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Isoquercitrin, the Major Constituent from *Jacquemontia pentantha* (Jacq.): Hepatoprotective Evaluation of the Extracts.

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ABSTRACT

The aqueous methanol crude extract and its defatted methanol fraction of *Jacquemontia pentantha* (Jacq.) (Family Convolvulaceae) aerial parts showed a significant hepatoprotective activity. Several chromatographic techniques of the methanol fraction led to the isolation of a major compound, identified as quercetin 3-*O*-*ß*-D-glucopyranoside (Isoquercitrin) along with kaempferol 3-*O*- *ß*-D-galactopyranoside for the first time from this species. The isolated compounds were identified by ^{1}H, ^{13}C NMR and ESI-MS spectroscopy.
Keywords: *Jacquemontia pentantha*, Convolvulaceae, Flavonoids, Hepatoprotective.

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INTRODUCTION

` Hepatitis is an inflammation of the liver. There are five types of hepatitis viruses, which are notified as A,B,C,D and E types. Types B and C lead to chronic disease in many people and cause of liver cirrhosis and cancer. *Jacquemontia pentantha* (Jacq.) (Family Convolvulaceae) is a herb distributed in many tropical and subtropical countries including the West African Region, America, tropical Africa, Asia and Australia [1]. Little studies are reported on *Jacquemontia pentantha*. Recently, we reported a new acylated flavonol glycoside and seven known flavonoid compounds from *J. pentantha* along with the anti-hyperglycemic activity, antioxidant activity, anti-inflammatory activity and antimicrobial activity of its extracts [2] . Continuing our study on this plant, this work involves the isolation and characterization of further two flavonol glycosides for the first time from this plant, as well as the evaluation of the hepatoprotective activity of the total extract and the flavonoids rich fraction of the plant.

MATERIALS AND METHODS

General

JEOL EX-500 MHz NMR spectrometer was used in NMR spectra. TMS is used as internal standard, mass spectra (±) ESI-MS: LCQ Advantage Thermo Finnigan spectrometer.

Plant sample collection

The unflowering aerial parts of *Jacquemontia pentantha* (Jacq.) were collected from El – Orman garden, Giza, Egypt in May 2013 and identified by Mm. Tressa Labib,Taxonomist, El- Orman garden, Giza, Egypt. A voucher specimens are deposited in the Herbarium, National Research Centre, with No. JP 455.

Animals

In this study we use adult male Albino rats of Sprague Dawely Strain of 130-150 g body weight, obtained from the animal house colony of National Research Centre (NRC), Egypt. They were kept on a standard laboratory diet and under the same hygienic conditions. All procedures concerning animals, treatment and experimentation were in accordance with the Guiding Principles in the Care and Use of Animals and were approved by the Experimental Animal Research Committee, NRC, Egypt.

Chemicals and kits

Transaminase kits (BioMerieux Co.): biodiagnostic kits for assessment of serum aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP). Silymarin: used as standard hepatoprotective drug. Carbon tetrachloride (analar). Doses of the tested materials were administrated orally by gastric tubes [3].

Extraction and isolation

The air-dried powdered unflowering aerial parts *of Jacquemontia pentantha* (1 kg) were extracted by percolation with 70% methanol till exhaustion. The solvent was evaporated under reduced pressure to yield 250 g extract. A part of the crude extract (200 g) was defatted by petroleum ether, then the residue was extracted by methanol to yield the methanol fraction (120 g). A part of the methanol fraction (100 g) was subjected to column chromatography (120 x 5 cm) on polyamide 6S (250 g). Similar fractions were collected after examination by paper chromatography to give six major fractions. Fraction 2 was subjected to Sephadex LH-20 CC using 40% methanol as eluent, to yield two compounds: compound **1 (**18 mg) which was purified on Sephadex LH-20 by using 50 % methanol as eluent and compound **2** (4g) (major constituent).

Determination of total phenolics and flavonoids

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The defatted MeOH extract of *J. pentantha* aerial parts was estimated for its total phenolics and flavonoids

The total phenolic content was determined by the method using Folin- Ciocalteu reagent (FCR) [4]. In this method 250 µl of the diluted extract was mixed to 1.25 ml diluted Folin–Ciocalteu reagent and 100µl of gallic acid was added. After 2 min, 1000 µl of saturated sodium carbonate (7.5 %) was added. After 2 h of incubation at room temperature, the absorbance at 760 nm was measured using UV-VIS spectrophotometer. Gallic acid was used for the standard calibration curve. The results were expressed as Gallic acid equivalent (GAE)/g dry weight of plants, and calculated as mean value ± SD (n = 3).

The total flavonoid was measured as colorimetric assay using aluminum chloride method [5]**.** A 100 µl aliquot of the diluted sample and standard solution of quercetin was added to 1ml volumetric flask containing 250 µl of distilled H_2O. Then 75 µl of 5% Na NO2 was added to the flask. After 5 min,75 µl of 10% Al Cl_3 was added. Then after 5 min, 500 µl of 1 M NaOH was added to the mixture. Absorbance of the mixture was determined at 410 nm versus prepared water blank. Total flavonoid content of the extract was expressed as mg quercetin equivalents (QE) / g dry weight.

Hepatoprotective activity assay:

Liver damage in rats was induced according to the method [6], by intraperitoneal injection of 5ml / kg of 25% carbon tetrachloride (CCl_4) in liquid paraffin. Animals **were** randomly divided into six groups each of 10 rats as follows:

Group (n=10)	Treatment
Group I	1 ml saline
Group II	70% methanol extract (50 mg/ Kg)
Group III	70% methanol extract(100 mg/ Kg)
Group IV	methanol extract (50 mg/ Kg)
Group V	methanol extract(100 mg/ Kg)
Group VI	Reference drug, silymarin (25 mg/ Kg)

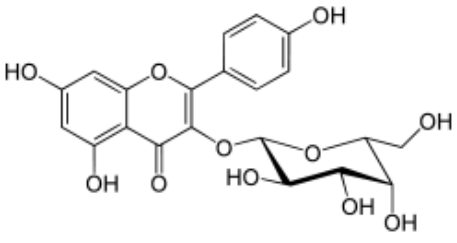
Administration of the drug was continued after liver damage for another one week. Followed by an overnight fast, whole blood was obtained from the retro orbital venous plexus through the eye canthus of anaesthetized rat. Blood samples were collected at zero time, 72 hours and after one week intervals. Serum was isolated by centrifugation. The levels of serum ALT, AST [7], ALP [8], were observed**.**

RESULTS AND DISCUSSION

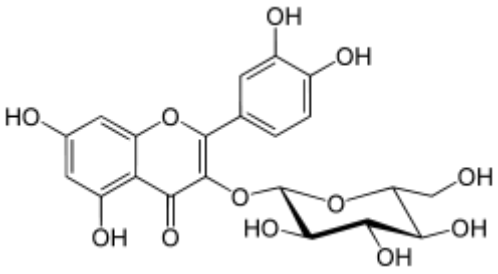
Total phenolics and flavonoids

The content of total phenolics in the extract was measured using FCR assay and is expressed in gallic acid equivalent (GAE), while the total flavonoids content was estimated using aluminum chloride method (expressed as quercetin equivalent, QE), The total phenolics content in *Jacquemontia pentantha* aerial parts was (8.49 ± 0.76), while the flavonoids content was (6.22 ± 0.25).

Phytochemical evaluation



Trifolin (1)



Isoquercitrin (2)

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Fig. 1

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Table (1): Effect of alc. ext. of *Jacquemontia pentantha* plant and silymarin drug on serum enzymes level (AST, ALT and ALP) in liver damaged rats (n=10)

Group	AST (u/L)			%	ALT (u/L)			%	ALP (u/L)			%
	Zero	72h	7d	Chg.	Zero	72h	7d	Chg.	Zero	72h	7d	Chg.
Control	38.8± 1.1	139.7±4.9*	145.6±6.2*	4.2	37.1±1.2	146.1±7.8*	151.2*±6.8*	3.5	7.4±0.1	71.3±3.4*	78.2±3.8*•	9.6
70% methanol extract (50mg)	38.5±1.2	82.3±3.7*	63.2±1.6*•	23.2	39.8±1.1	99.8±2.5*	77.5±0.1*•	22.3	7.5±0.1	43.6±1.9*	31.9±1.8*•	26.8
70% methanol extract (100mg)	39.4±1.5	60.2±2.1*	42.4±2.5*•	29.6	36.6±1.5	66.3±2.7*	50.8±2.5*•	23.4	7.3±0.1	31.3±2.1*	19.2±0.3*•	38.7
Methanol extract (50mg)	41.1±1.7	92.3±4.8*	85.4±4.1*	7.5	37.5±0.8	91.4±3.8	82.8±3.6*•	9.4	7.2±0.1	61.3±2.7*	49.2±1.5*•	19.7
Methanol extract (100mg)	39.6±1.4	73.4±3.2*	56.8±2.6*•	22.6	38.2±1.3	77.5±2.6*	63.4±2.9*•	18.2	7.6±0.1	56.9±1.2*	37.3±1.1*•	34.4
Silymarin 25 mg/kg	37.6±1.5	53.2±2.2*	37.2±1.4•	30.1	41.2±1.7	53.7±1.8*	39.9±1.4*	25.7	7.8±0.1	18.9±0.6*	7.6±0.1•	59.8

*Statistically significant from zero group at p. <0.01• Statistically significant from 72h after CCL_4 at P<0.01.

Investigation of the methanol extract of the unflowering aerial parts of *Jacquemontia pentantha* afforded two flavonol glycosides which were identified, applying UV, ESI-MS and NMR spectroscopic techniques, as kaempferol 3 –*O*-β-D- galactopyranoside (trifolin, **1**) and quercetin 3-*O*-ß-D-glucopyranoside (isoquercetin, **2**) (Fig. 1). Their spectroscopic data were in accordance with the previously reported data [9,10].

Kaempferol 3 –*O*-β-D- galactopyranoside (1):

yellow powder; ESI-MS, *m/z*: 447 $[M-H]^-$; UV λ_{max} nm(Methanol) : 268 (II), 350 (I); ^{1}H NMR (DMSO-d6, 500MHz) δ (ppm),12.62 (1H, s, OH-5), 8.07 (1H, d, *J* = 8 Hz, H-2'), 8.02 (1H, d, *J* = 8 Hz, H-6'),6.86 (2H, d, *J* = 8 Hz, H-3', 5'), 6.44 (1H, s, H- 8),6.21 (1H, s, H-6), 5.39 (1H, d, *J* = 8 Hz, H-1''), 3.5-4.7 (m, sugar protons); ^{13}C-NMR (DMSO-*d*6, 125 MHz) δ (ppm),156.4 (C-2), 133.6 (C-3), 177.4 (C-4), 161.2 (C-5), 98.7 (C-6), 164.2 (C-7), 93.7 (C-8), 156.7(C-9), 103.9 (C-10), 120.6 (C-1'), 130.9 (C-2'), 130.7 (C-6'), 115.0 (C-3'), 159.9 (C-4'), 115.2 (C-5'), 101.6 (gal-C-1), 71.19 (gal-C-2),73.08 (gal-C-3), 67.86 (gal-C-4), 75.86 (gal-C-5), 60.17 (gal-C-6).

Quercetin 3-*O*-ß-D-glucopyranoside (2):

yellow amorphous powder; ESI-MS *m/z* 463 $[M-H]^-$; UV λ_{max} nm(Methanol): 273 (II), 354 (I); ^{1}H NMR (DMSO- d6, 500MHz) δ (ppm), 12.60 (1H, s, OH-5), 6.18 (1H, d, *J* = 1.8 Hz, H-6), 6.38 (1H, d, *J* = 1.8 Hz, H-8), 7.66 (1H, d, *J* = 2.2 Hz, H-2'), 7.53 (1H, dd, *J* = 2.2, 8 Hz, H-6'), 6.82 (1H, d, *J* = 8 Hz, H-5'), 5.37 (1H, d, *J* = 7.8 Hz, glc-H-1), 3.2-3.7 (m, sugar protons); ^{13}C-NMR (DMSO-*d*6, 125 MHz) δ (ppm), 156.8 (C-2), 133.9 (C-3), 177.8 (C-4), 161.7 (C-5), 99.3 (C-6), 165.3 (C-7), 94.1 (C-8), 156.6(C-9), 104.1 (C-10), 121.5 (C-1'), 115.6 (C-2'), 145.3 (C-3') , 148.8 (C-4'), 116.3 (C-5'), 122.4 (C-6'),102.3 (glu-C-1), 71.6 (glu-C-2), 73.6 (glu-C-3), 68.3 (glu-C-4), 76.3 (glu-C-5), 60.6 (glu-C-6).

Bioassay evaluation

Treatment of rats with 70% methanol and methanol extracts of aerial parts of *Jacquemontia pentantha* showed significant decrease in serum AST, ALT and ALP as compared to reference drug (Silymarin). The results revealed that the 70% methanol extract (100mg) has been found to be promising hepatoprotective agent as it reduced the serum liver enzymes AST, ALT and ALP by 29.6, 23.5 and 38.7, respectively, compared to reference drug silymarin (30.1, 25.5 and 59.7), respectively. Next,70% methanol extract (50 mg/kg) with percentage of change were 23.2%, 22.3% and 26.8%, respectively . Followed, by methanol (100mg/kg) fraction with percentage of change were 22.6%, 18.2% and 34.4%, respectively. The least effective was the methanol (50 mg/kg) fraction, the percentage of change being 7.5%, 9.4% and 19.7%, respectively (Table 1).

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