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FLAVONOIDS FROM FLOWER OF *LINUM CAPITATUM* KIT

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Abstract. *A phytochemical investigation of the flowers of Linum capitatum Kit. (Linacea) of Serbian origin yielded five additional known flavonoids including: kaempferol, kaempferol-3-O-galactoside, rutin (quercetin-3-O-rutinosid), genistin (genistein-7-O-glucoside) and orientin (luteolin-8-C-glucoside). The characterization of these compounds was achieved by microanalysis, as well as various chromatographic and spectroscopic methods (UV/VIS and 1H NMR).*

Key words: *Linum capitatum Kit., flavonoid, etheric oil, extracts*

1. INTRODUCTION

Linum capitatum Kit. from Linacea family, is widespread on area of carbonate and silicate roks of south-east Europe [1]. Flavonoids are natural phenolic compounds, which appear as secondary metabolites of plants. Considering the fact that they are widely spread in plants, they have extreme importance from the phytogenetic aspect in clearing up the origin and evolution of plants[1].

The present paper describes isolation and structure elucidation of flavonols (kaempferol, kaempferol-3-*O*-galactoside, rutin), flavone (orientin) and isoflavone (genistin) from *Linum capitatum* Kit. flower.

2. EXPERIMENTAL

Plant material. *Linum capitatum* Kit. air dried plant material (flower) was collected in June 2001, in the mountain Varedenik at Vlasina Lake.

General. The UV/VIS spectra were recorded on a Perkin-Elmer Lambda 15 UV/VIS spectrophotometer. The FTIR spectra were recorded on a Michaelson Bomen MB-series spectrophotometer. The ^{1}H NMR spectra were obtained in DMSO-d_6 solution using tetramethilsilane (TMS) as internal standard, on a GEMINI-200 "HF NMR" spectrometer. Thin-layer chromatography was performed on silica gel 60 (Merck) and paper chromatography on Whatman No.3.

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Extraction of Linum capitatum Kit. flower using methanol. The air-dried plant material was extracted with petrolether to eliminate oil components from drugs. After that, the drug was extracted with methanol at room temperature. Methanolic extract was evaporated in vacuum at 50^0C.

Extraction liquid-liquid. Concentrated methanolic extract was diluted with methanol:*n*-buthanol (1:2) mixture. After that, water phase was extracted in successive steps. Both water and *n*-buthanol phase were analyzed using thin-layer and chromatography on paper.

Thin-layer chromatography on Silica gel. For analysis of the methanolic extract, thin-layer chromatography was used on silica gel, and with the following solvent systems: methanol : toluene = 30:70(v/v); chloroform : acetone = 15:1(v/v) and chloroform : acetone : *n*-butanol= 17:2:1(v/v).

Chromatography on Whatman No.3 paper. For this technique, as mobile phases were used systems: *t*-buthanol : acetic acid : water = (3:1:1, v/v), (system 1); acetic acid : water = (15:85, v/v), (system 2).

Isolation of etheric oil of Linum capitatum Kit. flower and it's chemical composition. The etheric oil of *Linum capitatum* Kit. flower was destilated using water bath at boiling temperature. The etheric oil was extracted using petrolether. The determination of chemical composition of etheric oil was performed on a HEWLETT PACKARD 5890 series II using Gas-chromatograph.

Extraction of bioactive components of Linum capitatum Kit. flower. The extraction of bioactive components (maceration method) were done using different solvents at room temperature for 24 hours. The drugs solvent ratio was 1:10. The followed extracts were gained by filtration: 95% ethanolic, 60% ethanolic, methanolic, 1-buthanolic, n-hexan, carbontetracloride and chloroform extract.

3. RESULTS AND DISCUSSION

The flavonoids were isolated from methanolic extract of *Linum capitatum* Kit. flower (Fig. 1).

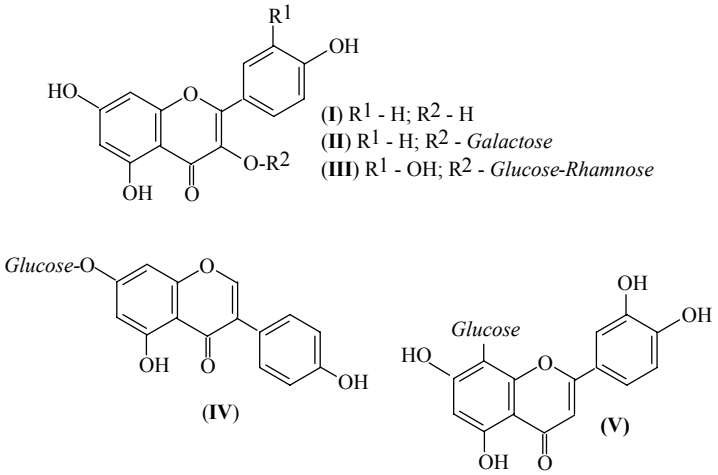

Fig. 1. Chemical structure of the isolated flavonoids and izoflavonoid

Kaempferol (I): Elemental analysis: *Calcd.* %C=68.70, %H=3.82, %O=27.48. *Found:* %C=68.59 %H=3.95, %O=27.46. Color of fluorescence at λ=366 nm is dark-violet and change color in yellow with ammonia. Rf values are: 0.58 for system 1, and 0.46 for system 2. UV/VIS data are shown in Table 1. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.96 (1H, *s*, OH-5), 12.79 (1H, *s*, OH-7), 10.44 (1H, *s*, OH-4'), 10.05 (1H, *s*, OH-3), 7.95 (2H, *d*, H-2', 6'), 6.88 (2H, *d*, H-3', 5'), 6.35 (1H, *d*, H-8), 6.23 (1H, *d*, H-6) [2-6]. Sugar component in flavonoid I was determined using acid hydrolysis. The sugar separates from aglicone using paper chromatography on Whatman No. 3 in ethylacetate-pyridine-water (12:5:4, v/v) solvent mixture. The sugar component ramnoglucose (rutinose) was identified by comparison Rf values with the authentic sample.

Table 1. UV/VIS spectra of flavonoids λ_{max}(nm

Solvent	I	II	III	IV	V
MeOH	268, 298*, 354	266, 298*, 351	259, 261*, 294*, 356	261, 331*	256, 267, 293+, 346
NaOMe	275, 378	275, 328, 401	271, 326, 408	273, 334	265, 305*, 334*, 405
$AlCl_3$	273, 302, 430	275, 304, 351, 402	273, 302, 432	275, 306*,381	272, 303*, 333, 426
$AlCl_3$+HCl	269, 299, 371*, 406	274, 302, 346, 397	271, 300, 362*, 401	305*, 382	262*,274, 356, 384
NaOAc	273, 326, 390	274, 317*, 382	271, 323, 393	272, 332	271, 325, 386
NaOAc+ H_3BO_3	263, 300, 381	267, 310*, 352	261, 297, 385	262, 333*	261, 302, 373

**curvature*

Kaempferol-3-*O*-galactoside (II): Elemental analysis: *Calcd.* %C=56.25, %H=4.46, %O=39.29. *Found:* %C=56.28, %H=4.51, %O=39.21. Color of fluorescence at λ=366 nm is dark-violet and change color in yellow with ammonia. Rf values are: 0.60 for system 1, and 0.44 for system 2. UV/VIS data are shown in Table 1. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.96 (1H, *s*, OH-5), 12.79 (1H, *s*, OH-7), 10.44 (1H, *s*, OH-4'), 7.93 (2H, *d*, H-2', 6'), 6.86 (2H, *d*, H-3', 5'), 6.34 (1H, *d*, H-8), 6.21 (1H, *d*, H-6), 5.65 (1H, *d*, H-1"), 3.30-3.90 (sugar protons, *m*) [2-6].

Thus, the knowledge gained from the spectroscopic data for kaempferol-3-O-galactoside suggest that positions C-5, C-7, C-4' are substituted with OH-group, while galactose (O-glycoside bound) is attached to C-3 position. 3.00-4.00 ppm suggest the presence of sugar protons (ramnoglucose), which methyl-protons are at 0.80 ppm [7,8].

Rutin (Quercetin-3-O-rutinosid) (III): Elemental analysis: *Calcd.* %C=53.11, %H=4.92, %O=41.97; *Found*: %C=53.22, %H=4.88, %O=41.90. Color of fluorescence at λ=366 nm is dark-violet, and change color in yellow with ammonia. Rf values are:

0.44 for system 1, and 0.56 for system 2. UV/VIS data are shown in Table 1. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.91 (1H, *s*, OH-5), 12.73 (1H, *s*, OH-7), 11. 12 (1H, *s*, OH-3'), 10.44 (1H, *s*, OH-4'), 7.20 (2H, *d*, H-2', 6'), 6.86 (2H, *d*, H-3', 5'), 6.40 (1H, *d*, H-8), 6.10 (1H, *d*, H-6), 5.30 (1H, *d*, H-1"), 3.00-4.00 (sugar protons, *m*), 0.80 (CH_3 from rhamnoze) [2-6]. Thus, the knowledge gained from the spectroscopic data for quercetin-3-rutinosid suggest that positions C-5, C-7, C-4' and C-5'are substituted with OH-group, while rutinose (O-glycoside bound) is attached to C-3 position.

Genistin (genistein-7-*O*-glucoside) (IV): Elemental analysis: *Calcd.* %C=58.47, %H=4.41, %O=37.12. *Found:* %C=58.39, %H=4.35, %O=37.16. Color of fluorescence at λ=366 nm is dark-violet and remain with ammonia. Rf values are: 0.60 for system 1, and 0.51 for system 2. UV/VIS data are shown in Table 1. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.91 (1H, *s*, OH-5), 10.43 (1H, *s*, OH-4'), 7.91 (2H, *d*, H-2', 6'), 7.60 (1H, s, H-2), 7.30 (2H, *d*, H-3', 5'), 6.30 (1H, *d*, H-8), 6.10 (1H, *d*, H-6), 4.92 (1H, *d*, H-1"), 3.30-3.90 (sugar protons, *m*) [2-6].

The spectroscopic data for genistin suggest that positions C-5, C-7, C-4' are substituted with OH-group.

Orientin (Luteolin-8-*C*-glucoside) (V) Elemental analysis: *Calcd.* %C=54.82, %H=4.55, %O=43.64. *Found:* %C=54.99, %H=4.46, %O=43.78. Color of fluorescence at λ=366 nm is dark-violet and remain with ammonia. Rf values are: 0.29 for system 1, and 0.15 for system 2. UV/VIS data are shown in Table 1. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 13.17 (1H, *s*, OH-5), 7.52 (2H, *d*, H-2', 6'), 6.86 (1H, *d*, H-5'), , 6.64 (1H, *s*, H-6), 6.26 (1H, *s*, H-3), 4.68 (1H, *d*, H-1"), 3.30-3.90 (sugar protons, *m*). [2-6].

The etheric oil was isolated as petrolether extract and its chemical composition was determined using gas chromatograph. The determination was done comparing the chromatograph with retention time of standards. Among the great number of components the most dominant is borneol (12.38%) and camphor (18.68%).

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FLAVONOIDI IZ CVETA BILJKE *LINUM CAPITATUM* KIT.

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Izvršeno je ispitivanje hemijskog sastava ekstrakata cveta biljke Linum capitatum Kit. (Linacea) sa područja južne Srbije. Izolovani su flavonoidi kempferol, kempferol-3-O-galaktozid, rutin (kvercetin-3-O-rutinozid), genistin (genistein-7-O-glukozid) iorientin (luteolin-8-C-glukozid). Identifikacija dobijenih flavonoidnih jedinjenja je urađena korišćenjem elementarne mikroanalize, hromatografskim i spektroskopskim metodama (UV/VIS i ^{1}H NMR).