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Investigation of Cytotoxicity and Antifungal Activities of Petroleum Ether and Aqueous Extracts of Leaves and Stems of *Kalanchoe pinnata* L. (Crassulaceae)

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Abstract: *Kalanchoe pinnata* L. (Crassulaceae) has traditionally been used in Bangladesh as medicinal plant for the treatment and prevention of different diseases. In the present study the petroleum ether and aqueous extracts of the leaves and stems of the plant were used to investigate the cytotoxicity and antifungal activities using brine shrimp lethality bioassay and agar disc diffusion method, respectively. Phytochemical screening was also carried out according to the standard procedures. In brine shrimp lethality bioassay, the LC_{50} (μg mL^{-1}) and LC_{90} (μg mL^{-1}) of the petroleum ether extract of *Kalanchoe pinnata* L. were 25.12 μg mL^{-1} and 177.83 μg mL^{-1}, respectively. The aqueous extract of the medicinal plant also showed lethality against the brine shrimp nauplii (LC_{50}: 25.12 μg mL^{-1} and LC_{90}: 173.78 μg mL^{-1}). Moreover, the petroleum ether and aqueous extracts of *Kalanchoe pinnata* L. were investigated for their antifungal activity against six fungal strains and the results (diameter of zone of inhibition) were compared with the activity of the commercially available standard drug, Griseofulvin (30 μg $disc^{-1}$). The petroleum ether and aqueous extracts of *Kalanchoe pinnata* L. (80 μg $disc^{-1}$) showed moderate activity against all tested fungal strains with zones of inhibition ranging from 1.50±0.00 mm to 2.67±0.24 mm and 1.67±0.24 mm to 2.83±0.24 mm, respectively. The study also revealed that the fungal strains, *Microsporum* spp. (zone of inhibition: 2.67±0.24 mm) and *Candida albicans* (zone of inhibition: 2.83±0.24 mm) were more susceptible to the petroleum ether and aqueous extracts of the medicinal plant, respectively. Results of different qualitative phytochemical tests showed the presence of alkaloid, glycoside, gums, saponins, reducing sugar and tannins in the petroleum ether crude extract of *Kalanchoe pinnata* L. while alkaloid, glycoside, steroid, saponins, tannins were present in the aqueous extract of the plant. These findings indicated that the petroleum ether and aqueous extracts of the plant possessed cytotoxic and antifungal activities.

Key words: *Kalanchoe pinnata,* bioassay, griseofulvin, brine shrimp, zone of inhibition

INTRODUCTION

Kalanchoe pinnata L. (Family: Crassulaceae) is grown all over the place in Bangladesh and the plant is commonly known as pathorkuchi. The plants are eaten to control diabetes and to dissolve kidney stones and taken for respiratory tract infections, as well as applied to wounds, boils and insect bites (Ghani, 2003). Hypertension (Lans, 2006), rheumatism and inflammation (Nayak *et al*., 2010) can also be treated by *Kalanchoe* species. Anthelmentic activity of the plant had been reported which was due to the presence of tannins (Majaz *et al*., 2011). Other activities reported for the plant or plant components included hepatoprotective activity (Yadav and Dixit, 2003) and anti-tumor promoting activity of bufadienolides isolated from leaves of the plant (Supratman *et al*., 2001). It was also useful in nephrotoxicity (Hartalka *et al*., 2007) and extreme allergic reaction due to quercitrin flavonoid isolated from the plant (Cruz *et al*., 2008). It was also observed that the plant possessed immunosuppressive (Bergmann *et al*., 2006), analgesic, anti-inflammatory, sedative and CNS depressant activity (Joseph *et al*., 2011). The aim of this research work was to investigate cytotoxicity and antifungal activity of petroleum ether and aqueous extracts of the leaves and stems of *Kalanchoe pinnata* L. This study was conducted on the leaves and stems of the plant because not much research work was done on the petroleum ether and aqueous extracts of these plant parts in the aspect of cytotoxicity and antifungal activities.

MATERIALS AND METHODS

Collection of plant materials: The leaves and stems of *Kalanchoe pinnata* (Crassulaceae) were collected from Pahartolly in Chittagong of Bangladesh in the month of

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January 2011 at day time. Fresh plant material were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Extraction of plant materials: About 100 g of powdered materials was taken in a clean, flat bottomed plastic container and soaked in 300 mL of petroleum ether. The container with its contents was sealed and kept for a period of 21 days accompanied by continuous shaking with the shaker. The whole mixture was filtered, collected and the filtrate was evaporated to dryness to give the crude dried extract of petroleum ether. The same procedure was applied to prepare aqueous extract of this medicinal plant using distilled water as a solvent.

Phytochemical screening: The petroleum ether and aqueous extracts of the leaves and stems of *Kalanchoe pinnata* L. (Crassulaceae) were subjected to preliminary screening as standard procedure to identify the presence of various phytoconstituents i.e., alkaloid, glycoside, steroid, gum, flavonoid, saponin, reducing sugar and saponin (Trease and Evans, 1989).

Fungal strains used: The test fungal strains investigated include 6 yeasts such as *Aspergillus niger, Blastomyces dermatitides, Candida albicans, Pityrosporum ovale, Trichophyton* spp. and *Microsporum* spp. All the fungal strains were obtained from Bangladesh Council of Scientific and Industrial Research (BCSIR), Chittagong, Bangladesh.

Cytotoxicity study and antifungal assay: The cytotoxic and antifungal activities of the petroleum ether and aqueous extracts of the medicinal plant were investigated by brine shrimp lethality bioassay (Meyer *et al*., 1982) and agar disc diffusion assay described by Bauer *et al*. (1966). Potato Dextrose Agar (PDA) medium was used to perform the antifungal activity. PDA medium was also used for subculture of the test organisms.

Statistical analysis: Three replicates of each sample were used for statistical analysis and the values were reported as Mean±SD (Standard Deviation).

RESULTS

Preliminary phytochemical analysis: Results of different qualitative phytochemical tests showed the presence of alkaloid, glycoside, gums, saponins, reducing sugar and tannins in the petroleum ether crude extract of *Kalanchoe pinnata* L. while alkaloid, glycoside, steroid, saponins, tannins were present in the aqueous extract of *Kalanchoe pinnata* L. Table 1 showed the results of the qualitative analysis of the petroleum ether and aqueous extract of *Kalanchoe pinnata* L.

Cytotoxic activity: In brine shrimp lethality bioassay, the petroleum ether and aqueous extract showed lethality against the brine shrimp nauplii. It showed different mortality rate at different concentrations (Table 2, 3). The LC_{50} (μg mL^{-1}) and LC_{90} (μg mL^{-1}) of the petroleum ether and aqueous extract of *Kalanchoe pinnata* L. from the best-fit line slope were deduced, respectively (LC_{50}: 25.12 μg mL^{-1} and LC_{90}: 177.83 μg mL^{-1}; LC_{50}: 25.12 μg mL^{-1} and LC_{90}: 173.78 μg mL^{-1}).

Antifungal activity: Table 4 showed the results of antifungal test. The antifungal potentials of the petroleum ether and aqueous extract of *Kalanchoe pinnata* L. were assessed against six fungal strains. The results (diameter

Table 1: Results of phytochemical analysis of petroleum ether and aqueous crude extracts of *Kalanchoe pinnata* L.

Name of extracts	Alkaloid	Glycoside	Steroid	Gum	Flavonoid	Saponin	Reducing sugar	Tannin
Pet. ether extract	+	+	-	+	-	+	+	+
Aq. extract	+	+	+	-	-	+	-	+

+: Present and -: Absent

Table 2: Brine shrimp lethality bioassay of petroleum ether extract of *Kalanchoe pinnata* L.

		No. of alive shrimp						
Conc. (μg μL^{-1})	Log Conc.	Test-1	Test-2	Test-2	Avg.	% of mortality	LC_{50} (μg mL^{-1})	LC_{90} (μg mL^{-1})
5	0.70	6	7	6	6.33	36.67	25.12	177.83
25	1.40	5	4	6	5.00	50.00		
50	1.70	4	3	4	3.67	63.33		
75	1.88	3	2	2	2.33	76.67		
100	2.00	3	2	2	2.33	76.67		
125	2.10	2	1	1	1.33	86.67		
150	2.18	2	1	1	1.33	86.67		
200	2.30	1	1	0	0.67	93.33		
250	2.40	0	1	0	0.33	96.67		
300	2.48	0	0	0	0.00	100.00		
400	2.60	0	0	0	0.00	100.00		
Blank	0.00	10	9	10	9.67	3.33		

Table 3: Brine shrimp lethality bioassay of aqueous extract of *Kalanchoe pinnata* L.

		No. of alive shrimp						
Conc. (μg μL^{-1})	Log Conc.	Test-1	Test-2	Test-3	Avg.	% of mortality	LC_{50} (μg mL^{-1})	LC_{90} (μg mL^{-1})
5	0.70	6	4	6	5.33	46.67	25.12	173.78
25	1.40	6	4	5	5.00	50.00		
50	1.70	4	6	4	4.67	53.33		
75	1.88	3	2	3	2.67	73.33		
100	2.00	3	1	2	2.00	80.00		
125	2.10	3	2	3	2.67	73.33		
150	2.18	1	2	1	1.33	86.67		
200	2.30	0	1	1	0.67	93.33		
250	2.40	0	0	0	0.00	100.00		
300	2.48	0	0	0	0.00	100.00		
400	2.60	0	0	0	0.00	100.00		
Blank	0.00	10	8	9	9.00	10.00		

Table 4: Screening of the petroleum ether (pet. ether) and aqueous (aq.) extract of *Kalanchoe pinnata* L. for anti-fungal activity against six fungal strains

	Zone of inhibition (mm)			
Tested fungi	Pet. ether extract of *K. pinnata* (80 μg disc^{-1})	Aq. extract of *K. pinnata* (80 μg disc^{-1})	Positive control [Standard drug, griseofulvin (30 μg disc^{-1})]	Negative control (Blank)
Aspergillus niger	2.33±0.47	2.17±0.24	11.02±0.43	-
Blastomyces dermatitides	1.83±0.62	2.00±0.41	12.15±0.54	-
Candida albicans	1.83±0.24	2.83±0.24	12.25±0.19	-
Pityrosporum ovale	1.50±0.00	2.00±0.41	14.68±0.29	-
Trichophyton spp.	2.17±0.24	1.67±0.24	12.92±0.72	-
Microsporum spp.	2.67±0.24	1.83±0.62	11.42±0.31	-

Data were represented as Mean±SD of triplicate determination; (-): no inhibition, SD: Standard deviation

of zone of inhibition) were compared with the standard drug, Griseofulvin (30 μg disc^{-1}). At 80 μg disc^{-1}, the petroleum ether extract of *Kalanchoe pinnata* L. exhibited antifungal activity with zone of inhibition ranging from 1.50±0.00 mm to 2.67±0.24 mm while the aqueous extract of *Kalanchoe pinnata* L. (80 μg disc^{-1}) showed antifungal activity with zone of inhibition ranging from 1.67±0.24 mm to 2.83±0.24 mm. Finally, it was concluded that petroleum ether extract of *Kalanchoe pinnata* L. was more effective against *Microsporum* spp. (2.67±0.24 mm) whereas *Candida albicans* was more susceptible to aqueous extract of the medicinal plant with zone of inhibition, 2.83±0.24 mm.

DISCUSSION

The findings showed the presence of potent bioactive phytoconstituents in the petroleum ether and aqueous crude extracts of *Kalanchoe pinnata* L. Majaz *et al.* (2011) reported the presence of steroid in the petroleum ether extract of the roots of *Kalanchoe pinnata* L. They also reported that aqueous extracts of the roots contained saponins, glycosides, flavonoids, tannins, carbohydrates and amino acids. On the other hand, the present study on the petroleum ether extracts of the leaves and stems showed the absence of steroid and flavonoid. Moreover, the presence of alkaloid, glycoside, steroid, saponin and tannin were identified in the aqueous extract of the leaves and stems of the same plant. The study also revealed the cytotoxic and antifungal activities of the plant. Biswas *et al.* (2011) studied on the ethanolic extracts of leaves and stems of *Kalanchoe pinnata* L. They reported that the ethanolic extract of leaves and stems of the plant showed cytotoxic activity with LC_{50}: 100 μg mL^{-1} and LC_{90}: 204.17 μg mL^{-1} while the cytotoxicity exhibited by the petroleum ether and aqueous extracts *Kalanchoe pinnata* L. showed more potent activity (LC_{50}: 25.12 μg mL^{-1} and LC_{90}: 177.83 μg mL^{-1} for petroleum ether extract; LC_{50}: 25.12 μg mL^{-1} and LC_{90}: 173.78 μg mL^{-1} for aqueous extract). The greater cytotoxic activity was indicated by lower LC_{50} (μg mL^{-1}) and LC_{90} (μg mL^{-1}) values. This may be due to the fact that the extracts from leaves and stems of *Kalanchoe pinnata* L. have greater solubility in petroleum ether and aqueous solvent. Moreover, the plant possessed antifungal activity. The more susceptible fungal strains to the petroleum ether and aqueous extracts of the medicinal plant were *Microsporum* spp. (zone of inhibition: 2.67±0.24 mm) and *Candida albicans* (zone of inhibition: 2.83±0.24 mm).

CONCLUSION

It was concluded that the crude petroleum ether and aqueous extracts possessed both cytotoxic and antifungal activities. The extracts also showed more potent cytotoxic activity than that of the crude ethanolic extract of the plant. Finally, more advanced research work is required to

isolate the bioactive constituents and to test the antifungal and cytotoxic properties of the compounds responsible for antifungal and cytotoxic activities.

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