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PHYTOCHEMICALS, ANTIOXIDANT ACTIVITY, AND CYTO-TOXICITY OF FIVE *KALANCHOE* SPECIES

MD ASHIKUR R LASKAR, MD UZZAL HOSSAIN*, SUBROTO KUMAR DAS, TANIA SULTANA, TASLIM SADIA, SONIA KHAN SONY AND MAHIN AFROZ

Department of Botany, University of Barishal, Barishal-8254, Bangladesh

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Abstract

Kalanchoe contains various bioactive compounds. Qualitative phytochemical screening of five species revealed strong, moderate, weak response to ethanol, methanol, and aqueous solvent extracts respectively. Ethanol extracts exhibited the highest antioxidant activity with *K. pinnata* (IC_{50} = 25.29 µg/ml). Total phenolic contents (TPC)) was highest in ethanol extracts of *K. pinnata* (154.82mg GAE/g). The LC_{50} conducted by brine shrimp lethality bioassay exhibited stronger cytotoxic activity in *K. pinnata* with LC_{50} = 64.44 µg/ml. The study also highlights the comparative presence of different phytochemicals, TPC, DPPH, and cytotoxic activity which will be helpful for phytochemical and pharmacological analysis in future.

Introduction

Bangladesh has a glorious history of traditional medical practices, including Ayurveda, Unani, folk medicine, and home remedies, which depends extensively on locally available plants. Species from *Kalanchoe* Adans. are exotic plant species. Around half of the discovered *Kalanchoe* species are indigenous to Madagascar, while the remaining others are spread over in East Asia, Arabia, India, and Africa. *Kalanchoe* species are used as medicinal herbs worldwide for treating multiple diseases, including kidney stones, rheumatoid arthritis, gastric ulcers, viral and bacterial infections, skin diseases, and cold ailments (Rajsekhar *et al*. 2016). In Bangladesh, *K. blossfeldiana* is locally used for cut and wound healing, *K. laciniata* for headache treatment and *K. pinnata* for blood dysentery, gall bladder and kidney stones (Ahmed *et al*. 2008).

Earlier phytochemical analysis revealed the presence of numerous secondary metabolites (Manan *et al*. 2015), anti-inflammatory, antioxidant and antibacterial activity in *K. pinnata K. blossfeldiana* and *K. laciniata* (Jassal *et al*. 2019). These species including *K. delagoensis*, *K. gastonis-bonnieri* also exist in Bangladesh (Ahmed *et al*. 2008). The present study involved experiments on five *Kalanchoe*s pecies *viz. K. blossfeldiana, K. delagoensis, K. gastonis-bonnieri, K. laciniata* and *K. pinnata*. The study aims to analyze comparative phytochemicals, antioxidant activity and LC_{50} in aqueous, ethanol and methanol solvents. These findings might be helpful to search for alternative species regarding specific phytochemicals for reducing pressure on particular species and future research on plant based new drug development.

Materials and Methods

Germplasm of *K. blossfeldiana* V. Poelln., *K. delagoensis* Eckl. & Zeyh., *K. gastonis-bonnieri* Raym.-Hamet & H. Perrier, *K. laciniata* (L.) DC. and *K. pinnata* (Lamk.) Pers. was collected from different areas of Bangladesh followed by identification with the help of Encyclopedia of Flora Fauna of Bangladesh (Ahmed *et al*. 2008) and Illustrated Handbook of Succulent Plants: Crassulaceae (Eggli 2003). The voucher specimens (Ref: BU/Botany/ Herbarium/Vol. 2/001-005)

*Author for correspondence: <muhossain@bu.ac.bd>.

were preserved in departmental herbarium of Botany, University of Barishal). Analytical grade chemicals and distilled water was utilized for the experiments.

The extracts for phytochemical analysis were prepared from leaves. 300g leaf samples were washed with tap water and then dried in air. The collected leaves were chopped into small pieces and later macerated using a mortar and pestle to obtain fresh leaf paste. 50g of leaf paste was dissolved separately into water, ethanol and methanol solvents. Leaf paste was conventionally digested in a solvent for three days at room temperature (RT) with occasional shaking and stirring using a mechanical shaker. After digestion, the mixture was filtered using double Ring filter paper, which was then concentrated at 60°C using mild heated evaporation in water bath. The resultant extract was stored in refrigerator for further phytochemical analysis. Methods followed to conduct different qualitative and quantitative test described at Table 1. Ascorbic acid and distilled water without plant extract were used as standard and control for DPPH and LC_{50} determination respectively. Statistical analysis mean, standard error, p-value and r-value were calculated using MS Excel.

Table 1. Methodological references of different qualitative and quantitative test.

Phytochemicals	Reference
Chlorophyll	Arnon 1949
Alkaloids	Harborne 1973
Steroids	Trease and Evans 1989
Phenolics and flavonoids	Awe and Sodipo 2001
Saponins and glycosides	Sofowora 1993
Tannins	Sofowora 1993
DPPH	Aoshima *et al*. 2004
IC_{50}	Brand-Williams *et al*. 1995
Total phenolic content	Mayer *et al*.1982
Cytotoxicity	Mayer *et al*.1982
LC_{50}	Finney 1971

Results and Discussion

Chlorophyll content is a critical indicator of photosynthetic efficacy and overall plant health. The species *K. pinnata* exhibited the highest Chl. a (0.502 ± 0.010 mg/g), Chl. b (0.198 ± 0.014 mg/g), and total chlorophyll (0.699 ± 0.022 mg/g) concentrations, while *K. gastonis-bonnieri* showed the lowest values (Table 2). The highest Chl. a:b ratio was 2.79 observed in *K. delagoensis*, suggesting a relatively greater proportion of Chl. a, whereas *K. gastonis-bonnieri* had the lowest (1.56) ratio (Table 2). These variations reflect species-specific differences in pigment composition, which may influence their photosynthetic efficiency and ecological adaptation. Earlier studies suggest that typical Chl. a/b for shade plants is about 1.6-2.2 and sunlight-exposed plants is 2.6-3.4 (Anderson 1986). This study has revealed the Chl. a/b in between 1.56-2.79 among five *Kalanchoe* species.

Qualitative phytochemicals screening revealed that alkaloids, flavonoids and phenolics are present in each solvent extract at different extents. The alkaloid test was more prominent in ethanol and methanol extracts.

Table 2. Comparative chlorophyll concentration in five *Kalanchoe* species.

Species	Chl. a(mg/g)	Chl. b(mg/g)	Total chl. (mg/g)	Ratio (a:b)
K. blossfeldiana	0.362±0.023	0.160±0.028	0.522±0.051	2.26
K. delagoensis	0.326±0.011	0.117±0.005	0.442±0.016	2.79
K. gastonis-bonnieri	0.146±0.021	0.094±0.003	0.240±0.023	1.56
K. laciniata	0.354±0.004	0.217±0.023	0.571±0.026	1.63
K. pinnata	0.502±0.010	0.198±0.014	0.699±0.022	2.54

All values are expressed as mean ± standard error for a given number of observations (n = 3).

Glycosides and saponins test in *K. delagoensis* showed negative results for each solvent. The species *K. delagoensis*, exhibited positive response in the tannin test for all solvent extracts except aqueous extract (Table 3). *K. gastonis-bonnieri* reflected negative result in steroids test while a completely opposite phenomenon has been observed in the case of *K. delagoensis* (Table 3). Methanol extracts of *K. pinnata*, ethanol extract of *K. lacinata*, *K. gastonis-bonnieri* and *K. delagoensis* showed the negative results in the terpenoid test (Table 3). These are probably related to the solubility of phytochemicals in particular solvents. Most of the findings of the present investigation align with previous research conducted (Manan *et al*. 2015). Based on findings it is clearly demonstrated that the *Kalanchoe* is a rich source of various bioactive phytochemicals. Therefore, the quantitative analyses for the antioxidant activity have been conducted.

Table 3. Comparative qualitative phytochemicals in three solvents extracts of *Kalanchoe* species.

Species	Solvent	Alkaloids	Tannins	Saponins	Flavonoids	Terpenoids	Glycosides	Phenolics	Steroids
K. blossfeldiana	Aqueous	+	+++	++	++	-	-	++	+
	Ethanol	+++	+++	++	+	++	+	++	+
	Methanol	++	+++	++	+	++	+	++	+
K. delagoensis	Aqueous	+	-	+	+	+	+	+	+
	Ethanol	++	+	+	++	+	+	+	+
	Methanol	+	+	+	++	-	+	+	++
K. gastonis-bonnieri	Aqueous	+	++	++	+	+	+	+	-
	Ethanol	+++	++	+	+++	-	+	++	-
	Methanol	++	++	+	++	+	+	++	-
K. laciniata	Aqueous	+	+++	+	++	+	+	+	-
	Ethanol	++	++	+	+++	-	++	+	++
	Methanol	+	+++	+	+	+	+	++	+
K. pinnata	Aqueous	+	++	+++	++	+	+	++	-
	Ethanol	+	+++	+	++	++	+	+	+
	Methanol	++	+++	+++	++	-	+	+	-

- = negative, + = weak, ++ = medium, +++ = strong response

The DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay is a widely used to evaluate the free radical scavenging activity and is important in assessing its therapeutic potential. DPPH-scavenging activities are typically associated with higher total phenol (Nabavi *et al*. 2008). Both the plant species and the extraction solvent expressed substantial variations in DPPH test. Ethanol extracts

demonstrated the highest antioxidant activity across all species, indicating its superior efficacy in extracting radical-scavenging compounds (Table 4). *K. delagoensis* exhibited the highest activity in ethanol (92.66 ± 0.22%) followed by methanol (92.3 ± 0.69%) extract. But the species *K. laciniata* displayed least activity in ethanol (26.29 ± 1.16%) and methanol extracts (24.04±1.15%). Similarly, ethanol extracts of *K. blossfeldiana* (80.51 ± 0.48%) and *K. pinnata* (78.7 ± 0.66%) also showed marked antioxidant potential. In contrast, aqueous extracts were generally the least effective, with *K. laciniata* (12.33 ± 1.45%) and *K. gastonis-bonnieri* (15.62 ± 2.88%). Methanolic extracts also showed moderate to high activity, particularly for *K. delagoensis*, *K. pinnata*, and *K. blossfeldiana* (Table 4). Overall, the DPPH antioxidant activity followed the general trend of ethanol > methanol > aqueous across most species. Ethanol and methanol despite being the alcoholic of solvents, the reasons for substantial variation in its DPPH activity of *K. blossfeldiana* and *K. gastonis-bonnieri* may be attributed to differences in molecular nature and structure of bioactive compounds, and is consistent with previously reported findings (Dai and Mumper 2010).

Table 4. Comparative DPPH and TPC of five *Kalanchoe* species in three solvents.

Species	Phytochemical	Solvents			Conclusion	
		Aqueous	Ethanol	Methanol	p-value	r-value
K. blossfeldiana	DPPH (%)	16.96±1.65	80.51±0.48	55.04±1.64		
	TPC (mg GAE/g)	4.88	91.94	32.30		
K. delagoensis	DPPH (%)	28.44±1.38	92.66±0.22	92.3±0.69		
	TPC (mg GAE/g)	4.88	72.61	48.24		
K. gastonis-bonnieri	DPPH (%)	15.62± 2.88	75.64±0.86	34.04±2.02		
	TPC (mg GAE/g)	21.27	62.64	24.12		
K. laciniata	DPPH (%)	12.33 ±1.45	26.29±1.16	24.04±1.15	0.0028	0.714^{**}
	TPC (mg GAE/g)	25.03	43.55	43.00		
K. pinnata	DPPH (%)	29.04± 0.16	78.7 ± 0.66	80.98±0.69		
	TPC (mg GAE/g)	5.09	154.82	76.12		
Correlation	r-value	-0.778^{NS}	0.475^{NS}	0.614^{NS}		
	p-value	0.121	0.419	0.271		

** significant at $p < 0.01$, NS-non significant. All values are expressed as mean ± standard error for a given number of observations (n = 3).

The half maximal inhibitory concentration (IC_{50}) is a key representation of the concentration required to inhibit 50% of the target activity, making it an important metric in antioxidant studies. The comparative study of IC_{50} of aqueous, ethanolic, and methanolic extracts are presented in Fig. 1. The species *K. delagoensis* and *K. pinnata* displayed particularly low IC_{50} values in ethanol (≈25-30 µg/ml), highlighting their antioxidant capacity. In contrast, water extracts demonstrated the highest IC_{50} values, especially in *K. blossfeldiana* (>550 µg/ml) and *K. gastonis-bonnieri*, suggesting limited antioxidant potential. Methanol extracts showed moderate scavenging activity. Previously scientists have reported significant antioxidant and oxidative radical scavenging activities in various *Kalanchoe* species (Bogucka-Kocka *et al*. 2018).

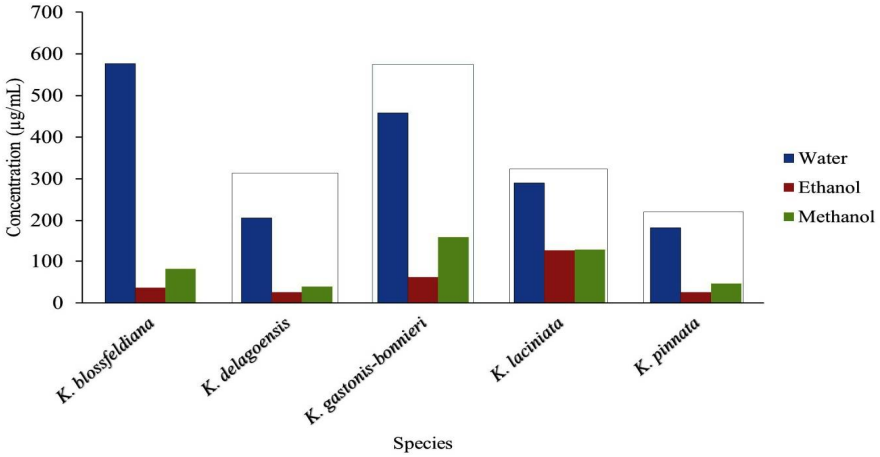

Fig. 1. Comparative IC_{50} of different solvent extracts of five *Kalanchoe* species.

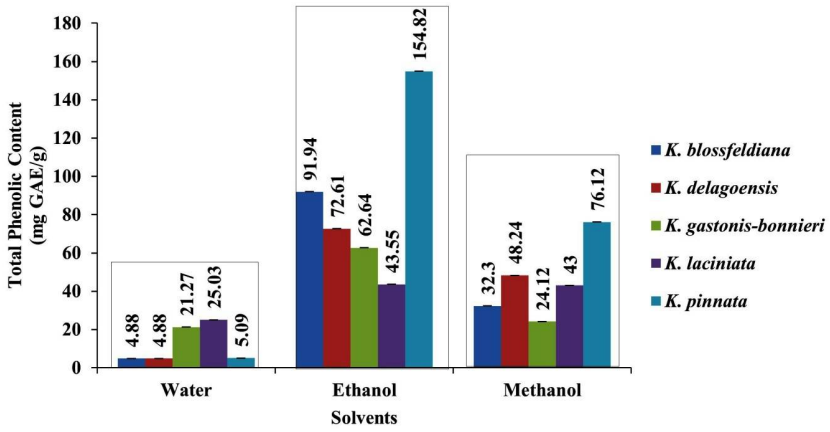

Fig. 2. Comparative total phenolic contents in three solvents extracts of *Kalanchoe* spp.
Results are expressed as mean ± SE for a given number of observations (n = 3).

Total phenolic content (TPC) of a plant refers to the concentration of phenolic compounds, which is important secondary metabolites known for their antioxidant, anti-inflammatory, and antimicrobial properties. Higher TPC is generally associated with stronger antioxidant activity, making it a valuable indicator in phytochemical and pharmacological research. Usually, TPC leads to better DPPH-scavenging activity (Nabavi *et al.* 2008). The present study found that ethanol consistently yielded the highest TPC values across all species, in which *K. pinnata* exhibiting the most pronounced (~155 mg GAE/g) phenolic accumulation followed by *K. blossfeldiana*, *K. delagoensis*, *K. gastonis-bonnieri*, and *K. laciniata* in descending order (Figs 2 and 3). Methanolic extracts showed intermediate phenolic content, with *K. pinnata* whereas aqueous extracts exhibited the lowest in all species (Figs 2 and 3), suggesting limited phenolic solubility in water. This comparative analysis underscores the solvent-dependent variability in phenolic extraction efficiency, highlighting ethanol as the most effective solvent and *K. pinnata* as the richest source

of phenolic compounds among the evaluated *Kalanchoe* spp. Earlier study reported that ethanol extract of *K. crenata* leaves has a TPC value of 0.98 ± 0.013 % (w/w) which is equivalent to 9.8 ± 0.13 mg GAE/ g (Bhatti *et al.* 2012). Compared to the previous result, the TPC obtained in this current study marginally varied. The reasons for these differences may be polarity degree, hydrogen-bonding capacity, and solvent matrix interactions and structural variation in phenolic compounds (Dai and Mumper 2010). The statistical correlation analysis revealed a strong positive and statistically significant correlation between TPC and DPPH activity ($r = 0.714$, $p < 0.01$), indicating that phenolic compounds significantly contribute to antioxidant activity across all extracts (Table 4).

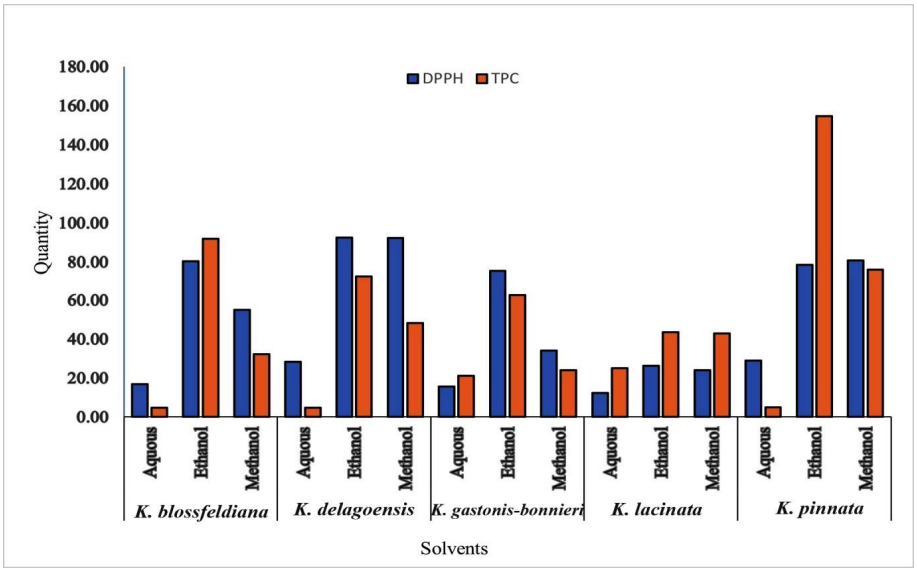

Fig. 3. Comparative DPPH and TPC in three solvents extract of *Kalanchoe* species.

The cytotoxicity studies used to evaluate the toxicity of plant extracts and the concentration required to cause 50% mortality in a test organism is lethal concentration 50%. A lower LC_{50} displays higher toxicity of the plant extract (Clarkson *et al*. 2004). This metric value provides a foundation for safety assessment in natural product-based research. The species *K. pinnata* exhibited the highest cytotoxicity, particularly in methanolic extract (~90 µg/ml), followed closely by its ethanolic among all tested solvent extracts (Fig. 4). On the other hand, *K. blossfeldiana* displayed the least toxicity, with its aqueous extract showing the maximum LC_{50} (~540 µg/ml), indicating poor lethality (Fig. 4). Previous study found that the ethanol extract of *K. pinnata* has an LC_{50} value of 100 µg/ml (Biswas *et al*. 2011) which is slightly higher than present observation (LC_{50}= 64.44 µg/ml).

In conclusion, consistently higher bioactivity was found in ethanol and methanol solvents extracts than water, reflecting the enhanced solubility and bioavailability of phytochemicals in organic solvents. This data reinforces the impact of polar solvent ethanol on extract efficacy and reveals *K. delagoensis* and *K. pinnata* as promising species for antioxidant screening. This comparative analysis also provides a scientific basis for selecting appropriate extraction methods in future phytopharmacological research involving *Kalanchoe* species.

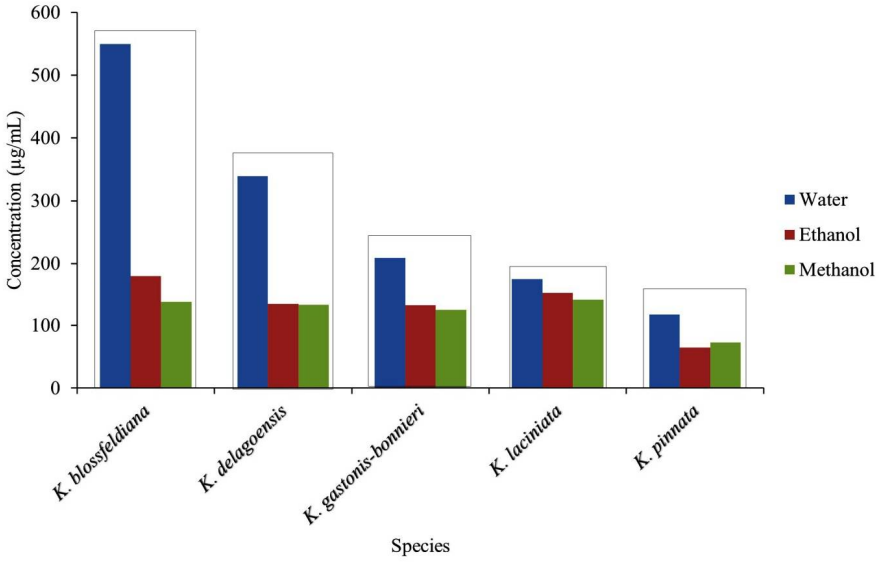

Fig. 4. Comparative LC_{50} of brine shrimp lethality bioassay for different extracts of *Kalanchoe* species.

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