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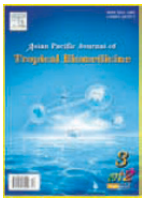

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Chemical and biological studies of *Kalanchoe pinnata* (Lam.) growing in Bangladesh

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ABSTRACT

Objective: To isolate compounds from *K. pinnata* and elucidate their structures and to explore preliminary antioxidant, antimicrobial, cytotoxic and thombolytic activities of extractives of the plant. **Methods:** The methanol extract of whole plant of *K. pinnata* has been subjected to different chromatographic separation and purification processes to isolate the secondary metabolites. The structures of the isolated compounds have been elucidated by extensive NMR studies. The free radical scavenging activity of the crude extract and its different Kupchan fractions were determined on stable radical DPPH. *In vitro* antimicrobial activity was determined by the disk diffusion method. Cytotoxicity screening has been performed against *Artemia salina*. Total phenolics content, membrane stabilizing activity and thombolytic activities were assessed by following established protocol. **Results:** The isolated compounds were identified as glut-5(6)-en-3-one, taraxerone, 3β-friedelanol, β-amyrin-3-acetate, 3,5,7,3',5'-pentahydroxyflavone and β-sitosterol. The chloroform soluble fraction showed potent antioxidant activity of (IC_{50}= 80.0 μg/mL) and significant cytotoxicity, while the crude extract demonstrated noticeable total polyphenol content (149.24 mg of GAE/gm of extractive), moderate membrane stabilizing activity and inhibition of clot lysis of blood. **Conclusions:** The obtained results rationalize the folkloric use of the plant and can be further investigated to isolate the active compounds responsible for the biological activities.

1. Introduction

Kalanchoe pinnata (Lam., syn. *Bryophyllum pinnatum*, *B. calycinum*; Local name: Pathorkuchi, Coughpatha; English name: Air plant; Family: Crassulaceae) is an herb found ubiquitously in Bangladesh. It has tall hollow stems, fleshy dark green leaves that are distinctly scalloped and trimmed in red, and bell-like pendulous flowers[1]. *Kalanchoe pinnata (K. pinnata)* has become naturalized in temperate regions of Asia, Australia, New Zealand, West Indies, Macaronesia, Mascarenes, Galapagos, Melanesia, Polynesia, and Hawaii. It is also widely distributed in the Philippines, where it is known as *katakataka* or *kataka-taka* which means astonishing or remarkable[1,2]. The leaves of *K. pinnata* have a variety of uses in the traditional system of medicine in Bangladesh. They are eaten for diabetes, diuresis, dissolving kinney stones, respiratory tract infections, as well as applied to wounds, boils, and insect bites[1]. It is useful for preventing alcoholic, viral and toxic liver damages. The aqueous extract of this plant have shown anti-inflammatory, anti-diabetic, anti-tumor and cutaneous leishmanicidal activities[3-6].

Previous phytochemical investigations of *K. pinnata* led to the isolation of bryophillin A that showed strong anti-tumor activity, and bersaldegenin-3-acetate and bryophillin C which exhibited insecticidal properties[4]. Besides, 1-octen-3-O-α-L-arabinopyranosyl-(1-6)-β-glucopyranoside[7], 24-alkyl-Δ-25-sterol[8], quercetin-3-O-α

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-L-arabinopyranosyl-(1-2)- α -L-rhamnopyranoside[9] have also been reported from this plant.

As a part of our systematic studies on medicinal plants of Bangladesh[10,11], we studied *K. pinnata* and we, herein, report isolation of compounds for the first time from this plant. The preliminary antioxidant, antimicrobial, cytotoxic activities are also reported here.

2. Materials and methods

2.1. General experimental procedure

NMR spectra were recorded using a Bruker AMX-400 (400 MHz for 1H and 100 MHz for ^{13}C) instrument in deuterated chloroform and the δ values for ^{1}H and ^{13}C spectra were referenced relative to the residual non-deuterated solvent signals.

2.2. Plant material

The whole plants of *K. pinnata* were collected from Narsingdi in November 2009 and was identified by the experts of Bangladesh National Herbarium where a voucher specimen (DACB Accession number-35468), representing this collection has been deposited.

2.3. Chemicals

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) and ascorbic acid were purchased from Sigma Chemical Co. Ltd, (St. Louis, MO, USA). Lyophilized streptokinase vials (1 500 000 I.U.) were obtained from Beacon Pharmaceutical Ltd. Bangladesh. All other chemicals and reagents were of analytical grade.

2.4. Extraction and isolation

The air dried and powdered material (450 g) was extracted with 2 L of methanol in a large flask at room temperature for 15 days with occasional shaking and stirring. The whole mixture was then filtered off through a filter paper and the filtrate thus obtained was concentrated at 40 ℃ with a rotary evaporator. A portion (5.0 g) of the concentrated methanol extract was fractionated by the modified Kupchan partitioning protocol[12] to afford petroleum ether (700 mg), carbon tetrachloride (400 mg), chloroform (900 mg) and aqueous (2.8 g) soluble materials.

A portion of the crude methanolic extract (10.0 g) was subjected to vacuum liquid chromatography (VLC) over silica gel 60H (100-200 mesh). The column was eluted with petroleum ether, followed by mixtures of petroleum ether and ethyl acetate, then with ethyl acetate and finally with ethyl acetate and methanol mixtures of increasing polarities. Depending on the TLC behaviors, fractions 1-4A and 13A-16 eluted with 3%-15% ethyl acetate in petroleum ether and 5%-30% methanol in ethyl acetate, respectively were selected for further investigation.

Fractions 1-4A were mixed together and further subjected to column chromatography over silica gel (Kieselgel, mesh 70-230) using petroleum ether and ethyl acetate mixtures of increasing polarities. A total of 50 fractions were collected. Depending on TLC behaviors, fractions 12, 17, 18, 20, 35 were selected for further purification. Evaporation of solvents from each of these provided white crystalline mass. Repeated washings with ethyl acetate allowed to remove the colored impurities and provided Glut-5(6)-en-3-one (compound 1, 5.6 mg), Taraxerone (compound 2, 6.1 mg), 3 β -Friedelanol (compound 3, 4.5 mg), β -Amyrin-3-acetate (compound 4, 6.5 mg) and 3,5,7,3 ' ,5 ' -Pentahydroxyflavonoe (compound 5, 4.3 mg). VLC fractions 13A-16 were bulked and subjected to gel permeation chromatography over Sephadex (LH-20) using *n*-hexane-dichloromethane-methanol (2:5:1) as the mobile phase. A total of 40 fractions were collected. Preparative thin layer chromatography of column fraction 25, over silica gel using 10% ethyl acetate in petroleum ether yielded β -Sitosterol (compound 6, 6.5 mg).

2.4.1. Glut-5(6)-en-3-one

White crystals, m.p. 208-210 ℃[13-14]; 1H NMR (400 MHz, $CDCl_3$): δ 5.69 (1H, m, H-6), 0.82 (3H, s), 0.96 (3H, s), 1.00 (3H, s), 1.03 (3H, s), 1.07 (3H, s), 1.17 (3H, s), 1.23 (3H, s), 1.24 (3H, s); 13C NMR (100 MHz, $CDCl_3$): see Table 1.

Table 1.

^{13}C NMR (100 MHz) spectral data of compounds 1, 2, 4 and 6 in $CDCl_3$.

Position	1	2	4	6
1	18.2	38.7	39.0	37.3
2	27.8	34.5	27.9	31.6
3	215.5	217.7	79.9	72.4
4	40.8	47.9	39.1	42.2
5	141.6	56.1	55.2	140.8
6	122.0	20.3	18.5	122.6
7	23.6	35.5	33.2	31.5
8	47.4	39.2	40.0	31.5
9	34.8	49.1	47.0	50.2
10	49.6	36.1	37.0	36.4
11	34.6	17.8	17.8	21.5
12	30.3	38.1	124.0	40.0
13	39.3	38.1	139.0	42.4
14	37.8	158.0	42.8	56.8
15	32.1	117.5	28.1	24.2
16	36.0	37.0	26.0	28.3
17	30.1	37.9	34.0	56.3
18	43.0	49.2	59.8	12.0
19	34.8	41.0	39.2	19.4
20	20.2	29.1	39.7	36.4
21	33.1	33.9	31.6	18.9
22	38.9	33.4	41.6	33.8
23	28.9	26.5	28.6	25.8
24	25.5	21.7	15.9	48.3
25	16.2	15.1	15.0	26.1
26	19.6	30.2	16.7	18.7
27	18.4	25.9	23.4	18.8
28	32.1	30.3	28.5	22.8
29	34.5	33.7	23.5	12.2
30	32.1	21.8	21.5	-