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Flavonoids of the leaves of Christ's thorn (*Paliurus spina-christi* Mill.)

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Two flavonoid glycosides were isolated from the methanolic extract of the leaves of *Paliurus spina-christi* Mill. (*Rhamnaceae*). The isolated flavonoids were identified as quercetin-3-O-glucoside (isoquercitrin) and quercetin-3-O-rhamnoglucoside (rutin) by means of thin-layer chromatography and UV spectroscopy.

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Paliurus spina-christi Mill. (*Rhamnaceae*) is a perennial thorny shrub widely spread on dry and rocky places (1). This plant grows in the Mediterranean region and Asia. In Yugoslavia this species is indigenous in the Adriatic coastal and island districts.

Christ's thorn grows up to a height of 3 m with greyish-brown to dark brown bark. On the branches small spines are arranged in opposite positions. One spine is long and flat and the other one is short and bent. The oval, smooth and asymmetric leaves with three costae are arranged alternately. Leaves are 2—4 cm long, and 1.5—3 cm wide. Numerous small, yellow flowers bloom in clusters at the axils in June. Fruits are wooden and discus, 1.5—3 cm wide; they are yellow-green at first and become yellow-brown in September (2). These fruits are used in folk medicine against diarrhoea and rheumatism (3, 4).

The aim of this study was to examine flavonoids of the leaves of *Paliurus spina-christi* Mill. because this plant is used in the traditional medicine of Yugoslavia, where it is called »drača«.

Chemical investigations of *Paliurus spina-christi* Mill. fruits indicated the presence of flavonoids (isoquercitrin, rutin and hyperoside) (3, 5, 6); up to now, data about investigations of leaves of *Paliurus spina-christi* Mill. could not be found in literature.

* Correspondence

EXPERIMENTAL

Plant material

The leaves of *Paliurus spina-christi* Mill. were collected during June 1986 in Uskoplje (Herzegovina). The plant material was identified on authenticity in »Dalmacijabilje«.

Extraction of the flavonoids

An amount of 200 g air-dried and coarsely powdered leaves was extracted with petrolether to separate chlorophyll and lipophilic substances. After that the drug was percolated successively with methanol. The methanolic extract was evaporated to a reduced volume. Other lipophilic contaminations were then separated by adding water-chloroform (1 : 1) (7). The aqueous-methanolic phase was concentrated to viscous consistence under reduced pressure.

Separation and identification of the flavonoids

The pre-adsorption technique (8) was used to separate the crude methanolic extract (30 g) by column chromatography on silica gel with ethyl acetate-formic acid-acetic acid-water (100 : 11 : 11 : 27) (9) as a solvent system. Fractions containing the same compounds were combined. Further separation was carried out on Sephadex LH-20 (Pharmacia) and Fractogel TSK (Merck) columns with methanol as a solvent.

Aglycones. — The isolated flavonoid glycosides G 1 and G 2 were hydrolyzed with 70% HCl for one hour (10). Then aglycones were extracted with ethyl acetate.

Aglycones were compared by TLC with quercetin, kaempferol, luteolin and apigenin using toluene-ethyl formiate-formic acid (5 : 4 : 1) as a solvent system (11). The aglycones of G 1 and G 2 appeared both at the Rf-value of quercetin (Rf = 0.51).

Sugars. — Sugars of the isolated flavonoids were identified by TLC in comparison with galactose, glucose, arabinose and rhamnose using chloroform-methanol-water (64 : 36 : 8) as a solvent system. Sugars were detected with thymol-sulfuric acid followed by heating at 120 °C for about 5 minutes (12). The flavonoid G 1 contained one sugar (Rf-value = 0.23) and the flavonoid G 2 contained two sugars (Rf-values are 0.23 and 0.43). The sugar of G 1 was identified as glucose and those of G 2 as glucose and rhamnose.

Glycosides. — The isolated glycosides G 1 and G 2 were identified by TLC (co-chromatography) in comparison with authentic substances (isoquercitrin and rutin). The respective Rf-values are 0.63 (G 1) and 0.38 (G 2) (solvent system I: ethyl acetate-formic acid-acetic acid-water, 100 : 11 : 11 : 27) (9) and 0.69 (G 1) and 0.44 (G 2) (solvent sistem II: ethyl acetate-formic acid-water, 65 : 15 : 20) (13). (Adsorbent: Kieselgel 60 F₂₅₄-Alufolien, Merck, Art. Nr. 5554. Detection: Naturstoff reagent-Polyethylenglycol 4000-UV_{365nm}) (9).

The R_f-values of the isolated flavonoid glycosides G 1 and G 2 were identical with those of the authentic substances.

Ultraviolet-visible absorption spectroscopy. — The identity of the flavonoid glycosides G 1 and G 2 was confirmed by UV spectroscopy (14) by means of a Kontron-Unikon 810 spectrophotometer. UV absorption maxima of the isolated flavonoids are given in Table I.

Table I. UV absorption maxima (nm) of the isolated flavonoid glycosides

Spectrum	Flavonoid G 1	Flavonoid G 2
MeOH	257, 269sh, 298sh, 358	258, 267sh, 299sh, 356
NaOMe	273, 331sh, 410	275, 329, 415
AlCl ₃	275, 433	275, 430
AlCl ₃ /HCl	268, 302sh, 364, 400	270, 299sh, 359sh, 401
NaOAc	272, 327, 392	268, 321, 386
NaOAc/H ₂ BO ₃	262, 379	264, 382

RESULTS AND DISCUSSION

From the methanolic extract of the leaves of *Paliurus spina-christi* Mill two flavonoid glycosides G 1 and G 2 were isolated by means of silicagel, Sephadex LH-20 and Fractogel TSK column chromatography.

The isolated flavonoid glycosides were hydrolyzed (with 7% HCl for one hour); both aglycones corresponded to quercetin chromatographically.

The sugars of the hydrolyzed glycosides were also identified by TLC. Flavonoid G 1 contained glucose and flavonoid G 2 glucose and rhamnose.

The flavonoid glycosides G 1 and G 2 were checked by co-chromatography and determined as isoquercitrin (G 1) and rutin (G 2). The chromatographic behaviour was verified by the results of the UV absorption spectroscopy.

The isolated flavonoids were identified as quercetin-3-O-glucoside (isoquercitrin) and quercetin-3-O-rhamnoglucoside (rutin).

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S A Ž E T A K

Flavonoidi listova drače — *Paliurus spina-christi* Mill.

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Dva flavonoidna glikozida izolirana su iz metanolnog ekstrakta listova drače — *Paliurus spina-christi* Mill. (*Rhamnaceae*). Izolirani flavonoidi su metodom kromatografije na tankom sloju i UV spektroskopije identificirani kao kvercetin-3-O-glukozid (izokvercitrin) i kvercetin-3-O-ramnoglukozid (rutin).

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