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## **Phytochemical Investigation with Assessment of Cytotoxicity and Antibacterial Activities of Chloroform Extract of the Leaves of *Kalanchoe pinnata***

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### **ABSTRACT**

*Kalanchoe pinnata* is used in traditional medicines for the treatment of several health problems in Bangladesh. The study was designed to investigate the cytotoxicity and antibacterial activities of the chloroform extract of the leaves of *Kalanchoe pinnata*. Qualitative phytochemical analysis was also performed for the identification of several phytochemical compounds such as alkaloids, glycosides, steroids, gums, flavonoids, saponins, reducing sugar, tannins and terpenoids. The study revealed that the plant extract contained all of the phytoconstituents except steroids, flavonoids and terpenoids. The agar disc diffusion technique was used to investigate the antibacterial activities of the plant extract at 500 µg disc<sup>-1</sup> against eight bacterial strains. The plant extract showed very low antibacterial activities with zone of inhibition ranging from 0.33±0.02 to 1.67±0.02 mm, while the maximum antibacterial action was recorded against *E. coli* with zone of inhibition of 1.67±0.02 mm but no sensitivity was found against *V. cholerae*. The antibacterial activity of the plant extract was compared with the standard drug, Levofloxacin which was used at 10 µg disc<sup>-1</sup>. Brine shrimp lethality bioassay was also done to find out the cytotoxic activity of the plant sample. The chloroform extract of the plant showed lethality against the brine shrimp nauplii (LC<sub>50</sub>: 125.89 and LC<sub>90</sub>: 234.42 µg mL<sup>-1</sup>. Finally, it was concluded that the chloroform extract of the plant leaves possessed considerable cytotoxic activity with less antibacterial potentials.

**Key words:** *Kalanchoe pinnata*, chloroform, cytotoxicity, antibacterial activity, phytochemical

### **INTRODUCTION**

Medicinal plants were used as the principal health care agent before the discovery of modern medicines (Shafaei *et al.*, 2011). The plants and phytochemical compounds are used in folk medicine for the treatment of different types of diseases (Pareta *et al.*, 2011). The medicinal plant *Kalanchoe pinnata* Linn belonging to the family of Crassulaceae is found in Bangladesh and the local name of the plant is patharkuchi (Ghani, 2003). Several pharmacological potentials such as immunosuppressive (Rossi-Bergmann *et al.*, 2006), anti-inflammatory and analgesic (Joseph *et al.*, 2011), hepatoprotective (Yadav and Dixit, 2003) and antitumor promoting activities (Supratman *et al.*, 2001) were reported. Inflammation of nephrons (Hartalka *et al.*, 2007), hypertension (Lans, 2006) and allergic conditions (Cruz *et al.*, 2008) can also be treated with the plant extract.

The ethanol extract of the leaves of *Kalanchoe pinnata* extracts possessed significant antibacterial activity (Biswas *et al.*, 2011) and the petroleum ether and aqueous extracts of the plant showed cytotoxicity and antifungal potentials (Chowdhury *et al.*, 2011). The present study was designed to find out the cytotoxic properties and antibacterial activities of the chloroform extract of the leaves of *Kalanchoe pinnata*. The preliminary phytochemical screening of the plant extract was also done.

## MATERIALS AND METHODS

**Collection and preparation of plant samples:** The leaves of the medicinal plant were collected in December 2009 at day time from Ramchandrapur, Magura, Bangladesh. The undesirable materials were separated from leaves and after that, the leaves were air-dried for three weeks. The plant parts were ground into fine powder and kept in an air-tight container until use. About 200 g of powder of the plant material was soaked in 1000 mL of chloroform in a glass container which was sealed and stored for three weeks followed by continuous shaking. Finally, the crude mixture was filtered and the obtained filtrate was completely evaporated to give a gummy concentrate as crude chloroform extract.

**Preliminary phytochemical screening:** The freshly prepared chloroform extract of *Kalanchoe pinnata* was qualitatively tested for the presence of phytochemical compounds such as alkaloids, glycosides, steroids, gums, flavonoids, saponins, reducing sugar, tannins and terpenoids. The standard procedures as preliminary screening (Trease and Evans, 1989) were used to identify the presence of different phytoconstituents in the plant extract.

**Test of antibacterial activity and cytotoxicity of the plant extract:** The cytotoxic activity of the crude extract was investigated by brine shrimp lethality bioassay (Bauer *et al.*, 1966) and agar disc diffusion method (Meyer *et al.*, 1982) was used to determine the growth of inhibition of the bacterial strains.

**Statistical analysis:** Three replicates of each sample were used for statistical analysis and the results of the experiment were expressed as mean $\pm$ standard deviation (SD).

## RESULTS

Table 1 showed the results of phytochemical screening of the chloroform extract of the plant which revealed the presence of alkaloids, glycosides, gums, saponins, tannins and reducing sugars but the extract did not reveal the presence of steroids, flavonoids and terpenoids.

**Cytotoxic activity:** The results of investigation of cytotoxic activity (Table 2) showed that the chloroform extract of the plant possessed moderate level of general toxicity in the brine shrimp lethality bioassay (LC<sub>50</sub>: 125.89 and LC<sub>90</sub>: 234.42  $\mu\text{g mL}^{-1}$ ).

**Antibacterial activity:** Antibacterial activity of the chloroform extract was investigated against eight bacterial strains. The results of antibacterial test showed that the crude extract showed very low level of antibacterial activity against *B. subtilis*, *B. megaterium*, *S. aureus*, *E. coli*, *P. aeruginosa*, *S. typhi* and *S. dysenteriae* with zone of inhibition between 0.33 $\pm$ 0.02 to

Table 1: Results of phytochemical analysis of the chloroform extract of the leaves of *Kalanchoe pinnata*

Types of phytoconstituents	Observations
Alkaloids	+
Glycosides	+
Steroids	-
Gums	+
Flavonoids	-
Saponins	+
Reducing sugars	+
Tannins	+
Terpenoids	-

+: Present and -: Absent

Table 2: Brine shrimp lethality bioassay of the chloroform extract of the leaves of *Kalanchoe pinnata*

<i>Kalanchoe pinnata</i> ( $\mu\text{g } \mu\text{L}^{-1}$ )	Log Conc.	Average No. of alive shrimp	Mortality (%)	LC <sub>50</sub> ( $\mu\text{g mL}^{-1}$ )	LC <sub>90</sub> ( $\mu\text{g mL}^{-1}$ )
5	0.70	9.33	6.67	125.89	234.42
25	1.40	8.33	16.67		
50	1.70	7.33	26.67		
75	1.88	6.67	33.33		
100	2.00	6.00	40.00		
125	2.10	5.00	50.00		
150	2.18	3.33	66.67		
200	2.30	2.00	80.00		
250	2.40	0.67	93.33		
300	2.48	0.00	100.00		
350	2.54	0.00	100.00		
400	2.60	0.00	100.00		
450	2.65	0.00	100.00		
500	2.70	0.00	100.00		

Table 3: Screening for antimicrobial activity of the chloroform extract of the leaves of *Kalanchoe pinnata*

Bacterial strains	Negative control (Blank)	Diameter of zone of inhibition	
		Chloroform extract of <i>Kalanchoe pinnata</i> (500 $\mu\text{g disc}^{-1}$ )	Standard drug, levofloxacin (10 $\mu\text{g disc}^{-1}$ )
<b>Gram (+)</b>			
<i>B. subtilis</i>	-	1.59±0.02	33.83±1.03
<i>B. megaterium</i>	-	0.33±0.02	32.60±0.43
<i>S. aureus</i>	-	1.26±0.03	32.77±0.56
<b>Gram (-)</b>			
<i>E. coli</i>	-	1.67±0.02	34.08±0.51
<i>P. aeruginosa</i>	-	1.02±0.02	32.60±0.43
<i>S. typhi</i>	-	1.62±0.05	32.98±0.43
<i>S. dysenteriae</i>	-	1.23±0.02	32.12±0.44
<i>V. cholerae</i>	-	-	33.50±0.41

Data were represented as Mean±SD of triplicate determination, -: No inhibition

1.67±0.02 mm. The highest zone of inhibition or antibacterial activity was found against *E. coli* (1.67±0.02 mm) while no antibacterial activity was found against *V. cholerae*. The zone of inhibition of the plant extract is shown in Table 3.

## DISCUSSION

Alkaloids, glycosides, gums, saponins and reducing sugars were identified in the chloroform extract of the leaves of *Kalanchoe pinnata* while Chowdhury *et al.* (2011) reported the presence of alkaloid, glycoside, gums, saponins, reducing sugar and tannins in the petroleum ether extract of the leaves of *Kalanchoe pinnata*. They also reported that the aqueous extract of the same plant contained alkaloids, glycosides, steroids, saponins, tannins. On the other hand, the ethanol extract of the medicinal plant possessed alkaloids, glycosides, steroids, gums, flavonoids, saponins, reducing sugars and tannins (Biswas *et al.*, 2011). The reducing sugars, tannins and glycosides present in the extract may be responsible for antibacterial activity and cardiac stimulatory potentials (Sofowora, 1993; Hagerman *et al.*, 1998).

From literature review, it is evident that the phytochemical compounds play an important role in the therapeutic effects of the plants such as flavonoids for free radical scavenging potentials and antinociceptive activities (Zakaria *et al.*, 2006). Alkaloids and tannins have also significant effect in inhibition of pain perception (Uche and Aprioku, 2008; Rahman *et al.*, 2011; Ramprasath *et al.*, 2006). Moreover, tannins could prevent infectious diseases (Agbafor *et al.*, 2011).

The obtained results also showed the cytotoxic activity against brine shrimp nauplii with  $LC_{50}$ : 125.89 and  $LC_{90}$ : 234.42  $\mu\text{g mL}^{-1}$ . In comparison with the cytotoxic activities of the extract of petroleum ether ( $LC_{50}$ : 25.12 and  $LC_{90}$ : 177.83  $\mu\text{g mL}^{-1}$ ) and aqueous extract ( $LC_{50}$ : 25.12 and  $LC_{90}$ : 173.78  $\mu\text{g mL}^{-1}$ ) of the leaves of the plant reported by Chowdhury *et al.* (2011) and that of ethanol extract ( $LC_{50}$ : 100 and  $LC_{90}$ : 204.17  $\mu\text{g mL}^{-1}$ ) by (Biswas *et al.*, 2011), the chloroform extract possessed less cytotoxic potentials. In addition to cytotoxic properties, the chloroform extract showed very low antibacterial activities showing zone of inhibition ranging from  $0.33\pm 0.02$  to  $1.67\pm 0.02$  mm while significant antibacterial activity was found in the ethanol extract of the leaves of the plant with zone of inhibition between  $6.0\pm 0.35$  to  $8.2\pm 0.22$  mm (Biswas *et al.*, 2011).

## CONCLUSION

The results obtained from this study suggested that the chloroform extract of the leaves of *Kalanchoe pinnata* might contain anticancer and antibacterial compounds. However, more research work is needed to isolate the active chemical compounds with their chemical structures elucidation.

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