Chapter 4

Phytochemical screening and TLC profiling of flowers of *Kalanchoe pinnata* (Crassulaceae)

Anuranjita Singh[1], Gireesh Tripathi[2], Nihar Ranjan Nayak[3], Nibedita Jena[4*] and Sanjeet Kumar[4]

[1]Greenland Public School, Ranchi, Jharkhand, India

[2]Department of Pharmacy, Shri Krishna University, Village Chowka Sagar Road, Chhatarpur, Madhya Pradesh, India

[3]Department of Botany, Maa Manikeshwari University, Bhawanipatna, Odisha, India

[4]Ambika Prasad Research Foundation, Odisha, India

[*]Email-Id: nibeditajena838@gmail.com

ORCID: https://orcid.org/0009-0000-0238-0412

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ABSTRACT

This study investigates the secondary metabolites present in *Kalanchoe pinnata* flowers using various extraction methods and chromatographic techniques. The qualitative analysis revealed the presence of tannins, saponins, phenols, reducing sugars, and alkaloids in the ethanol and aqueous extracts. The TLC analysis showed varying Rf values for the detected compounds, indicating the presence of different compounds with varying polarities. The results suggest that *K. pinnata* flowers may have potential medicinal properties, warranting further research into their bioactive compounds and potential applications.

Keywords: Bioactive compounds, flowers, medicinal uses

INTRODUCTION

Kalanchoe pinnata (Lam.) Pers., a plant species known for its medicinal properties, has been studied for its secondary metabolites, which are bioactive compounds that can have various biological effects (Nascimento *et al.,* 2023). The plant has been traditionally used in folk medicine for treatment of various health problems (Hernández-Caballero et al., 2022).

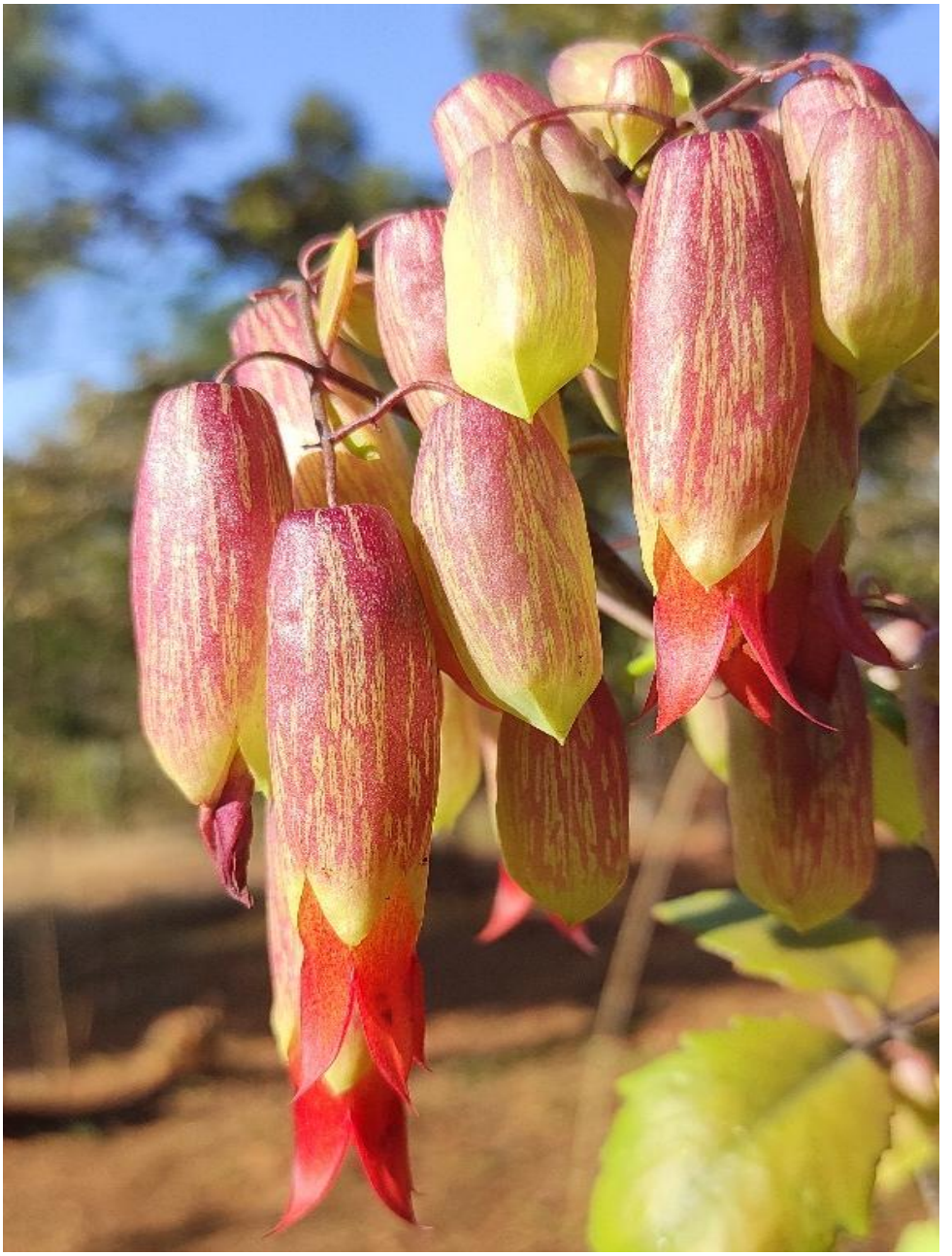

Figure 1: Flowers of *K. pinnata*

However, the specific bioactive compounds present in the flowers of *K. pinnata* have not been extensively studied. The flowers of *K. pinnata* (Figure 1) may be a

rich source of secondary metabolites which can be shown to have potential medicinal properties. Therefore, it is essential to screen the flowers of *K. pinnata* for their bioactive compounds to explore their potential medicinal uses. The screening of *K. pinnata* flowers for their bioactive compounds is necessary to identify the specific compounds responsible for their medicinal values. This knowledge can be used to develop new drugs or herbal remedies that can be used to treat various diseases. Furthermore, the identification of bioactive compounds in *K. pinnata* flowers can also provide insights into the plant's potential uses in traditional medicine and its potential interactions with other medications.

METHODOLOGY

The flowers of *K. pinnata* were collected from Khordha district of Odisha, India (Figure 2). The plant species was identified by the authors (Nayak and Kumar, 2023).

Figure 2: Collected flowers of *K. pinnata* for experimental work

The Soxhlet extraction method was adopted using n-hexane, ethanol, and distilled water for phytochemical analysis. Detection of nine secondary metabolites were conducted using standard methods (Devi *et al.,* 2023; Marndi *et al.,* 2024; Mety *et al.,* 2024; Mehra *et al.,* 2025; Roy *et al.,* 2025; Sampatrao *et al.,* 2025; Yadav *et al.,* 2025).

Test for tannin: 1 ml of flower extract was taken in a test tube and 3-5 drops of 10% lead acetate solution were added. The gelatinous precipitate formation confirmed the presence of tannin.

Test for saponin: About 1 ml of distilled water was added to 1 ml of the flowers extract and shaken well. The formation of persistent froth confirmed the presence of saponin.

Test for flavonoids: About 1 ml of the flowers extract was taken. About 2 ml of NaOH solution and 3-5 drops of dilute HCl were added to it. The colour initially turned to an intense yellow colour with NaOH solution and later became colourless which confirmed the presence of flavonoids.

Test for terpenoids: To 1 ml of the flowers extract, 5-6 drops of chloroform were added. Then 4-6 drops of concentrated sulphuric acid were added to it. The reddish-brown interface confirmed the presence of terpenoids.

Test for phenols: About 1 ml of the flowers extract was taken. A few drops of 5% ferric chloride solution were added. The dark bluish-black appearance confirmed the presence of phenolic compounds.

Test for reducing sugars: About 1 ml of the flowers extract was taken. 2 drops of Fehling's solution A followed by Fehling's solution B were added and kept in the water bath for a few minutes. The presence of red-orange precipitate confirmed the presence of reducing sugars.

Test for steroids: To 1 ml of the flowers extract, about 1 ml of chloroform followed by 1 ml of concentrated sulphuric acid were added to it. The appearance of upper red and lower yellow with green fluorescence provides the presence of steroids.

Test for alkaloid: About 1 ml of the flowers extract was taken. About 3-4 drops of Dragendroff's reagent was added to it. The formation of reddish-brown precipitate confirmed the presence of alkaloids.

Test for carbonyl compounds: About 1 ml of flowers extract was taken and about 3-4 drops of 2,4-Dinitrophenylhydrazine (DNPH) reagent was added. The formation of yellow crystals confirmed the presence of carbonyl compounds.

TLC (Thin Layer Chromatography) analysis

TLC plates were prepared using silica gel powder on 6 cm glass slides. Slides were washed with clinical laboratory detergent and dried. Clean and dried slides were wiped with ethyl acetate for removing surface adherents. 3 g of silica was taken in 20 ml of distilled water and slurry was prepared by constantly stirring, and was finally poured over the slides and slides were left undisturbed until the silica layer on slides was dried. Then the slides were activated at 50° C for 15-20 minutes before running the TLC. Methanol: chloroform (1:1), ethyl acetate: chloroform (1:9) and ethyl acetate: distilled water: acetic acid (8:1:1) were used as mobile phases. Then spots were marked, the RF (Retention Factor) values were recorded and visualized under UV chamber (Mishra and Bhatnagar, 2024).

RESULTS AND DISCUSSION

The qualitative analysis of secondary metabolites in *K. pinnata* flowers revealed the presence of various bioactive compounds in different extracts (Table 1; Figures 3-5). The ethanol and aqueous extracts showed the presence of tannins, saponins, phenols, reducing sugars, and alkaloids, while the n-hexane extract did not show any detectable compounds. The presence of these compounds suggests that *K. pinnata* flowers may have potential medicinal properties. The TLC analysis of *K.*

pinnata flowers using different mobile phases showed varying Rf values for the detected compounds (Figure 6). The chloroform: methanol (1:1) mobile phase showed two distinct spots with Rf values of 0.68 and 0.81, while the ethyl acetate: chloroform (1:9) mobile phase showed a single spot with an Rf value of 0.9. The ethyl acetate: water: acetic acid (8:1:1) mobile phase showed a single spot with an Rf value of 0.66.

Table 1: Qualitative analysis of secondary metabolites of *Kalanchoe pinnata* flowers using different extracts

Bioactive compounds	Extracts		
	Non-polar ⟶		Polar
	n-Hexane	Ethanol	Aqueous
Tannin	Not detected	Detected	Detected
Saponin	Not detected	Detected	Detected
Flavonoids	Not detected	Not detected	Not detected
Terpenoids	Not detected	Detected	Not detected
Phenols	Not detected	Detected	Detected
Reducing sugars	Not detected	Detected	Detected
Steroid	Not detected	Not detected	Not detected
Alkaloid	Not detected	Detected	Detected
Carbonyl compounds	Not detected	Not detected	Not detected

Table 2: TLC analysis of *Kalanchoe pinnata* flowers using different mobile phases

Mobile phases	Extracts	Rf value of spots	
		S_1	S_2

Chloroform: Methanol (1:1)	Hydroethanolic aqueous	0.68	0.81
Ethyl acetate: chloroform (1:9)	Hydroethanolic aqueous	0.9	Nil
Ethyl acetate: water: acetic acid (8:1:1)	Hydroethanolic aqueous	0.66	Nil

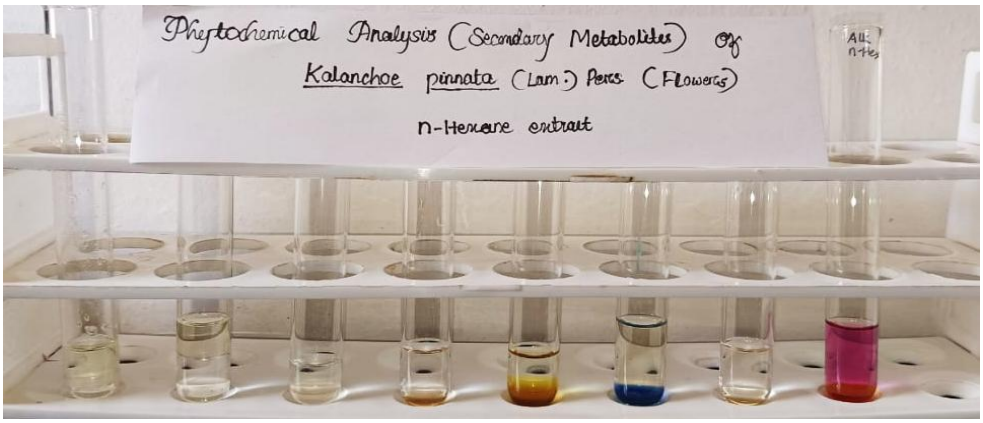

Figure 3: Detection of secondary metabolites of flowers using n-hexane extract

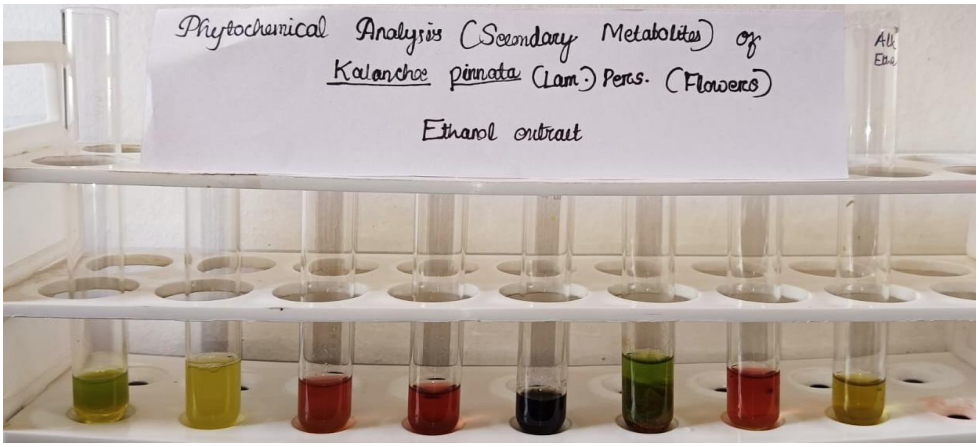

Figure 4: Detection of secondary metabolites of flowers using ethanol extract

Figure 5: Detection of secondary metabolites of flowers using aqueous extract

Figure 6: Spots on TLC under UV light; a) (Chloroform: methanol) hydroethanolic extract; b) (Ethyl acetate: chloroform) hydroethanolic extract of *K. pinnata* flowers

These results indicate the presence of different compounds with varying polarities in the *K. pinnata* flowers (Table 2). Other researchers documented the medicinal potentials of plant parts of *K. pinnata*. Rahman *et al.,* (2019) reviewed on the leaves of *K. pinnata* that are known to have numerous anti-ulcer compounds such as steroids, quercetin, pyruvate and have antitumor and antibacterial potentials. Quintero et al., (2021) had studied that the leaf extract of *K. pinnata* have significant antioxidant and anti-inflammatory activity. Rajini and Sujatha, (2023) have reported that *K. pinnata* (whole plant) possesses various pharmacological activities like antioxidant, anticancer, antidiabetic and antimicrobial due to the presence of numerous bioactive compounds that are alkaloids, phenols, glycosides, carbohydrate etc. The above reports indicated that less works have carried out on flowers.

CONCLUSION

Present study on the secondary metabolites of *Kalanchoe pinnata* flowers revealed the presence of various bioactive compounds, including tannins, saponins, phenols, and alkaloids. The TLC analysis showed varying Rf values for the detected compounds, indicating the presence of different compounds with varying polarities. These findings suggest that *K. pinnata* flowers may have potential medicinal properties, warranting further research into their bioactive compounds and potential applications. The study contributes to the understanding of the plant's chemical composition and its potential uses in traditional medicine.

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