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POLYPHENOLIC COMPOUNDS FROM *Rubus saxatilis*

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The genus *Rubus* comprises more than 700 species distributed throughout all continents except Antarctica [1]. *Rubus saxatilis* L. (Rosaceae), commonly known as "stone bramble", is a perennial plant growing widely, preferring light, deciduous forests throughout Poland and also distributed in other countries of Europe and Asia [2–4].

Previous chemical studies of *R. saxatilis* fruits reported by Novruzov [5] proved the presence of sugars, pectins, carboxylic acids, anthocyanins, catechins, as well as vitamin C. The contents and fatty acids composition of stone bramble seed oil have been reported [6, 7]. The considerable quantity of amino acids, particularly *L*-tryptophan, was affirmed in the leaves of *R. saxatilis* [8]. In addition, the content of mineral components has previously been determined from the leaves of *R. saxatilis* [9, 10]. In our earlier phytochemical investigation of *R. saxatilis* leaves we reported the identification of phenolic acids, flavonoids, and methyl brevifolincarboxylate – a compound isolated for the first time from the genus *Rubus* [11]. Recently, the content of flavonoids, tannins, and ellagic acid in the leaves of *R. saxatilis* using spectrophotometric and HPLC methods has been reported [12]. Berries of stone bramble are used fresh and for production of jam and berry juices [13]. In continuation of research on *Rubus* species we isolated three pure phenolic compounds from the aerial parts of *R. saxatilis*.

Plant material was purified and then successively extracted with methanol. Solvent evaporation under reduced pressure yielded a residue which was diluted with water and exhaustively partitioned between diethyl ether and ethylacetate. The ethylacetate extracts were chosen for this study. Initial isolation of compounds from the ethylacetate extract was carried out by chromatography on polyamide eluting with increasing polarity H_2O–MeOH solvent mixtures. Elution with H_2O–MeOH (3:2 v/v) gave a mixture of flavonoid compounds (mixture A). Further elution with MeOH yielded pure compound **1**. Repeated chromatography of mixture A on a polyamide column eluting with EtOAc–MeOH (3:2 v/v) afforded compound **2** and mixture B. The polyamide column chromatography of mixture B, eluting with C_6H_6–MeOH (8.5:1.5 v/v), led to the isolation of compound **3**. Compounds **2** and **3** were further purified on Sephadex LH-20 using MeOH as a eluent. Those compounds **1–3** were isolated for the first time from *R. saxatilis* leaves.

Ellagic acid (**1**) $C_{14}H_6O_8$, mp > 360°C, UV spectrum (MeOH, λ_{max}, nm): 254, 304, 351, 364 [14, 15].

Hyperin (**2**) (quercetin 3-O-β-D-galactoside), $C_{21}H_{20}O_{12}$, mp 236–238°C, UV spectrum (MeOH, λ_{max}, nm): 256, 268 sh, 296 sh, 359; +NaOMe: 270, 325 sh, 408; +$AlCl_3$: 271, 304 sh, 337 sh, 437; +$AlCl_3$/HCl: 267, 301 sh, 359 sh, 406; +NaOAc: 269, 368; +NaOAc+ H_3BO_3: 260, 297 sh, 377; ^{1}H NMR (200 MHz, DMSO-d_6, δ, ppm, J/Hz): proton signals at 12.62 (1H, s, H-OH-5), 7.66 (1H, dd, J_1 = 2.0, J_2 = 8.4, H-6′), 7.52 (1H, d, J = 2.1, H-2′), 6.81 (1H, d, J = 8.5, H-5′), 6.40 (1H, d, J = 1.9, H-8), 6.20 (1H, d, J = 1.9, H-6), 5.37 (1H, d, J = 7.5, H-1″ galactose), 5.10–4.40 (m, 4H, OH-sugar), .65–3.31 (m, 4H, H-sugar); ^{13}C NMR (50 MHz, DMSO-d_6, δ, ppm) carbon signals at 156.27 (C-2), 133.45 (C-3), 177.50 (C-4), 161.20 (C-5), 98.63 (C-6), 164.09 (C-7), 93.46 (C-8), 156.19 (C-9), 103.88 (C-10), 121.06 (C-1′), 115.15 (C-2′), 144.80 (C-3′), 148.43 (C-4′), 115.90 (C-5′), 122.00 (C-6′), 101.70 (C-1″), 71.10 (C-2″), 73.10 (C-3″), 67.90 (C-4″), 75.80 (C-5″), 60.10 (C-6″) [16–18].

Trifolin (**3**) (kaempferol 3-O-β-D-galactoside), $C_{21}H_{20}O_{11}$, mp 256–257°C, UV spectrum (MeOH, λ_{max}, nm): 252, 265 sh, 292 sh, 348; +NaOMe: 273, 324, 397; +$AlCl_3$: 272, 352, 399; +$AlCl_3$/HCl: 271, 344, 399; +NaOAc: 271, 354; +NaOAc+ H_3BO_3: 266, 351; ^{1}H NMR (200 MHz, DMSO-d_6, δ, ppm, J/Hz): proton signals at 12.60 (1H, s, H-OH-5), 8.07 (2H, d, J = 8.8, H-2′ and H-6′), 6.86 (2H, d, J = 8.8, H-3′ and H-5′), 6.43 (1H, d, J = 1.9, H-8), 6.21 (1H, d, J = 1.9, H-6), 5.40 (1H, d, J = 7.5, H-1″ galactose), 3.66–3.28 (m, 6H, H-sugar + H_2O); ^{13}C NMR (50 MHz, DMSO-d_6, δ, ppm) carbon signals at 156.32 (C-2), 133.20 (C-3), 177.50 (C-4), 161.18 (C-5), 98.68 (C-6), 164.17 (C-7), 93.65 (C-8), 156.36 (C-9), 103.91 (C-10), 120.84 (C-1′), 130.96 (C-2′), 115.04 (C-3′), 159.93 (C-4′), 115.04 (C-5′), 130.96 (C-6′), 101.63 (C-1″), 71.18 (C-2″), 73.06 (C-3″), 67.86 (C-4″), 75.75 (C-5″), 60.17 (C-6″) [16–18].

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R_f values of aglycones (TLC) after acid hydrolysis of **2** and **3** were identified with quercetin and kaempferol, respectively. R_f values of sugars (TLC) after hydrolysis of **2** and **3** were identified with a standard of galactose.

The presence, in the genus *Rubus*, of derivatives of kaempferol and quercetin glycosylated derivatives may have some chemotaxonomic implications. There is, in fact, a general homogeneity regarding the flavonoids present inside the genus, most of them being 3-O-glycosylated flavonols in which the aglycone moiety is often represented by quercetin and kaempferol. The presence of free flavonoid aglycones, in particular kaempferol and quercetin, has been reported in the leaves of *R. idaeus*, *R. coreaneum*, *R. sanctus*, *R. ulmifolius*, as well as *R. plicatus* [14, 15, 19–22]. Except for the leaves, qualitative and quantitative analysis of flavonols and other phenolics in the berries of the genus *Rubus* were performed [23–25]. In *R. phoenicolasius* the presence of quercetin methylether derivatives was reported [26]. Similarly to flavonol aglycones, the glycosylated derivatives have been used as chemotaxonomic markers in the *Rubus* genus. In both fruits and leaves of *R. idaeus*, *R. fruticosus*, *R. ulmifolius*, *R. sanctus*, *R. coreanum*, *R. amabilis*, *R. alceaefolius*, and *R. coriifolius*, the occurrence of flavonol monoglycosides was observed. Glycosylation at C-3 of those compounds has been found to be the most frequent substitution. The presence of 3-O-glucosides, 3-O-galactosides, 3-O-glucuronides, and rarely 3-O-arabinosides has been reported. The flavonol diglycosides with C-3 and C-3,7 substitutions which occur in minor amounts are not so often reported. Generally, acylated flavonoids, such as tiliroside, are known to be characteristics of *Rubus* species and are considered valuable chemotaxonomic markers [11, 20, 22, 27–37]. Moreover, flavone glycosides, especially apigenin and luteolin 7-O-glucoside, were identified in some species of the genus *Rubus* [33, 38]. The presence of ellagitannins in many species of the genus has an important meaning and has been described as the main phenolics in leaves, seeds, fruits, or nuts, and their by-products as juices and jams. Mullen et al. [25, 39] have shown that free ellagic acid levels are very low in raspberries and that high levels of this compound have been detected after acid hydrolysis of extracts from ellagitannins, mainly sanguiin H-6 [15, 40–46]. From a chemotaxonomic point of view, the identification in *R. saxatilis* of ellagic acid and quercetin and kaempferol 3-O-β-D-galactopyranosides further confirms the general chemical homogeneity regarding this class of compounds inside the genus.

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