PHARMACIA

Pharmacia 71: 1–12
DOI 10.3897/pharmacia.71.e124248

Review Article

The genus *Rubus* L.: An insight into phytochemicals and pharmacological studies of leaves from the most promising species

Reneta Gevrenova[1], Dimitrina Zheleva-Dimitrova[1], Vessela Balabanova[1]

1 *Faculty of Pharmacy, Medical University-Sofia, Sofia, Bulgaria*

Corresponding author: Reneta Gevrenova (rgevrenova@pharmfac.mu-sofia.bg)

Received 30 March 2024 ♦ **Accepted** 7 May 2024 ♦ **Published** 20 June 2024

Citation: Gevrenova R, Zheleva-Dimitrova D, Balabanova V (2024) The genus *Rubus* L.: An insight into phytochemicals and pharmacological studies of leaves from the most promising species. Pharmacia 71: 1–12. https://doi.org/10.3897/pharmacia.71.e124248

Abstract

Rubus L. species (Rosaceae) are traditionally used worldwide for their food and medicinal properties. Although raspberries and blackberries are well-known fruits, the leaves hold significant but often overlooked value. The review is focused on the phytochemicals and pharmacological studies on leaves from the most promising taxa. Through a comprehensive search of the MEDLINE, Scopus, and Web of Science databases, numerous research articles were identified. The studies revealed over 160 diterpenoids, triterpenoid acids, saponins, ellagitannins, phenolic and acylquinic acids, and flavonoids in the discussed *Rubus* species. These compounds contribute to the leaves' protective effects, including astringent, hypoglycemic, and wound healing activity. Moreover, *Rubus* leaves are used for relieving diarrhea as well as in the treatment of ulcerative colitis, owing to their anti-inflammatory and antioxidant properties. This review highlights *R. sanctus*, *R. ibericus*, and *R. chingii*, along with *R. idaeus*, as prospective raw materials for therapeutic applications.

Keywords

Rubus species, leaves, phytochemical composition, pharmacological activity

Introduction

The genus *Rubus* L. comprises over 700 species, including raspberries, blackberries, and related hybrids (Graham and Brennan 2018). There are some difficulties in the classification of the taxon due to hybridization, but molecular studies have made a significant contribution to the phylogeny of the genus *Rubus*. A detailed list of species, subgenera, and sections is given by Skirvin et al. (2005). There are 12 subgenera; the two subgenera of greatest economic importance are Idaeobatus and Eubatus. Idaeobatus includes the European red raspberry (*R. idaeus* L. subsp. *idaeus*), the North American raspberry (*R. idaeus* subsp. *strigosus* Michx.), and the black raspberry (*R. occidentalis* L.). The cultivation of raspberries began in the 1920s and then developed intensively in the period 1981–2001, when 100 cultivars were produced.

The traditional medicinal uses, nutraceutical significance, bioactive components, and economical exploitation of *Rubus* species related to the fruits are the subject of literature reviews by Patel et al. (2004), Verma et al. (2014), Zia-Ul-Haq et al. (2014), Bhatt et al. (2023), and Tao et al. (2023). The progress of the pharmacological activity, chemical composition, and quality control of *R. chingii* and *R. idaeus* are summarized in the literature reviews by Yu et al. (2019) and Tao et al. (2023), respectively. Zia-Ul-Haq et al. (2014) and Verma et al. (2014) summarize information on nutritional composition, pharmacological research,

and agronomic practices and processing of *R. fruticosus* berries. Raspberry and blackberry are high-value crops that are sought after as functional foods and are rich sources of carbohydrates, amino acids, anthocyanins, and ellagic acid. Raspberry and blackberry leaves may be considered byproducts of berry cultivation. However, they remain understated, and their importance is unrecognized. The aforementioned reviews emphasize the ethnomedicinal application of *Rubus* species leaves. They are renowned for the relief of diarrhea, menstrual cramps, and labor pain (Ferlemi and Lamari 2016; Tao et al. 2023). Scarce data on the phytochemicals and biological activity of *R. fruticosus* leaves were summarized by Zia-Ul-Haq et al. (2014) and Verma et al. (2014), where Ferlemi and Lamari (2016) reviewed qualitative and quantitative data on the phenolic compounds in raspberry and blackberry leaves. As highlighted in recent studies, the protective effects of raspberry and blackberry leaves have been demonstrated in a number of experimental models, with evidence suggesting that they are astringent, antioxidant, and anti-inflammatory agents. Noteworthy progress has been made in understanding the mechanisms underlying the anti-inflammatory, antimicrobial, and hypoglycemic effects of *Rubus* leaves. There is no survey summarizing the phytochemicals and pharmacological studies on *Rubus* species leaves.

This work aims to evaluate the recent progress of secondary metabolites and pharmacological studies on *Rubus* leaves, with a focus on the most promising species. The MEDLINE, Scopus, and Web of Science databases were searched to identify research papers.

The leaves of *Rubus* species – an alternative source of bioactive compounds

An overview of the main classes of secondary metabolites, with a special focus on leaf composition and their distribution in *Rubus* species, is presented in Table 1.

Diterpenoids and triterpenoids

The diterpenoid glycoside rubusoside (1) has been isolated from *R. chingii* and *R. suavissimus* leaves, and it is 115 times sweeter than sugar (Fig. 1). The aforementioned compound is a major diterpenoid glycoside in the *R. suavissimus* leaves, along with other kaurane-type diterpenoid glycosides (10–20) (Chaturvedula et al. 2012). The rubusoside's aglycone is the diterpenoid *ent*-13-hydroxykaur-16-ene-19-oic acid, known as steviol. More than 15 diterpenoid glycosides have been isolated from the *R. chingii* leaves, mainly labdane-type goshonosides (2–8) (Yu et al. 2019) and kaurane-type (9–20) (Zhang et al. 2017).

Pentacyclic triterpenoids of oleanane- and ursane-types have been reported (Table 1, Fig. 1). Exclusively in *R. moluccanus* aerial parts, the oleanane-type analogs rubonic (21) and rubusic acids (22) have been isolated (Patel et al. 2004). Ursolic acid (29) was accompanied by its various derivatives: 3-oxo analog 7 α-hydroxy-3-oxo-12-ursen-28-oic (rubinic) acid (25) as well as 3, 19-dihydroxy and 2, 3, 19-trihydroxy analogs of usrolic acid: pomolic (31), euscaphic (30), and tormentic acid (28) (Patel et al. 2004; Haq et al. 2014; Yu et al. 2019). Taking into consideration the stereoisomerism (α/β mainly at C-3), pairs of isomers were observed among the triterpenoid acids.

Triterpenoid saponins have been isolated from the aerial parts of *Rubus* taxa, including 28-glucosyl esters of triterpenoid acids, mainly of the ursane-type mono-, di-, tri-, and tetrahydroxyurs-12-ene-28 acids (Table 1). Their analogs with an additional double bond at C-18 (di- and trihydroxyurs-12, 18-diene-28-oic acid) were found, as well as derivatives with a supplementary carboxyl group (2α, 3β, 19α-trihydroxyurs-12-ene-23, 28-dicarboxylic acid). Among them were suavissimoside R1 (35) and niga-ichinoside F1 (36) (Table 1). An ursane-type triterpenoid saponin 2α, 3α, 19α, 24 tetrahydroxyurs-12-ene-28-carboxylic acid-28-*O*-β-glucopyranosyl ester (37) was isolated from *R. sanctus* (Tuzlacı and Gürkan 2004). A series of triterpenoid acids along with their hexosyl esters were tentatively identified in *R. sanctus* and *R. ibericus* leaves by LC-Orbitrap-HRMS (Zengin et al. 2019). Thus, triterpenoid acids were ascribed to trihydroxyurs-12-en-28 carboxylic acid and tetrahydroxyurs-12-en-28 carboxylic acid, accompanied by analogs with an additional carboxylic group: dihydroxyurs-12-en-23, 28 dicarboxylic acid, and trihydroxyurs-12-en-23, 28 dicarboxylic acid. It should be noted the presence of isobaric pairs assigned as either urs-12-en or olean-12-en possessing α/β C-3 hydroxyl group (Zengin et al. 2019), as was observed in *R. crataegifolius* and *R. aleaefolius* leaves (Nam et al. 2007; Guo et al. 2013).

Ellagitannins

Rubus species are especially rich sources of ellagitannins (ET) and ellagic acid (57) conjugates (Macierzynski et al. 2020). ET are hydrolysable tannins, esters of hexahydroxydiphenic acid (HHDP) with a polyol, usually glucose (Pouysegu et al. 2011). According to Okuda's classification, ET from the *Rubus* genus refers to the type II hydrolysable tannins containing HHDP (Okuda et al. 2000). The characteristic monomer of *Rubus* ET is galloyl-bis-HHDP glucoside (61). Oligomeric ET sanguine H-6 (56) (with 2 galloyl-bis-HHDP-glucose units) and lambertianin C (51) (with 3 galloyl-bis-HHDP-glucose units) were among the first ETs to be thoroughly characterized (Tanaka et al. 1993). ET, together with ellagitannin monomers and EA (57), were commonly found in the leaves of *Rubus* species (Table 1). The sugar esters 3, 6-di-*O*-caffeoyl-glucose, and 1-*O*-caffeoyl-hexose, together with the ET 2,3-HHDP-glucose, bis-2,3,4,6-HHDP-glucose (pedunculagin) (53) and 2,3-*O*-HHDP-4,6-*O*-sanguisorbyl-glucose, were isolated from an hydroalcoholic extract of *R. sanctus* aerial parts (Hussein et al. 2003). In addition, sanguine-10 isomers were annotated by LC-HRMS in both *R. sanctus* and *R. ibericus* leaves for the first time, along with bis-HHDP-hexose (unpublished data of the authors, R.G.).

Table 1. Main classes of secondary metabolites and compounds in *Rubus* leaves.

COMPOUNDS	*RUBUS* SPECIES	REFERENCES
1.1. Diterpenoids		
13-*O*-β-D-glucosyl-steviol-β-D-glucosyl ester (rubusoside) (**1**)	*R. suavissimus*, *R.chingii*	Tanaka et al. 1981
Labdane-type diterpenoid glycosides		
3α, 15, 18-ent-labda-8(17),13-dien-18-*O*-β-glucopyranoside (goshonoside 1) (**2**), goshonoside 2-5 (**3**–**6**), goshonoside 6–7 (**7**–**8**)	*R. suavissimus*, *R.chingii*, *R. foliolus*, *R.chingii*	Patel et al. 2004; Yu et al. 2019; Zhang et al. 2017
Ent-Kaurane-type diterpenoid glycosides		
ent-16α,17-dihydroxy-kauran-19-oic acid 16β,17-dihydroxy-3-one-kauran-17-O-β-glucopyranoside (sugeroside) (**9**); Suavioside A (**10**), B (**11**), C1 (**12**), D1 (**13**), D2 (**14**), E-J (**15**–**20**)	*R. suavissimus*, *R. chingii*	Ohtani et al. 1992; Zhang et al. 2017; Chaturvedula et al. 2012
1.2. Triterpenoids		
Oleanane-type triterpenoid acids		
3,7-diketo-olean-12-ene-28-oic (rubonic) acid (**21**)	*R. moluccanus*	Patel et al. 2004
3β,7α-dihydroxyolean-12-ene-28-oic (rubusic) acid (**22**)	*R. moluccanus*	Patel et al. 2004
iexosapogenin A (**29**), barrinic acid (**30**), arjunolic acid (**23**), hydroxygypsogenic acid (**24**)	*R. ibericus*, *R. sanctus*	Zengin et al. 2019
Ursane-triterpenic acids		
7 α-hydroxy-3-oxo-12-ursen-28-oic (rubinic) acid (**25**)	*R. fruticosus*	Zia-Ul-Haq et al. 2014
7α-hydroxyursolic (rubitic) acid (**26**)	*R. fruticosus*	Zia-Ul-Haq et al. 2014
2 α,3β,19α-trihydroxyurs-12-ene-23,28-oic acid (**27**)	*R. aleaefolius*	Guo et al. 2013
2 α,3β,19α-trihydroxyurs-12-ene-28-oic (tormentic acid) (**28**)	*R. pinfaensis*, *R. moluccanus*, *R. ellipticus*, *R. sieboldii*, *R. cochichinensis*, *R. aleaefolius*	Patel et al. 2004; Guo et al. 2013
3β-hydroxyurs-12-en-28-oic (ursolic acid) (**29**)	*R. pinfaensis*, *R. fruticosus*,	Patel et al. 2004;
2 α,3α,19α-trihydroxyurs-12-ene-28-oic (euscaphic) acid (**30**)	*R. pinfaensis*, *R. sieboldii*, *R. chingii*, *R. aleaefolius*	Patel et al. 2004; Guo et al. 2013
3β,19α-dihydroxyurs-12-ene-28-oic (pomolic) acid (**31**) 3-*O*-*p*-coumaroyl-pomolic acid (**32**)	*R. aleaefolius*	Guo et al. 2013
2α-hydroxyursolic acid (**33**)	*R. fruticosus*,	Patel et al. 2004;
19α- hydroxyursolic acid (**34**)	*R. coreanus*, *R. microphylus*	Patel et al. 2004
Triterpenoid saponins		
2 α,3β,19α-trihydroxyurs-12-ene-23, 28-dicarboxylic acid-28-*O*-β-D-glucopyranosyl ester (Suavissimoside R1) (**35**)	*R. crataegifolius*, *R. aleaefolius*	Guo et al. 2013
23-hydroxytormentic acid-28-*O*-β-D-glucopyranosyl ester (niga-ichigoside F1) (**36**)	*R. imperialis*, *R. microphyllus*, *R. suavissimus*, *R. ellipticus*, *R. crataegifolius*	Patel et al. 2004; Tonin et al. 2016;
2α,3α,19α,23-tetrahydroxyurs-12-ene-28-oic acid 28-*O*-β-D-glucopyranosyl ester (niga-ichigoside F2) (**37**)	*R. sanctus*	Tuzlacı and Gürkan 2004
ilexosapogenin A-hexoside (**38**), barrinic acid-hexoside (**39**), hydroxygypsogenic acid-hexoside (**40**), arjunolic acid-hexoside (**41**)	*R. ibericus*, *R. sanctus*	Zengin et al. 2019
Dimeric glucosyl ester of oxidized in a ring A 19α-hydroxyursolic acid (coreanoside F1) (**42**)	*R. coreanus*	Patel et al. 2004
3β,19α-dihydroxyurs-12-ene-24, 28-dicarboxylic acid-28-*O*-(6'-*O*-methyl)-β-D-glucopyranosyl ester (**43**); 2α,3β,19α-tri-hydroxyurs-12-ene-24 (**44**); 28-dicarboxylic acid-28-*O*-(3'-*O*-methyl)-β-D-glucopyranosyl ester (**45**); 2 α,3β,19α-trihydroxyurs-12-ene-24, 28-dicarboxylic acid-28-*O*-(6'-*O*-methyl)-β-D-glucopyranosyl ester (**46**)	*R. pileatus*	Patel et al. 2004
1α,2α,3β,19α-tetrahydroxyurs-12-ene-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**47**); 2α,3β,19α, 24-tetrahydroxyurs-12-ene-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**48**)	*R. xanthocarpus*	Patel et al. 2004
1.3. Ellagitannins		
Lambertianin A (**49**), B (**50**) – ellagitannin dimers	*R. lambertianus*	Patel et al. 2004
Lambertianin C (**51**) – ellagitannin trimer	*R. lambertianus*, *R. crataegi-folius*, *R. chingii*, *R. parvi-folius*, *R. palmatus*, *R. idaeus*, *Rubus sanctus* and *R. ibericus*	Spinola et al. 2019; Yu et al. 2019; Zengin et al. 2018
Lambertianin D (**52**) – ellagitannin tetramer	*R. lambertianus*, *R. crataegifolius*, *R. chingii*, *R. parvifolius*, *R. palmatus*, *R. idaeus*, and others	Patel et al. 2004; Spinola et al. 2019; Yu et al. 2019
Ellagitannin monomers: pedunculagin (**53**), rubiphenol (**54**), sanguine H-2 (**55**)	*R. lambertianus*, *R. eleaefolius*, *R. caesius*, *R. ulmifolius*	Patel et al. 2004; Spinola et al. 2019
Sanguine H-6 (**56**) - ellagitannin dimer	*R. lambertianus*, *R. crataegifolius*, *R. parvifolius*, *R. palmatus*, *R. idaeus* and others	Patel et al. 2004
Ellagic acid (EA) (**57**), EA-pentoside (**58**), EA-hexoside (**59**), EA-deoxyhexoside (**60**)	*R. grandofolius*, *Rubus sanctus* and *R. ibericus*	Zengin et al. 2019
Galloyl-bis-hexahydroxyphenoylhexoside (**61**)	*Rubus sanctus* and *R. ibericus*	Zengin et al. 2019

COMPOUNDS	*RUBUS* SPECIES	REFERENCES
1.4. Flavonoids		
***Flavonols*:** kaempferol (**62**), quercetin (**63**), myricetin (**64**)**,** isorhametin (**65**), kaempferide (**66**), ***Glycosides of kaempferol* (*Km*):** astragalin (**76**), Km 3-*O*-galactoside (trifoline) (**68**), Km 3-*O*-β-D-(6"-*O*-(E)-p-coumaryl)-glucoside (tiliroside) (**69**), Km-3-*O*-rutinoside (**70**), Km-3-*O*-glucuronide (**71**), Km 3-*O*-(6'-acetyl)-glucoside (**72**), Km-*O*-dihexoside (**73**), Km-2"-*O*-pentosylhexoside (**74**), Km-*O*-caffeoylhexoside (**75**), Km 3-*O*-(2"-*O*-hexosyl)-hexoside (**76**), Km *O*-pentoside (**77**), Km 3-*O*-[6"-*O*-(3-hydroxy-3-methylglutaryl)]-hexoside (**78**), Km 7-*O*-(6"-*O*-rhamnosyl)-hexoside (**79**), Km 3-*O*-(2"-*O*-pentosyl)-hexuronide (**80**) ***Glycosides of quercetin* (*Qu*):** Qu 3-galactoside (hyperoside) (**81**), Qu 3-glucuronide (miquelianin) (**82**), rutin (**83**), Qu *O*-pentoside (**84**), Qu-*O*-acetylhexoside (**85**), quercitrin (**86**), Qu-methoxyhexoside (**87**), isoquercitrin (**88**), Qu-3-[6"-(3-Hydroxy-3-methylglutaryl)-galactoside] (**89**), Qu 3-*O*-(2"-*O*-rhamnosyl)-hexuronide (**90**), Qu 3-*O*-(6"-*O*-coumaroyl)-hexoside (**91**), Quercetagetin-7-*O*-glucopyranoside (**92**), Qu-3-methyl ether (**93**) ***Glycosides of isorhamnetin* (*Isorh*)** Isorh-glucuronide (**94**), Isorh-hexoside (**95**), Isorh 7-*O*-glucuronopyranoside (**96**), Isorh-pentoside (**97**) ***Flavan 3-ols*:** catechin (**98**), epicatechin (**99**), epigallocatechin (**100**), gallocatechin (**101**) and isomers, epicatechin gallate (**102**), methylgallate (**103**), proanthocyanidin dimer (type B) (**104**) ***Flavones*:** apigenin-and luteolin-7-*O*-glucuronide (**105**, **106**)**,** Luteolin (Lu) (**107**), Lu-*O*-dihexoside (**108**), Lu-O-hexuronide (**109**), Lu-7-O-glucoside (**110**), 7, 3', 4'-trihydroxyflavone (**111**) ***Flavanones*:** naringin (**112**) ***Anthocyanins*:** cyanidin 3-*O*-glucoside (**113**)	*R. fruticosus*, *R. ulmifolius*, *R. hirsutus*, *R. idaeus*, *R. caesius*, *R. grandifolius*, *R. suavissimus*, *R. corchorifolius*, *R. sanctus*, *R. ibericus*, 26 wild *Rubus* species 22 accessions of the Bulgarian raspberry germplasm collection *R. idaeus* cultivars (Willamette, Tulameen, Meeker) and *R. fruticosus* cultivar Cacanska Bestrna *R. sanctus* and *R. ibericus*	Patel et al. 2004; Chaturvedula et al. 2012; Gevrenova et al. 2013; Oszmiański et al. 2015; Ferlemi and Lamari 2016; Pavlovic et al. 2016; Spinola et al. 2019; Grochowski et al. 2020; Tian et al. 2021 ; Zengin et al. 2019
1.5. Phenolic acids and derivatives		
***Hydroxybenzoic acids*:** gallic (**114**), gentisic (**1115**), vanillic (**116**), *p*-hydroxybenzoic (**117**), protocatechuic (**118**), dihydroxybenzoic acid-hexoside/pentosylpentoside (**119**, **120**), gallocatechin gallate (**121**), epigallocatechin gallate (**122**), hexosides of gallic, protocatechuic and gentisic acid (**123**-**125**), ***Hydroxycinnamic acids*:** caffeic acid (**126**), caffeic acid-pentoside (**127**), caffeic acid-hexoside (**128**), caffeic acid-*O*-galloylhexoside (**129**), ferulic acid (**130**), ferulic acid-hexoside (**131**), *p*-coumaric acid (**132**), *m*-, *p*-coumaric acid (**133**, **134**) and their hexosides (**135**, **136**), dicaffeoyl-hexoside (**137**), caffeoyl-dihexoside (**138**)	*R. fruticosus*, *R. grandifolius*, *Rubus sanctus*, and *R. ibericus*, 26 wild species *Rubus*, *R. idaeus* cultivars (Willamette, Tulameen, Meeker) and *R. fruticosus* cultivar Cacanska Bestrna	Ferlemi and Lamari 2016; Spinola et al. 2019; Zengin et al. 2019; Oszmiański et al. 2015; Pavlovic et al. 2016
***Caffeoylquinic* (*CQA*)*, feruloylquinic* (*FQA*)*, p-coumaroylquinic* (*p-CoQA*)*acids*:** chlorogenic (5-CQA) (**139**), neochlorogenic (3-CQA) (**140**), methyl-4(5)-O-caffeoylquinate (**141**), methyldicaffeylquinate (**142**), dicaffeoylthreonic acid (**143**), feruloyl-tartaric acid (**144**), coumaroylhexaric acid (**145**), 4-CQA (**146**), 1-CQA (**147**), 3,4-diCQA (**148**), 3,5-diCQA (**149**), 4,5-diCQA (**150**), 3-p-CoQA (**151**), 4-p-CoQA (**152**), 5-p-CoQA (**153**), 1-FQA (**154**), 3-FQA (**155**), 4-FQA (**156**), 5-FQA (**157**), 3-F-5-CQA (**158**), 4-F-5-CQA (**159**), 1-C-5-FQA (**160**), 3-C-5-FQA (**161**), 3,4,5-triCQA (**162**)	*R. idaeus* cultivars (Willamette, Tulameen, Meeker) and *R. fruticosus* cultivar Cacanska Bestrna, *Rubus sanctus* and *R. ibericus*	Pavlovic et al. 2016; Zengin et al. 2019
1.6. Others		
methylbrevifolin carboxylate (**163**)	*R. caesius*	Grochowski et al. 2020

The study of *R. grandifolius* leaves showed that they consist mainly of ellagitannins (44–49%) (Spinola et al. 2019), which correlates with the LC-QTOF/MS analysis of 26 wild *Rubus* species (Oszmiański et al. 2015). In the latter study, the total content of phenolic compounds varied from 83 mg/g dry weight (dw) (*R. austroslovacus*) to 334.24 mg/g (*R. perrobustus*). The highest amount was found for the ET, from 51.59 mg/g dw (*R. austroslovacus*) to 255.01 mg/g in *R. wimmerianus* (the average value in the studied species was 165.84 mg/g) (Oszmiański et al. 2015). Thus, ETs were the predominant compounds in the leaf extracts. Raspberry leaves are particularly rich in ET and gallotannins, ranging from 2.6% to 6.9% (w/w), and the main compound is ellagic acid (up to 2.53%) (Gudej and Tomczyk 2004).

Among the ET, the dimers sanguine H-6 (56), H-10, and the trimers lambertianin D (52) and C (51), as well as methyl gallate, have been identified (Ferlemi and Lamari 2016). Brevifolin derivatives, including brevifolincarboxilic acid and methylbrevifolin, have been annotated in the *R. sanctus* and *R. ibericus* leaves (unpublished data of the authors, R.G.). Brevifolin analogs have been rarely discovered in *Rubus* taxa, i.e., *Rubus idaeus var.* reveille leaves (Yang et al. 2020), *R. caesius* (Grochowski et al. 2020), and *R. saxatilis* (Gudej et al. 1998). The biogenesis of brevifolincarboxilic acid was related to the transformation of DHHDP (dehydrohexahydoxydiphenoyl) residue (Pouysegu et al. 2011).

Flavonoids

Raspberry and blackberry leaves contain a variety of phenolic compounds, including hydroxybenzoic and hydroxycinnamic acids and their derivatives, flavonols,

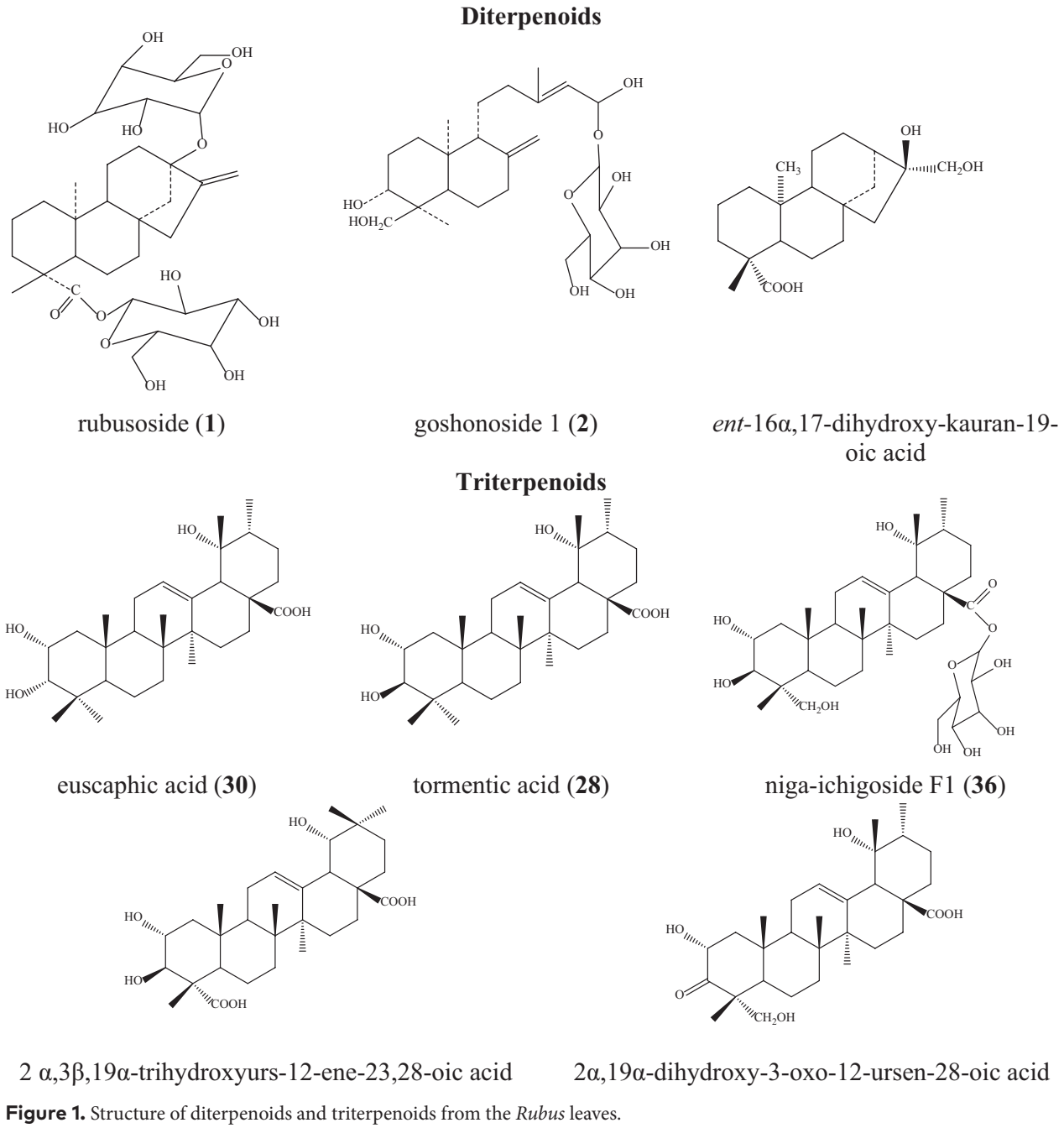

Figure 1. Structure of diterpenoids and triterpenoids from the *Rubus* leaves.

flavanols, anthocyanins, ET, and proanthocyanidins (Ferlemi and Lamari 2016).

Raspberry leaves are an especially rich source of flavonols such as kaempferol (Km) (62), quercetin (Qu) (63), and their respective glycosides, achieving up to 1.05% (Gudej and Tomczyk 2004). *R. grandifolius* leaves contained flavonols (12–16%), flavanols (2–10%), and flavones (0.28–0.32%) (Spinola et al. 2019). The content of flavonols and flavones including Qu (63), Km (62), luteolin (Lu) (108) and apigenin (Api) (105) derivatives has been estimated from 8.68 mg/g in the leaves of *R. macrophyllus* to 61.27 mg/g in *R. crispomarginatus* (average value in the studied species as 35.17 mg/g) (Oszmiański et al. 2015). The distribution of flavonoids showed that only 7 flavonoids (out of 17) were found in all assayed species in the aforementioned study. Rarely occur Km-3-*O*-rhamnosylglucoside-7-*O*-rhamnoside (in 9 of 26 species); Qu-3-[6”-(3-hydroxy-3-methylglutaryl)-galactoside] (89) (13 species); and Qu 3-*O*-pentoside (84) in 14 species. Km 3-*O*-glucuronide (71) (average content 9.23 mg/g) and Qu 3-*O*-glucuronide (miquelianin) (82) (7.00 mg/g) are in the greatest quantity.

Gudej and Tomczyk (2004) established the highest content of aglycones after hydrolysis in the *R. nessensis* leaves (1.06% dw) and the lowest in the *R. fruticosus* leaves (0.34% dw) (HPLC). Generally, the quantity of flavonoids in the *R. idaeus* leaves is significantly higher than that in the fruits, where flavonoids present a small extent of the bioactive compounds; leaf flavonoids range from 0.46% to 1.05% (w/w) (Ferlemi and Lamari 2016).

Rutin (83) was the most abundant flavonol, ranging between 157.25 and 585.89 mg/kg in the leaves of raspberry and blackberry cultivars (Pavlovic et al. 2016). Among the flavan-3-ols, a higher content of catechin (98) has been assessed in the raspberry leaves (up to 1913 mg/kg) compared to the blackberry leaves (739 mg/kg). The results from the aforementioned study suggest that the cultivar "Willamette" leaves, especially rich in flavan-3-ols, could be used as a substitute for *Camelia sinensis* tea (Pavlovic et al. 2016).

Galloyl esters of flavonols were isolated from the hydroalcoholic extract of *R. sanctus* leaves: Km-, Qu-, and myricetin-3-*O*-(6"-galloyl)-β-D-galactopyranoside (Badr et al. 2009). Coumaroylhexoside derivatives of flavonols are present in *Rubus* leaves, including Qu and Km 3-*O*-(6"-*O*-p-coumaroyl)-glucoside (tiliroside) (69) (Pavlovic et al. 2016). In the study of 22 accessions of Bulgarian raspberry germplasm collection, the highest content of isoquercitrin (88), tiliroside (69), and hyperoside (81) was assessed in the Bulgarian elite clones: 1.70, 0.60, and 0.97 mg/kg, respectively (Gevrenova et al. 2013). Despite a great variety of flavonoids annotated in *R. sanctus* and *R. ibericus* leaves by LC-HRMS, the profiles are dominated by Qu- and Km-hexuronide and tiliroside (69) (Zengin et al. 2019), as was seen in wild species and cultivars (Guo et al. 2013; Oszmianski et al. 2015; Pavlovic et al. 2016; Grochowski et al. 2018, 2020). Both flavonols, miquelianin (82) and tiliroside (69), are considered chemophenetic markers in the *Rubus* genus (Grochowski et al. 2020).

Phenolic acids

Derivatives of caffeic, *p*-coumaric, and ellagic acid were typical for the *Rubus* leaves, with an average content of 28.74 mg/g (Ferlemi and Lamari 2016). EA (57) was found to be the main phenolic acid in the leaves of raspberry and blackberry cultivars, ranging between 1574 and 2875 mg/kg dw (Pavlovic et al 2016). The leaves of the wild *Rubus* species were especially rich in *p*-coumaric acid (132) and neochlorogenic acid (140) (Oszmiański et al. 2015). The leaves of raspberry cultivars possessed a higher quantity of chlorogenic (139) and p-hydroxybenzoic acid (117) (up to 169.74 and 63.73 mg/kg dw, respectively) in comparison with the blackberry accessions (Pavlovic et al. 2016). Caffeic acid (126) was the predominant phenolic acid in the leaves of a Bulgarian raspberry germplasm collection, being up to 1.43 mg/g dw in a Bulgarian elite clone (Gevrenova et al. 2013), while in other studies of raspberry leaves, a significantly lower content of caffeic acid (126) was reported (0.55 mg/g dw) (Ferlemi and Lamari 2016). In-depth studies of the phenolic compounds by LC-HRMS revealed a variety of phenolic acids and derivatives in the *Rubus* leaf extracts (Pavlovic et al. 2016; Zengin et al. 2019). Thus, numerous hydroxycinnamic acid esters with hexaric, quinic, tartaric, and treonic acids were annotated. Accordingly, a series of acylquinic acids were tentatively identified in *R. sanctus* and *R. ibericus* leaves, dominated by monoacylquinic acids (neochlorogenic (140), 4-caffeoylquinic (146), and 3-*p*-coumaroylquinic acid (151), and diacylquinic acids (3,5- and 4,5-dicaffeoylquinic acid (149 and 150)) (Zengin et al. 2019). In this study, the feruloylquinic and feruloylcaffeoylquinic acids were minor compounds.

Waxes

The waxes form a microcrystalline layer on the leaf surface and include long-chain esters (*R. idaeus*); in the young leaves, there are more C23 and C25 homologues and less C29. The main components are primary alcohols, monounsaturated alcohols C24-C34, their acetates, long-chain saturated esters C36-C54, and fatty acids C12-C32 (Patel et al. 2004).

Ethnobotanical data

Hummer (2010) published detailed ethnobotanical data on the use of the species from ancient times to the present, revealing the usage of *Rubus* leaves as plastered to constrain shingles, head scurf, prolapsed eyes, and hemorrhoids.

Young shoots of *Rubus* species are traditionally used to heal wounds and insect bites (Süntar et al. 2011). *R. fruticosus* aerial parts are applied for cough; the juice of the fruits is recommended for colitis; and the decoction of the roots is recommended for diarrhea and dysentery (Verma et al. 2014). The leaf decoction of the species is used as a mouthwash for inflammation of the gums, throat, and mouth ulcers; a poultice of the leaves is applied to abscesses and skin ulcers as an astringent (Zia-Ul-Haq et al. 2014). There are data on the application of leaves, roots, and fruits of *R. ibericus* (synonym *R. discolor*) in nephritis and prostatitis, and the leaves for wound healing and diarrhea (Veličković et al. 2016). *R. caesius* leaves are used in folk medicine for diarrhea, inflammation, mainly of the mouth and throat, and as a hypoglycemic agent (Groshowski et al. 2020).

In Traditional Chinese Medicine (TCM), *R. chingii* is used for sterility, impotence, back pain, and impaired vision (Bakar et al. 2016). It is applied as a tonic for enuresis, micturition, and kidney deficiency (Yu et al. 2019; Tao et al. 2023). The species is included in a number of Chinese medical monographs, including "Compendium of Materia Medica," "Bencao Mengquan," and others. (Yu et al. 2019). Alcoholic infusions and decoctions of *R. grandifolius* leaves, twigs, and fruits have a protective effect on diabetes, sore throat, and diuretic effects (Spínola et al. 2019); *R. parvifolius* roots are used for hepatitis (Xu et al. 2017).

Recently, the phytotherapists recommended raspberry for diarrhea, nausea, and vomiting. Raspberry

is called „a panacea for pregnancy“: it soothes morning sickness, prevents abortion, and leaves extract relieves labor pains (Patel et al. 2004). Raspberry leaves are used as infusions, gargle solutions, and gargles, according to Flora Health (Strik 2007). Despite the protective effect of tannins, they damage the liver in large amounts. Blackberry leaf infusion used in non-specific acute diarrhea (up to 4.5 g daily as tea or other supplement) is approved by Commission E, the German regulatory agency for medicinal plants (Oszmiański et al. 2015). The monograph issued by the EMA accepts the traditional use of raspberry leaves for the symptomatic relief of spasms, the treatment of mild forms of throat and mouth inflammation, and diarrhea (Committee on Herbal Medicinal Products (HMPC)). The traditional use of *R. idaeus* and *R. fruticosus*, relevant medicinal products, and biological activity are summarized in Table 2. The main protective effects of some *Rubus* species are presented in Table 3.

In recent years, the European Medicine Agency (EMA) has approved tinctures and extracts of *Rubus idaeus* leaves as herbal medicinal products (as well as those of *Ribes nigrum* and *Arctostaphylos uva-ursi*) based on their traditional use (Committee on Herbal Medicinal Products 2012) and published a monograph on raspberry leaves. They have been included in the British Pharmacopoeia since 1983 (Ferlemi and Lamari 2016).

Table 2. Biological activity and application of *R. idaeus* and *R. fruticosus* leaves in medical practice (according to Ferlemi and Lamari (2016)).

	***R. idaeus* (rapberry)**	*R. fruticosus* **(blackberry)**
EMA	***Traditional medicinal products for***: Symptomatic relief of minor spasms associated with menstrual period Symptomatic treatment of mild forms of mouth and throat inflammation Symptomatic treatment of mild diarrhea	
Application in traditional medicine	Stimulation of the birth process	Mouthwash against gums, throat inflammation, and mouth ulcers
	Relief of menstrual cramps	Against respiratory problems
	Relief of diarrhea	Astringent agent
	Astringent agent	In anemia, diarrhea, dysentery, cystitis, and hemorrhoids
	Anti-inflammatory agent (mouth, throat)	
	Chronic skin conditions	
	Treatment of conjunctivitis	
In vitro/in vivo	Antioxidant activity	Antidiabetic/ hypoglycemic activity Antibacterial activity Anesthetic and anti-inflammatory activity
Clinical study	Indications that it facilitates labor	

Phytopharmacological studies of extracts and compounds from *Rubus* species. Protective effects of extracts and compounds from the leaves of *Rubus* species.

Mostly, the phytopharmacological studies have been conducted with crude extracts, without relevant data on the sample preparation and standardization of the extracts. The established pharmacological effects are largely associated with phenolic compounds, well known for radical-scavenging activity, which is a key point in many pathological conditions and metabolic diseases (Zia-Ul-Haq et al. 2014). Although many of the applications in traditional medicine have been confirmed, *in vivo* preclinical and clinical studies are necessary to confirm their efficacy and safety.

Antibacterial activity

A comparison of the antibacterial activity of different plant parts of *R. fruticosus* on a panel of bacterial strains (Table 3) shows the following order of activity based on the minimum inhibitory concentration: stem > root > leaves > fruits (Riaz et al. 2011). No activity was found on strains of pathogenic fungi. The preparations obtained from the leaves of *R. idaeus* L. and *R. fruticosus* L. could complement classical antibiotics.

Antidiabetic activity

In the model of insulin-independent diabetes, water and butanol fractions of 70% alcoholic extract of *R. fruticosus* leaves restored blood glucose levels (Zia-Ul-Haq et al. 2014). A hypoglycemic effect was observed in normal rats, indicating that regulatory mechanisms cannot rapidly normalize glucose levels (Jouad et al. 2002). Aqueous extracts of the leaves also have an anti-hyperglycemic effect, which can be associated to a certain extent with the content of chromium and zinc. At 5 g/kg daily, the aqueous extract reduced hyperglycemia by 50% in an alloxan-induced diabetes model in rabbits (Zia-Ul-Haq et al. 2014).

In a comparative study of the inhibitory effects of methanol extracts of *R. grandifolius* leaves and fruits towards α-glucosidase and α-amylase, the leaves showed higher activity with an IC_{50} of 0.11-0.15 mg/ml compared to that of fruits (0.61–0.68 mg/ml) (positive control acarbose) (Spínola et al. 2017). The assays were performed with α-glucosidase from brewer's yeast and rats, and the extracts were less active on the mammalian enzyme; the IC_{50} was about 9 times higher. The trend toward higher activity of the leaf extracts was also observed in the inhibition of α-amylase and pancreatic lipase. Blood sugar control with plant polyphenols is associated with their ability to bind to proteins and therefore modulate glucose breakdown by inhibiting en-

Table 3. Protective effects of plant substances and secondary metabolites from *Rubus* leaves.

RUBUS SPECIES	PROTECTIVE EFFECTS	REFERENCES
R. fruticosus	Astringent effect of infusions, decoctions, and compresses for wounds and bruises; diarrhea and hemorrhoids; leaves poultice in abscesses. Hemostatic action.	Verma et al. 2014
Antithrombotic and anti-complementary activity		
70% ethanol fraction of the aqueous extract of *R. chingii* and flavonoids isolated from it	The extract and the isolated flavonoids quercetin, kaempferol, and tiliroside (2 mg/ml) have antithrombotic activity (*in vitro* and *in vivo*). Activates the partial thromboplastin time; inhibitory activity on thrombin.	Han et al. 2012
Cytotoxic and antitumor effects		
R. pileatus, *R. xanthocarpus*	Anticancer and antibacterial activity.	Patel et al. 2004
Polysaccharides from *R. chingii*	Polysaccharides in the leaves inhibited the proliferation of the MCF-7 breast carcinoma cell line at 2 mg/ml by 48.48 ± 0.55% and 66.30 ± 0.61% for 48 h and 72 h (*in vitro*), respectively.	Zhang et al. 2015
R. sanctus methanol extract	Significant inhibition of spontaneous migration of the HCT116 cell line suggests a potential protective effect against the migration and invasion capacities of human colon cancer cells.	Zengin et al. 2019
Hypoglycemic and hypolipidemic effects		
Aqueous extract of *R. chingii*	The extract lowers blood sugar levels in an alloxan-induced diabetes model in rats and in hyperglycemic patients. It improves the lipid profile.	Verma et al. 2014; Patel et al. 2004
R. fruticosus; *R. ellipticus*; *R. sanctus*	At a dose of 5 g/kg daily, the aqueous extract reduced hyperglycemia by 50% in an alloxan-induced diabetes model in rabbits.	Zia-Ul-Haq et al. 2014
	The extract shows low α-amylase inhibition and prominent α-glucosidase inhibitory activity.	Zengin et al. 2019
Fraction rich in 19α-hydroxyursane-type triterpenoids from *R. crataegifolia*	The fraction (at 30 and 60 mg/kg) reduced abdominal adipose tissue in rats, triglycerides, phospholipids, and total lipids, as well as total cholesterol and LDL, and increased HDL.	Nam et al. 2007
Leaf and fruit extracts of *R. grandifolius*	Inhibit α-glucosidase, β-glucosidase, α-amylase, lipase, and aldose reductase.	Spínola et al. 2019
Neuroprotective activity		
Hexane extract of *R. brasiliensis*	The extract (300 mg/kg) has a hypnotic, anticonvulsant, and muscle-relaxing effect (*in vivo*); GABAA receptors have a key role in these effects. The action is similar to that of a benzodiazepine.	Nogueira and Vassilieff 2000
Ethanol extracts of *R. idaeus*	Extracts and three lignans from the rhizomes have *in vitro* protective effects in a SH-SY5Y cell model of H_2O_2-induced neurodegeneration.	Xu et al. 2017
Ethanol extract of *R. coreanus*	It showed inhibitory activity against acetylcholinesterase *in vitro* and exerted memory ameliorating effects *in vivo.*	Kim et al. 2013
Ethyl acetate and methanol extracts from *R. sanctus* and *R. ibericus*	Enzyme inhibitory activity on the acetyl and butyryl cholinesterases: up to 3.30 and 2.49 mg galantamine equivalents/g extract.	Zengin et al. 2019
Suavisimoside R1 from *R. parvifolius*	At a dose of 100 μM/L, there are protective effects on dopaminergic neurons and a protective effect on Parkinson's disease.	Yu et al. 2008
Anti-inflammatory activity		
Polysaccharides from *R. chingii*	Anti-inflammatory activity on LPS-stimulated RAW264.7 macrophages by reducing NO formation and increasing TNF-α, iNOS, and IL-6 gene expression (*in vitro*).	Zhang et al. 2015
A fraction enriched in 19α-hydroxyursane-type triterpenoids from *R. coreanus*	Protective effects in a murine model of colitis: reduced cytokines and macrophage infiltrates in tissues. In LPS-stimulated macrophages, RAW 264.7 suppressed the formation of NO, PGE2, and cytokines through the NF-κB and p38 MAPK signaling pathways.	Shin et al. 2014
Niga-ichigoside F1 (from *R. imperialis*) and methanol extract	Anti-inflammatory activity in a model of LPS-induced inflammatory processes; early-healing effect of triterpenoid saponin in *in vivo* models	Tonin et al. 2016
R. hirtus, *R. sanctus*	Ethanol and aqueous extracts of *R. sanctus* have antinociceptive activity against p-benzoquinone-induced abdominal contraction in mice. The extracts have anti-inflammatory activity in a carrageenan-induced inflammation model.	Erdemoglu et al. 2003
R. sanctus, *R. hirtus*, and their hybrid *n*-butanol and aqueous fractions	Anti-inflammatory activity in a model of carrageenan-induced inflammation. Side effects related to irritation of the gastric mucosa.	Akcos et al. 1998
Hexane, chloroform, ethyl acetate, and methanol extracts of *R. sanctus*	The effect on wound healing was established in two rat models: incision and excision. A 1% methanol extract showed higher activity compared to madecasol (proliferation, re-epithelialization, and collagen fibers). Preclinical research.	Süntar et al. 2011
Niga-ichigoside F1 (from the ethyl acetate extract of *R. imperialis*)	antinociceptive activity with an ID_{50} of 2.6 (first phase) and 2.7 (second phase) mg/kg, (ip), respectively.	Patel et al. 2004
R. ellipticus	Protective effects in colitis, antiprotozoal activity	Patel et al. 2004
Antimicrobial activity		
Ethanol extracts of *R. fruticosus*	Strains: *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus*, *Proteus mirabilis*, *Bacillus subtilis*, *Citrobacteri* sp., *Pseudomonas aeruginosa*	Zia-Ul-Haq et al. 2014
R. ulmifolius and isolated ellagitannins	Antibacterial activity on *Helicobacter pylori*.	Martini et al. 2009
Antioxidant activity		
Polysaccharides from *R. chingii*	*In vitro* antioxidant activity (DPPH assay). $IE_{50.}$ 754.33 μg/ml (fruit polysaccharides); 671.39 μg/ml (leaves).	Zhang et al. 2015

RUBUS SPECIES	PROTECTIVE EFFECTS	REFERENCES
Methanol extract of *R. ulmifolius*	Methanol extract (300 mg) and flavonoid fraction have antioxidant and antipyretic activity. No toxicity up to 6 g/kg (p.o).	Ali et al. 2017
Hydroalcoholic extracts of 26 wild blackberries	Reducing capacity (FRAP): *R. pedemontanus* (192.91 mmol TE/g dm) and *R. parthenocissus* (192.53 mmol TE/g dm). Radical scavenging activity - (ABTS) of *R. pedemontanus* (212.69 mmol TE/g dm).	Oszmiański et al. 2015
Extracts from *R. ibericus* (synonym of *R. discolor*)	DPPH - $IC_{50:}$ 17.76 μg/ml (aqueous extract); ABTS - $IC_{50:}$ 4.76 μg/ml (aqueous extract); FRAP – 2.96 μmol Fe^{+2}/ mg dw (aqueous extract).	Velickovic et al. 2016
R. chingii, *R. coreanus*, *R. crataegifolius*, *R. foliosus*, and *R. fruticosus*	Tonic agents	Patel et al. 2004; Verma et al. 2014
Saponin mixture and niga-ichigoside F1 from *R. parvifolius*	Protective effects in a mouse model of fatigue; mechanisms included slowing the accumulation of urea and lactic acid; a decrease in triglycerides; and an increase in LDH and liver glycogen. The accumulation of lactic acid and glycogen in the muscles is reduced, and the formation of cytokines is suppressed.	Chen et al. 2013
Aqueous, ethyl acetate, and methanol extracts of *R. sanctus* and *R. ibericus*	The aqueous extract showed high phenolic content and antioxidant activity, while the ethyl acetate and methanol extracts showed potent enzyme inhibitory activity.	Zengin et al. 2019

zymes (Edirisinghe and Burton-Freeman 2016). Although inhibition of digestive enzymes is a therapeutic approach in the treatment of diabetes and obesity, strong inhibition of α-amylase is not recommended. Based on that, extracts with a weak inhibitory effect on α-amylase and a strong effect on α-glucosidase are most suitable to avoid excessive breakdown of undigested starch in the colon, which is associated with abdominal pain and flatulence. Another study revealed *R. sanctus* aqueous extract to be a promising candidate, showing low α-amylase inhibition and prominent α-glucosidase inhibitory activity (Zengin et al. 2019). *R. grandifolius* leaf extracts also revealed higher inhibitory activity than fruits on aldose reductase (the first enzyme of the polyol pathway), as well as more pronounced anti-glycation activity (Spínola et al. 2017).

Antioxidant activity

The antioxidant potential of *Rubus* species fruits and leaves has been evaluated (Oszmiański et al. 2015; Pavlovic et al. 2016; Spínola et al. 2017; Zengin et al. 2019). Generally, leaves have higher activity than fruits in many of the assays, and the results could be related to the phenolic composition patterns (Spínola et al. 2017). Accordingly, the total content of phenolic compounds in *R. grandifolius* (HPLC-DAD) was 92.96–97.47 mg/g dry extract (berries) and 118.01–137.41 mg/g (leaves). Antioxidant activity was correlated with the total phenols ($r \geq 0.929$), as it was found for different blackberry species (Oszmiański et al. 2015). Flavonols have the main contribution to the antioxidant effect ($r \geq 0.947$), followed by hydroxycinnamic acids ($r \geq 0.943$) and ellagitannins ($r \geq 0.884$). The content of ellagitannins and anthocyanins (in the fruits) is thought to play a role in the observed effects.

The *in vitro* antioxidant activity of ethanolic and aqueous extracts of leaves of *R. ibericus* was investigated by different assays (DPPH, ABTS, H_2O_2, O^2, metal chelating activity, reducing power, and inhibition of lipid peroxidation) (Keser et al. 2015). Despite the lower total flavonoids in *R. ibericus* leaves originating from Serbia (35.63 and 24.49 mg QE/g extract in aqueous and ethanol extracts, respectively, *versus* 74.54 and 92.75 QE/g in the aforementioned study), high radical scavenging activity and reducing power were observed (Velickovic et al. 2015).

Another study revealed the importance of solvent selection in the sample preparation of *R. sanctus* and *R. ibericus* extracts (Zengin et al. 2019). Thus, the aqueous extracts manifested the highest radical scavenging activity (DPPH and ABTS), reducing power (FRAP and CUPRAC), and metal chelating capacity. According to sPLS-DA analysis, the main parameters for the extracts differentiation were total flavonoids, metal chelating activity, and antioxidant potential in the phospho-molibdenum assay. It should be noted that ethylacetate and methanol extracts from *R. sanctus* and *R. ibericus* leaves inhibited, to the highest extent, acetyl- and butyrylcholinesterase, tyrosinase, α-glucosidase, and α-amylase.

In a model of CCl_4-induced hepatotoxicity in isolated hepatocytes, the methanol-aqueous extract of *R. sanctus* (100 mg/ml) showed hepatoprotective activity: it restored the levels of the antioxidant marker GSH, the enzymes lactate dehydrogenase (LDH) by 40%, alanine aminotransferase (ALAT) by 30%, and aspartate aminotransferase (ASAT) by 20% compared to the CCl_4-treated group (Badr et al. 2009). Antioxidant effects were found regarding lipid peroxidation and TBARS (thiobarbituric acid reactive substances) formation in isolated hepatocytes.

Neuropharmacological activity

In a comparative neuropharmacological study of methanol extracts from different plant parts of *R. fruticosus*, the following order of activity on the CNS was found: fruits > roots > leaves > stems (Riaz et al. 2014). All extracts have an anxiolytic effect without observing sedative muscle relaxing effects.

Anti-inflammatory activity

The flavonol glucoside tiliroside (*R. chingii*) at a concentration of 100 μg/ml revealed the highest inhibitory activity of the assayed flavonoids on NO formation in LPS-stimulated RAW 264.7 macrophages, which was very close to the effect of dexamethasone (50 μg/ml). Leaf and fruit polysaccharides also produced a dose-dependent inhibition of NO uptake (2–400 μg/ml) in the same macrophage cell line by suppressing TNF-α, iNOS, and IL-6 gene expression (Zhang et al. 2015).

Polar and butanol fractions of an extract from *R. sanctus* aerial parts exerted anti-inflammatory activity in a carrageenan-induced inflammation model. Side effects related to irritation of the gastric mucosa have also been reported (Akcos et al. 1998). In a further study, ethanol and aqueous extracts of *R. sanctus* (aerial parts) revealed antinociceptive activity against *p*-benzoquinone-induced abdominal contraction in mice. The extracts have anti-inflammatory activity in a carrageenan-induced inflammation model (Erdemoglu et al. 2003).

Another study on *R. sanctus* methanol extract showed anti-inflammatory activity in an experimental model of ulcerative colitis, revealing significant blunting effects on LPS-induced levels of markers of oxidative stress and tissue damage such as nitrites, MDA, and LDH. Besides, *R. sanctus* methanol extract displayed a significant inhibition of spontaneous migration of human colon cancer cells HCT116, thus suggesting a potential protective effect against migration and invasion capacities of human colon cancer (Zengin et al. 2019). Ethyl acetate extracts from *R. sanctus* reduced the LPS-induced serotonin (5-hydroxytriptamine) steady state levels, which play a pro-inflammatory role in ulcerative colitis.

Different polarity extracts of *R. sanctus* aerial parts (hexane, chloroform, ethyl acetate, and methanol extract) were assayed in two wound healing models in rats: an incisional and an excisional wound model (Suntar et al. 2015). The 1% methanol extract has the highest activity. The mechanism of action included the formation of fibroblasts and collagen fibers in the granulation tissue and epithelization. The effects could be attributed to flavonoids and ellagitannins. The viability of collagen fibrils was increased by inhibiting lipid peroxidation (Suntar et al. 2015).

Toxicity

Few data exist on the safety of *Rubus* extracts. In an acute toxicity test of *R. chingii* leaves, a dose of 20 mg/kg/day did not induce toxicity for 2 weeks (Yu et al. 2019). Over 90 days, oral doses of 2.5, 5, and 10 g/kg produced no significant differences in body weight, blood biochemical parameters, pathology, or histopathology. Mutagenic and genotoxic effects have not been established.

Protective effects of *R. idaeus* leaves in pregnancy

Based on the traditional use of raspberry leaves to relieve labor pains, Jing et al. (2010) studied the effects in rats and reported that leaf extracts did not affect oxytocin-induced contacts and lacked a direct effect on uterine contact. In a study of 150 pregnant women, no significant differences were found between groups in terms of blood loss, blood pressure, length of pregnancy, ease of labor, or relief of labor pains (Simpson 2001). A recent review on using raspberry leaf in pregnancy to facilitate labor revealed that there is a lack of evidence to inform the practice (Bowman et al. 2021).

The discussed *Rubus* species afford a rich source of ellagitannins and ellagic acid conjugates, flavan-3-ols and flavonols, di- and triterpenoids, phenolics, and acylquinic acids. Accordingly, their health-promoting benefits, including an astringent effect, oxidative stress prevention, and inhibition of key enzymes in neurodegenerative and metabolic conditions, have been attributed to the ellagitannin monomers and oligomers, catechin, miquelianin, tiliroside, oleanane-, and ursane-type triterpenoids.

Conclusions

Despite the ethnopharmacological data, the leaves of *Rubus* species are used relatively rarely, in contrast to the fruits, which are considered a "functional food." The leaves of several *Rubus* species are renowned for their ethnomedicinal use as astringent, anti-inflammatory, wound healing, and antidiabetic agents for the relief of menstrual cramps, diarrhea, morning sickness during pregnancy, and labor pain. Most recent studies afford new insights on wild species and cultivars in terms of phytochemical assessment of extracts emphasizing di- and triterpenoids, hydrolyzable tannins (ellagitannins), phenolic acids, and flavonoids. Comprehensive metabolite profiling of *R. idaeus*, *R. fruticosus*, *R. chingii*, *R. sanctus*, *R. ibericus*, and numerous raspberry and blackberry cultivars afforded a more extended view on the bioactive compounds and the mode of action of the taxa. Hyphenated analytical techniques allowed for the dereplication and annotation of hundreds of secondary metmetabolites, highlighting a variety of ellagitannins and ellagic acid conjugates, hydroxycinnamic acid esters with hexaric, quinic, tartaric, and treonic acids, flavan-3-ols, and flavonols together with triterpenoid acids and saponins.

The antibacterial activity of the herbal drugs from the *Rubus* leaves was associated with ellagitannins. The flavonoids and di- and triterpenoids could be related to the anti-inflammatory effects (inhibition of cytokines) and antidiabetic activity (targets are various signaling pathways in the pancreas, liver, and skeletal muscles, β-cells, and insulin sensitivity in the peripheral tissues). Besides evoking an antioxidant response, *Rubus* leaf extracts or compounds exerted moderate or low inhibition on α-amylase and prominent inhibitory activity towards α-glucosidase, which gives rise to further interest in the herbal drugs as promising candidates for the management of hyperglycemia. Because of the downregulation of nitrite, malondialdehyde, lactate dehydrogenase, and serotonin levels, the markers of oxidative stress and tissue damage may be therapeutic targets for the treatment of ulcerative colitis. Moreover, *R. sanctus* extract displayed a protective effect against the migration and invasion capacity of human colon cancer cells.

In conclusion, the review of the scientific literature on *Rubus* species highlights the potential of leaves as a source of bioactive compounds with diverse health benefits. *In vitro* studies gave promising results; well-designed and targeted trials are needed to evaluate the health-promoting application of *Rubus* extracts. The raspberries and blackberry leaves have been "rediscovered" as sources of secondary metabolites for prevention and treatment.

Further investigations are necessary to assess the efficacy of secondary metabolites that are responsible for the observed *in-vitro* effects of *Rubus* using *in-vivo* models. It is anticipated that this review will offer a concise and current compilation of data to scientific researchers interested in research on the genus *Rubus*.

Acknowledgments

This research was funded by the European Union's Next-GenerationEU economic recovery package via the National Recovery and Resilience Plan of the Republic of Bulgaria, project No. BG-RRP-2.004-0004-C01.

References

Akcos Y, Yesilada E, Ezer N (1998) Anti-inflammatory activity of some Turkish *Rubus* species, Hacettepe univ eczacı fak. derg. 18: 33–38.

Ali N, Shaoi M, Shah SWA, Shah I, Shuaib M (2017) Pharmacological profile of the aerial parts of *Rubus ulmifolius* Schott. BMC Complementary Medicine and Therapies 17: 59. https://doi.org/10.1186/s12906-017-1564-z

Badr AM, El-Demerdasha E, Khalifaa AE, Ghoneim AI, Ayoub NA, Abdel-Naim AB (2009) *Rubus sanctus* protects against carbon tetrachloride-induced toxicity in rat isolated hepatocytes: isolation and characterization of its galloylated flavonoids. Journal of Pharmacy and Pharmacology 61: 1511–1520. https://doi.org/10.1211/jpp/61.11.0011

Bakar MFA, Ismail NA, Isha A, Ling ALM (2016) Phytochemical composition and biological activities of selected wild berries (*Rubus moluccanus* L., *R. fraxinifolius* Poir., and *R. alpestris* Blume). Evidence-Based Complementary and Alternative Medicine 16: 2482930. https://doi.org/10.1155/2016/2482930

Bhatt SC, Naik B, Kumar V, Gupta AK, Kumar S, Preet MS, Sharma N, Rustagi S (2023) Untapped potential of non-conventional *Rubus* species: bioactivity, nutrition and livelihood opportunities. Plant Methods 19: 114. https://doi.org/10.1186/s13007-023-01094-y

Bowman R, Taylor J, Muggleton S, Davis D (2021) Biophysical effects, safety and efficacy of raspberry leaf use in pregnancy: a systematic integrative review. BMC Complementary Medicine and Therapies 21: 56. https://doi.org/10.1186/s12906-021-03230-4

Chaturvedula VSP, Miguel RIS, Prakash I (2012) Chemical constituents from the polar fraction of *Rubus suavissimus*. Organic Chemistry Current Research 1: 1. https://doi.org/10.4172/2161-0401.1000101

Chen J, Wang X, Cai Y, Tang M, Dai Q, Hu X, Huang M, Sun F, Liu Y, Xia P (2013) Bioactivity-guided fractionation of physical fatigue-attenuating components from *Rubus parvifolius* L. Molecules 18: 11624–11638. https://doi.org/10.3390/molecules180911624

Committee on Herbal Medicinal Products [HMPC] (2012) Community Herbal Monograph on Rubus *idaeus* L., Folium. European Medicines Agency: London, UK.

Edirisinghe I, Burton-Freeman B (2016) Anti-diabetic actions of berry polyphenols- review on proposed mechanisms of action. Journal of Berry Research 6: 237–250. https://doi.org/10.3233/JBR-160137

Erdemoglu N, Kupeli E, Yesilada E (2003) Anti-inflammatory and anti-nociceptive activity assessment of plants used as remedy in Turkish folk medicine. Journal of Ethnopharmacology. 89: 123–129. https://doi.org/10.1016/S0378-8741(03)00282-4

Ferlemi A-V, Lamari FN (2016) Berry Leaves: An alternative source of bioactive natural products of nutritional and medicinal value. Antioxidants 5: 17. https://doi.org/10.3390/antiox5020017

Gevrenova R, Badjakov I, Nikolova M, Doichinova I (2013) Phenolic derivatives in raspberry (*Rubus* L.) germplasm collection in Bulgaria. Biochemical Systematics and Ecology 50: 419–427. https://doi.org/10.1016/j.bse.2013.06.002

Graham J, Brennan R (2018) Introduction to the *Rubus* genus. In: Graham J, Brennan R (Eds) Raspberry. Springer, Cham, 16 pp. https://doi.org/10.1007/978-3-319-99031-6_1

Grochowski D, Strawa JW, Granica S, Tomczyk M (2020) Secondary metabolites of *Rubus caesius* (Rosaceae). Biochemical Systematics and Ecology 92: 104111. https://doi.org/10.1016/j.bse.2020.104111

Gudej J, Tomczyk M, Urban E (1998) Analysis of chemical composition of *Rubus saxatilis* L. leaves. Herba Polonica 44: 340–344.

Gudej J, Tomczyk M (2004) Determination of flavonoids, tannins and ellagic acid in leaves of *Rubus* L. species. Archives of Pharmacal Research 27: 1114–1119. https://doi.org/10.1007/BF02975114

Guo YL, Tang TX, Yang DP, Cen S, Zhu L-p, Xu X (2013) Polyphenols and triterpenoids in *Rubus aleaefolius* Poir leaves. Plant Science Journal 31: 191–197. https://doi.org/10.3724/SP.J.1142.2013.20191

Han N, Gu Y, Ye C, Cao Y, Liu Z, Yin J (2012) Antithrombotic activity of fractions and components obtained from raspberry leaves (*Rubus chingii*). Food Chemistry 132: 181–185. https://doi.org/10.1016/j.foodchem.2011.10.051

Hummer KE (2010) Rubus pharmacology: antiquity to the present. HortScience 45: 1587–1591. https://doi.org/10.21273/HORTSCI.45.11.1587

Hussein SAM, Ayoub NA, Nawwar MAM (2003) Caffeoyl sugar esters and an ellagitannin from *Rubus sanctus*. Phytochemistry 63: 905–911. https://doi.org/10.1016/S0031-9422(03)00331-5

Jing Z, Pistilli MJ, Holloway AC, Crankshaw DJ (2010) The effects of commercial preparations of red raspberry leaf on the contractility of the rat's uterus *in vitro*. Reproductive Sciences 17: 494–501. https://doi.org/10.1177/1933719109359703

Jouad H, Maghrani M, Eddouks M (2002) Hypoglycaemic effect of *Rubus fructicosis* L. and *Globularia alypum* L. in normal and Streptozotocin-induced diabetic rats. Journal of Ethnopharmacology 81: 351–356. https://doi.org/10.1016/S0378-8741(02)00118-6

Keser S, Çelik S, Turkoglu S, Yilmaz Ö, Turkoglu I (2015) Antioxidant properties of *Rubus discolor* L. extracts and protective effects of its flower extract against hydrogen peroxide induced oxidative stress in wistar rats. Turkish Journal of Pharmaceutical Sciences 12: 187–206.

Kim CR, Choi SJ, Oh SS, Kwon YK, Lee NY, Park GG, Kim Y-J, Heo HJ, Jun WJ, Park Ch-S, Shin D-H (2013) *Rubus coreanus* Miquel inhibits acetylcholinesterase activity and prevents cognitive impairment in a mouse model of dementia. Journal of Medicinal Food 16: 785–792. https://doi.org/10.1089/jmf.2012.2663

Kotula M, Kapusta_duch J, Smolen S, Doskocil I (2022) Phytochemical composition of fruits and leaves of raspberries (*Rubus idaeus* L.) – conventional vs. organic and those wild grown. Applied science 12: 11783. https://doi.org/10.3390/app122211783

Macierzynski J, Sojka M, Kosmala M, Karlińska E (2020) Transformation of oligomeric ellagitannins, typical for *Rubus* and *Fragaria* genus, during strong acid hydrolysis. Journal of Agricultural and Food Chemistry 68: 8212–8222. https://doi.org/10.1021/acs.jafc.0c02674

Martini S, D'Addario C, Colacevich A, Focardi S, Borghini F, Santucci A, Figura N, Rossi C (2009) Antimicrobial activity against *Helicobacter pylori* strains and antioxidant properties of blackberry leaves (*Rubus ulmifolius*) and isolated compounds. International Journal of Antimicrobial Agents 34: 50–59. https://doi.org/10.1016/j.ijantimicag.2009.01.010

Nam J-H, Jung H-J, Tapondjou LA, Lee, K-T, Choi J-W, Kim W-B, Park H-J (2007) The anti-hyperlipidemic effect and constituents of the 19α-hydroxyursane-type triterpenoid fraction obtained from the leaves of *Rubus crataegifolius*. Natural Product Sciences 13:152–159.

Nogueira E, Vassilieff VS (2000) Hypnotic, anticonvulsant and muscle relaxant effects of *Rubus brasiliensis*. Involvement of GABA(A)-system. Journal of Ethnopharmacology 70: 275–280. https://doi.org/10.1016/S0378-8741(99)00205-6

Ohtani K, Aikawa Y, Kasai R, Chou W-H, Yamasaki K, Tanaka O (1992) Minor diterpene glycosides from sweet leaves of *Rubus suavissimus*. Phytochemistry 31: 1553–1559. https://doi.org/10.1016/0031-9422(92)83105-8

Okuda T, Yoshida T, Hatano T (2000) Correlation of oxidative transformations of hydrolyzable tannins and plant evolution. Phytochemistry 55: 513–529. https://doi.org/10.1016/S0031-9422(00)00232-6

Oszmiański J, Wojdyło A, Nowicka P, Teleszko M, Cebulak T, Wolanin M (2015) Determination of phenolic compounds and antioxidant activity in leaves from wild *Rubus* L. species. Molecules 20: 4951–4966.

Park JH, Oh SM, Lim SS, Lee YS, Shin H-K, Oh Y-S, Choe N-H, Park JHY, Kim J-K (2006) Induction of heme oxygenase-1 mediates the anti-inflammatory effects of the ethanol extract of *Rubus coreanus* in murine macrophages. Biochemical and Biophysical Research Communications 351: 146–152. https://doi.org/10.1016/j.bbrc.2006.10.008

Patel AV, Rojas-Vera J, Dacke CG (2004) Therapeutic constituents and actions of *Rubus* species. Current Medicinal Chemistry 11: 1501–1512. https://doi.org/10.2174/0929867043365143

Pavlović A V, Papetti A, Zagorac DČD, Gašić UM, Mišić DM, Tešić ŽL, Natić MM (2016) Phenolics composition of leaf extracts of raspberry and blackberry cultivars grown in Serbia. Industrial Crops and Products 87: 304–314. https://doi.org/10.1016/j.indcrop.2016.04.052

Pouysegu L, Deffieux D, Malik G, Natangeloab A, Quideau S (2011) Synthesis of ellagitannin natural products. Natural Product Reports 28: 853–874. https://doi.org/10.1039/c0np00058b

Riaz M, Ahmad M, Rahman N (2011) Antimicrobial screening of fruit, leaves, root and stem of *Rubus fruticosus* L. Journal of Medicinal Plant Research 5: 5920–5924.

Riaz M, Zia-Ul-Haq M, Ur-Rahman N, Ahmadi M (2014) Neuropharmacological effects of methanolic extracts of *Rubus fruticosus* L. Turkish Journal Of Medical Sciences 44: 454–460. https://doi.org/10.3906/sag-1211-1

Shin JS, Cho EJ, Choi HE, Seo J-H, An H-J, Park H-J, Cho Y-W, Lee K-T (2014) Anti-inflammatory effect of a standardized triterpenoid-rich fraction isolated from *Rubus coreanus* on dextran sodium sulfate-induced acute colitis in mice and LPS-induced macrophages. Journal of Ethnopharmacology 158: 291–300. https://doi.org/10.1016/j.jep.2014.10.044

Simpson M (2001) Raspberry leaf in pregnancy: Its safety and efficacy in labor. Journal of Midwifery & Women's Health 46: 51–59. https://doi.org/10.1016/S1526-9523(01)00095-2

Skirvin RM, Motoike S, Coyner M, Norton MA (2005) *Rubus* spp. cane fruit. In: Litz RE (Ed.) Biotechnology of fruit and nut crops. CABI, Wallingford, 566–583.

Spínola V, Pinto J, Llorent-Martínez EJ, Tomás H, Castilho PC (2019) Evaluation of *Rubus grandifolius* L. (wild blackberries) activities targeting management of type-2 diabetes and obesity using in vitro models. Food and Chemical Toxicology 123: 443–452. https://doi.org/10.1016/j.fct.2018.11.006

Strik BC (2007) Berry crops: Worldwide area and production systems. In: Zhao Y (Ed.) Berry Fruit Value AddedProducts for Health Promotion, 1st edn.; CRC: Boca Raton, FL, USA, 3–49. https://doi.org/10.1201/9781420006148.ch1

Süntar I, Koca U, Keleş H, Akkol EK (2011) Wound healing activity of *Rubus sanctus* Schreber (Rosaceae): preclinical study in animal models. Evidence-Based Complementary and Alternative Medicine 2011: 816156. https://doi.org/10.1093/ecam/nep137

Tao Y, Bao J, Zhu F, Pan M, Liu Q, Wang P (2023) Ethnopharmacology of *Rubus idaeus* L.: A critical review on ethnobotany, processing methods, phytochemicals, pharmacology and quality control. Journal of Ethnopharmacology 302: 115870. https://doi.org/10.1016/j.jep.2022.115870

Tanaka T, Tachibana H, Nonaka G-I, Nishioka I, Hsu F-L, Kohda H, Tanaka O (1993) Tannins and related compounds. CXXII. New Dimeric, Trimeric and Tetrameric Ellagitannins, Lambertianins A-D, from *Rubus lambertianus* Chemical and Pharmaceutical Bulletin 41: 1214–1220. https://doi.org/10.1248/cpb.41.1214

Tonin DT, Thiesen LC, Nunes ML, Broering MF, Donato MP, Goss MJ, Petreanu M, Niero R, Machado ID, Santin JR (2016) *Rubus imperialis* (Rosaceae) extract and pure compound niga-ichigoside F1: wound healing and anti-inflammatory effects. Naunyn-Schmiedeberg's Archives of Pharmacology 389: 1235–1244. https://doi.org/10.1007/s00210-016-1285-8

Tuzlacı E, Gürkan EM (2004) A pentacyclic triterpenoid from *Rubus sanctus*. Acta Pharmaceutica Turcica 46: 185–188.

Veličković IZ, Grujić SM, Marin PD (2016) Antioxidant properties of *Rubus discolor* leaf extracts. Zbornik Matice Srpske za Prirodne Nauke 2016(131): 189–196. https://doi.org/10.2298/ZMSPN1631189V

Verma R, Gangrade T, Punasiya R, Ghulaxe C (2014) *Rubus fruticosus* (blackberry) use as an herbal medicine. Pharmacognosy Reviews 8: 101–104. https://doi.org/10.4103/0973-7847.134239

Wójcik E (1989) Phytochemical investigation of *Rubus plicatus* blackberry. Acta Poloniae Pharmaceutica 46: 386–390.

Xu Y, Li L-Z, Cong Q, Wang W, Qi X-L, Peng Y, Song S-J (2017) Bioactive lignans and flavones with in vitro antioxidant and neuroprotective properties from *Rubus idaeus* rhizome. Journal of Functional Foods 32: 160–169. https://doi.org/10.1016/j.jff.2017.02.022

Yang J, Cui J, Han H, Chen J, Yao J, Liu Y (2020) Determination of active compounds in raspberry leaf extracts and the effects of extract intake on mice. Journal of Food Science and Technology 40: 124–131. https://doi.org/10.1590/fst.35518

Yu G, Luo Z, Wang W, Li Y, Zhou Y, Shi Y (2019) *Rubus chingii* Hu: A Review of the Phytochemistry and Pharmacology. Frontiers in Pharmacology 10: 799. https://doi.org/10.3389/fphar.2019.00799

Zengin G, Ferrante C, Senkardes I, Gevrenova R, Zheleva-Dimitrova D, Menghini L, Orlando G, Recinella L, Chiavaroli A, Leone S, Brunetti L, Picot-Allain CMN, Rengasamy KRR, Mahomoodally MF (2019) Multidirectional biological investigation and phytochemical profile of *Rubus sanctus* and *Rubus ibericus*. Food and Chemical Toxicology 127: 237–250. https://doi.org/10.1016/j.fct.2019.03.041

Zhang TT, Lu C, Jiang JG, Wang M, Wang D-M, Zhu W (2015) Bioactivities and extraction optimization of crude polysaccharides from the fruits and leaves of *Rubus chingii* Hu. Carbohydrate Polymers 130: 307–315. https://doi.org/10.1016/j.carbpol.2015.05.012

Zia-Ul-Haq M, Riaz M, De Feo V, Jaafar HZE, Moga M (2014) *Rubus fruticosus* L.: Constituents, biological activities and health related uses. Molecules 19: 10998–11029. https://doi.org/10.3390/molecules190810998