

Full Length Research Paper

Acaricidal activity of *Chrozophora oblongifolia* on the two spotted spider mite, *Tetranychus urticae* Koch

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The acaricidal activity of *Chrozophora oblongifolia* (Delile) Spreng. (Euphorbiaceae) extracts collected from Dakhla Oasis, Western Desert of Egypt was examined against larvae and adult females of *Tetranychus urticae* Koch (Acari: Tetranychidae). Acaricidal activity-guided isolation of methylene chloride, ethyl acetate, butanol fractions resulted in separation and identification of *p*-hydroxybenzaldehyde (1), 3,5-dimethoxy-4-hydroxybenzaldehyde (2), scopoletin (3), amentoflavone (4), apigenin 7-O- β -D- glucopyranoside (5) and apigenin 7-O- β -D-[2",6"-bis(4-hydroxy-E-cinnamoyl)] glucopyranoside (6). The isolated compounds were identified by MS and NMR spectral analyses. The susceptibility of the larvae and adult females of *T. urticae* Koch to the tested isolated compounds revealed that apigenin 7-O- β -D-[2",6"- bis(4-hydroxy-E-cinnamoyl)] glucopyranoside (6) and apigenin 7-O- β -D- glucopyranoside (5) isolated from butanol fraction (most effective fraction) exhibited a high degree of acaricidal activity using leaf-dipping technique against larvae after 7- days of exposure, respectively.

Key words: Acaricidal activity, phytochemistry, *Tetranychus urticae* Koch, *Chrozophora oblongifolia*, Euphorbiaceae.

INTRODUCTION

Two-spotted spider mite (*Tetranychus urticae* Koch) is a phytophagous pest that causes significant yield losses in many agricultural crops in Egypt, including cotton, vegetables, fruits and ornamentals (Dawidar et al., 2014). *T. urticae* feeds by puncturing cells and draining the

contents, producing a characteristic yellow-brown speckling on the leaf surface. They also produce silk webbing which is clearly visible at high infestation levels (Salman et al., 2014). The overuse of synthetic acaricides can lead to serious adverse effects against humans and

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the environment, as well as non-target organisms including beneficial insects and mites that prey on pests (Geng et al., 2014). Because of this problem, green pesticides of botanical sources were considered to be a safer alternative approach to the widest spread synthetic one and have great demand by several scientists (Koul et al., 2008).

Chrozophora oblongifolia (Delile) Spreng. (Euphorbiaceae) was recorded in Western Desert (Alshamy, 2016), Eastern Desert (Salama et al., 2014; Shaltout et al., 2010), Gabel Elba (Abd El-Ghani and Abdel-Khalik, 2006) and South Sinai (Abdel Ghani and Amer, 2003). It is a perennial, erect shrub, subshrub or woody herb up to 1 m high with inflorescences up to 1.5 cm long and described as a much-branched under a shrub, with stems stout and woody below, but sometimes herbaceous and dying after flowering in the first year; stems rather harshly white or tawny stellate-pubescent. Leaves are distinctly petioled, ovate-rhomboid or oblong to lanceolate (Ahmed et al., 2014). *C. oblongifolia* extracts exhibited strong antihepatotoxic (Abdel-Sattar et al., 2014), antioxidant antiviral and antimicrobial activities (Kamel et al., 2016). This phytochemical investigation of *C. oblongifolia* led to isolation and identification of phytochemicals 1-octacosanol, lupeol, *p*-hydroxybenzoic acid, methyl gallate and amentoflavone (Kamel et al., 2016).

The objective of this study was to investigate the phytochemistry and the acaricidal activity of *C. oblongifolia* extracts and secondary metabolites.

MATERIALS AND METHODS

Instruments

NMR spectra were recorded on a Bruker AMX 400 and 500 instrument standard pulse sequences operating at 400 and 500 MHz in ¹H-NMR and ¹³C-NMR were recorded at 125 MHz. Chemical shifts are given in δ (ppm) relative to TMS as internal standard material and the coupling constants (*J*) are in Hz. HSQC, HMBC (H-H) COSY and NOESY were recorded at 500 MHz. GC/MS analysis was performed on a Varian GC interfaced to Finnegan SSQ 7000 Mass selective Detector (SMD) with ICIS V2.0 data system for MS identification of the GC components.

Chemicals

Columns chromatography (CC) was performed using silica gel F254 (230-400 mesh) or polyamide 6. Thin layer chromatography and preparative TLC were performed on silica gel (Kieselgel 60, F 254) of 0.25 mm thickness. Solvents of hexane, methylene chloride, ethyl acetate, butanol and methanol were obtained from Adwic Company.

Plant material

C. oblongifolia (Delile) Spreng was collected in Dakhla: Tennida

veillage (Latitude 25° 30.518 N and Longitude 029° 19.964 E) on April, 2016 and identified by the second author according to Boulos (2000) and Boulos (2009).

Extraction and isolation

The air dried powdered whole plant (1 kg) was macerated in a mixture of organic solvents of methylene chloride/methanol (1:1), the filtrate was evaporated to its 1/3 volume and then diluted by water and exhaustively extracted by hexane, then methylene chloride, ethyl acetate and finally by butanol. All the extracts were collected, dried over anhydrous sodium sulphate and evaporated to give hexane fraction (13.71 g), methylene chloride fraction (2.41 g), ethyl acetate fraction (3.74 g) and butanol fraction (4.42 g).

The methylene chloride fraction (2.41 g) was chromatographed over silica gel column chromatography using mixtures of hexane/ethyl acetate and methylene chloride/methanol of increasing polarities. The effluents were combined into ten sub-fractions based on their TLC pattern. Sub-fraction III was further purified on silica gel preparative TLC developed by a mixture of hexane/toluene/ethyl acetate (65:25 v/v) to afford a pure compound (1) (190 mg, $R_f = 0.16$). Sub-fraction V was purified on silica gel preparative TLC using a mixture of toluene/ethyl acetate (84:16 v/v) as a mobile phase and give compound (2) (160 mg, $R_f = 0.13$). Sub-fraction VIII have been separated on PTLC silica gel plates using a mixture of toluene/ethyl acetate (77:23 v/v) to yield compound (3) (80 mg, $R_f = 0.32$).

Ethyl acetate fraction (3.74 g) was fractionated by chromatography on polyamide 6 CC. The column was eluted with water, water-methanol (1:1), methanol, methanol-acetone (1:1), acetone, acetone-ammonium hydroxide (1:1) and ammonium hydroxide. The obtained fractions (500 ml of each fraction) gave ten sub-fractions according to their TLC pattern step gradient. Sub-fraction VIII was chromatographed on PTLC silica gel plates using ethyl acetate-methanol-water (8.5:1.2:0.3) as a developing system to afford compound (4) (225 mg, $R_f = 0.52$).

Butanol fraction (4.35 g) was separated by polyamide 6 CC using the same previous method to give ten subfractions. Separation of subfraction III was applied on PTLC Silica gel plates using EtOAc-methanol-H₂O (20:4:1) as eluting system to afford compound (5), (201mg, $R_f = 0.31$). Subfraction VIII was purified on silica gel PTLC developed by EtOAc-methanol-H₂O (42:7:1) to give compound (6), (105 mg, $R_f = 0.36$). (Figure 1)

p-Hydroxy benzaldehyde (1)

Yellow powder, ¹H-NMR (CDCl₃): δ_H 7.81 (1H, d, *J* = 8.5 Hz, H-2/H-6); 6.95 (1H, d, *J* = 8.5 Hz, H-3/H-5); 9.87 (1H, s, CHO).

3,5-Dimethoxy-4-hydroxybenzaldehyde (2)

Yellow solid, ¹H-NMR (CDCl₃): δ_H 9.81 (1H, s, CHO); 7.15 (2H, s, H-2/H-6); 6.10 (1H, br.s); 3.97 (6H, s, 2 OCH₃); EI-MS; *m/z* (rel. int.) 182 (100%) [M⁺], 181 (4.2%) [M-H]⁺, 167 (14%) [M-CH₃]⁺, 151 (3%) [M-OCH₃]⁺, 139 (7%) [C₇H₇O₃]⁺, 111 (16%) [C₇H₆O₂]⁺.

Scopoletin (3)

Colourless oily material, ¹H-NMR (CDCl₃): δ_H 7.60 (1H, d, *J* = 9.5 Hz, H-4); 6.92 (1H, s, H-5); 6.85 (1H, s, H-8); 6.27 (1H, d, *J* = 9.5 Hz, H-3); 3.96 (3H, s, OCH₃).

Amentoflavone (4)

Amorphous yellow powder, $^1\text{H-NMR}$ (CD_3OD): Unity I: δ_{H} 6.61 (s, 1H, H-3); 6.26 (s, 1H, H-6); 7.65 (d, 2H, J = 8.8 Hz, H-2'/H-6'); 6.61 (d, 2H, J = 8.8 Hz, H-3'/H-5'); Unity II: δ_{H} 6.61 (s, 1H, H-3); 6.14 (d, 1H, J = 2.1 Hz, H-6); 6.15 (d, 1H, J = 2.1 Hz, H-8); 8.23 (d, 1H, J = 2.3 Hz, H-2'); 7.09 (d, 1H, J = 8.7 Hz, H-5'); 7.89 (dd, 1H, J = 8.7 and 2.3 Hz, H-6'). $^{13}\text{C-NMR}$ (CD_3OD): Unity I: δ_{C} 166.5 (C-2); 103.1 (C-3); 183.7 (C-4); 163.0 (C-5); 103.2 (C-6); 165.5 (C-7); 104.9 (C-8); 156.6 (C-9); 108.3 (C-10); 121.6 (C-1'); 129.3 (C-2'/C-6'); 116.8 (C-3'/C-5'); 162.5 (C-4'); Unity II: δ_{C} 166.5 (C-2); 103.5 (C-3); 183.9 (C-4); 163.6 (C-5); 100.5 (C-6); 163.6 (C-7); 95.4 (C-8); 159.4 (C-9); 108.3 (C-10); 123.2 (C-1'); 132.8 (C-2'); 124.5 (C-3'); 162.2 (C-4'); 120.4 (C-5'); 127.8 (C-6').

Apigenin 7-O- β -D- glucopyranoside (cosmosiin) (5)

Amorphous yellow powder, $^1\text{H-NMR}$ (DMSO-d_6): δ_{H} 7.86 (2H, d, J = 8.4 Hz, H-2' /H-6'); 6.89 (2H, d, J = 8.4 Hz, H-3' /H-5'); 6.62 (1H, s, H-3), 6.81 (1H, d, J = 2 Hz, H-8); 6.49 (1H, d, J = 2 Hz, H-6); 5.09 (1H, d, J = 6.8 Hz, H-1''); 3.96 (1H, dd, J = 11.6, 1.2 Hz, H-6''b); 3.70 (1H, dd, J = 11.6, 5.6 Hz, H-6''a); 3.60-3.37 (4H, m, H-2'',3'',4'',5'').

Apigenin 7-O- β -D-[2'',6''-bis(4-hydroxy-E-cinnamoyl)]glucopyranoside (anisofolin-B) (6)

Yellow powder; $^1\text{H-NMR}$ (CD_3OD): δ_{H} 6.6 (1H, s, H-3); 6.47 (1H, d, J = 1.8 Hz, H-6); 6.78 (1H, d, J = 1.8 Hz, H-8); 7.84 (2H, d, J = 8.4 Hz, H-2' /H-6'); 6.91 (2H, d, J = 8.4 Hz, H-3' /H-5'); 7.44 (2H, d, J = 8.6 Hz, H-2''' /H-6'''); 6.79 (2H, d, J = 8.6 Hz, H-3''' /H-5'''); 7.60 (1H, d, J = 16 Hz, H-7'''); 6.31 (1H, d, J = 16 Hz, H-8'''); 7.17 (2H, d, J = 8.5 Hz, H-2'''' /H-6'''); 6.61 (2H, d, J = 8.5 Hz, H-3'''' /H-5'''); 7.50 (1H, d, J = 15.9 Hz, H-7'''''); 6.26 (1H, d, J = 15.9 Hz, H-8'''''); 5.07 (1H, d, J = 7.1 Hz, H-1''); 3.54 (1H, m, H-2''); 3.50 (1H, m, H-3''); 3.40 (1H, m, H-4''); 3.65 (1H, m, H-5''); 3.72 (1H, m, H-6''a); 4.25 (1H, m, H-6''b); $^{13}\text{C-NMR}$ (CD_3OD): δ_{C} 164.7 (C-2); 104.0 (C-3); 184.0 (C-4); 160.1 (C-5); 101.2 (C-6); 163.2 (C-7); 96.1 (C-8); 156.4 (C-9); 107.0 (C-10); 122.8 (C-1'); 129.6 (C-2'/C-6'); 117.1 (C-3'/C-5'); 160.4 (C-4'); 127.1 (C-1''); 131.1 (C-2''/C-6''); 116.8 (C-3''/C-5''); 158.9 (C-4''); 146.3 (C-7''); 114.8 (C-8''); 169.0 (C-9''); 126.9

(C-1'''); 131.0 (C-2'''/C-6'''); 116.7 (C-3'''/C-5'''); 158.7 (C-4'''); 147.0 (C-7'''); 114.4 (C-8'''); 166.7 (C-9'''); 101.6 (C-1''); 74.1 (C-2''); 74.7 (C-3''); 71.3 (C-4''); 77.8 (C-5''); 62.4 (C-6'').

Maintenance of spider mite colony

Colony of spider mite, *T. urticae* Koch was reared under laboratory condition ($25\pm 2^\circ\text{C}$ and $60\pm 5\%$ R.H) at plant protection research institute branch, Dakahlia Governorate. This colony was isolated from heavily infested castor oil plant leaves and reared on fresh one. These leaves were cleaned and placed on moisten cotton wool pad in Petri dishes. This colony was left for one year under the precious conditions in order to get a homogenous and sensitive colony. Spider mites individual were transferred to the leaves by the aid of fine camels hair brush. Breeding leaves were changed twice weekly at the summer and once weekly at the winter. Adding water was done twice daily to prevent escaping of *T. urticae* individuals.

Assessment of acaricidal activity

In this respect, laboratory experiments were conducted to evaluate the activity of tested plant extracts and its isolated compounds against *T. urticae* mobile stages (larvae and adult females). The leaf-dip technique was used (Dittrich, 1962).

The indication of mortality was chosen as the failure of mites to respond positively by leg movement followed light brooding with a fine brush. Mortality percentages were determined and corrected by using Abotts (1925) formula and they are statistically analyzed to estimate LC_{50} , LC_{90} and slope values according to Finney (1971). Toxicity index was computed for different extracts and their isolated compounds by comparing these materials with the most effective extracts or isolated compounds using Sun's (1950) equation.

$$\text{Toxicity index} = \frac{\text{LC}_{50} \text{ of compound A}}{\text{LC}_{50} \text{ of compound B}} \times 100$$

Where, A is the most effective compound; B is the tested compound.

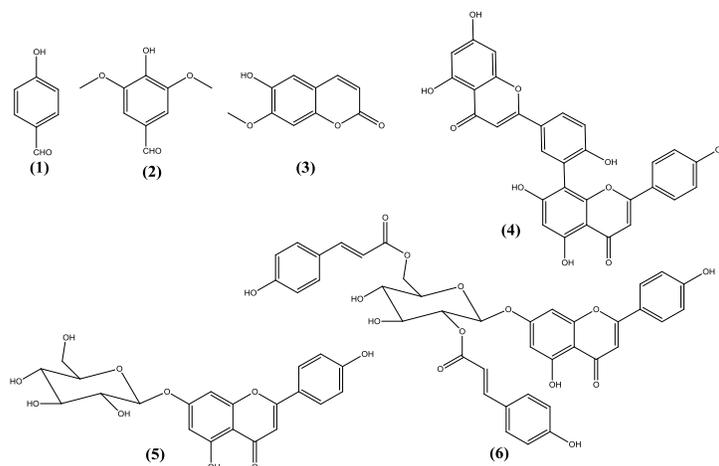


Figure 1. structures of the isolated compounds.

RESULTS AND DISCUSSION

The whole plant parts of *C. oblongifolia* were processed to give four different fractions of different polarities: hexane, methylene chloride, ethyl acetate and butanol fractions. These fractions were screened for their acaricidal activity against the larvae and adult females (mobile stages) of *T. urticae* after 7-days of exposure to find out the most effective fractions and search for its bioactive ingredients. Table 1 revealed that the most effective fractions were butanol, ethyl acetate and methylene chloride against both larvae and adult females of *T. urticae* after 7-days of treatment, respectively.

Chromatographic separation using column and thin layer chromatography of these fractions resulted in isolation of six compounds, which were characterized by MS and NMR spectroscopy. Three shikimates were isolated from methylene chloride fraction and identified as *p*-hydroxy benzaldehyde (1), 3,5-dimethoxy-4-hydroxybenzaldehyde (2) and scopoletin (3). Compound (1) gave the characteristic ¹H-NMR spectrum possessing the same substitution pattern of AA'BB' system of *p*-hydroxy benzaldehyde, which was confirmed by comparing its spectra with those reported by Riaz et al. (2013). EI-MS spectrum of compound (2) showed a molecular ion peak at *m/z* 182 corresponding to [C₉H₁₀O₄]. The fragmentation pattern as well as ¹H-NMR spectral data suggested that compound (2) is 3,5-dimethoxy-4-hydroxybenzaldehyde which was compared with the previously published by Tripathi et al. (2010) and found to be the same. The ¹H-NMR spectrum of compound (3) showed two doublets with coupling constant 9.5 Hz at δ 6.27 and 7.60 ppm characteristic of coumarins, in addition to two aromatic singlets protons at δ 6.92 and δ 6.85 ppm and methoxyl group singlet at δ 3.96 ppm which was characteristic to scopoletin. Coumarin (3) was assigned by comparison its ¹H-NMR data with those reported by Dawidar et al. (2009).

Examination of ¹H and ¹³C-NMR spectra of compound (4) which was isolated from ethyl acetate fraction revealed that it belongs to a biflavonoid of apigenin moiety which identified as amentoflavone. This biflavonoid was isolated and characterized previously from the same plant species by Kamel et al. (2016).

Compounds (5) and (6), isolated from butanol fraction were found to be belong to apigenin 7-O-glycosides, as established by ¹H and ¹³C-NMR spectra, with the sugar anomeric proton signals at δ 5.09 (1H, d, J=6.8 Hz) and δ 5.08 ppm (1H, d, J=7.1 Hz), respectively which were assigned to glucopyranosyl moiety for both, indicating that compound (5) is apigenin 7-O-β-D- glucopyranoside (cosmosiin). Additionally, the spectrum of 6 contained signals of two *p*-coumaroyl moieties. So, structure of compound (6) was characterized as apigenin 7-O-β-D-[2'',6''- bis(4-hydroxy-E-cinnamoyl)]glucopyranoside by H-H COSY, NOESY, HSQC and HMBC. Compounds (5)

and (6) were previously isolated from *Chrozophora plicata* by Riaz et al. (2014).

It is worthwhile mentioning here, that this is the first report of *p*-hydroxybenzaldehyde (1), 3,5-dimethoxy-4-hydroxybenzaldehyde (2), scopoletin (3), cosmosiin (5) and apigenin 7-O-β-D-[2'',6''- bis(4-hydroxy-E-cinnamoyl)] glucopyranoside (6) from *C. oblongifolia*.

Acaricidal effect of *C. oblongifolia* fractions and isolated compounds to larvae and adult females of *Tetranychus urticae* (Koch) after 7-days of exposure

Results in Table 1 showed the toxic action of plant extracts to larvae and adult females of *T. urticae* after 7-days of exposure. The butanol fraction was the most effective at the LC₅₀ and LC₉₀ levels, followed by ethyl acetate fraction, methylene chloride fraction and hexane fraction for both larvae and adult females using leaf-dipping technique. Comparing the slopes values, butanol fraction flattest toxicity line and methylene chloride fraction had the steepest one in case of larvae and fluctuated by increasing from hexane fraction to ethyl acetate fraction in case of adult females. The other fractions lines came between these two fractions (Table 1).

On the basis of the toxicity index it was observed that the butanol fraction was the most effective fraction against larvae and adult females of *T. urticae* after 7-days of treatment followed by ethyl acetate fraction, methylene chloride fraction and hexane fraction was the least toxic fraction.

The acaricidal efficiency of any plant extract depends up on the chemical constituents of every extract contains. The trial to separate and isolate the major effective metabolites and searching for promising acaricidal compounds is our main goal. Table 2 showed the susceptibility of the larvae and adult females of *T. urticae* to the tested isolated compounds. Taking the toxicity index in consideration, data revealed that anisofolin-B (6) which belong to flavonoids glycosides isolated from butanol fraction (most effective fraction) exhibited a high degree of efficiency against larvae after 7-days of exposure, followed by cosmosiin (5), amentoflavone (4), *p*-hydroxy benzaldehyde (1), 3,5-dimethoxy-4-hydroxybenzaldehyde (2) and scopoletin (3). The LC₅₀ values were 109.68, 120.40, 209.90, 1016.04, 1536.96 and 3834.16 ppm, respectively. However, cosmosiin (5) which belong to flavonoids glycosides isolated from butanol fraction (most effective fraction) was the most effective at the LC₅₀ level against adult females of *T. urticae*, followed by anisofolin-B (6), amentoflavone (4), *p*-hydroxy benzaldehyde (1), 3,5-dimethoxy-4-hydroxybenzaldehyde (2) and scopoletin (3). The LC₅₀ values of these tested isolated compounds were: 237.68, 278.59, 380.03, 1101.26, 1738.95 and 7943.96 ppm,

Table 1. Toxicity of plant fractions against larvae and adult females of *T. urticae* after 7 days of treatment.

Plant extract	Larvae				Adult females						
	LC ₅₀ (ppm) and confidence limits at 95%		LC ₉₀ (ppm) and confidence limits at 95%		Slope	Toxicity index at LC ₅₀ value	LC ₅₀ (ppm) and confidence limits at 95%		LC ₉₀ (ppm) and confidence limits at 95%	Slope	Toxicity index at LC ₅₀ value
Hexane fraction	1639.77 1070.72 3793.93		10413.89 6268.28 82671.00		1.596±0.339	12.6	2120.72 1304.34 8030.00		12978.32 4564.91 370964.21	1.629±0.446	14.7
Methylene chloride fraction	674.27 528.18 900.32		2488.75 1648.32 5021.33		2.260±0.338	39.3	674.27 528.18 900.32		2488.75 1648.32 5021.33	2.260±0.338	46.4
Ethyl acetate fraction	457.20 357.65 590.54		1705.19 1177.92 3116.66		2.242±0.320	45.3	546.63 432.77 705.31		1889.09 1314.59 3409.95	2.380±0.337	57.2
Butanol fraction	206.91 123.81 295.13		1514.35 880.79 4712.40		1.483±0.302	100.0	312.72 221.63 424.01		1800.25 1119.84 4266.58	1.686±0.284	100.0

Table 2. Toxicity of isolated compounds against larvae and adult females of *T. urticae* after 7-days of treatment.

Fractions	Isolated compounds	Larvae				Adult females						
		LC ₅₀ (ppm) and confidence limits at 95%		LC ₉₀ (ppm) and confidence limits at 95%		Slope	Toxicity index at LC ₅₀ value	LC ₅₀ (ppm) and confidence limits at 95%		LC ₉₀ (ppm) and confidence limits at 95%	Slope	Toxicity index at LC ₅₀ value
Methylene chloride	p-Hydroxy benzaldehyde (1)	1016.04 603.94 6708.65		5842.19 1887.09 6143.00E+2		1.687±0.532	10.8	1101.26 644.21 8530.98		5854.29 1893.08 7299.29E+2	1.766±0.567	21.6
	3,5-Dimethoxy-4-hydroxybenzaldehyde (2)	1536.96 1125.26 2392.70		8817.61 4675.19 31190.19		1.689±0.304	7.1	1738.95 1265.19 2799.73		9746.64 5072.11 36431.75	1.712±0.313	13.7
	Scopoletin (3)	3834.16 2516.01 5486.59		23152.48 23152.48 56252.40		1.641±0.280	2.9	7943.96 3185.74 1896.53E+1		2902.17 E+2 86773.13 33942.72E+2	0.820±0.163	3.0
Ethyl acetate	Amentoflavone (4)	209.90 137.53 332.94		2336.59 1011.23 18882.46		1.225±0.280	52.3	380.03 276.29 615.36		2290.46 1157.72 9747.82	1.642±0.318	62.5
		Butanol	Cosmosiin (5)	120.40 66.41 179.17		1345.72 660.34 7665.67		1.223±0.281	91.1	237.68 169.73 351.52		1716.94 890.89 6905.86
Anisofolin-B (6)	109.68 60.80 160.56			1073.68 568.08 4657.66		1.294±0.284	100.0	278.59 209.57 394.51		1450.24 844.01 4109.39	1.789±0.312	85.3

respectively.

According to the toxicity assay, it was found that the flavonoid glycosides (5) and (6) were the most effective compounds and considered to be one of the active ingredients followed by biflavonoid compound (4), this is in agreement with the previous studies reported for other flavonoids glycosides isolated from butanol fraction of *Polygonum equisetiforme* which was the most effective fraction against the larvae and adult females of *T. urticae* reported by Dawidar et al. (2014).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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