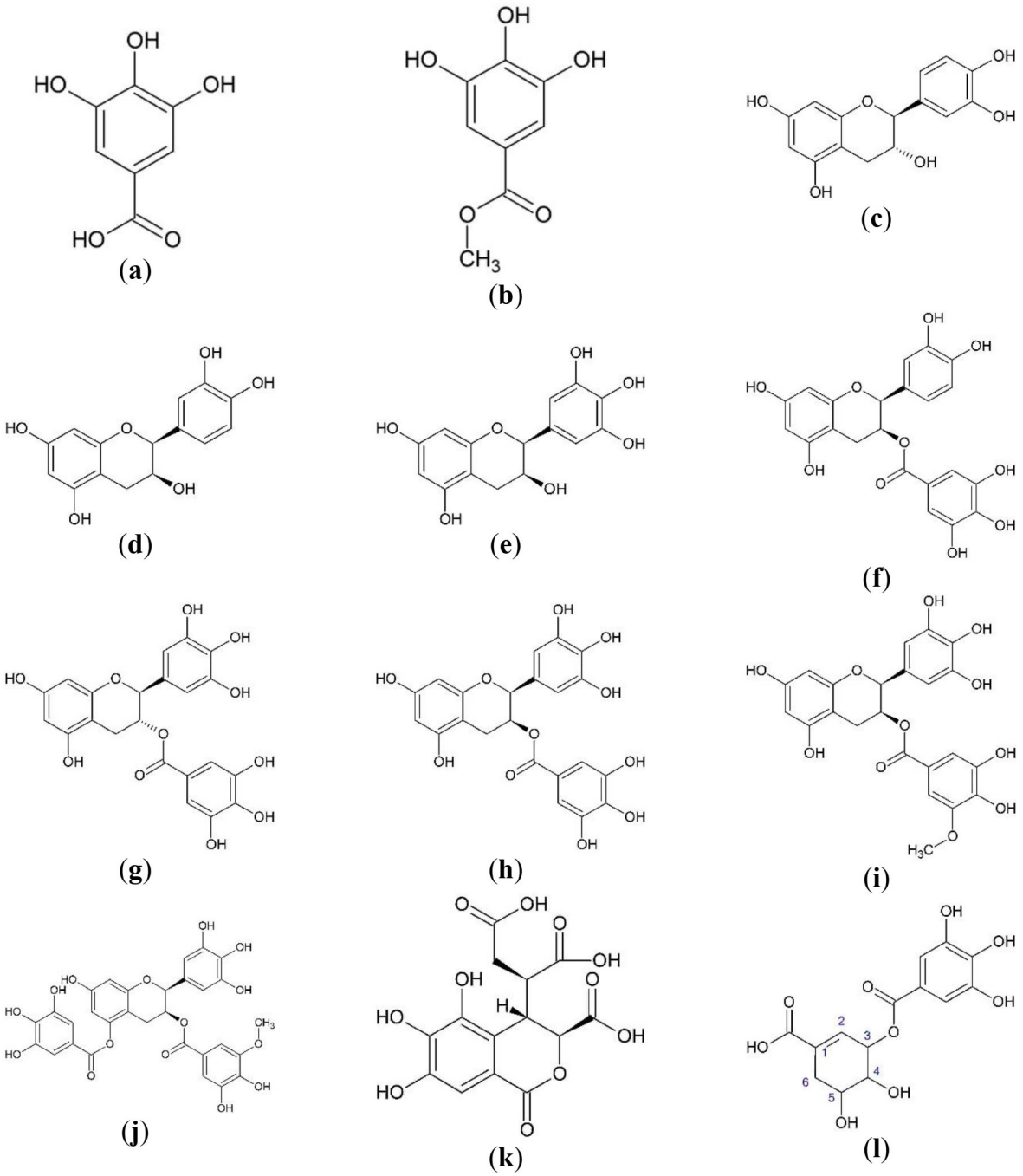


Figure 2 Tannins from *Arctostaphylos uva-ursi* (L.) Spreng.: (a) gallic acid, (b) methylgallate, (c) (+)-catechin (d) (-)-epicatechin (e) (-)-epigallocatechin (f) (-)-epicatechin gallate (g) (+)-gallocatechin gallate (h) (-)-epigallocatechin gallate (i) (-)-epigallocatechin methylgallate (j) (-)-epigallocatechin digallate (k) chebulagic acid (1) 3-0-galloylshikimic acid.

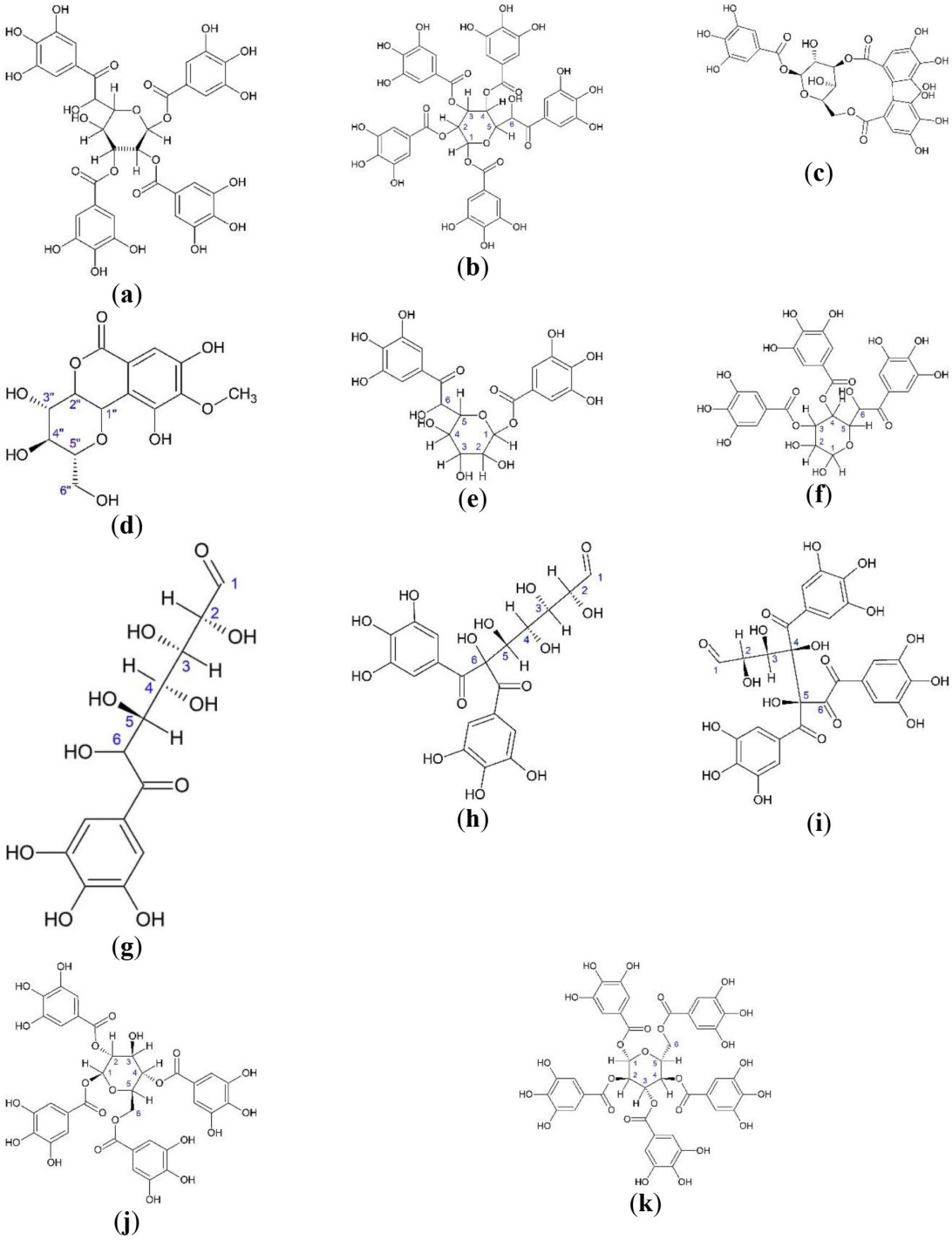


Figure 3 Glycosides of tannins from *Arctostaphylos uva-ursi* (L.) Spreng.: (a) 1,2,3,6-tetra-O-galloyl-beta-D-glucose (b) 1,2,3,4,6-penta-O-galloylglucose (c) corilagin (d) bergenin (e) 1,6-di-O-galloylglucose (f) 3,4,6-tri-O-galloylglucose (g) monogalloylglucose (h) digalloylglucose (i) trigalloylglucose (j) tetragalloylglucose (k) pentagalloylglucose

Figure 4 Antocyanes from *Arctostaphylos uva-ursi* (L.) Spreng.: (a) cyanidin (b) delphinidin

stems > leaves > fruits. Metals accumulated in higher amounts in the roots compared to the aerial parts of the plants. The authors concluded that *Arctostaphylos uva-ursi* can be recognized as a source of Cr and it is possible to use it in complex therapy and for the prevention of diabetes and atherosclerosis.

Pharmacological Activity

Bearberry leaves are included in the Pharmacopoeias of the Russian Federation (XIV edition), Belarus (II edition), Japan (XVII edition), European Pharmacopoeia (10th edition), British Pharmacopoeia (BP 2020) monographs.

Extracts obtained from the bearberry raw material are widely used in both traditional and alternative medicines. Data about toxic effects of bearberry on the liver, kidneys, stomach and pancreas can be found in some outdated sources. To follow up on this, de Arriba *et. al.,* investigated the toxic effects of the arbutin metabolite hydroquinone. The authors did not detect any toxic effects caused by the arbutin breaking down into hydroquinone in low amounts. Most of the arbutin was excreted unchanged in the urine (34). The best known therapeutic activity of bearberry is its effects on the genitourinary system, usually expressed as diuretic, nephrolytic and antibacterial effects. Grases *et. al.,* determined that an extract from *Arctostaphylos uva-ursi* prevented the development of possible risk factors of nephrolithiasis such as acidic urine pH, increased urinary excretion of calcium ions, phosphate ion, and citrate ion (35). It should be noted that such changes were accompanied by a marked increase in diuresis in female Wistar rats. At the same time, it is important to understand that the bearberry extracts do not for practical purposes affect the excretion of electrolytes in the urine (there was a slight increase in the elimination of ionized sodium) (36). It is also

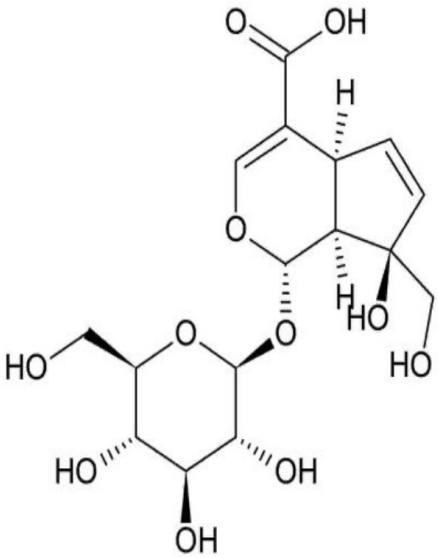


Figure 5 Iridoide monotropein fro**m** *Arctostaphylos uva-ursi* (L.) Spreng.

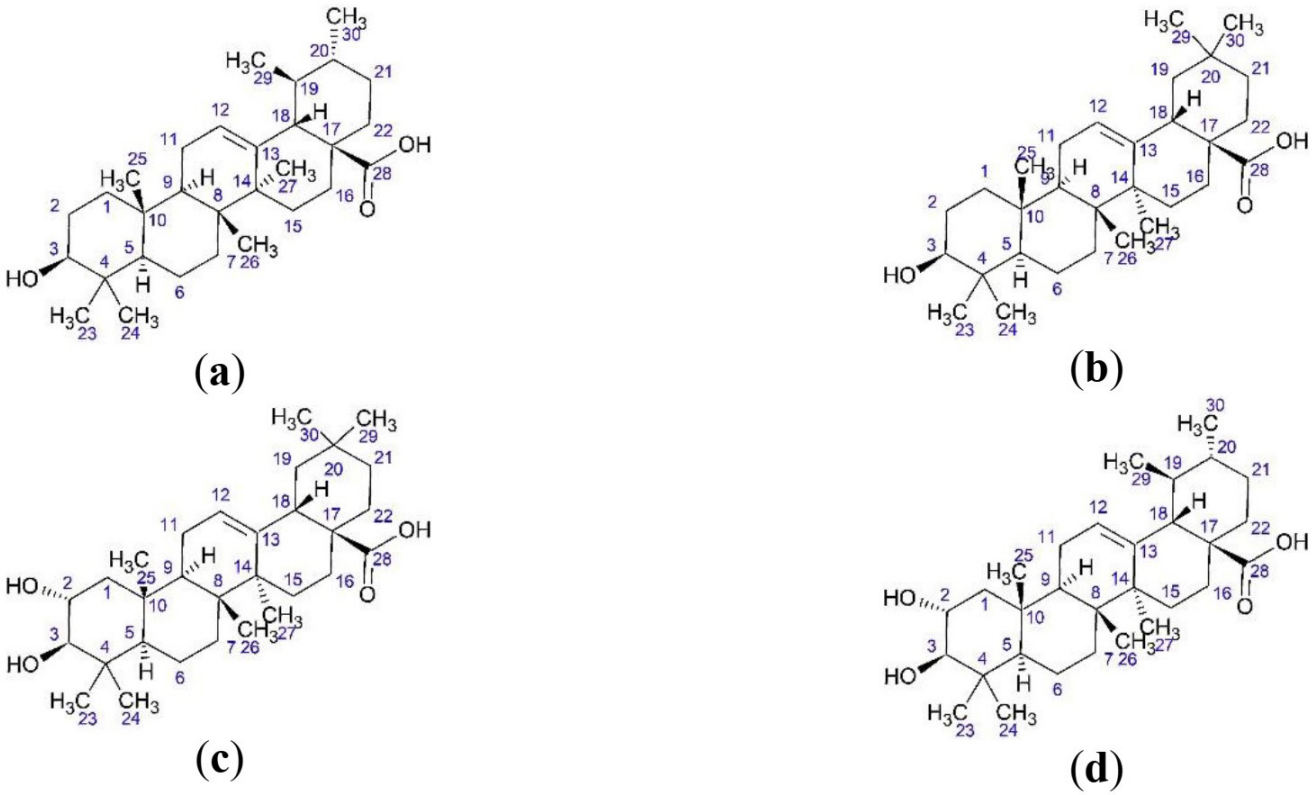


Figure 6 Triterpene acids from *Arctostaphylos uva-ursi* (L.) Spreng.: (a) ursolic acid (b) oleanolic acid (c) maslinic acid (d) corosolic acid

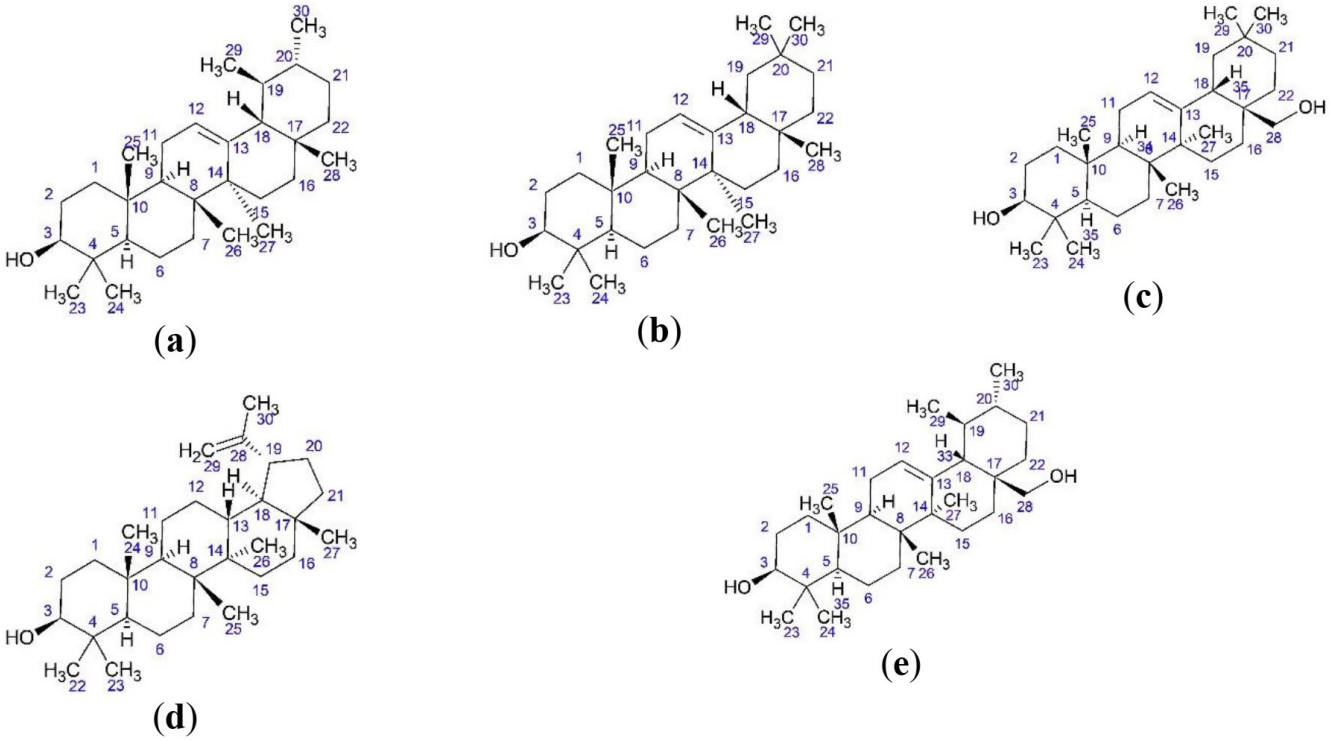


Figure 7 Triterpenes from *Arctostaphylos uva-ursi* (L.) Spreng.: (a) a-amyrin (b) -amyrin (c) erythrodiol (d) lupeol (e) uvaol

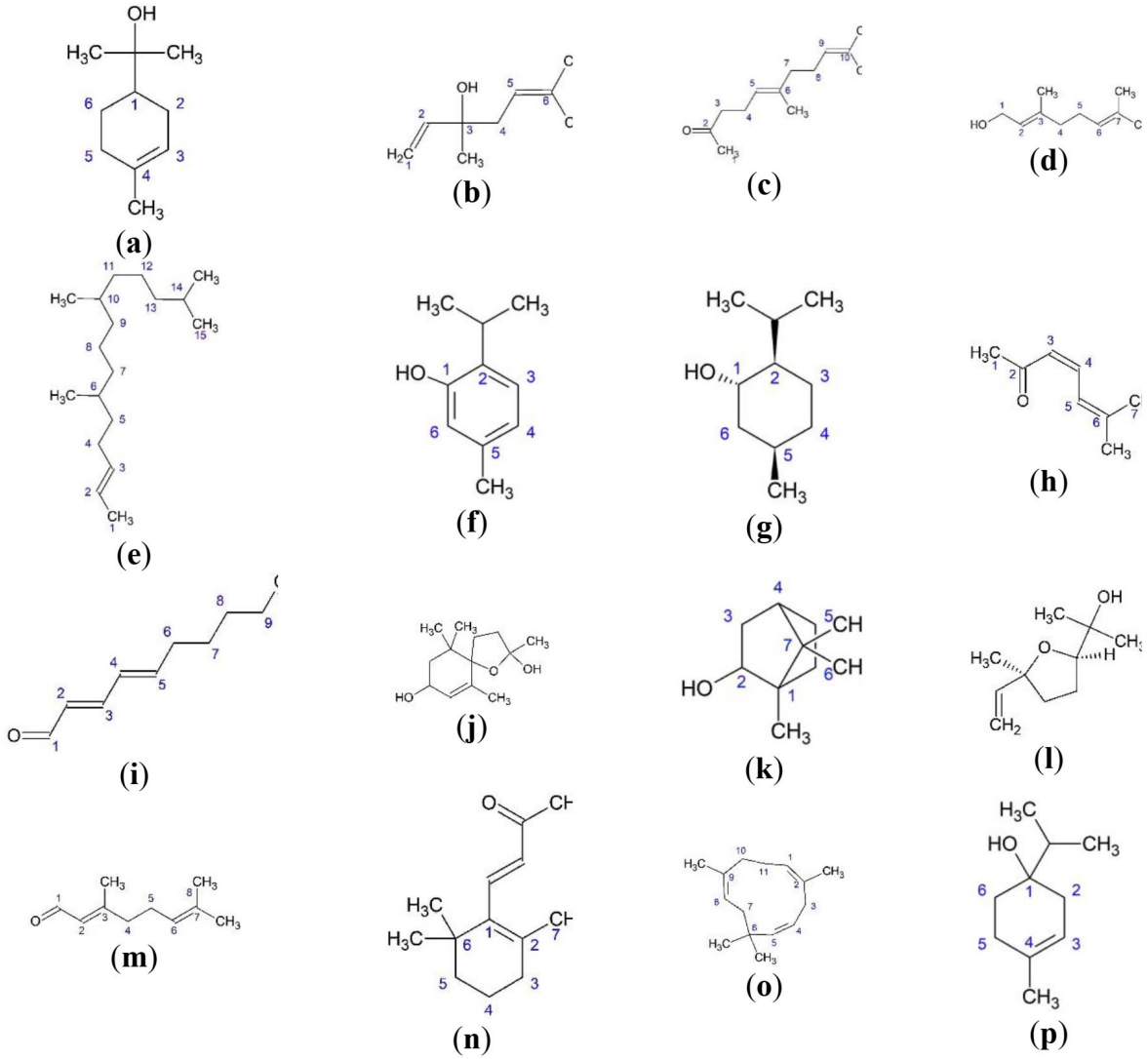


Figure 8 Essential oil from leaves of *Arctostaphylos uva-ursi* (L.) Spreng.: (a) a-terpineol; (b) linalool; (c) geranyl acetone; (d) geraniol; (e) hexahydrofarnesyl acetone; (f) thymol; (g) isomenthol; (h) 6-methyl-3,5-heptadien-2-one; (i) (E, E)-2,4-decadienal; (j) riesling acetal; (k) bomeol; (1) cis-linalool oxide; (m) geranial; (n) E-B-ionone; (o) a-humulene; (p) terpinen-4-ol

worth noting that a course of an aqueous extract of bearberry leaves increased the activity of endogenous antioxidant defense enzymes of the glutathione family in the kidneys, which can have a positive effect on their function (37). Further studies showed the positive effect of extracts obtained from bearberry on bacterial cystitis. Larsson *et. al.,* showed that the use of an aqueous extract of bearberry (standardized for the content of arbutoside and methylarbutoside) as an adjuvant therapeutic agent helped to reduce the manifestations of recurrent bacterial cystitis in women, and statistically significant differences were found versus the placebo group (23%) (38). In this study, a month-long course of treatment was prescribed, which presented difficulties, since it was known that the bearberry contains hydroquinone, which in high concentrations has a carcinogenic effect. Therefore, the recommended course of treatment with bearberry phyto-preparations is no more than 14 days (39). However, a recent study on the pharmacology of bearberry extracts conducted by Park *et. al.* showed that extracts from bearberries do not have significant

toxic effects in humans and do not enter into clinically significant interactions with either endogenous or exogenous compounds (40).

Additionally, the high level of toxicological safety of the bearberry extracts was confirmed by Saeed *et. al.,* (41). The study was carried out on rabbits of both sexes both which received an aqueous ethanol extract of bearberry leaves at a low dose (25 mg/kg per day), over a long period of time (90 days). The results of the study determined that animals treated with the bearberry extract had no significant changes in their biochemical, hematological parameters, functional activity of the heart, kidneys and liver, as well as the lipid profile compared to the control group, which suggests there was little systemic toxicity in the bearberry extracts (41).

The effectiveness of an aqueous extract of bearberry in combination with ibuprofen as an alternative therapeutic approach to the treatment of cystitis has been confirmed in a series of clinical studies. Work by Afshar *et. al.*, showed that adding bearberry extract at a dose of 105 mg (in terms of arbutin) 3 times a day in combination with ibuprofen to standard therapy with fosfomycin (3 g per day) significantly improved the clinical status of patients with bacterial cystitis (42). At the same time, when using an alternative phytotherapy approach to auxiliary therapy, the authors recommend reducing the dose of the antibacterial drug, which made the treatment safer and reduced the drug load on the patient (42). Also a study by Moore *et. al.*, demonstrated that, despite the fact that the bearberry extract administered in isolation, does not significantly affect the course of cystitis in women, adding this extract to antibiotic therapy significantly increased the safety of therapy and allowed one in seven women who participated in the study to refuse the antibacterial agent in the future (43). However, the use of ibuprofen as an adjuvant to standard antibiotic therapy had a more pronounced therapeutic effect than the use of bearberry extract.

The positive effect of extracts of bearberry on changes in the functional activity of the kidneys has been confirmed by many authors and can be associated with a high content of arbutin in the raw material and diuretic activity (34). However, other potential positive pharmacological effects, such as direct antibacterial action should not be discounted. Extracts obtained from bearberry raw materials have a fairly wide range of antibacterial effects, including against resistant strains and pathogens of particularly dangerous infections. Work carried out by Cybulska *et. al.*, showed that an extract obtained from bearberry leaves suppressed the growth of resistant cultures of *N. gonorrhoeae* with a minimum inhibitory concentration range of 0.25-32 micrograms/ml (44). The activity against *N. gonorrhoeae* strains resistant to a wide range of antibacterial drugs was studied, including: tetracycline, β-lactams, streptomycin, erythromycin, azithromycin, and ciprofloxacin.

According to Holopainen *et. al.,* extracts of the above ground parts of bearberry showed antimicrobial activity against gram-negative bacteria such as *E. coli* and *Proteus vulgaris* (45). The effect is probably related to the presence of arbutin and methylarbutin. According to Annuk *et. al.*, an aqueous extract of the bearberry leaves showed antimicrobial activity against *Helicobacter pylori*, due to the presence of tannins (46). Also, extracts from bearberry exhibited antibacterial activity against rare, but highly pathogenic bacteria. Tolmacheva *et. al.*, determined that an aqueous ethanol extract of bearberry leaves at sufficiently low concentrations suppressed the growth of a bacterial culture of the wild strain of *C. violaceum* ATCC 31532 (47).

The presence of arbutin and hydroquinone as the predominant compounds in bearberry raw material may be the basis for the use of bearberry extracts in dermatological practice as an anti-inflammatory or depigmenting agent. A study by Kubo *et. al.* (1990) showed that the use of a 50% ethanol extract from bearberry leaves at a dose of 100 mg/kg (orally) significantly reduced the exudative phase of the inflammatory reaction caused by application of picryl chloride to the skin (14). At the same time, the effectiveness of bearberry extract was slightly inferior to the effect of prednisone, but increased its activity when used in combination. In addition, the work of Matsuda *et. al.,* established a pronounced anti-inflammatory

activity of an extract from bearberry (48). Thus, in the model of carrageenan edema and adjuvant arthritis, the use of an aqueous extract of bearberry at a dose of 50 mg/kg (orally, in terms of arbutin) significantly reduced the severity of inflammatory edema in rats. It is also worth noting that the administration of an extract obtained from raw bearberry to animals suppressed a type IV hypersensitivity reaction (48). According to recent data, the anti-inflammatory activity of extracts of bearberry raw materials may be associated with the suppression of intracellular pro-inflammatory signal transduction mediated by the activation of toll-like receptors of subtypes 2 and 4. Reducing the intensity of inflammatory reaction on the basis of the use of bearberry extracts, mediated reduction in leukocyte migration in inflammation and decreased the intensity of reactions of NF-kB pathways (49). In addition, the anti-inflammatory properties may be based on the ability of bearberry extracts to coagulate inflammatory exudate proteins, which is accompanied by a significant reduction in edema (50).

The depigmenting activity of bearberry extracts has been investigated in a number of experimental studies. Matsuda *et. al.,* (1992) studied the anti-tyrosinase properties of a 50% methanol extract from bearberry leaves (15). At the same time, the authors found that the extract showed high inhibitory activity against fungal tyrosinase and significantly reduced the synthesis of melanin. Anti-tyrosinase properties were also established for a 50% ethanol extract from bearberry leaves (17). It is worth noting that an aqueous extract of bearberry leaves is part of a patented combination product intended for skin whitening (51).

It is possible to use bearberry extracts as a means of correcting metabolic disorders that occur in diabetes. Swanston-Flatt *et. al.,* carried out a study examining the possibility of applying a phytotherapy approach to the treatment of experimental diabetes mellitus in mice (52). As a result, it was found that the addition of a bearberry leaves extract to a standard diet contributed to a decrease in polydipsia, hyperphagia and a decrease in blood glucose concentration.

The antioxidant and neuroprotective properties of plant extracts are widely known. These types of pharmacological activity of bearberry raw material extracts have been studied in several experimental studies.

Chandler *et. al.,* showed that a bearberry leaf extract, at a dose of 100 mg/ml suppressed the formation of TNF-α and excess amounts of nitric oxide, which prevented the death of mouse n-11 neuronal cells (53). However, in this case, the total extraction from bearberry was inferior in terms of pharmacological activity to individually isolated compounds-apigenin and diosmetin. The antioxidant properties of a methanol extract of bearberry leaves were studied by Mohd Azman *et. al.*, in 2016, in the ABTS and methoxyl radical generation model. As a result, a significant inhibition of the formation of these radicals was found when adding bearberry extract to the medium at the rate of 100 mg/ml (54).

According to Amarowicz and Pegg bearberry leaf extract exhibits antiproliferative properties in human carcinoma cell cultures (55). In this study, fresh bearberry leaves were extracted using 80% ethyl alcohol. The data obtained showed that the extract inhibited the proliferation of five human carcinoma cell lines, namely MCF-7-breast, HT-29-colon, DU-145-prostate, SK-MEL-5-skin, and MDA-MB-435-skin carcinomas. The authors associated antiproliferative activity with gallotannins present in bearberry leaves and induction of a cascade of apoptotic reactions (55). The types of pharmacological activity of extracts obtained from bearberry raw materials are shown in Table 3.

DISCUSSION

A review of the literature suggests that there are two species of the genus *Arctostaphylos* Adans. namely *A. caucasica* Lipsch. and *A. uva-ursi* (L.) Spreng. The taxonomic status of *A. caucasica* Lipsch. is currently challenging as some scientists consider it conspecific to *Arctostaphylos uva-ursi* (L.) Spreng. and others regard it as subspecies and a third group holds to the validity

of the species. Thus, a molecular investigation of these species are expedient for determining the authenticity when used for medicinal purposes which are included in the Pharmacopoeias of different countries.

The main compound used for the standardization of bearberry leaves is arbutin. According to the literature flavonoids, hydroxycinnamic acids, saponins etc. accumulate in different parts of the plant. Researchers in different countries have carried out phytochemical analysis and established that qualitative and quantitative content of bearberry leaves is variable. Phytochemical analysis of the bearberry leaves from different habitats may clarify the content of biological active substances. Together with phenologlycosides, flavonoids and tannins are two classes of substances that should be standardized.

Several studies have shown that extracts from bearberry leaves have a wide range of pharmacological activites including antibacterial, diuretic, nephrotic, antioxidant, antiproliferative, depigmenting, anti-inflammatory, antidiabetic, and neuroprotective properties. There is need for continued experimental work aimed at the expansion of the types of biological activity and detection of detail mechanisms of effects extracts from bearberry raw material. For example, the increasing activity of endogenous antioxidant enzymes from kidneys of animals without a pathological background may be a precondition for investigation of nephroprotective features of aqueous bearberry extract used to treat various kidney diseases. The advanced evaluation of diuretic activity of bearberry extracts with determination of mechanisms of effect is needed.

Table 3 Pharmacological activity of extracts obtained from the bearberry

ACTIVITY	EXTRACT	RAW MATERIAL	STUDY TYPE	DOSE	REFERENCE
Antibacterial	Aqueous	Leaves	Clinical	NA	(38)
	Aqueous	Leaves	Clinical	105 mg per day (for arbutin)	(42)
	Aqueous	Leaves	Clinical	NA	(43)
	Water-ethanol	Leaves	Preclinical *in vitro* for resistant strains of *N. gonorrhoeae*	0,25-32 μg/ml	(44)
	Water-ethanol	Aerial part	Preclinical in vitro for resistant strains: *E. coli* и *Proteus vulgaris*.	50 mg/ml, 100 mg/ml	(45)
	Water-ethanol	Leaves	Preclinical *in vitro* for resistant strains *Helicobacter pylori*	180 μg/ml	(46)
Nephrolytic	Aqueous	Leaves	Preclinical *in vivo*	1 g/kg, *per os*	(35)
Diuretic	Aqueous	Leaves	Preclinical *in vivo*	100 mg/kg, *per os*	(36)
Depigmenting	Water-ethanol	Leaves	Preclinical *in vivo*	100 mg/kg, *per os*	(14)
	Methanolic	Leaves	Preclinical *in vitro,* tyrosinase test	NA	(15)
	Water-ethanol	Leaves	Preclinical, *in vitro,* tyrosinase test	NA	(17)
Anti-inflammatory	Water-ethanol	Leaves	Preclinical, *in vivo*	100 mg/kg, *per os*	(14)
	Aqueous	Leaves	Preclinical, *in vivo*	50 mg/kg, *per os* (in term of arbutin)	(48)
Antidiabetic	Water-ethanol	Leaves	Preclinical, *in vivo*	1g/kg	(52)
Neuroprotective	Water-ethanol	Leaves	Preclinical, *in vitro,* glial cell culture N-11	100 mg/ml	(53)
Antioxydant	Water-ethanol	Leaves	Preclinical, *in vitro,* ABTS и metoxyl-radicals	100 mg/ml	(54)
Antiproliferative	Water-ethanol	Leaves	Preclinical, *in vitro,* cell lines: MCF-7; HT-29; DU-145; SK-MEL; MDA-MB-435.	NA	(55)

NA = Not Available

CONCLUSION

The limited range of research on the antioxidant activity *in vitro* confirms the need to examine antioxidant activity also *in vivo*. The effects of biologically active substances from the bearberry extracts on the main pathological pro-inflammatory mechanisms (TLR-receptors, Nf-kB, TNF-α) may be a pre-condition for further investigation of the neuroprotective activity implemented via inhibitory of inflammatory reaction in the brain tissue. In addition, studies on the antidiabetic effects of *Arctostaphylos uva-ursi* extracts could allow expansion of the range of safe agents for complex therapy of diabetes and metabolic syndrome.

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REFERENCES

1 The Plant List. The Royal Botanic Gardens, Kew and Missouri Botanical Garden. http://www.theplantlist.org. Accessed 21 August 2021.

2 Takhtadjan A. Flowering Plants. Second ed. Springer Science & Business Media; 2009. p. 192.

3 Czerepanov SK. Vascular plants of Russia and neighboring countries (within the former USSR). Russian Edition. Word and Family-95, Saint-Petersburg; USSR, 1995. p. 424.

4 Tuniyev BS, Aliev KhU, Timukhin IN. Landscape-cenotic description of habitats of caucasian bearberry - *Arctostaphylos caucasica* Lipschitz in the greater Caucasus. Botanical Herald of the North Caucasus. Makhachkala, 1: 81-92, 2015.

5 Byng JW, Chase MW, Christenhusz MJM, Fay MF, Judd WS, Mabberley DJ et al. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Bot. J. Linn. Soc, 181: 1–20, 2016.

6 Komarov VL, Shishkin BK. Flora of the USSR. In: Komarov VL, Shishkin BK, Bobrov EG, editors. Volume 18. Publishing of Academy of Sciences of USSR, Leningrad, USSR, 1952. p. 83-84.

7 Abisheva LN, Belenovskaya LM, Bobileva NS, Bikova OP, Kondratenkova TD, Kudrova (Yakobson) EL et al. Useful wild plants of Russia. In: Budancev AL, Lesiovskaya EE, editors. SPCPA, St. Petersburg, Russian Federation, 2001. p. 252-253.

8 Keller E. Eine cerimetrische Arbutinbestimmung. Archiv der Pharmazie, 292 (8-9): 477-479, 1959.

9 Keller E. Beiträge zur maßanalytischen Bestimmung von Arbutin in Bärentraubenblättern. Archiv der Pharmazie 293(12): 1079-1084, 1960.

10 Jahodár L, Leifertova I. The evaluation of p-methoxyphenol in the leaves of *Arctostaphylos uva-ursi*. Pharmazie 1979; 34: 188-189.

11 Sticher O, Soldati F, Lehmann D. Hochleistungsflussigchromotographische Trennung und quantitative Bestimmung von Arbutin, Methylarbutin, Hydrochinon und Hydrochinonmonomethylaether in *Arctostaphylos*–, *Bergenia*–, *Calluna*–und *Vaccinium* Arten. Planta Medica 1979; 35(03): 253-261.

12 Karikas GA, Euerby MR, Waigh RD. Isolation of piceoside from *Arctostaphylos uva-ursi*. Planta medica, 53(03): 307-308, 1987.

13 Linnenbrink N, Kraus, L. *In Vitro*-Cultures of *Arctostaphylos*-Species. III: Phytochemical Comparison of *in Vitro*-Cultures and Outdoor Plants. Planta medica, 52(06): 511-511, 1986.

14 Kubo M, Ito M, Nakata H, Matsuda H. Pharmacological studies on leaf of *Arctostaphylos uva-ursi* (L.) Spreng. I. Combined effect of 50% methanolic extract from *Arctostaphylos uva-ursi* (L.) Spreng. (bearberry leaf) and prednisolone on immuno-inflammation. Yakugaku Zasshi = Journal of the Pharmaceutical Society of Japan, 110(1): 59-67, 1990.

15 Matsuda H, Nakamura S, Shiomoto H, Tanaka T, Kubo M. Pharmacological studies on leaf of *Arctostaphylos uva-ursi* (L.) Spreng. IV. Effect of 50% methanolic extract from *Arctostaphylos uva-ursi* (L.) Spreng. (bearberry leaf) on melanin synthesis. Yakugaku Zasshi: Journal of the Pharmaceutical Society of Japan, 112(4): 276-282, 1992.

16 Matsuo K, Kobayashi M, Takuno Y, Kuwajima H, Ito H, Yoshida T. Anti-tyrosinase activity constituents of *Arctostaphylos uva-ursi*. Yakugaku zasshi: Journal of the Pharmaceutical Society of Japan, 117(12):1028-1032, 1997.

17 Matsuda H, Higashino M, Nakai Y, Iinuma M, Kubo M, Lang FA. Studies of cuticle drugs from natural sources. IV. Inhibitory effect of some *Arctostaphylos* plants on melanin biosynthesis. Biol. Pharm. Bull, 19 (1): 153-156, 1996.

18 Parejo I, Viladomat F, Bastida J, Codina C. A single extraction step in the quantitative analysis of arbutin in bearberry (*Arctostaphylos uva-ursi*) leaves by high-performance liquid chromatography. Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques, 12(5): 336-339, 2001.

19 Lamien-Meda A, Lukas B, Schmiderer C, Franz C, Novak J. Validation of a quantitative assay of arbutin using gas chromatography in *Origanum majorana* and *Arctostaphylos uva-ursi* extracts. Phytochemical Analysis, 20(5): 416-420, 2008.

20 Alam P, Alqasoumi SI, Shakeel F, Abdel-Kader MS. HPTLC

densitometric analysis of arbutin in bulk drug and methanolic extracts of *Arctostaphylos uva-ursi*. Natural Product Research, 25(17): 1671-1675, 2010.

21 Roznyatovskaya AA, Senchenko SP, Kharakhashyan AA. Development and validation of the arbutin qualitative analysis in cowberries leaves and bearberry leaves using the capillary electrophoresis method. Modern problems of science and education, 6, 2014.

22 Kurkin VA, Ryazanova TK, Platonov IA, Pavlova LV. Quantitative determination of arbutin in the leaves of *Arctostaphylos uva-ursi* (L.) Spreng. Khimija rastitel'nogo syr'ja, 1: 95-100, 2015.

23 Shimizu M, Shiota S, Mizushima T, Ito H, Hatano T, Yoshida T et al. Marked Potentiation of Activity of β-Lactams against Methicillin-Resistant *Staphylococcus aureus* by Corilagin. Antimicrob Agents Chemother, 45(11): 3198-201, 2001.

24 Chauhan B, Yu C, Krantis A, Scott I, Arnason JT, Marles RJ et al. *In vitro* activity of uva-ursi against cytochrome P450 isoenzymes and P-glycoprotein. Canadian journal of physiology and pharmacology, 85(11): 1099-1107, 2007.

25 Olennikov DN, Chekhirova GV. 6 "-Galloylpicein and other phenolic compounds from *Arctostaphylos uva-ursi*. Chemistry of Natural Compounds, 49(1): 1-7, 2013.

26 Panusa A, Petrucci R, Marrosu G, Multari G, Gallo FR. UHPLC-PDA-ESI-TOF/MS metabolic profiling of *Arctostaphylos pungens* and *Arctostaphylos uva-ursi*. A comparative study of phenolic compounds from leaf methanolic extracts. Phytochemistry, 115: 79-88, 2015.

27 Wähner C, Schoönert J, Friedrich H Zur kenntnis des gerbstoffs der bärentraubenblatter (*Arctostaphylos uva-ursi* L.). Pharmazie. Jahrg, 29, H.9: 616-617, 1974.

28 Olennikov DN, Nazarova AV. Polysaccharides from *Arctostaphyllos uva-ursi*. Chemistry of Natural Compounds, 45(5): 702-704, 2009.

29 Jahodar L, Leifertova I, Lisa M. Investigation of iridoid substances in *Arctostaphylos uva-ursi*. Die Pharmazie,; 33(8): 536-537, 1978.

30 Takada, K., Nakane, T., Masuda, K., & Ishii, H. Ursolic acid and oleanolic acid, members of pentacyclic triterpenoid acids, suppress TNF-α-induced E-selectin expression by cultured umbilical vein endothelial cells. Phytomedicine, 17(14), 1114-1119, 2010.

31 Caligiani A, Malavasi G, Palla G, Marseglia A, Tognolini M, Bruni R. A simple GC–MS method for the screening of betulinic, corosolic, maslinic, oleanolic and ursolic acid contents in commercial botanicals used as food supplement ingredients. Food chemistry, 136(2): 735-741, 2013.

32 Radulović N, Blagojević P, Palić R. Comparative Study of the Leaf Volatiles of *Arctostaphylos uva-ursi* (L.) Spreng. And *Vaccinium vitis-idaea* L. (Ericaceae). Molecules, 15(9): 6168-85, 2010.

33 Afanasyeva LV, Ayushina TA. Accumulation and distribution of microelements in *Arctostaphylos uva-ursi*. Khimiya Rastitel'nogo Syr'ya, 3: 123–128, 2018.

34 de Arriba SG, Naser B, Nolte KU. Risk assessment of free hydroquinone derived from *Arctostaphylos Uva-ursi* folium herbal preparations. International journal of toxicology, 32(6): 442-453, 2013.

35 Grases F, Melero G, Costa-Bauza A, Prieto R, March JG. Urolithiasis and phytotherapy. International Urology and Nephrology, 26(5): 507-511, 1994.

36 Beaux D, Fleurentin J, Mortier F. Effect of extracts of *Orthosiphon stamineus* Benth, *Hieracium pilosella* L., *Sambucus nigra* L. and *Arctostaphylos uva-ursi* (L.) Spreng. in rats. Phytotherapy Research, 13(3): 222-225, 1999.

37 Vranješ M, Popović BM, Štajner D, Ivetić V, Mandić A, Vranješ D. Effects of bearberry, parsley and corn silk extracts on diuresis, electrolytes composition, antioxidant capacity and histopathological features in mice kidneys. Journal of Functional Foods, 21: 272-282, 2016.

38 Larsson B, Jonasson A, Fianu S. Prophylactic effect of UVA-E in women with recurrent cystitis: a preliminary report. Current therapeutic research, 53(4): 441-443, 1993.

39 Yarnell E. Botanical medicines for the urinary tract. World journal of urology, 20(5): 285-293, 2002.

40 Park JB, Kim D, Min JS, Jeong S, Cho DY, Zheng YF et al. Identification and characterization of in vitro inhibitors against UDP-glucuronosyltransferase 1A1 in uva-ursi extracts and evaluation of in vivo uva-ursi-drug interactions. Food and Chemical Toxicology, 120: 651-661, 2018.

41 Saeed F, Jahan N, Ahmad M. In vivo evaluation & safety profile evaluation of *Arctostaphylos uva-ursi* (L.) Spreng. extract in rabbits. Pakistan journal of pharmaceutical sciences, 27(6): 2197-2205, 2014.

42 Afshar K, Fleischmann N, Schmiemann G, Bleidorn J, Hummers-Pradier E, Friede T et al. Reducing antibiotic use for uncomplicated urinary tract infection in general practice by treatment with uva-ursi (REGATTA)–a double-blind, randomized, controlled comparative effectiveness trial. BMC complementary and alternative medicine, 18(1): 1-8, 2018.

43 Moore M, Trill J, Simpson C, Webley F, Radford M, Stanton L et al. Uva-ursi extract and ibuprofen as alternative treatments for uncomplicated urinary tract infection in women (ATAFUTI): a factorial randomized trial. Clinical Microbiology and Infection, 25(8): 973-980, 2019.

44 Cybulska P, Thakur SD, Foster BC, Scott IM, Leduc RI, Arnason JT, Dillon JAR. Extracts of Canadian first nations medicinal plants, used as natural products, inhibit *Neisseria gonorrhoeae* isolates with different antibiotic resistance profiles. Sexually transmitted diseases, 38(7): 667-671, 2011.

45 Holopainen M, Jahodar L, Seppänen-Laakso T, Laakso I, Kauppinen V. Antimicrobial activity of some Finnish Ericaceous plants. Acta Pharmaceutica Fennica, 97(4): 197-202, 1988.

46 Annuk H, Hirmo S, Türi E, Mikelsaar M, Arak E, Wadström T. Effect on cell surface hydrophobicity and susceptibility

of Helicobacter pylori to medicinal plant extracts. FEMS Microbiology Letters, 172(1): 41-45, 1999.

47 Tolmacheva AA, Rogozhin EA, Deryabin DG. Antibacterial and quorum sensing regulatory activities of some traditional Eastern-European medicinal plants. Acta Pharmaceutica, 64(2): 173-186, 2014.

48 Matsuda H, Tanaka T, Kubo M. Pharmacological studies on leaf of *Arctostaphylos uva-urs*i (L.) Spreng. III. Combined effect of arbutin and indomethacin on immuno-inflammation. Yakugaku zasshi: Journal of the Pharmaceutical Society of Japan, 111(4-5): 253-258, 1991.

49 Schink A, Neumann J, Leifke AL, Ziegler K, Fröhlich-Nowoisky J, Cremer C et al. Screening of herbal extracts for TLR2- and TLR4-dependent anti-inflammatory effects. PLoS One,13(10) :e0203907, 2018.

50 Naczk M, Pegg RB, Amarowicz R. Protein-precipitating capacity of bearberry-leaf (*Arctostaphylos uva-ursi* L. Sprengel) polyphenolics. Food chemistry 2011; 124(4): 1507-1513. doi: 10.1016/j.foodchem.2010.08.003

51 National Center for Biotechnology Information. PubChem Patent Summary for US-5980904-A. https://pubchem.ncbi.nlm.nih.gov/patent/US-5980904-A. Accessed 21 August 2021.

52 Swanston-Flatt SK, Day C, Bailey CJ, Flatt PR. Evaluation of traditional plant treatments for diabetes: studies in streptozotocin diabetic mice. Acta diabetologia Latina, 26(1): 51-55, 1989.

53 Chandler D, Woldu A, Rahmadi A, Shanmugam K, Steiner N, Wright E. et al. Effects of plant-derived polyphenols on TNF-α and nitric oxide production induced by advanced glycation endproducts. Molecular nutrition & food research, 54(S2): S141-S150, 2010.

54 Mohd Azman NA, Gallego MG, Segovia F, Abdullah S, Shaarani SM, Almajano Pablos MP. Study of the Properties of Bearberry Leaf Extract as a Natural Antioxidant in Model Foods. Antioxidants (Basel), 5(2): 11, 2016.

55 Amarowicz R, Pegg RB. Inhibition of proliferation of human carcinoma cell lines by phenolic compounds from a bearberry-leaf crude extract and its fractions. J. Funct. Foods, 5: 660–667, 2013.