ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

A New Acylated Flavonol Triglycoside and Bioactivities of *Jacquemontia pentantha* (Jacq.).

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ABSTRACT

A new flavonoid, named quercetin 3-*O*-(2-*E*-caffeoyl-ß-D-xylopyranosyl)–(1→2)–α–L–rhamno-pyranosyl–(1→2)–ß–D–glucopyranoside**,** was isolated from the methanol extract of *Jacquemontia pentantha* (Jacq.) (Family Convolvulaceae) aerial part. Its structure was elucidated by spectroscopic analysis including ^{1}H, ^{13}C- NMR, HMQC, HMBC, and ESI-MS. The known flavonoids, quercetin 3-*O*-ß- xylo-pyranoside, quercetin 3-*O*-α-L- rhamnopyranoside, quercetin-3-*O*-β-galacto-pyranoside (hyperin), isorhamnetin 3-*O*-β- D-glucopyranoside**,** kaempferol 3-*O*-β-D-glucopyranoside (astragalin), quercetin and kaempferol, were also isolated and characterized. The 70% methanol crud extract and its successive methanol fraction showed significant anti-hyperglycemic activity, antioxidant activity and anti-inflammatory activity. Determination of the median lethal dose (LD_{50}) revealed that 70% methanol total extract was safe. The antimicrobial activity of the extracts was evaluated against Gram positive, Gram negative bacteria, yeast and fungi.

Keywords: *Jacquemontia pentantha*, Convolvulaceae, Flavonoids, Antihyperglycemic, Antioxidant, Anti-inflammatory, Antimicrobial**.**

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ISSN: 0975-8585

INTRODUCTION

Convolvulaceae**,** known commonly as the bindweed or morning glory family of flowering plants, which includes 57 genera and about 1600 species, widely cultivated for their colorful funnel-shaped flowers and heart-shaped leaves [1]. The family is widespread in both tropical and temperate areas [2]. Convolvulacea includs many economic uses, edible crops, ornamentals, medicinal plants, and some serious weeds. The genus *Jacquemontia* involves 120 species. They are distributed neotropical occurring mainly in America, but also in tropical Africa, Asia and Australia [3]. *Jacquemontia pentantha* is attractive ornamental, fast growing twining climber, distributed in many tropical and subtropical countries including the West African Region**.** [4]. Chemically, Convolvulaceae species are characterized by the presence of flavonoids, terpenoids [5] and alkaloids [6]. Bioactivities so far detected in many species of family Convolvulaceae are antioxidant [7], antimicrobial [8], anti-inflammatory [9] and antidiabetic [10-11]. Nothing is reported on *Jacquemontia pentantha* (Jacq.). This study aimed at investigation of chemical constituents and pharmacological activity of *Jacquemontia pentantha* aerial part, cultivated in Egypt, in order to support the possibility of its uses as a natural resource in therapeutics and to demonstrate the correlation between chemical composition and bioactivity.

MATERIALS AND METHODS

General

NMR spectra were recorded on a JEOL EX-500 MHz NMR spectrometer using TMS as internal standard, mass spectra (±) ESI-MS: LCQ Advantage Thermo Finnigan spectrometer.

Plant sample collection

The aerial part of *Jacquemontia pentantha* (Jacq.) (Family Convolvulaceae) were collected from El–Orman garden, Giza, Egypt in May 2013. The plant was identified by Mm. Tressa Labib, Taxonomist, El- Orman garden,Giza, Egypt. The plant samples were air–dried, powdered and kept for phytochemical and bioactivity studies.

Animals

Adult male Albino rats of Sprague Dawely Strain of 130-150 g body weight. Albino mice of 25-30 g body weight were used in this study, obtained from the animal house colony of National Research Centre (NRC), Egypt. All animals were kept under the same hygienic conditions and on a standard laboratory diet. 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

Glutathione kit (Wak Company-Germany) for the assessment of antioxidant activity. Indomethacin (Epico, Egyptian Int. Pharmaceutical Industries Co.). Metformin (Chemical Industries Development, Giza, ARE). Vitamin E (Pharco Pharmaceutical Co.), Carrageenan (Sigma Co.). Alloxan (Sigma Co.). Thiophenicol (Sanofi Aventis, France) used as an antibacterial agent and Treflucan (Egyptian International Pharmaceutical Industries Company, EIPICO) used as an antifungal agent. Nutrient agar medium was purchased from Lab M limited (Lancashire BL9 6AS, United Kingdom) and potato dextrose agar medium was purchased from Becton, Dickinson and company Sparks (MD 21152, USA). Doses of the tested materials were administrated orally by gastric tubes [12].

Microorganisms

Microorganisms used in the present study were obtained from the culture collection of Chemistry of Microbial and Natural Product Department, NRC, Dokki, Cairo, Egypt. The bacterial strains used were the gram positive bacteria, *Staphylococcus aureus* and *Bacillus subtilills*, the gram negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*. The fungi used were *Aspergillus niger*, *Fusarium solani* and *Alternaria alternata*.

The Yeast used was *Candida albicans*. All bacteria were cultured in nutrient agar medium at 37°C for 24 h prior to use while the fungi was cultured on potato dextrose agar at 37°C for 24 h then suspended in potato dextrose broth prior to use.

Extraction and isolation

The air-dried powdered aerial part *of Jacquemontia pentantha* (1200g) was extracted with 70% methanol till exhaustion. The methanol extract was evaporated under reduced pressure to yield 279.47 g extract (23% yield) and was fractionated into petroleum ether, chloroform and methanol fractions. The successive extracts were separately evaporated under reduced pressure to yield 13.85 g, 0.81 g, 140 g residues, respectively. The methanol fraction (140 g) was subjected to column chromatography on polyamide 6S. The column was eluted with distilled water followed by water/methanol step –gradient. Finally, the column was eluted with methanol to ensure complete elution. The separated fractions resulted from the column were examined by PC using solvent systems (BAW and 15% ACOH) and visualization under UV light. Similar fractions were collected to give six major fractions which were subjected to Sephadex LH-20 columns to give , quercetin 3-*O*-(2-*E*-caffeoyl-ß-D-xylopyranosyl)–(1→2)–α–L–rhamno-pyranosyl–(1→2)–ß–D–glucopyranoside (**1**) (12 mg), in addition to seven known flavonoids, namely quercetin 3-*O*-ß- xylo-pyranoside, quercetin 3-*O*-α-L –rhamnopyranoside, quercetin-3-*O*-β-galacto-pyranoside (hyperin), isorhamnetin 3-*O*-β- D-glucopyranoside**,** kaempferol 3-*O*-β-D-glucopyranoside (astragalin), quercetin and kaempferol which were isolated for the first time from this plant.

Preparation of extract for bioactivities

The air-dried powdered aerial part of *Jacquemontia pentantha* (500 g) was extracted by 70% methanol. The methanol extract was evaporated under reduced pressure to give 105 g extract and was fractionated into petroleum ether, chloroform and methanol fractions. The extracts were separately evaporated under reduced pressure to yield 5 g, .28g and 40 g, respectively. The extract were kept in tightly sealed sample tubes for the biological study.

Method for evaluation Median Lethal Dose (LD_{50})

Determination of LD_{50} was carried out according to Karber G [13]. For acute toxicity study, groups of ten mice of both sex (20 – 30 g) were used. Several doses at equal logarithmic intervals were chosen , each dose was injected in a group of 10 animals by subcutaneous injection. The mice were then observed for24 hrs. and symptoms of toxicity and mortality of rats in each group were recorded and the LD_{50} was calculated using the following equation :

$$LD_{50} = D_m - \Sigma (Z \times d) / n$$

Where:
D_m = Highest dose which kills all animals in the groups
Z = The mean of dead animals in two successive groups
D = The constant factor between two successive groups
N = The number of animals in each of the dose levels

Method for evaluation of antihyperglycemic activity:

Male albino rats of the Sprague Dawely Strain (130- 140 g) were injected intraperitoneally with alloxan (150 mg/ kg body weight) to induce diabetes mellitus [14]. Hyperglycemia was assessed by measuring blood glucose after 72 hrs and after 2 and 4 weeks intervals [15].

Group (n=6)	Treatment
Group I	Positive control
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, cidophage (100 mg/ Kg)

At the end of each study period, blood samples were collected from the retro orbital venous plexus through the eye canthus of anaesthetized rats after an overnight fast. Serum was isolated by centrifugation and the blood glucose level was measured [15].

Method for evaluation of antioxidant activity:

Male albino rats of the Sprague Dawely Strain (130- 140 g) were injected intraperitoneally with alloxan (150 mg/ kg body weight) to induce diabetes mellitus [14]. Forty two rats were divided into seven groups each of six animals:

Group (n=6)	Treatment
Group I	Negative control
Group II	Positive control
Group III	Reference drug, vitamin E (7.5 mg/kg)
Group IV	70% methanol extract(100 mg/ Kg)
Group V	70% methanol extract (50 mg/ Kg)
Group VI	methanol extract(100 mg/ Kg)
Group VII	methanol extract(50 mg/ Kg)

After seven days, blood samples were collected from the rats.

Determination of blood glutathione: Glutathione in blood was determined according to the method of Beutler E. [16].

Glutathione (GSH) concentration in blood = A_{sample} x 66.66 mg/dl

Method for evaluation of anti-inflammatory activity:

This effect was determined according to the method described by Winter [17]**.** Sixty male albino rats, weighing 130- 150 g were divided into six groups, each of ten animals:

Group (n=6)	Treatment
Group I	Control
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, Indomethacin (20 mg/Kg)

One hour later, all the animals received a subplantar injection of 0.1 ml of 1% carrageenan solution in saline, in the right hind paw .One, two, three and four hours after drug administration, edema thickness were measured by the caliber.

The % edema were calculated .

$$\% \text{ Edema} = \frac{\text{Paw diameter after carrageenan} - \text{Paw diameter before carrageenan}}{\text{Paw diameter before carrageenan}} \times 100$$

Statistical analysis

The obtained data were analyzed by using the student ``t`` test [18].

Antimicrobial assay

Antimicrobial activity of plant extracts (70 % methanol and methanol) was performed by the agar diffusion technique [19-20]. Six different concentrations of each extract were prepared and individually tested against gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), yeast (*Candida albicans*), and fungi *Aspergillus niger* , *Fusarium solani* , and *Alternaria alternate*. Microbial suspension of tested bacteria and fungi spread on nutrient agar (NA) and potato dextrose agar (PDA), respectively. After the media had cooled and solidified, the discs were applied on the inoculated agar plates and incubated for 24 h at 30 ºC for bacteria and 72 h at 28 ºC for fungi. Thiophenicol and Treflucan were used as positive controls for antibacterial and antifungal activity in a concentration of 100 µg/disc. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition around the disc in millimeters (mm) and compared with that of the controls.

Minimum inhibitory concentration

The minimum inhibitory concentration of the crude extracts (70 % methanol and methanol) were evaluated at the final concentrations; 1000, 500, 250, 125 and 62.5 µg. And the final concentrations of positive controls tested were; 50, 25, 12.5, 6.25, 3.13 µg. The lowest concentration showing inhibition zone around the disc was taken as the minimum inhibitory concentration (MIC).

RESULTS AND DISCUSSION

Phytochemical evaluation

The methanol extract of *Jacquemontia pentantha* was subjected to column chromatography of polyamide 6S. Several chromatographic runs on Sephadex LH-20 led to isolation of a new acylated flavonol glycoside (**1**), along with seven known flavonoids. The known flavonoids, quercetin 3-*O*-ß- xylo-pyranoside [21-22], quercetin 3-*O*-α -L –rhamnopyranoside [23], quercetin-3-*O*-β- galacto-pyranoside (hyperin) [22], isorhamnetin 3-*O*-β- D- glucopyranoside [24], kaempferol 3-O-β-D-glucopyranoside (astragalin) [25], quercetin and kaempferol [26], were all identified by comparison of their UV, ^{1}H and ^{13}C NMR with literature data. The ESI-MS spectrum of compound **1** showed a molecular ion peak at *m/z* 903[M-1]$^{-}$, corresponding to $C_{41}H_{44}O_{23}$. The UV spectrum of the compound showed maximal absorbance peaks at λmax 341, 265nm, which suggested a flavonol structure. The ^{1}H-NMR spectrum of compound **1** exhibited two aromatic hydrogen doublet signals with 'meta coupling' at δ 6.18 with (J = 1.8 Hz) for H- 6 and at δ 6.38 with (J= 1.8 Hz) for H- 8 of the A ring of the flavonol skeleton. Furthermore, it showed a doublet signal at δ 7.66 with (J= 2.2 Hz) for H- 2', a doublet signal at δ 6.84 with (J= 8 Hz) for H- 5', a doublet signal at δ 7.65 with (J= 2.2 & 8Hz) for H- 6' of the B ring. The spectrum showed three anomeric proton signals at δ 5.52 (1H, d, J=7.6 Hz, glu-H-1), δ 5.34 (1H, br s, rha-H-1), δ 4.19 (1H, d, J=6.7 Hz, xyl-H-1) (Table 1). The absence of a specific signal for an olefinic hydrogen at C-3 suggested that the compound was a flavonol glycoside. It also showed the (*E*)- caffeoyl signals at δ 7.26 (1H, d, J=15.7 Hz), δ 6.04 (1H, d, J=15.7 Hz). A methyl signal observed at δ 1.05 (d, J = 6.1Hz). ^{13}C-NMR spectrum of compound **1** showed 41 carbon resonances, 15 of which for a flavonol nucleus and six for glucose moiety, six for rhamnose moiety, five for xylose and nine for (*E*)- caffeoyl signals (Table 2). The hydrogenated carbons were assigned using HSQC experiment. The anomeric carbon signal at δ 99.9 ppm was observed to have a cross peak with the anomeric proton signal at δ 5.52 ppm, also the anomeric carbon signal at 108.6 ppm was observed to have a cross peak with the anomeric proton signal at 5.34 ppm in the HSQC spectrum. The glycosidic linkages were detected from the HMBC cross-peaks. A correlation between anomeric signal δ 5.52 of ß-D- glucose and C-3 (δ 133.2) of quercetin was observed, therefore ß-D- glucose was determined to be linked to quercetin at the C-3. The correlation between anomeric signal δ 5.34 of rhamnose and C-2 of glucose at δ 74.1 indicated that rhamnose was attached to C-2 of glucose. A correlation between anomeric signal δ 4.19 of ß-D- xylose and C-2 of rhamnose at δ 68.4 indicated that ß-D- xylose was attached to C-2 of rhamnose. Also the cross peak observed between H-C-2 xylose at δ 4.19 and C(1)-caff at 166.2 revealed the (*E*)- caffeoyl to be at C (2) of the xylose moiety. The relative stereochemistry of compound **1** was suggested by comparison of coupling constant values with those of the reference compounds [27-28]. Based on the accumulated data above, the compound **1** was identified as Quercetin-3-*O*-(2-*E*- caffeoyl-ß-D-xylopyranosyl)-(1→2)–α–L–rhamnopyranosyl–(1→2)–ß–D–glucopyranoside (Fig. 1). To our knowledge, this compound **1** is reported here for the first time from nature.

ISSN: 0975-8585

Table 1: ^{1}H - NMR data (DMSO-*d*6) for compound 1 δ in ppm, J in Hz.

H	δ_H	H	δ_H
A-ring		Xylose	
6	6.18 (d, J=1.8)	1''''	4.19 (d, J= 6.7)
8	6.38 (d, J= 1.8)	2''''	4.37
B-ring		(*E*)- Caffeoyl	
2'	7.66 (d, J= 2.2)	3	7.29(d, J= 15.9)
5'	6.84 (d, J= 8)	2	6.17(d, J= 15.9)
6'	7.65(J= 2.2 & 8)		
Glc			
1''	5.52 (d, J=7.6)		
2''-6''	3.4 - 3.7 (m)		
Rha			
1'''	5.34 (brs)		
6	1.05 (d, J= 6.1,3H)		
2'''-5''''	3.2 – 3.9 (m)		

Table 2: ^{13}C - NMR data (DMSO-*d*6) for compound 1 δ in ppm

C	δ_C	C	δ_C
2	156.8	1'''	98.7
3	133.2	2'''	68.4
4	177.7	3'''	70.5
5	160.2	4'''	71.8
6	99.1	5'''	70.3
7	161.2	6'''	17.9
8	93.4	1''''	100.4
9	156.6	2''''	73.5
10	104.4	3''''	73.9
1'	120.9	4''''	69.8
2'	115.7	5''''	67.5
3'	144.4	(*E*)-Caffeoyl	
4'	149.4	1'	121.2
5'	116.1	2'	114.5
6'	121.9	3'	144.9
1''	99.9	4'	148.4
2''	74.1	5'	115.8
3''	76.5	6'	121.1
4''	69.7	3	145.5
5''	77.5	2	114.7
6''	60.2	1	166.2

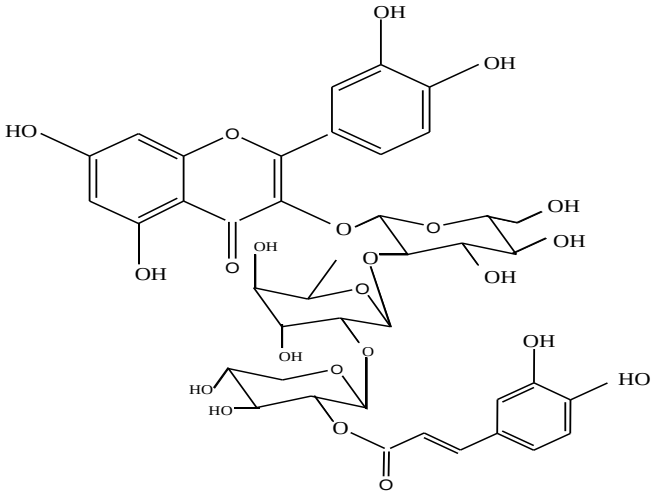

Figure 1: Structure of compound 1

ISSN: 0975-8585

Bioassay evaluation

The 70 % methanol and methanol extracts of the aerial part of *Jacquemontia pentantha* were tested for their different pharmacological activity.

Study acute toxicity (LD_{50}) of 70% methanol extract of *Jacquemontia* is safe up to 7.100 g / Kg. (Table 3). $LD_{50} = D_m - \Sigma (Z \times d) / n$

$= 8000 - \frac{9000}{} = 7100$ mg / kg $=$ 7.100 g /kg.

Table 3: LD_{50} of 70% methanol total extract

Dose mg/kg	No of animal	No of dead animal	Z	D	Zd	LD_{50}
8000	10	10	-	-	-	7100
7500	10	7	8.5	500	4250	mg/kg
7000	10	4	5.5	500	2750	
6000	10	-	2	1000	2000	
					9000	

$LD_{50} = D_m - \Sigma (Z \times d) / n$

The antihyperglycemic activity showed that the 70 % methanol total extract (100 mg/kg) possess significantly antihyperglycemic activity as it showed a decrease in serum glucose level by 31.5 % and 58.04 % respectively after 2 and 4 weeks , this result nearly similar to the reference drug metformin (35.8 % and 67.3 %) , followed by methanol extract (100 mg/kg) then 70% methanol total extract (50 mg/kg) with percentage of change 24.7% and 52.7% and15.3%, 42.5% after 2 and 4 weeks respectively. The least effective was the methanol extract (50 mg/kg) with percentage of change 14.6% and 37.95% after 2 and 4 weeks, respectively, (Table 4).

Table 4: Evaluation of antihyperglycemic activity

Grp. Time	Diab non treated	Diab. Treated with 70% methanol extract (100 mg/kg) *Jacq.*		Diab. Treated with methanol extract (100mg/kg) *Jacq.*		Diab. Treated with 70% methanol extract (50 mg/kg) *Jacq.*		Diab. Treated with methanol extract (50 mg/kg) *Jacq.*		Diab. Treated with MetFormin (100 mg/kg)	
	M±S.E	M±S.E	% of change	M±S.E	% of change	M±S.E	% of change	M±S.E	% of change	M±S.E	% of change
Zero	249.8±7.2	268.4±9.2	____	261.2±8.9	____	255.4±8.9	____	259.8±9.5	____	267.2±9.1	_____
2w	258.3±7.6	183.8±7.4 *	31.5	196.7±8.6 *	24.7	216.6±9.6 *	15.3	221.9±8.2 *	14.6	171.5±7.4*	35.8
4w	266.7±8.9	112.6±5.3 *	58.0	123.5±6.1 *	52.7	146.8 ±9.4	42.5	161.5±7.8 *	37.9	87.3 ±2.9*	67.3

* Statistically significant from zero time at $p < 0.01$

Antioxidant activity results revealed that the 70% methanol extract (100 mg/kg) of induced highly antioxidant activity, producing percentage of change 2.7 % nearly similar to the reference drug, vitamin E, (percentage of change: 1.4 %), followed by 70% methanol extract (50 mg/kg), methanol extract (100 mg/kg) with percentage of change 8.2 % and 8.8 % respectively. The least effective was the methanol extract(50 mg/kg) , the percentage of change being 19.5% (Table 5).

ISSN: 0975-8585

Table 5: Evaluation of antioxidant activity

Control	Blood glutathione (mg%)	% change from control
Control (1ml saline)	36.4±1.3	_____
Diabetic	21.8±0.5*	40.1
Diabetic + Vitamin E (7.5m/kg)	35.9±1.2	1.4
Diabetic +70% methanol extract (50 mg/kg) *Jacquemontia pentantha*	33.4±1.3	8.2
Diabetic+ 70% methanol extract (100 mg/kg) *Jacquemontia pentantha*	35.4±1.4	2.7
Diab. + 100% methanol extract (50 mg/kg) *Jacquemontia pentantha*	29.3±0.9*	19.5
Diab+ 100% methanol extract (100 mg/kg) *Jacquemontia pentantha*	33.2±1.1	8.8

* Statistically significant different from control group at $p < 0.01$

The anti-inflammatory activity results showed that the 70% methanol extract (100 mg/kg) is the most potent extract as it reduced the edema by 10.1% after 4 hours, this result similar to the reference drug indomethacin (10.1 % change) after 3 hours. Next the methanol (100 mg/kg) extract and then 70% methanol extract (50 mg/kg) with percentage of change were 14.2% and 16.8% respectivily. The least effective extract was the methanol (50 mg/kg) extract, the percentage of change being 18.1% (Table 6).

Table 6: Evaluation of anti - inflammatory activity

	Zero	1 h		2 h		3 h		4 h	
	Paw diameter (mm)	Paw diameter (mm)	% edema change (mm)	Paw diameter (mm)	% edema change (mm)	Paw diameter (mm)	% edema change (mm)	Paw diameter (mm)	% edema change (mm)
Control	3.41±0.09	4.63±0.1*	-----	4.72±0.1*	------	4.86±0.12*	------	4.98±0.1*	-----
70% methanol (50 mg/kg) *Jacq.*	3.52±0.07	4.34±0.1*	23.3	4.28±0.1*	21.6	4.16±0. 1*	18.2	4.07±0.1*	16.8
70% methanol (100 mg/kg) *Jacq.*	3.48±0.08	4.31±0.1*	23.8	4.05±0.09*	16.4	3.91±0.08*	12.4	3.83±0.04*	10.1
Methanol (50 mg/kg) *Jacq.*	3.61±0.04	4.49±0.1*	24.4`	4.42±0.1*	22.4	4.35±0.1*	20.5	4.26±0.1*	18.1
Methanol (100mg/kg) *Jaq.*	3.59±0.05	4.44±0.14	23.7	4.22±0.1*	17.5	4.15±0.1*	15.6	4.10±0.1*	14.2
Indomethacin	3.56±0.08	4.26±0.09*	20.2	3.99±0.06*	12.1	3.92±0.01*	10.1	3.83±0.01*	7.6

* Statistically significant from zero time at $p < 0.01$

Antimicrobial activity evaluation

Antibacterial testing of the different concentrations of the extracts 70% methanol and methanol are shown in (Table 7), revealed that the methanol extract showed inhibitory activity against the gram positive bacteria *B. subtilis* and the gram negative bacteria *P. aeruginosa* with zone of inhibition of 9 mm and minimum inhibitory concentrations (MIC) 62.5 µg (Table 8**).** The results also indicated that, all different concentrations of the extract 70% methanol exhibited a wide range of antibacterial activity against gram positive and gram negative bacteria, with zones of inhibition range from 6 to 9mm. In addition, the extract 70% methanol showed moderate activity against *C. albicans* with zone of inhibition of 7 mm, and minimum inhibitory concentrations (MIC) 500 µg (Table 8).

ISSN: 0975-8585

Table 7: Antimicrobial activity of different concentrations of the aerial part of Jacquemontia *pentantha* extracts (70% and 100%methanol).

Entry	Different conc. (mg/5µL DMSO/disc)	Gram positive bacteria		Gram negative bacteria		Yeast	Fungi		
		B. subtilis ATCC6633	*S. aureus* ATCC29213	*E. coli* ATCC25922	*P.aeruginosa* ATCC27953	*C.albicans* ATCC10321	*A.niger* NRRL363	*F.solani* NRC15	*A.alternata* NRC43
Methanol	**2.0**	9	N.A.	N.A.	9	N.A.	N.A.	N.A.	N.A.
	1.0	8	N.A.	N.A.	9	N.A.	N.A.	N.A.	N.A.
	0.5	8	N.A.	N.A.	9	N.A.	N.A.	N.A.	N.A.
	0.25	7	N.A.	N.A.	8	N.A.	N.A.	N.A.	N.A.
	0.125	7	N.A.	N.A.	7	N.A.	N.A.	N.A.	N.A.
	0.063	6	N.A.	N.A.	6	N.A.	N.A.	N.A.	N.A.
70% Methanol	**2.0**	8	7	9	8	7	N.A.	N.A.	N.A.
	1.0	8	6	9	7	7	N.A.	N.A.	N.A.
	0.5	7	6	8	7	6	N.A.	N.A.	N.A.
	0.25	7	N.A.	6	6	N.A.	N.A.	N.A.	N.A.
	0.125	7	N.A.	6	6	N.A.	N.A.	N.A.	N.A.
	0.063	6	N.A.	N.A.	6	N.A.	N.A.	N.A.	N.A.
Thiophenicol	100 µg/disc	22	11	15	14	N.A.	N.A.	N.A.	N.A.
Treflucan	100 µg/disc	N.A.	N.A.	N.A.	N.A.	8	13	11	20

[a] Antimicrobial activity expressed as inhibition diameter zones in millimeters (mm) of the tested different concentrations against the pathological strains based on the agar diffusion technique.
[b] N.A. No activity.

Table 8: Minimal inhibitory concentration (µg/disc) against the pathological strains.

Entry	Extracts	Gram positive bacteria		Gram negative bacteria		Yeast
		B. subtilis ATCC6633	*S. aureus* ATCC29213	*E. coli* ATCC25922	*P.aeruginosa* ATCC27953	*C.albicans* ATCC 10321
Plant	100% Methanol	62.5	-	-	62.5	-
	70% Methanol	62.5	500	125	62.5	500
control	Thiophenicol	3.13	3.13	25	25	-
	Treflucan	-	-	-	-	25

REFERENCES

[1] Austin DF. Systematics Ann Missouri Bot Gard 1973; 60: 306–412.
[2] Austin DF. Convolvulaceae (morning glory family) 1997.
[3] Choisy JD. Jacquemontia Choisy, Mémoires de la Société de Physique et d'Histoire Naturelle de Genève 1837; 6: 476.
[4] Burkill HM. The useful plants of west tropical Africa 1985.
[5] Eich E. Solanaceae and Convolvulaceae: Secondary Metabolites –Biosynthesis, Chemotaxonomy, Biological and Economic Significance Springer-Verlag Berlin – Heidelberg 2008; pp. 637.
[6] Leistner E, Steiner U. Fungal origin of ergoline alkaloids present in dicotyledonous plants Convolvulaceae. In: Anke, T. and Weber D. (eds.) Physiology and Genetics, 1st ed., The Mycota XV, Springer-Verlag Berlin – Heidelberg 2009; pp. 197-208.
[7] Mahmood N, Moore PS, Tommasi ND, Simone FD, Colman S, Hay AJ, Pizza C. Antiviral Chem Chemother 1993; 4: 235-240.
[8] Panaccione DG. FEMS Microbiol Lett 2005; 251: 9-17.
[9] Ferreira AA, Amaral FA, Duarte IDG, Oliveira PM, Alves RB, Silveira D, Azevedo AO, Raslan DS, Castro MSA. J Ethnopharmacol 2006; 105: 148-153.
[10] Jayaweera DMA. In: Medicinal plants (indigenous and exotic) used in Ceylon. Part 11, National Science Council, Colombo, Sri Lanka 1982; pp. 99.
[11] Malalavidhane S, Wickramasinghe SM, Jansz ER. Phytother Res 2001;15: 635-637.

[12] Paget G , Barnes E. Toxicity tests in evaluation of drug activities sited in the laboratory rats. London: Academic Press 1964; pp. 135- 60.
[13] Karber G. Arch Exp Pathol Pharmacol 1931; 162: 480-487.
[14] Eliasson SG , Samet TM. Life Sciences 1969; 8 : 493- 498.
[15] Trinder P. Am Clin Biochem 1969; 6: 24.
[16] Beulter E, Duran O, Kelly B. J Lab Clin Med 1963; 61: 882- 888.
[17] Winter GA, Risley EA, Nuss GW. Proc Soc Exp Biol Med 1962; 111: 544- 547.
[18] Spedecor WG , Cochran GW. J Lab Clin Med 1982; 61: 882- 888.
[19] Domig JK, Mayrhofer S, Zitz U, Mair C, Petersson A, Amtmann E, Mayer KH, Kneifel W. Int J Food Microbiol 2007; 120:191 .
[20] Ajaiyeoba OE, Onocha AP, Nwozo OS, Sama W. Fitoterapia. 2003; 74: 706.
[21] Yan X, Murphy BT, Hammon GB, Vinson JA, Neto CC. J Agric Food Chem 2002; 50 (21) : 5844-5849.
[22] Milena Kalegari, et al. Brazilian Journal of Pharmaceutical Sciences 2011; 47 (3) .
[23] Jung MJ, Kang SS, Jung HA, Kim GJ, Choi JS. Arch Pharm Res 2004; 27: 593-599.
[24] Dong-Mei Wang, Wen-Jun Pu, Yong-Hong Wang, Yu-Juan Zhang, Shan-Shan Wang. Molecules 2012; 17: 4595-4603.
[25] Clifford W Beninger, George L. Hosfield , Mark J Bassett. J Amer Soc Hort Sci 1999; 124 (5) : 514–518.
[26] Mabry TJ, Markham KR, Thomas MB. The Systematic Identification of Flavonoids. Berlin: Springer Verlag 1970.
[27] Jiang – hai Lu, Yi Liu, Yu – ying Zhao, Guang – zhong Tu. New Flavonoids from Oxytropis myriophylla 2004; 52 (2) : 276 - 278.
[28] Yan – Ping Zou, Chang – Heng Tan, Bao – De Wang, Shan – Hao Jiang, Da– Yuan Zhu. Flavonoid Glycosides from anunculus chinensis BGE 2007 ;90 : 1940 - 1945.