Deterrent activity of hops flavonoids and their derivatives against stored product pests

SUPPLEMENTARY MATERIALS

Jacek Jackowskia, Jarosław Popłońskib,, Kamila Twardowskaa, Joanna Magiera-Dulewicza, Michał Hureja and Ewa Huszczab

aDepartment of Plant Protection, Wrocław University of Environmental and Life Sciences, pl. Grunwaldzki 24a, 53-363 Wrocław, Poland,

bDepartment of Chemistry, Wrocław University of Environmental and Life Sciences, ul. Norwida 25, 50-375 Wrocław, Poland

Corresponding author: Jacek Jackowski, e-mail: [jacek.jackowski@up.wroc.pl](mailto:jacek.jackowski@up.wroc.pl)

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**Section A:**



Fig. S1. Deterrent activity of xanthohumol and seven related compounds in three species of test insects



Fig. S2. Response of the test insects and their instars to deterrent activity of xanthohumol and seven related compounds

**Section B:**

a) Xanthohumol flavone (**FXN**) - To a solution of iodine (38 mg, 0.3mmol) in 10 mL of anhydrous pyridine **IXN** (106.2 mg, 0.3mmol) was added and the reaction mixture was stirred at 90°C. After 3 hours the reaction was cooled in ice bath and stopped by addition of 10 mL of water and 2 mL of saturated solution of sodium thiosulfate, while still stirring vigorously. Pyridine was neutralized with ice-cold 1M HCl and the mixture was extracted with ethyl acetate (3 x 40 mL). Then the organic phase was washed with water (70 mL), dried over MgSO4,evaporated and purified by column chromatography (eluent: chloroform-methanol 5:1) to afford xanthohumol flavone (**FXN**). Yield 55%, white-yellow amorphous powder; UV (MeOH) λmax 268, 335nm; 1H NMR (600 MHz, methanol-*d*4) δ: 1.67 (3H, s, H-4’’), 1.81 (3H, s, H-5’’), 3.54 (2H, d, *J* = 6.7 Hz, H-1’’), 3.86 (1H, s, C5-OCH3), 5.23 (1H, t, *J* = 6.7 Hz, H-2’’), 6.43(1H, s, H-6), 6.52 (1H, s, H-3), 6.91 (2H, m, *J* = 8.8 Hz, H-3’, H-5’), 7.78 (2H, m, *J* = 8.8 Hz, H-2’, H- 6’); 13C NMR (150 MHz, methanol-*d4*) δ: 18.24 (C-5’’), 22.71 (C-1’’), 25.92 (C-4’’), 56.09 (C5-OCH3), 96,60 (C-6), 106.04 (C-3), 108.09 (C-10), 109.32 (C-8), 116.67 (C-3’, C-5’), 123.36 (C-1’), 123.43 (C-2’’), 128.81 (C- 2’, C-6’), 132.51 (C-3’’), 158.23 (C-9), 159.57 (C-5), 161.67 (C-7), 161.68 (C-4’), 163.29 (C-2), 180.58 (C=O); HR ESI-MS m/z: 351.1217 [M-H]- (calcd for C21H20O5 - H, 351.1232).

b) 1”,2”-Dihydroxanthohumol K (**DHXN K**) - To a solution of **XN** (354 mg) in 99 mL of methylene chloride 1 mL of trifluoroacetic acid was added and the reaction mixture was stirred at room temperature for 12 h. The reaction was stopped by a slow addition of saturated NaHCO3 while stirring the reaction mixture vigorously, until its color changed to yellow. Then the organic phase was washed with water (3 × 70 mL), dried over MgSO4, evaporated and purified by column chromatography (eluent: hexan-acetone 1:1) to afford 1”,2”-dihydroxanthohumol K (**DHXN K**) . Yield 71%, orange amorphous powder; UV (MeOH) λmax 331nm; 1H NMR (600 MHz, acetone-*d*6) δ: 1.22 (6H, s, H-4’’, H-5’’), 1.75 (2H, t, *J*= 6.7 Hz, H-2’’), 2.63 (2H, t, *J*= 6.7 Hz, H-1’’), 3.62 (3H, s, C6’-OCH3), 6.19 (1H, s, H-5’), 6.77 (1H,d, *J*= 16.0 Hz, H-α), 6.88 (2H, m, *J*= 8.6 Hz, H-3, H-5), 7.23 (1H, d, *J*= 16.0 Hz, H-β), 7.48 (2H, m, *J*= 8.6 Hz, H-2, H-6); 13C NMR (150 MHz, acetone-*d*6) δ: 17.39 (C-1’’), 26.80 (C-4’’, C5’’), 32.61 (C-2’’), 55.87 (C6’-OCH3), 75.07 (C-3’’), 92.01 (C-5’), 101.98 (C-3’), 111.52 (C-1’), 116.72 (C-3, C-5), 127.52 (C-1), 127.62 (C-α), 130.82 (C-2, C-6), 144.26 (C-β), 153.72 (C-2’), 157.41 (C-6’), 157.56 (C-4’), 160.42 (C-4), 194.46 (C=O); HR ESI-MS m/z: 353.1384 [M-H]- (calcd for C21H22O5 - H, 353.1389).

**Section C: Analytical procedures and spectroscopic data**

The products of reactions were separated by column chromatography on silica gel 60 (230–400 mesh, Merck) using chloroform/methanol (9:1 v/v), hexane/acetone (1:1 v/v) or hexane/tetrahydrofuran (1:1 v/v) as eluents.

TLC was carried out on Merck silica gel 60, F254 (0.2 mm thick) plates with chloroform/methanol (9:1 v/v) or hexane/acetone (1:1 v/v) as eluents. HPLC was performed on a Waters 2695 Aliance instrument with a photodiode array detector Waters 2996 using the analytical HPLC column Cosmosil Cholester 5 µm (4.6 x 250 mm) at the flow rate of 1 ml/min. A linear solvent gradient from 45% to 95% *aq* MeOH containing 0.05% HCOOH over 39 min was used.

NMR spectra (1H NMR, 13C NMR, DEPT 135°, COSY, HMQC, HMBC) were recorded on a MHz DRX Bruker Avance™ 600 (600 MHz) instrument in acetone-d*6* or methanol-d4. UV spectra were run on a Spectrophotometer Cintra 303, GBC, in methanol. HR-ESI-MS spectra were taken on a Bruker micrOTOF-Q.



Figure 1: 1H NMR Spectrum of DHXN – α,β-dihydroxanthohumol.



Figure 2: 13C NMR Spectrum of DHXN – α,β-dihydroxanthohumol.



Figure 3: 1H NMR Spectrum of FXN – xanthohumol flavone.



Figure 4: 13C NMR Spectrum of FXN – xanthohumol flavone.Figure : 1H NMR Spectrum of XN C - xanthohumol C.



Figure 6: 13C NMR Spectrum of XN C - xanthohumol C.



Figure 7: 1H NMR Spectrum of DHXN C – 1’’,2’’-dihydroxanthohumol C.



Figure 8: 13C NMR Spectrum of DHXN C - 1’’,2’’-dihydroxanthohumol C.



Figure 9: 1H NMR Spectrum of DHXN K – 1’’,2’’-dihydroxanthohumol K.



Figure 10: 13C NMR Spectrum of DHXN K - 1’’,2’’-dihydroxanthohumol K.