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PHENOLIC GLYCOSIDES OF *Juncus acutus* AND ITS ANTI-ECZEMATIC ACTIVITY

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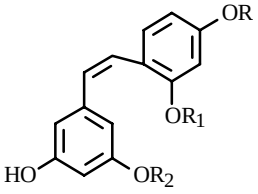
The total alcohol extract of Juncus acutus *L. showed significant anti-eczematic activity. The isolation of the five phenolic glycosides which responsible for this activity were isolated and identified as oxyresveratrol 2*-O-β-*D-glucopyranoside (**1**), resveratrol 3′,4′*-O,O′-*di*-β-*D-glucopyranoside (**2**), markhamioside F (**3**), canthoside B (**4**), and caffeic acid glucorhamnoside (**5**). The toxic effect of the alcohol extract of the plant was studied on mice to determine their LD_{50}, which proved to be nontoxic up to 3000 mg/kg body weight. The anti- eczematic activity of the isolated compounds was tested in mice and showed variable effect. Compounds **3** and **4** were found to have the highest activity; they cured eczema by 90 and 100% respectively.*

Key words*:* phenolic glycosides, anti-eczematic, *Juncus acutus*.

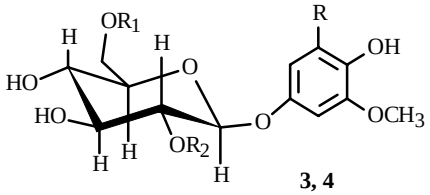
Juncaceae is a very large family distributed all over the world; it holds a rather unique position among angiosperms. However, very little is known about its chemical constituents. Red pigments in juncaceae were studied in 1975 by some worker and they reported the presence of glycosides of luteolinidin and 3-deoxyanthocyanidine but no ordinary anthocyanidines [1]. Seed fats of the family juncaceae were shown to be somewhat similar to one another in fatty acid composition [2]. Dehydrojuncusol and the uncommon simple phenanthrene derivative have been isolated from roots of *Juncus roemerianus* [3]. Concerning the biological activity of *Juncaceae*, phenolic compounds of dihydrophenanthrine derivatives showed very good activity as an antimicrobial against gram positive and gram negative bacteria and some fungi [4]. The chemistry and pharmacological use of Juncaceae have been little studied. Our plant under study is *Juncus acutus* L. This plant has been mentioned by bedewing in the treatment of infection and inflammation, but there is no scientific background on this, so our study was carried out to investigate the chemical content and biological activity of this plant. From *Juncus acutus* L. we identified five substances (**1–5**).

The ^{13}C NMR spectrum of compound **1** indicated the presence of one *β*-D-glucopyranosyl unit together with 14 carbon signals for the aglycone moiety. Acid hydrolysis gave glucose, and the ^{1}H NMR showed the signals of an ABX aromatic ring system in addition to two oleofinic proton at 7.21(1H, d, J = 16.4 Hz) and 6.83 (1H, d, J = 16.4 Hz). The spectral data of the aglycone part was similar to those published for oxyresveratrol [5] except for the additional signal for sugar. From this data and by comparing with published [5] data this compound was identified as oxyresveratrol 2-*O*-*β*-D-glucopyranoside.



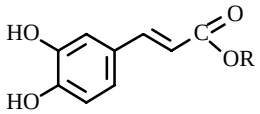
1, 2

1: R = H, R_1 = *β*-D-Glc*p*, R_2 = H
2: R = *β*-D-Glc*p*, R_1 = H, R_2 = *β*-D-Glc*p*



3, 4

3: R = H, R_1 = H, R_2 = Apiose
4: R = OCH_3, R_1 = Apiose, R_2 = H



5

5: R = Glucose-Rhamnose

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TABLE 1. Anti-eczematic Activity of Alcohol Extract (% w/w) of *Juncus acutus* and Its Isolated Compounds

Compound	Number of cured mice/day				
	4	5	6	7	% recovery
Alcohol 2	1	2	2	1	60
Alcohol 4	2	3	2	2	80
Alcohol 6	3	3	3	1	100
1	1	2	3	1	60
2	1	1	3	2	70
3	2	3	3	1	90
4	2	4	3	1	100
5	1	3	1	1	50
Betamethasone*	2	3	2	1	80

*0.1% w/w.

The ^{1}H NMR for compound **2** showed signals of the 1,3,5-trisubstituted aromatic ring at 6.79 (1H, br. s), 6.60 (1H, br. s), and 6.40 (1H, br. s), and the *para*-disubstituted aromatic ring at 7.53 (2H, d, J = 8.6 Hz) and 7.03 (2H, d, J = 8.6 Hz). Acid hydrolysis of this compound gave glucose when tested on TLC with an authentic reference. Therefore this compound was identified [5] as resveratrol 3′,4′-*O*,*O*′-di-β-D-glucopyranoside.

Compound **3:** The ^{1}H NMR spectrum revealed the presence of a set of the ABX system at 6.68 (1H, d, J = 2.7 Hz, H-2), 6.60 (1H d, J = 8.8 Hz, H-5), and 6.47 (1H, dd, J = 8.8, 2.7 Hz, H-6), and a methoxy signal at 3.72 (3H, s, 3-OMe). In addition, there are two anomeric sugars for glucose. This is confirmed by comparative TLC using system (b) for sugar after hydrolysis and by comparing with published data [6]. This compound was identified as markhamioside F.

The ^{1}H NMR and ^{13}C NMR spectral data for compound **4** revealed the presence of a tetrasubstituted symmetrical aromatic ring, and two equivalent methoxy groups together with the ab-apiofuranosyl-(1→6)-*O*-β-D-glucopyranosyl unit. The chemical shifts of the aglycone part were the same as published [7] data for the compound *koaburaside*. The two methoxy groups are located at C3 and C5; therefore this compound was identified as canthoside B [7].

Compound **5** showed the typical signal for the caffeoyl moiety [8]. The glycosidic part upon hydrolysis gave two sugars, rhamnose and glucose. This compound was identified as caffeic acid glucorhamnoside.

The alcohol extract of *Juncus acutus* was effective in mice at concentrations of 4 and 6%, it cured eczema by 80 and 100% respectively, while the isolated compounds showed variable activity (Table 1) at a concentration of 2%.Compounds **3** and **4** were the most effective; they cured eczema by 90 and 100% respectively within 7 days.

EXPERIMENTAL

Plant. The aerial parts of *Juncus acutus* L. were collected from the Siwa Oassis during 2001–2002. The identity of this plant was verified by Prof. N. El-Hadidi, Professor of Botany, Botany Dept., Faculty of Science, Cairo University and by comparison with the plant description in the Flora of Egypt [9]. A voucher specimen of the titled plant was kept in the herbarium of the Desert Research Center. The plant sample was air-dried in shade, reduced to a fine powder, packed in tightly closed containers, and stored for phytochemical and biological studies.

Extraction and Isolation. The defatted powder of plant aerial parts (1 kg) was extracted in a Sohxlet apparatus with 90% ethanol. The ethanolic extract was concentrated under reduced pressure to yield (250g) of dry extract. Silica Gel G was used for thin layer chromatography (TLC, precoated plates) and column chromatography (CC). **Systems**: a) ethyl acetate–methanol–water (30:5:4) and b) ethyl acetate–methanol–acetic acid–water (65:15:10:10); were used for developing the chromatoplates. Visualization of chromatograms was achieved under UV before and after exposure to ammonia vapor or by spraying [10] with ferric chloride. TLC examination of the extract using solvent system “a” and visualizing reagent revealed the presence of 5 spots.

For the isolation of these spots, the alcohol extract (25 g) was applied to the top of a column chromatographic column packed with silica gel (240 gm) and eluted gradually with chloroform–methanol–ethyl acetate, then reapplied on preparative thin layer chromatography (system a).

Bands corresponding to each compound were separately extracted with methanol, concentrated, and submitted to a column of Sephadex LH-20 eluted with methanol–water, where compounds **1–5** were isolated.

Acid Hydrolysis. Five milligram of each glycoside was refluxed with methanolic H_2SO_4 (5 mL MeOH, 5 mL H_2O, 1 mL H_2SO_4) for 3 hr. The reaction mixture was diluted with water and the released aglycone extracted with ether. The aqueous layer was neutralized, and extracted with pyridine, and the concentrated pure sugar residue was dissolved in 10% propanol-2 for identification.

Apparatus. FAB-MS (chn N 29 Ng 5526) ver Ion Uic22 for mass spectra. Varian 600 MHz spectrophotometer for ^{1}H NMR, ^{13}C NMR (TMS as internal standard).

Oxyresveratrol 2-*O*-*β*-D-glucopyranoside (1), amorphous powder (80 mg). ^{1}H NMR (CD_3OD, δ, J/Hz): 7.39 (1H, d, J = 9.3, H-6), 7.21 (1H, d, J = 16.4, H-*α*), 6.83 (1H, d, J = 16.4, H-*β*), 6.58 (1H, d, J = 2.4, H-3), 6.55 (1H, dd, J = 9.3, 2.4, H-5), 6.44 (2H, d, J = 2.0, H-2′, 6′), 6.14 (1HH, t, J = 2.0, H-4′), 4.855 (1H, d, J = 7.3, H-1 Glc), 3.89 (1H, dd, J = 12.3, 1.7, H-6 Glc), 3.68 (1H, dd, J = 12.3, 4.9, H-6 Glc).

^{13}C NMR (DMSO-d_6, δ): 120.4 (C-1), 156.9 (C-2), 105.1 (C-3), 159.6 (C-4), 109.4 (C-5), 128.5 (C-6), 142.1 (C-1′), 106.1 (C-2′), 159.3 (C-3′), 103.2 (C-4′), 159.3 (C-5′), 106.1 (C-6′), 124.4 (C-*α*), 128.2 (C-*β*), 102.1 (G-1), 74.9 (G-2), 78.1 (G-3), 71.3 (G-4), 77.9 (G-5), 62.4 (G-6). FAB-MS, *m/z*: 406 (M) (100), 226 (70).

Resveratrol 3′,4′-*O*,*O*′-*di*-*β*-D-glucopyranoside (2), amorphous powder (100 mg). ^{1}H NMR (CD_3OD, δ, J/Hz): 7.53 (2H, d, J = 8.6, H-2), 7.39 (1H, d, J = 9.3, H-6), 7.1 (1H, d, J = 16.4, H-*β*), 7.03 (2H, d, J = 8.6, H-3′, 5′), 6.98 (1H, d, J = 16.4, H-*α*), 6.79 (1H, br. s, H-2), 6.60 (1H, br. s, H-6), 6.58 (1H, d, J = 2.4, H-3), 6.55 (1H, dd, J = 9.3, 2.4, H-5), 6.44 (2H, d, J = 2.0, H-6), 6.40 (1H, br. s, H-4), 4.89 (1H, d, J = 7.3, H-1 Glc), 4.80 (1H, d, J = 7.6, H-1/Glc).

^{13}C NMR (DMSO, δ): 13.2 (C-1), 103.3 (C-2), 159.2 (C-3), 105.0 (C-4), 158.1 (C-C-5), 107.4 (C-6), 130.9 (C-1′), 127.9 (C-2′), 116.7 (C-3′), 157.3 (C-4′), 116.7 (C-5′), 127.9 (C-6′), 126.9 (C-*α*), 128.3 (C-*β*), 100.4 (G-1), 100.9 (G-1′), 7.4 (G-2,2′), 76.6 (G-3), 76.7 (G-3′), 69.8 (G-4,4′), 77.1 (G-5), 77.2 (G-5′), 60.9 (G-6,6′), FAB-MS, *m/z*: 552 (100) (M), 472 (60), 292 (40).

Markhamioside F (3), amorphous powder (110 mg). ^{1}H NMR (CD_3OD, δ, J/Hz): 6.68 (1H, d, J = 2.7, H-2), 6.60 (1H, d, J = 8.8, H-5), 6.47 (1H, dd, J = 8.8, 2.7, H-6), 3.72 (3H, s, 3-OMe), Apiose [5.36 (1H, d, J = 1.5, H-1), 4.02 (1H, d, J = 9.5, H-4), 3.88 (1H, d, J = 1.5, H-2), 3.70 (1H, d, J = 9.5, H-4), 3.50 (2H, s)], Glucose [4.70 (1H, d, J = 7.8, H-1), 3.40 (1H, dd, J = 9.4, 7.8, H-2), 3.54 (1H, dd, J = 9.3, 8.0, H-3), 3.23 (1H, m, H-5), 3.80 (1H, dd, J = 12.2, 2.0, H-6)].

^{13}C NMR (DMSO-d_6, δ): 153.1 (C-1), 103.9 (C-2), 150 (C-3), 143.2 (C-4), 117.0 (C-5), 110.0 (C-6), 56.6 (OMe), Apiose [110.9 (C-1′), 78.6 (C-2′), 80.9 (C-3′), 75.8 (C-4′), 66.4 (C-5′), Glucose [102.8 (C-1′), 79.1 (C-2′), 78.3 (C-3′), 71.9 (C-4′), 79.1 (C-5′), 62.9 (C-6′)]. FAB-MS, *m/z*: 434 (M), 288 (70), 108 (50).

Canthoside B (4), amorphous powder (130 mg), ^{1}H NMR (CD_3OD, δ, J/Hz): 6.51 (1H, s, H-2), 6.51 (1H, s, H-6), 3.84 (3-OMe), 3.84 (5-OMe), Apiose [5.1 (1H, d, J = 2.5, H-1), 4.02 (1H, d, J = 2.5, H-4), 3.88 (1H, d, J = 1.5, H-2), 3.70 (1H, d, J = 9.5, H-4), 3.50 (2H, s, H-5)], Glucose [4.90 (1H, d, J = 7.8, H-1), 3.69 (1H, dd, J = 9.0, 7.8, H-2), 3.54 (1H, dd, J = 9.3, 8.0, H-3), 3.45 (1H, dd, J = 8.8, 8.6, H-4), 3.56 (1H, m, H-5), 3.71 (1H, dd, J = 10.3, 2.0, H-6)].

^{13}C NMR (DMSO, δ): 152.5 (C-1), 97.3 (C-2), 149.8 (C-3), 132.6 (C-4), 149.8 (C-5), 79.1 (C-6), 56.9 (3-OMe), 56.9 (5-OMe), Apiose [110.8 (C-1′), 77.9 (C-2′), 80.9 (C-3′), 74.9 (C-4′), 65.8 (C-5′), Glucose [103.8 (C-1′), 75.1 (C-2′), 70.9 (C-3′), 71.9 (C-4′), 77.1 (C-5′), 68.9 (C-6′)]. FAB-MS, *m/z*: 464 (M) (100), 318 (60), 138 (50).

Compound 5: amorphous powder (60 mg). ^{1}H NMR (CD_3OD, δ, J/Hz): 7.048 (1H, d, J = 16, H-7), 7.00 (1H, s, H-2), 6.98 (1H, d, J = 8, H-6), 6.85 (1H, d, J = 8 Hz, H-5),6.35 (1H, d, J = 16, H-8), Glucose [4.70 (1H, d, J = 7.8, H-1), 3.40 (1H, dd, J = 9.4, 7.8, H-2), 3.54 (1H, dd, J = 9.3, 8.0, H-3), 3.23 (1H, m, H-5), 3.80 (1H, dd, J = 12.2, 2.0, H-6)], rhamnose [5.4 (1H, d, J = 2, H-1″ rhamnose anomeric sugar proton), 3–4 (m, remaining sugar protons) and 1.1 (3H, d, J = 6, CH_3)].

^{13}C NMR (DMSO, δ): 125.8 (C-1), 115.8 (C-2), 146.6 (C-3), 148.6 (C-4), 116.4 (C-5), 121.8 (C-6), 148.6 (C-7), 114.8 (C8), 166.1 (C-9), rhamnose [101.3 (C-1) 70.6 (C-2), 70.4 (C-3), 71.9 (C-4), 70 (C-5), and 17.6 (C-6)], Glucose [102.8 (C-1′), 74.1 (C-2′), 79.3(C-3′), 70.9(C-4′), 74.6 (C-5′), 60.9 (C-6′)]; MS, *m/z*: 522 (M), 390 (60%), 210 (50).

Biological Activity. The LD_{50} of the investigated extract was determined following Finney's method [11], from which the therapeutic index was also calculated.

2-Anti-eczematic Activity [12].

I. Preparation of the Extract and Compounds. The alcohol extract (residue removed) was prepared as an ointment using 10% lanolin in Vaseline (as ointment base) in a concentration of 2, 4, and 6% w/w, and the isolated compounds in a concentration of 2% only. Dinitrochlorobenzene (DNCB) prepared in a acetone in concentration of 2% was used for inducing eczema.

II. Eczema Formation and Treatment. Nineteen groups of mice (10 mice each) were sensitized by local application of 2% DNCB. Eczema was formed after 5 days. Each group of eczematic mice was treated once daily with the ointments (extract and the five compounds) and compared with the standard 0.1% w/w betamethasone ointment.

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REFERENCES

1. A. Fredga, G. Bendz, and A. Apell (1974). Red pigment in Juncaceae family. *Nobel Symp*., 194, 25, 121 (through chemical abstract, 82, 82918, 1975).
2. M. Isobel Morice, *J. Food Agr*., **18** (3), 129 (1967).
3. H. Sarkar, M. Zerezghi, and B. Jundabrata, *Phytochemistry*, **27** (9), 3006 (1989).
4. D. Chapatwala, A. Rmando, and M. Howard, *Life Sci.,* **29** (19), 1997 (1981).
5. K. Tripetch, S. Kotomi, K. Ryoji, and Y. Kazuo, *Chem. Pharm. Bull.,* **50** (6), 863 (2002).
6. K. Tripetch, K. Ryoji, and Y. Kazuo, *Phytochemistry*, **59**, 55 (2002).
7. K. Tripetch, K. Ryoji, and Y. Kazuo, *Phytochemistry*, **61**, 461 (2002).
8. H. M. Grace and M. Salwa Nofal, *Bull. Fac. Pharm. Cairo Uni*., **39** (1), 345 (2001).
9. B. Loutfy, *Flora of Egypt*, **II**, al Hadara Publishing, Cairo, Egypt, 2000, 249.
10. E. Stahl, *Thin-Layer Chromatography*, 1969, 2nd ed., George Allon and Unwirid, London, Springer, Berlin.
11. D. J. Finney, *Statistical Methods in Biological Assay,* Charles, Griffin and Company Ltd., London, 1964, 97.
12. S. El-Dondity, T. Khalifa, H. Ammar, and M. Ashour, *Az. J. Pharm. Sci*., **24**, 230 (1999).