Chapter 8

Qualitative study of bioactive compounds of *Justicia quinqueangularis* J. Koenig ex Roxb. flowers through TLC profiling & phytochemical screening

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ABSTRACT

Present study investigates the phytochemical composition of *Justicia quinqueangularis* flowers, revealing the presence of various bioactive compounds, including tannins, saponins, phenols, reducing sugars, and alkaloids. The TLC analysis confirms the presence of these compounds, suggesting potential therapeutic applications. The findings provide a foundation for further research into the medicinal properties of *J. quinqueangularis* and its possible uses in traditional medicine.

Keywords: Acanthaceae, bioactive compounds, unexplored plants

INTRODUCTION

Justicia quinqueangularis is a plant species belonging to the Acanthaceae family (Carneiro *et al.,* 2023; Figure 1). The plant is known for its diverse range of bioactive compounds, which have been reported to possess therapeutic properties (Correa and Alcantara, 2012). Despite its widespread use, there is limited scientific evidence on the phytochemical composition of *J. quinqueangularis*, highlighting the need for further research (Kumar *et al.,* 2023). Phytochemicals are naturally occurring

compounds found in plants that have been shown to have various health benefits (Fais and Era, 2024). These compounds can be used to develop new medicines, and their study is essential for understanding the therapeutic properties of plants (Chihomvu et al., 2024). The analysis of phytochemicals in *J. quinqueangularis* can provide valuable insights into its potential medicinal uses (Nasir *et al.,* 2024). This study aims to investigate the phytochemical composition of *J. quinqueangularis* flowers, with a focus on identifying the presence of bioactive compounds (Altemimi *et al.,* 2017). The study employed various analytical techniques, including thin-layer chromatography (TLC), to detect and identify the phytochemicals present in the plant (Sureshkumar, 2021). The findings of this study will contribute to the understanding of the medicinal properties of *J. quinqueangularis* and its potential uses in traditional medicine (Kokunang *et al.,* 2011).



Figure 1: Flower of *J. quinqueangularis*

METHODOLOGY

The flowers of *J. quinqueangularis* were collected from the Athagarh division of Cuttack district in Odisha, India (Figure 2). The plant species was identified by the authors (Figures 1-2). The Soxhlet extraction method was adopted using n-hexane, ethanol and distilled water for phytochemical analysis. The detection of nine secondary metabolites was conducted using standard methods (Devi *et al.,* 2023; Marndi *et al.,* 2024; Das *et al.,* 2025; Malwal *et al.,* 2025).



Figure 2: Collected flowers of *J. quinqueangularis* for experimental work

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 to it. 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 flower extract and shaken well. The formation of persistent froth confirmed the presence of saponin.

Test for flavonoids: About 1 ml of the flower 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 flower 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 flower 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 flower 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 flower extract, added 1 ml of chloroform followed by 1 ml of concentrated sulphuric acid into it. The appearance of upper red and lower yellow with green fluorescence indicates the presence of steroids.

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

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

TLC (Thin layer chromatography) analysis

TLC plates were prepared on 6 cm glass slides using silica gel powder (SILICA GEL G, CAS 112926-00-8, Spectrochem Pvt. Ltd.). 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 constant stirring, and finally was poured over the slides and slides were left undisturbed till the drying of the silica layer. Then, the slides were activated at 50^{o} 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, RF (Retention Factor) values were recorded and visualized under UV chamber (Kumar and Jena 2014; Mishra and Bhatnagar 2024).

RESULTS AND DISCUSSION

The phytochemical analysis of *Justicia quinqueangularis* flowers revealed the presence of various secondary metabolites. The hydro-ethanolic extract showed the presence of tannins, saponins, phenols, reducing sugars, and alkaloids. These bioactive compounds have been known to possess various therapeutic properties, including antioxidant, anti-inflammatory, and antimicrobial activities. The TLC analysis of *J. quinqueangularis* flowers using different mobile phases further confirmed the presence of various bioactive compounds. The acetone extract showed the presence of compounds with different Rf values, ranging from 0.89 to 0.94, depending on the mobile phase used. The results suggest that *J. quinqueangularis* flowers contain a diverse range of bioactive compounds with potential therapeutic applications. There are less or no reports on bioactive compounds of *J. quinqueangularis* flower, but some researchers have documented the bioactive compounds of the genus *Justicia*. The ethanol leaf extract of *J. quinqueangularis* showed potent antioxidant and hepatoprotective properties (Suresh *et al.,* 2021). Methanolic extracts of *J. adhatoda* leaves showed significant

cytotoxic effects (Nasir *et al.,* 2024). The hydroalcoholic extract of *J. pectoralis* and its main constituents, coumarin and umbelliferone, were studied for possible anti-edaema activity (Renjana, 2014).

Table 1: Detection of secondary metabolites of *Justicia quinqueangularis* flowers resin using different solvents

Bioactive compounds	Hydroethanolic extract
Tannin	Detected
Saponin	Detected
Flavonoids	Not detected
Terpenoids	Not detected
Phenols	Detected
Reducing sugar	Detected
Steroid	Not detected
Alkaloid	Detected
Carbonyl compound	Not detected

Table 2: TLC analysis of *Justicia quinqueangularis* flowers using different mobile phases

Mobile phases	Extracts	Rf value of spots	
		S_1	S_2
Chloroform: methanol (1:1)	Acetone	0.89	0.91
Ethyl acetate: chloroform (1:9)	Acetone	0.93	Nil
Ethyl acetate: water: acetic acid (8:1:1)	Acetone	0.94	Nil

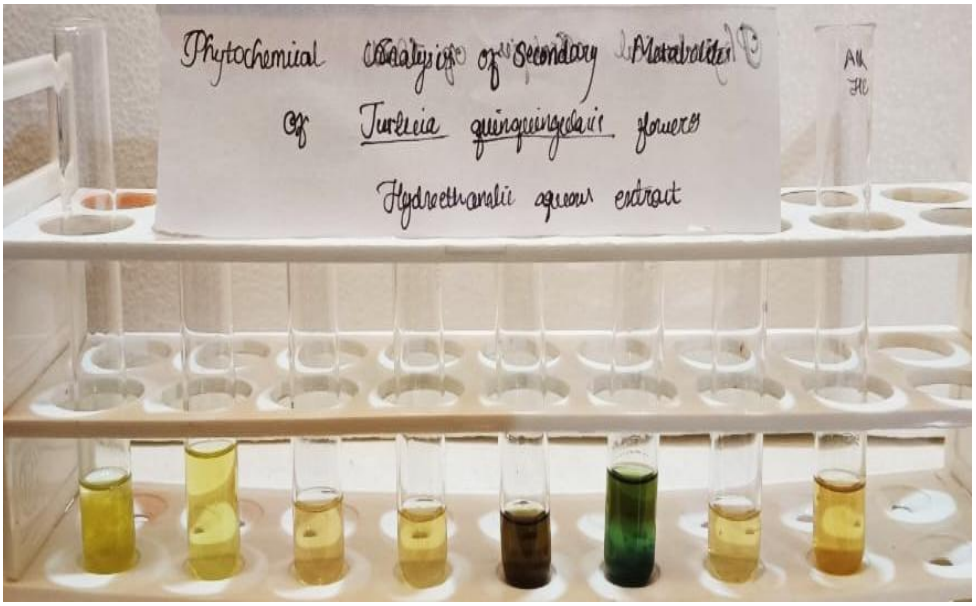


Figure 3: Detection of secondary metabolites of *J. quinqueangularis* using hydroethanolic extract

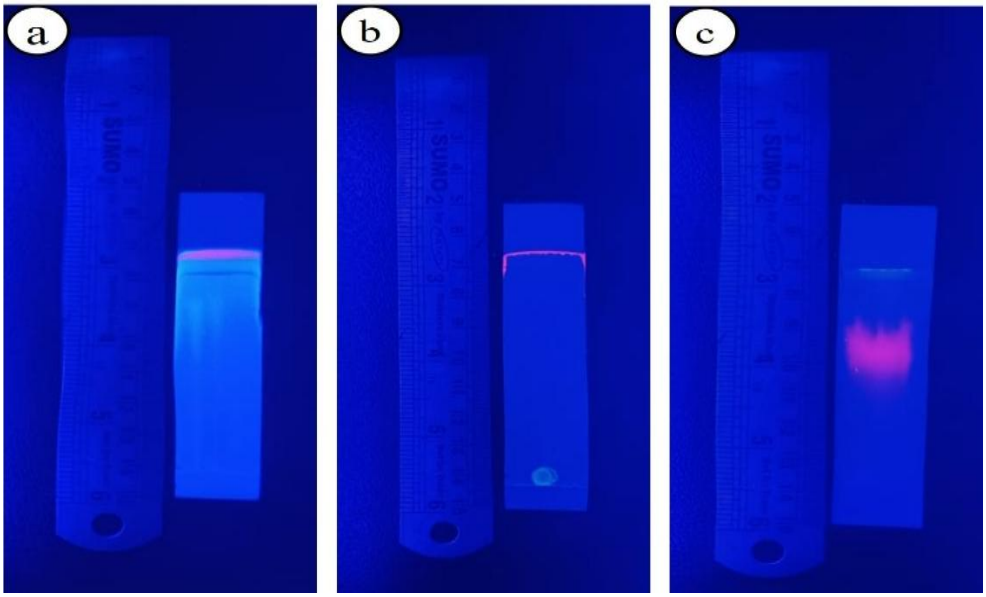


Figure 4: Spots on TLC under UV light; a) (Chloroform: methanol); b) (Ethyl acetate: chloroform); c) (Ethyl acetate: water: acetic acid) using acetone extract

CONCLUSION

This study highlights the presence of various bioactive compounds in *Justicia quinqueangularis* flowers, including tannins, saponins, phenols, reducing sugars, and alkaloids. The TLC analysis confirms the presence of these compounds, suggesting potential therapeutic applications. These findings provide a foundation for further research into the medicinal properties of *J. quinqueangularis* and its possible uses in traditional medicine.

REFERENCES

Altemimi A, Lakhssassi N, Baharlouei A, Watson DG and Lightfoot DA. (2017). Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. Plants (Basel, Switzerland). 6(4): 42. DOI: 10.3390/plants6040042

Carneiro MRB, Sallum LO, Martins JLR, Peixoto JC, Napolitano HB and Rosseto LP. (2023). Overview of the *Justicia* Genus: insights into its chemical diversity and biological potential. Molecules. 28(3): 1190. DOI: 10.3390/molecules28031190

Chihomvu P, Ganesan A, Gibbons S, Woollard K and Hayes MA. (2024). Phytochemicals in drug discovery-a confluence of tradition and innovation. International Journal of Molecular Sciences. 25(16): 8792. DOI: 10.3390/ijms25168792

Correa GM and de Alcantara AF. (2012). Chemical constituents and biological activities of species of *Justicia* - a review. Brazilian Journal of Pharmacognosy. 22(1): 220-238.

Das A, Thanvi HA, Jena N and Kumar S. (2025). Phytochemical screening of fruits of *Holoptelea integrifolia* (Roxb.) Planch. In: *Plants and Secondary Metabolites, Volume 5*. Ambika Prasad Research Foundation, Odisha, India.

Devi RS, Satapathy KB and Kumar S. (2023). Validation of tribal claims for formulation of future drugs through evaluation of ethno-pharmacological values of *Ludwigia adscendens*. Medicinal Plants. 15(4): 691-697.

Fais A and Era B. (2024). Phytochemical composition and biological activity. Plants (Basel, Switzerland). 13(3): 331. DOI: 10.3390/plants13030331

Fokunang CN, Ndikum V, Tabi OY, Jiofack RB, Ngameni B, Guedje NM, Tembe-Fokunang EA, Tomkins P, Barkwan S, Kechia F, Asongalem E, Ngoupayou J, Torimiro NJ, Gonsu KH, Sielinou V, Ngadjui BT, Angwafor F, Nkongmeneck A, Abena OM, Ngogang J, Asonganyi T, Colizzi V, Lohoue J and Kamsu-Kom. (2011). Traditional medicine: past, present and future research and development prospects and integration in the National Health System of Cameroon. African Journal of Traditional, Complementary and Alternative Medicines: AJTCAM. 8(3): 284-295. DOI: 10.4314/ajtcam.v8i3.65276

Kumar A, Nirmal P, Kumar M, Jose A, Tomer V, Oz E, Proestos C, Zeng M, Elobeid T, Sneha K and Oz F. (2023). Major phytochemicals: recent advances in health benefits and extraction method. Molecules (Basel, Switzerland). 28(2): 887. DOI: 10.3390/molecules28020887

Kumar S and Jena PK. (2014). Chromatographic, antibacterial and FT-IR analysis of *Dioscorea pentaphylla* L. tuber extracts. Plant Science Research. 36(1&2): 83-90.

Malwal M, Jena N, Gyani G, Lal S and Kumar S. (2025). Phytoconstituents of fruits of Marking nut tree (*Semecarpus anacardium* L.f.). In: *Plants and Secondary Metabolites. Volume 5*. Ambika Prasad Research Foundation, Odisha, India.

Marndi S, Barik M and Kumar S. (2024). Phytochemical and cytotoxicity analysis of *Eulophia explanata* Lindl. (Tuber extracts). African Journal of Biological Sciences. 6(4): 2751-2756.

Mishra S and Bhatnagar S. (2024). Comparative analysis of methanol extract (leaf) of three species of *Sansevieria* using chemical and biological parameters. World Journal of Pharmaceutical Life Science. 10(2): 116-118.

Nasir M, Ramash R, Fatima H, Ashraf S, Munir I, Asghar S, Adnan M, Masood A and Chaudhari SK. (2024). phytochemical characterization and assessment of crude extracts from *Justicia adhatoda* for phytotoxic and cytotoxic activity. Scientifica, 2024: 1374346. DOI: 10.1155/2024/1374346

Renjana PK. (2014). Cytogenetic and phytochemical aspects of members of Acanthaceae aspects of members of Acanthaceae used as the drug ‘Sahachara’ in Ayurveda submitted to University of Calicut, Kerala, India.

Suresh K, Ahad HA and Satyanarayana SV. (2021). Antioxidant activities and hepatoprotective potential of ethanol leaf extract of *Justicia quinqueangularis* against selected hepatotoxins induced hepatotoxicity in albino wistar rats. 33(40A): 24-34.

Sureshkumar V. (2021). Phytochemical screening and thin layer chromatography profiling of various extracts of *Achyranthes aspera* and *Cissus quadrangularis*. The Journal of Phytopharmacology. 10(4): 225-229.