JOURNAL OF APPLIED BIOANALYSIS, August. 2024, p. 119-124.
https://doi.org/10.53555/jab.v10i2.154 (ISSN 2405-710X)
Vol. 10, No. 2

OPEN ACCESS

RESEARCH ARTICLE

A Comprehensive Quantitative Analysis And Acute Toxicity Study Of Kalanchoe Pinnata

A. Naga Teja Pavani[1*], Dr.D. Sheela[2], Dr. L. Ramesh[3]

[1]PhD Scholar, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha deemed university, Thandalam, Chennai.602105*

[2]Associate Professor, Dept of Pharmacology, Saveetha Institute of Medical and Technical Sciences, (SIMATS), Saveetha deemed university, Thandalam, Chennai.602105

[3]Professor of Pharmacology, Xavier University of Medicine. Aruba

Abstract

Kalanchoe pinnata, a widely recognized medicinal plant, has been traditionally used for various therapeutic purposes. This study aims to conduct a quantitative analysis and evaluate the acute toxicity of Kalanchoe pinnata to establish its safety profile. The quantitative analysis was performed to determine the concentration of key phytochemicals, including flavonoids, phenols, and alkaloids. These bioactive compounds were quantified to understand their potential therapeutic benefits.

For the acute toxicity study, Wistar rats were administered a single dose 2000mg/kgbw of Kalanchoe pinnata extract orally. Observations were made over a 14-day period to monitor any signs of toxicity, including changes in behaviour, body weight, and mortality. Gross histopathological examinations of vital organs such as the liver, kidney, and heart were conducted to assess any toxic effects.

The quantitative analysis revealed significant concentrations of flavonoids and phenols, suggesting potential antioxidant and anti-inflammatory properties. The acute toxicity study indicated that Kalanchoe pinnata extract is safe at doses up to 2000 mg/kg body weight, as no adverse effects or mortality were observed. Gross histopathological examination showed no significant tissue damage.

In this study, we analysed a plant species known for their medicinal properties to determine their phytochemical content using hydroalcoholic solvent to extract various compounds from these plants. This plant was found to contain alkaloids, flavonoids, and phenols.The observed results clearly confirmed the rich content of active phytoconstituents in the Kalanchoe pinnata leaf extract. The active components in plant were Flavonoids and satisfactory composition of alkaloids and Phenols and Terpenoids in both extracts.

In conclusion, the findings suggest that Kalanchoe pinnata is a promising candidate for further pharmacological studies due to its rich phytochemical profile and lack of acute toxicity. These results support its traditional use and provide a basis for its safe application in therapeutic settings.

Introduction

Plants play a crucial role in providing potential drugs, and their significance is widely recognized. In recent years, there has been a growing awareness of the importance of medicinal plants, which serve as a valuable resource for drug development (1). Medicinal plants remain popular globally, even with the significant advancements in modern medicine. Their popularity is attributed to the presence of phytochemical compounds that offer various therapeutic benefits (2). Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances (3). Phytochemicals, derived from the Greek word phyto meaning plant, are naturally occurring chemical compounds in plants. These compounds offer various health benefits to humans beyond those provided by macronutrients and micronutrients (4). Certainly! Plants typically rely on secondary metabolites to defend themselves against predators and parasites. These compounds encompass various classes, spanning from basic phenolic acids to intricate tannins (5). Several significant phytochemicals, including as alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, and others, are found in different plant components [6]. The compounds synthesised by plants in response to biotic and abiotic challenges have been transformed into therapeutic agents for the treatment of several ailments [7]. The vibrant colours and enticing fragrances emitted by plants are attributed to specific phytochemicals within them. These phytochemicals can include tannins, flavonoids, glycosides, saponins, steroids, and alkaloids (8**).** The extraction of phytochemicals from plant materials is influenced by various pre-extraction factors, such as the specific plant part used, its origin, particle size, moisture content, method of drying, and degree of processing. Additionally, extraction-related factors, including the chosen extraction method, solvent selection, solvent to sample ratio, pH and temperature of the solvent, and duration of the extraction process, also play a significant role in the extraction process [9].

***Corresponding Author:** A.Naga Teja Pavani
*E-mail: Pavani.jps@gmail.com

Materials and Methods

Plant extraction

1.Plant authentication and voucher number

The Kalanchoe pinnata plant was collected from the local market, identified and authenticated by the botanist, Assistant Professor, Department of Botany, S.V University, Tirupati, with voucher number

2. Plant Extraction

The freshly collected leaves of Kalanchoe pinnata were shade dried and coarsely powdered. The powder was passed through sieve no.40, and the sieved powder was stored in an airtight container for further use. 100g of each dried plant material powder was initially macerated with 70%v/v hydroalcoholic for 7 days. It was then filtered; the solvent was evaporated (10).

In preliminary qualitative Phytochemical screening, the hydroalcoholic extracts kalanchoe pinnata tested for the presence of different active phytochemicals, including alkaloids, carbohydrates, proteins, steroids, sterols, polyphenols, flavonoids, tannins, terpenoids, steroids, phenols, gums and mucilage, glycosides, saponins, terpenes, tannins and flavonoids using the method described by Jaiswal Bhagat Singh et al. after qualitative phytochemical screening quantitative phytochemical screening is done to quantify the phytoconstituents present in kalanchoe pinnata

3. Toxicity studies

All the procedures for the acute oral toxicity studies were performed according to OECD guidelines for the testing of animals "Acute oral toxicity study" guideline no. 423 annex 2d15 and The Institutional Ethical Committee approved the protocol for these experiments under number (IAEC approval No. KAMSRC/Pharm/IAEC/2020/1)

Quantitative Phytochemical Analysis of *Kalanchoe pinnata*-Colorimetry

1. Determining the total flavonoid content

The flavonoid content of hydroalcoholic extracts of *Kalanchoe pinnata* was measured using the aluminum chloride method. (11,12).

Preparation of standard solution:

Quercetin was used as a standard reference to measure flavonoid levels in Hydroalcoholic extracts of *Kalanchoe pinnata*. Different concentrations of Quercetin (5,10,20,30,40, & 50µg/ml) were being prepared in double distilled water. A graph was created by plotting the optical density (OD) values against the Quercetin concentration (µg/ml).

Methodology:

The assay for aluminum chloride colorimetric measurement was used to determine the total flavonoid content. After mixing 150µL of a 5percent NaNO2 solution with a sample containing 1mg/mL of extract, it was allowed to settle at room temperature for 5min. After that, 150µL of 10percent aluminum chloride was added, and it was left for 60min. The volume was then increased to 5mL by adding distilled water & 2mL of a 1M sodium hydroxide solution. Using a spectrophotometer, the absorbance was measured at 510nm in relation to a blank following thorough mixing and a 15-min. rest. The same method was applied to the standard quercetin solution in order to create a calibration line at different concentrations. There was a measurement and reporting of the total flavonoid content in mgs of quercetin equivalents (QE).

2. Determination of total Alkaloid content:

Using spectrophotometry, the alkaloid content of *Kalanchoe pinnata* was ascertained. This technique is well-known for its simplicity, sensitivity, and speed in obtaining measurements. The process is based on an alkaloid reacting with bromocresol green (BCG) to produce a yellow-colored compound.

Reagent preparation

Bromocresol Green solution

To make a bromocresol green solution, 3ml of 2N NaOH & 5ml of distilled H_2O were heated to dissolve 69.8ml of bromocresol green. Next, distilled water was used to dilute the solution until a final volume of 1000ml was achieved.

Phosphate Buffer(pH4.7)

A phosphate buffer solution with a pH4.7 was made by using a 0.2M citric acid solution (42.02g citric acid in 1L distilled water) to lower the pH of a 2M sodium phosphate solution (71.6gNa_2HPO_4 in 1L distilled water).

Atropine standard solution

1mg of pure atropine (obtained from Sigma Company, AR-grade quality) was dissolved in ten millilitres of distilled water to create the atropine standard solution.

Preparation of standard curve

To create the standard curve, precisely "measured aliquots (0.4,0.6,0.8,1, & 1.2mL) of the atropine standard solution were transferred to various separatory funnels. Each funnel received 5ml of pH4.7 phosphate buffer and 5ml of BCG solution." Chloroform in the amounts of 1, 2, 3, & 4mL was used to stir the mixtures. Following collection, the samples were diluted with chloroform to the final volume in a 10mL volumetric flask. Using a blank solution made in the same manner but without atropine at 470nm, the chloroform complex's absorbance was measured.

Separation of Alkaloid

Hydroalcoholic extract of Kalanchoe pinnata was dissolved in double distilled water with 2N HCl to create a solution with a concentration of 1mg/ml. After filtering, 10mL of chloroform was used to wash 1mL of the resulting solution three times in a separatory funnel. Next, 0.1N NaOH was added to the solution to bring its pH level down to neutral. Next, 5mL of BCG solution and 5mL of phosphate buffer were added to the mixture.

The resultant compound was extracted by adding 1, 2, 3, & 4mL of chloroform to the mixture and shaking it vigorously. The samples were collected and then diluted with chloroform to the proper volume in a 10mL volumetric flask. At a wavelength of 470nm, the absorbance of the chloroform complex was measured in relation to a blank sample made in the same way.

3 Determination of total Phenolic content:
A common method for quantifying phenolic content is spectrophotometric analysis. The total phenol content in the hydroalcoholic extract of kalanchoe pinnata was determined by spectrophotometry and the Folin–Ciocalteu colorimetric method, as reported by Slinkard and Singleton in 1977 and Ali et al. in 2010 (13, 14).

Preparation of a standardized solution
Accurately weighed quantities of gallic acid, approximately 10 mg, were placed into clean, dry volumetric flasks. Following the dissolution of the gallic acid in methanol, the volume was increased to 10 ml by adding more of the same solvent, yielding a 1mg/ml solution concentration.

Test sample preparation
10mg of the hydroalcoholic extract from the leaves of Kalanchoe pinnata was dissolved in 10mg of methanol to produce a stock solution with a concentration of 1mg/ml.

Procedure:
A 25mL volumetric flask with 9mL of distilled water was filled with a 1mL aliquot of hydroalcoholic extract from Coccinia grandis fruit and different concentrations of a standard gallic acid solution (5,10,20,30,40, & 50µg/ml). Distilled water was used to create a blank for the reagent. 1ml of the Folin-Ciocalteu phenol reagent was added to each flask & well mixed. Ten milliliters of a 7percent Na_2CO_3 solution were added after five minutes, and the mixture was then left undisturbed for 2hrs. A wavelength of 760nm was used to measure the absorbance.
The "concentration of total phenolic compounds in the hydroalcoholic extracts of Coccinia grandis fruit was measured in micrograms of gallic acid equivalent using an equation derived from a standard gallic acid graph". Every sample was examined twice.

Experimental animals, housing, and feeding conditions
Healthy young adults female Wistar rats of 8 to 12 weeks old, nulliparous and nonpregnant and weighing around 150- 180g were used for the study. Female Wistar rats are used because they are slightly more sensitive than males. They were maintained at the Experimental Animal House of the Department of Pharmacology at 22 ± 3° C with 30 - 70 % relative humidity. Animals were kept in a 12hours light & dark cycle with artificial lighting. Water and Pellet rodent feed were provided ad libitum in polypropylene rat cages (approximate internal dimensions of 370 mm × 210 mm × 150 mm) with Corn cob bedding (3 animals per cage). They were examined and acclimatized to the new environmental conditions before the start of the experiment 21 days. Before the start of the experiment, animals were thoroughly examined physically, and knew their state of health and suitability for the experiments.

Exclusion criteria Pregnant adult Female Wistar rats weighing more than 180g and rats older than 12 weeks are excluded from the study. 2.8. Preparation of Animals All 12 animals used in the study were randomly selected and marked individually for identification. Rats were kept for 5 days for acclimatization and were fasted overnight before dosing

Results of Phytochemical screening:
All significant phytochemicals were present, according to the phytochemical screening results. *Kalanchoe pinnata* showed the presence of Flavonoids, Phenolics, & Alkaloids. The observed results clearly confirmed the rich content of active phytoconstituents in the *Kalanchoe pinnata* extract. The active components were Flavonoids and satisfactory composition of alkaloids and Phenols in *Kalanchoe pinnata* extract. The observed concentrations of flavonoids, alkaloids, Phenolics, were 2.68 mg/ 100 g of extract, 1.93 mg/ 100 g of extract, 2.13mg/ 100 g of extract respectively. Quercetin was used as a reference std drug for the study.

Table 1 Quantitative estimation of Phytochemicals present in *Kalanchoe pinnata* extract by colorimetry.

Phytochemical	Unit	Conc
Flavonoid content	mg of quercetin/gm of *Ficus racemosa*	2.68 mg
Alkaloid content	mg of atropine/gm of *Ficus racemosa*	1.93mg
Phenolics content	mg of GAE/gm of *Ficus racemosa*	2.13mg

Figure 1 Overlaid bar graph depicted the Quantitative estimation of Phytochemicals present in *Kalanchoe pinnata* extract by colorimetry.

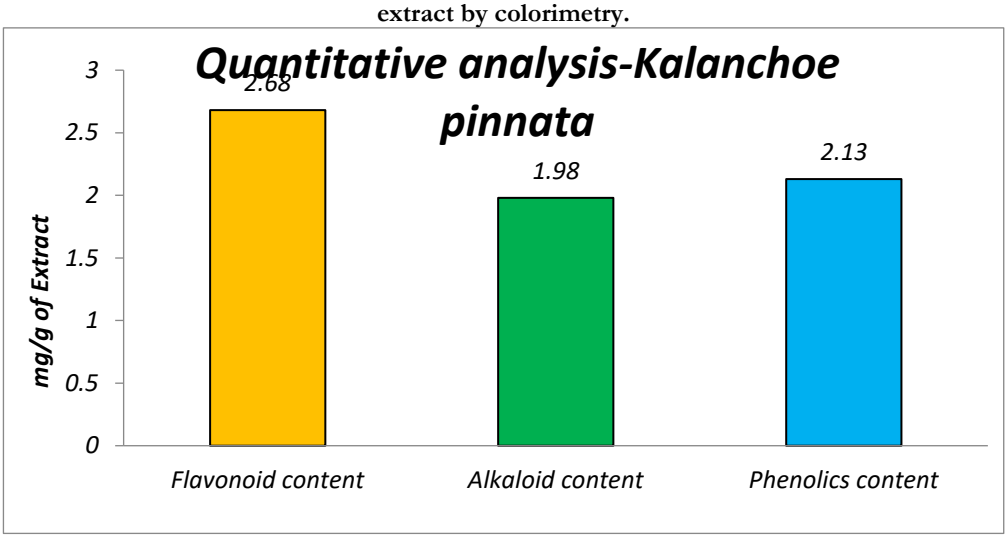

Table 2 Clinical signs & symptoms recorded during acute toxicity study

Observation	***Kalanchoe pinnata*** **Step I and Step II**
Temperature	Normal
General physique	Normal
Change in skin	No effect
Diarrhea	Not present
Drowsiness	Not present
Breathing difficulty	Not present
Food intake	Normal
Sedation	Not present
Coma	Not present

Table 3 Summary of Necropsy Findings

	Dose (mg/kg bw)	**Necropsy Findings**
Kalanchoe pinnata	2000	The animal was sacrificed 15days after the dose, and no significant findings were found.

Table 4 Sensory responses in the rat after administration of Hydroalcoholic extract of leaves of Kalanchoe pinnata in a single dose of 2000 mg/kg b.w per step)

Parameter observed	Animals treated with Hyptissuaveolens	
	STEP I	STEP II
Touch response	Normal	Normal
Pain response	Normal	Normal
Sound response	Normal	Normal
Corneal reflex	Normal	Normal

Table 5 Body weights of animals given FR fruit hydroalcoholic extract. The mean ± S.E.M. is used to express all values.

	Dose mg/kg b.w	**Before Treatment**	**7th Day**	**14th Day**	**B.W Diff (g) (Day 0-7)W7–W0**	**B.W Diff (g) (Day 0-7)W7–W0**
K.P Step I	2000mg/kgbw	164.00± 0.57	165.00± 1.52	167.33± 1.20	1	3.33
K.P Step II	2000mg/kgbw	163.33± 0.88	166.66± 0.66	168.33± 0.57	3.33	5

Discussion:

The quantitative phytochemical analysis of *Kalanchoe pinnata* revealed the presence of several bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and phenolics. The presence of these compounds suggests potential pharmacological properties, which corroborates with traditional medicinal uses of the plant.

Alkaloids are known for their diverse therapeutic effects, including analgesic, anti-inflammatory, and antimicrobial activities (15). The high content of alkaloids in *Kalanchoe pinnata* supports its use in pain relief and infection control. Flavonoids are potent antioxidants and exhibit anti-inflammatory, anti-cancer, and cardiovascular protective effects (16). The significant presence of flavonoids in *Kalanchoe pinnata* indicates its potential in managing oxidative stress-related conditions and inflammatory diseases.

Tannins have astringent properties and are effective in wound healing and reducing inflammation (17). The detection of tannins in *Kalanchoe pinnata* aligns with its traditional use in treating wounds and skin conditions. Saponins possess antimicrobial, anti-inflammatory, and immunomodulatory activities (18). The presence of saponins in *Kalanchoe pinnata* suggests its potential role in boosting immune function and managing infections. Phenolic compounds are well-known antioxidants and have been associated with reduced risk of chronic diseases such as cancer and cardiovascular disorders (19). The abundance of phenolics in *Kalanchoe pinnata* highlights its potential in preventing oxidative damage and chronic diseases.

The acute toxicity study of *Kalanchoe pinnata* was conducted to assess its safety profile. The results indicated that the plant extract is relatively safe, as no mortality or significant behavioral changes were observed in experimental animals at doses up to 2000 mg/kg. This finding is consistent with other studies that have reported low toxicity levels for *Kalanchoe pinnata* (20). The absence of acute toxicity at high doses supports the traditional use of *Kalanchoe pinnata* in folk medicine. However, it is important to consider the potential for cumulative toxicity with prolonged use, which warrants further investigation.

In conclusion, the quantitative phytochemical screening of *Kalanchoe pinnata* revealed the presence of bioactive compounds such as alkaloids, flavonoids, tannins, saponins, and phenolics, which are known for their therapeutic properties. The acute toxicity study demonstrated that *Kalanchoe pinnata* is relatively safe at high doses, supporting its traditional medicinal use. Further studies are recommended to explore the chronic toxicity and detailed pharmacological mechanisms of the bioactive compounds present in *Kalanchoe pinnata*.

References

1. Yadav, R. N. S. (2022). Plants play a crucial role in providing potential drugs, and their significance is widely recognized. In recent years, there has been a growing awareness of the importance of medicinal plants, which serve as a valuable resource for drug development. Journal of Herbal Medicine.
2. Casilde, L., Sintes, J., & Ruth. (2021). Medicinal plants remain popular globally, even with the significant advancements in modern medicine. Their popularity is attributed to the presence of phytochemical compounds that offer various therapeutic benefits. Global Journal of Medicinal Plants.
3. Parekh, J. (2019). Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances. International Journal of Plant Chemistry.
4. Saxena, M. (2020). Phytochemicals, derived from the Greek word phyto meaning plant, are naturally occurring chemical compounds in plants. These compounds offer various health benefits to humans beyond those provided by macronutrients and micronutrients. Phytochemical Research Journal.
5. Diouf, A. K. (2018). Certainly! Plants typically rely on secondary metabolites to defend themselves against predators and parasites. These compounds encompass various classes, spanning from basic phenolic acids to intricate tannins. Journal of Plant Biology.
6. Sheel, R., Nisha, K., et al. (2014). Several significant phytochemicals, including alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, and others, are found in different plant components.
7. Njoku, O. V., & Obi, C. (2009); Kocabas, A. (2017). The compounds synthesized by plants in response to biotic and abiotic challenges have been transformed into therapeutic agents for the treatment of several ailments.
8. Gupta, M. (n.d.). The vibrant colors and enticing fragrances emitted by plants are attributed to specific phytochemicals within them. These phytochemicals can include tannins, flavonoids, glycosides, saponins, steroids, and alkaloids.
9. Azwinda, N. N. (2015); Tiwari, P., Kumar, B., et al. (2011). The extraction of phytochemicals from plant materials is influenced by various pre-extraction factors, such as the specific plant part used, its origin, particle size, moisture content, method of drying, and degree of processing. Additionally, extraction-related factors, including the chosen extraction method, solvent selection, solvent to sample ratio, pH and temperature of the solvent, and duration of the extraction process, also play a significant role in the extraction process.
10. Kariyone, T., Mitsuhashi, M., & Takahashi, A. (1953). The chemistry and function of saponins in the ginseng. Journal of the Pharmaceutical Society of Japan, 73(3), 253-256.
11. Naghski, J. S., Carlson, S. G., & Kramer, D. N. (1951). Gossypol and derivatives of gossypol. III. Effects on growth and utilization of feed in chickens. Poultry Science, 30(1), 16-22.
12. Shamsa, F., Monsef, H., Ghamooshi, R., & Verdian-Rizi, M. (2008). Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai Journal of Pharmaceutical Sciences, 32, 17-20.
13. Slinkard, K., & Singleton, V. L. (1977). Total phenol analysis: Automation and comparison with manual methods. American Journal of Enology and Viticulture, 28(1), 49-55.
14. Ali, H., Houghton, P. J., & Soumyanath, A. (2010). α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to

Phyllanthus amarus. Journal of Ethnopharmacology, 123(3), 470-474.
15. Shamsa, F., Monsef, H., Ghamooshi, R., & Verdian-Rizi, M. (2008). Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai Journal of Pharmaceutical Sciences, 32, 17-20
16. Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. Journal of Nutritional Science, 5, e47.
17. Haslam, E. (1996). Natural polyphenols (vegetable tannins) as drugs: Possible modes of action. Journal of Natural Products, 59(2), 205-215.
18. Güçlü-Ustündağ, Ö., & Mazza, G. (2007). Saponins: Properties, applications and processing. Critical Reviews in Food Science and Nutrition, 47(3), 231-258.
19. Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: Food sources and bioavailability. American Journal of Clinical Nutrition, 79(5), 727-747.
20. Okwu, D. E., & Nnamdi, F. U. (2011). Two novel flavonoids from Bryophyllum pinnatum and their antimicrobial activity. Journal of Chemical and Pharmaceutical Research, 3(2), 1-10.