"Preliminary phytochemical profiling of selected species of *Dicliptera* Juss. (Acanthaceae) from Peninsular India"

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Abstract

Phytochemical investigations provide essential baseline information for evaluating the chemical diversity and bioactive potential of medicinally important plant taxa. The present study reports a qualitative phytochemical screening of selected species of *Dicliptera* Juss. (Acanthaceae) collected from Peninsular India. Methanolic extracts of eleven species were prepared using Soxhlet extraction and subjected to standard confirmatory phytochemical tests for major classes of secondary metabolites. The screening revealed appreciable interspecific variation in phytochemical composition. Flavonoids, alkaloids, glycosides, triterpenoids, sterols, tannins, and saponins were detected in varying combinations across the studied taxa, whereas carbohydrates, proteins, and lipids were largely absent. Among the investigated species, *Dicliptera paniculata* (Forssk.) I.Darbysh., *D. srisailamica* Rasingam, Nethaji & Susmitha, *D. nasikensis* Lakshmin. & B.D.Sharma, and *D. cuneata* Nees exhibited comparatively broader qualitative phytochemical profiles, while *D. beddomei* C.B.Clarke and *D. foetida* (Forssk.) Blatt. showed lower diversity of metabolites. The consistent occurrence of flavonoids and alkaloids across all species suggests their conserved presence within the genus. The present findings provide baseline phytochemical data for *Dicliptera* species from Peninsular India and underscore the need for further quantitative, chromatographic, and bioactivity-guided studies to characterize individual phytoconstituents and assess their biological relevance.

***Index Terms*— Acanthaceae; *Dicliptera*; preliminary phytochemical screening; TLC; secondary metabolites; Peninsular India**

I. Introduction

Plants have historically constituted a fundamental source of therapeutic agents, with their medicinal significance largely arising from the presence of diverse secondary metabolites that perform ecological, physiological, and pharmacological functions (Harborne, 1998; Wink, 2015). Qualitative phytochemical screening serves as a crucial preliminary approach for the detection of major classes of bioactive constituents and for identifying promising taxa for subsequent, detailed chemical and pharmacological investigations (Trease & Evans, 2009; Sofowora, 1993). Such baseline assessments are particularly valuable for plant groups that are taxonomically well studied but remain under explored from a phytochemical perspective, as they help bridge the gap between systematic knowledge and chemical characterization (Harborne, 1998; Heinrich et al., 2018).

Although the genus *Dicliptera* Juss. (Acanthaceae) is taxonomically well studied within the family Acanthaceae and exhibits considerable morphological and geographical diversity, particularly in Peninsular India, phytochemical information for the majority of Indian species remains limited and fragmented, revealing a substantial gap in chemical characterization (Hooker, 1885; Balkwill et al., 1996; Daniel, 2006; Harborne, 1998; Trease & Evans, 2009). Peninsular India represents an important center of diversity for *Dicliptera*, harbouring several widespread as well as endemic and regionally restricted taxa, many of which have not yet been subjected to systematic phytochemical or pharmacological investigation (Hooker, 1885; Balkwill et al., 1996; Sofowora, 1993). Notably, *Dicliptera paniculata*, *D. verticillata* (Forssk.) C. Chr., and *D. bupleuroides* Nees are traditionally employed in indigenous medicinal systems for the treatment of fever, infections, respiratory ailments, and inflammatory conditions, suggesting the presence of biologically active constituents (Kirtikar & Basu, 1935; Chopra et al., 1956). Available phytochemical studies on a five to six species have demonstrated the occurrence of diverse classes of secondary metabolites, including alkaloids, flavonoids, tannins, terpenoids, saponins, phenolics, triterpenoids, sterols, glycosides, and coumarins, many of which are associated with antioxidant, antimicrobial, anti-inflammatory, and anticancer activities (Harborne, 1998; Trease & Evans, 2009; Wink, 2015). In this context, systematic qualitative phytochemical screening serves as an essential preliminary approach for documenting major classes of secondary metabolites and for prioritizing Indian *Dicliptera* taxa for detailed chemical and pharmacological investigations (Harborne, 1998; Trease & Evans, 2009). Among the species investigated, *D. roxburghiana* Nees shows rich antioxidant and anticancer potential (Ahmad et al., 2013; 2021); *D. verticillata* exhibits antimalarial activity (Akpakpan et al., 2017); *D. ghatica* Santapau demonstrates antioxidant and anti-inflammatory properties (Gaikar et al., 2021);

D. bupleuroides possesses antimicrobial and antidiabetic effects (Rehman et al., 2017); and *D. paniculata* displays notable antioxidant and antimicrobial activity (Krishna & Drisya, 2018).

However, phytochemical data for most Indian *Dicliptera* species remain scarce, revealing a significant research gap. Since Peninsular India hosts several widespread and endemic taxa, systematic phytochemical screening is essential to explore their medicinal potential.

II. Materials and methods

a) Plant material Collection and Authentication

Eleven species of *Dicliptera* were collected from different localities in Peninsular India **"Table. 1"**. The collected specimens were identified using standard taxonomic literature, and voucher specimens were prepared and deposited in the S.S.G.M. college herbarium for future reference. Only healthy aerial plant parts were selected for phytochemical analysis to ensure uniformity across taxa.

b) Preparation of plant extracts

The collected plant materials were shade-dried at ambient temperature and subsequently pulverized into a coarse powder. For extraction, 25 g of the powdered material was mixed with 100 mL of methanol and subjected to continuous shaking at 60 °C for 3 h. The resulting mixture was initially filtered through muslin cloth to remove coarse debris, followed by filtration through Whatman filter paper to obtain a clear extract. The filtrate was then concentrated on a hot water bath until a semi-solid residue was obtained, yielding the crude methanolic extract. The prepared extracts were finally stored in airtight containers at low temperature until further qualitative phytochemical analyses were carried out (Harborne, 1998, Trease & Evans, 2009, Azwanida, 2015)

c) Qualitative Phytochemical Screening

Preliminary qualitative phytochemical screening was carried out using various standard confirmatory tests mentioned to detect major classes of secondary metabolites, including triterpenoids, sterols, glycosides, flavonoids, alkaloids, saponins, tannins, carbohydrates, proteins, and lipids. All tests were performed following established protocols described in the classical phytochemical manuals of Harborne (1998) and Trease and Evans (2009), and the results were recorded as present (+) or absent (–).

Table 1- Locations and voucher specimen details of eleven *Dicliptera* species form Peninsular India

Sr. No.	Species	Locality	Voucher specimens
1.	*D. baphica* Nees	Ghat section, 8th hairpin Wayanad churam Mattikunnu, Wayanad district, Kerala.	SSG-10
2.	*D. beddomei* C.B.Clarke	Slopes of Uma Maheswaram Temple, Rangapur, Nagarkurnool district, Telangana.	SSG-11
3.	*D. cuneata* Nees	Near Gudalur, Ooty-Gudalur road, Nilgiris district, Tamil Nadu.	SSG-09
4.	*D. foetida* (Forssk.) Blatt.	Kaas Road, Yekiv, Satara district, Maharashtra	SSG-05
5.	*D. ghatica* Santapau	Mahabaleshwar, Satara district, Maharashtra.	SSG-07
6.	*D. heterostegia* C.Presl	Sangameshwar, Ratnagiri district, Maharashtra.	SSG-08
7.	*D. leonotis* Dalzell ex C.B.Clarke	Anjeneri hill, Nashik district, Maharashtra.	SSG-02
8.	*D. nasikensis* Lakshmin. & B.D.Sharma	Near village Umbrale Bk. Nashik district, Maharashtra.	SSG-03
9.	*D. paniculata* (Forssk.) I.Darbysh.	Dharangaon Road, Murshatpur, Ahilyanagar district, Maharashtra	SSG- 13
10.	*D. srisailamica* Rasingam, Nethaji & Susmitha	Mallela Thirtham Waterfall, Srisailam forest, Nagarkurnool district, Telangana	SSG-12
11.	*D. verticillata* (Forssk.) C. Chr.	S.S.G.M. College Campus, Kopargaon, Ahilyanagar district, Maharashtra.	SSG-04

The extract was tested for the presence of bioactive compounds by using the following standard methods:

1. ***Test for triterpenoids (Liebermann–Burchard test)***

2 mL of the plant extract was mixed with 2 mL of chloroform ($CHCl_3$) in a test tube. Glacial acetic acid (1 mL) was carefully added along the side wall, followed by the slow addition of 2–3 mL of concentrated sulfuric acid (H_2SO_4). The development of a deep reddish coloration was taken as a positive indication of triterpenoids.

2. ***Test for sterols (Liebermann–Burchard test)***

The same procedure as described for triterpenoids was followed for sterol detection . The appearance of a green-colored layer was considered indicative of the presence of sterols.

3. ***Test for glycosides (Keller–Killiani test)***

1 mL of the extract was treated with 2–3 mL of glacial acetic acid, followed by the addition of a few drops of ferric chloride ($FeCl_3$). Concentrated sulfuric acid (2 mL) was then carefully added along the side of the test tube. The formation of a blue color in the upper layer with a brown ring at the interface was considered indicative of glycosides.

4. ***Test for flavonoids (Ferric chloride test)***

Add 1 mL of ferric chloride ($FeCl_3$) solution to 1 mL of the plant extract. The formation of a deep green precipitate or coloration was taken as a positive result.

5. Test for alkaloids (Wagner's test)

Treat 2 mL of the extract with 2 mL of Wagner's reagent. The formation of a brown or reddish-brown precipitate indicated the presence of alkaloids.

6. Test for proteins (Ninhydrin test)

Add 2 mL of ninhydrin reagent to 1 mL of the extract. The appearance of an indigo or violet coloration upon heating indicated the presence of amino acids or proteins.

7. Test for carbohydrates (Barfoed's test)

2 mL of Barfoed's reagent was added to 2 mL of the extract and the mixture was heated. The formation of a brick-red precipitate was considered indicative of monosaccharides.

8. Test for saponins (Foam test)

Saponins were detected by vigorously shaking 1 mL of the extract with 2–3 mL of distilled water. The persistence of stable froth for approximately 10 minutes was recorded as a positive result.

9. Test for lipids (Soap formation test)

Treat 2 mL of the extract with 0.5 N alcoholic potassium hydroxide (KOH) and a drop of phenolphthalein indicator, followed by heating in a water bath for approximately 30 minutes. The formation of soap or partial neutralization of alkali indicated the presence of lipids.

10. Test for tannins (Ferric chloride test)

Add 1–2 drops of ferric chloride ($FeCl_3$) solution to 1 mL of the extract. The appearance of a bluish-green or brown coloration was taken as evidence of tannins.

d) Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) was employed profiling of phytochemical constituents in the plant extract. A sample solution (1 mg mL^{-1}) was prepared by dissolving 1 mg of the crude extract in 1 mL of ethyl acetate. The mobile phase consisted of ethyl acetate and hexane in an 8:12 (v/v) ratio and was selected to achieve optimal separation based on compound polarity. Prior to development, the TLC chamber was saturated with solvent vapours for 15 min. The sample was applied as small spots on a pre-marked TLC plate using a capillary tube, and the plate was developed by capillary action. After development, the plate was air-dried, visualized under UV light and the retention factor (Rf) values were calculated as the ratio of the distance travelled by each compound to that of the solvent front.

III. Results and Discussion

Table 2- Qualitative Phytochemical Screening of eleven Species of Dicliptera Juss. from Peninsular India

Sr. No	Name of species	Name of Tests									
		Triterpenoids	Sterols	Glycosides	Flavonoids	Alkaloids	Proteins	Carbohydrates	Saponins	Lipids	Tannins
1	*D. baphica* Nees	-	+	+	+	+	-	-	-	-	+
2	*D. beddomei* C.B. Clarke.	-	-	+	+	+	-	-	-	-	+
3	*D. cuneata* Nees.	+	+	+	+	+	-	-	-	-	+
4	*D. foetida* (Forssk.) Blatt.	-	-	+	+	+	-	-	-	-	+
5	*D. ghatica* Santapau	+	+	+	+	+	-	-	-	-	+
6	*D. heterostegia* Nees	+	_	+	+	+	-	-	+	-	+
7	*D. leonotis* Dalzell ex C.B Clarke	+	+	-	+	+	-	-	+	-	+
8	*D.nasikensis* Lakshmin. & B.D. Sharma	+	+	+	+	+	-	-	-	-	+
9	*D. paniculata* (Forssk.) I. Darbysh.	+	+	+	+	+	-	-	-	-	+
10	*D. srisailamica* Rasingam, Nethaji & Susmitha	+	+	+	+	+	-	-	-	-	+
11	*D. verticillata* (Forssk.) C. Chr.	+	+	-	+	+	-	-	-	-	+

Qualitative Phytochemical Screening

Qualitative phytochemical screening of eleven *Dicliptera* species revealed the presence of multiple classes of secondary metabolites, including flavonoids, alkaloids, tannins, triterpenoids, sterols, glycosides, saponins, and proteins, while carbohydrates and lipids were not detected under the present assay conditions **"Table. 2".** The predominance of secondary metabolites over primary metabolites is consistent with earlier preliminary phytochemical studies in *Dicliptera* and other members of Acanthaceae, where organic solvent extracts preferentially recover bioactive secondary compounds (Akpakpan et al., 2017; Gaikar et al., 2021).

Flavonoids, alkaloids, and tannins were detected in all eleven species (11/11), indicating that these compounds are highly conserved within the genus *Dicliptera*. Such universal occurrence suggests that these metabolites may represent core chemical features of the genus, a pattern previously reported for selected *Dicliptera* species and related taxa within Acanthaceae (Rehman et al., 2017; Sawadogo et al., 2006). Triterpenoids, sterols, and glycosides were recorded in eight species, indicating moderately widespread but not universal distribution, whereas saponins and proteins were comparatively rare. Saponins were restricted to *D. heterostegia* and *D. leonotis*, while proteins were detected exclusively in *D. ghatica*. Carbohydrates and lipids were absent in all investigated species, which may reflect either their low abundance in the methanolic extracts or the limited sensitivity of qualitative assays for these metabolite classes. Similar absence of carbohydrates and lipids have been reported in other preliminary phytochemical screenings using comparable methodologies (Azwanida, 2015).

Species-wise Phytochemical Richness

Based on the number of positive qualitative tests (out of ten phytochemical groups), seven species—*D. cuneata, D. ghatica, D. heterostegia, D. leonotis, D. nasikensis, D. paniculata,* and *D. srisailamica*—exhibited the highest phytochemical richness, each showing six positive classes. Two species (*D. baphica* and *D. verticillata*) displayed intermediate diversity with five positive classes, whereas *D. beddomei* and *D. foetida* showed comparatively lower diversity with four detected classes.

This ranking reflects qualitative phytochemical breadth rather than quantitative concentration and should therefore be interpreted as an indicator of relative chemical diversity among taxa. Comparable interspecific variation in phytochemical richness has been documented in other multi-species surveys within Acanthaceae and underscores the importance of species-level evaluation rather than genus-level generalization (Gaikar et al., 2021; Rehman et al., 2017).

Compound-wise Distribution Pattern

Among the metabolite classes detected, flavonoids, alkaloids, and tannins showed the highest prevalence (100% occurrence), followed by triterpenoids, sterols, and glycosides (each present in eight species). Saponins (two species) and proteins (one species) were infrequent, while carbohydrates and lipids were entirely absent. The consistent presence of flavonoids, alkaloids, and tannins suggests their potential value as chemotaxonomic indicators within *Dicliptera*, a concept previously proposed for secondary metabolites in Acanthaceae (Sawadogo et al., 2006).

Flavonoids and tannins are widely recognized for their antioxidant and antimicrobial relevance, whereas alkaloids contribute to diverse biological activities, including antimalarial and anti-inflammatory effects reported for some *Dicliptera* species (Akpakpan et al., 2017; Rehman et al., 2017). The recurrent detection of these compounds across all taxa indicates a broad biosynthetic capacity within the genus.

Interpretation and Phytochemical Implications

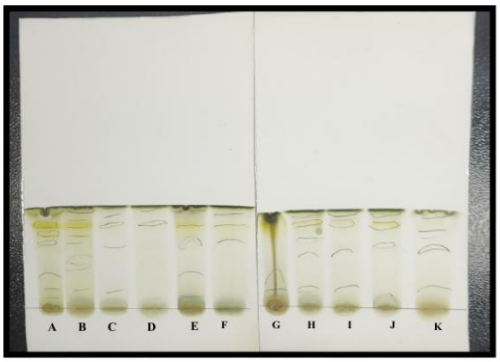
The overall distribution pattern reveals substantial qualitative similarity among the investigated *Dicliptera* species, particularly with respect to flavonoids, alkaloids, and tannins. At the same time, species-specific differences were evident for less common metabolite classes such as saponins and proteins. The occurrence of triterpenoids, sterols, and glycosides in the majority of species suggests the presence of lipophilic terpenoid and glycosidic constituents that may contribute to the medicinal relevance reported for the genus in ethnobotanical literature.

Species such as *D. heterostegia* and *D. leonotis*, which uniquely tested positive for saponins, represent promising candidates for future bioactivity-guided investigations, particularly in relation to membrane-active, antifungal, or cytotoxic properties commonly associated with saponins. The detection of proteins exclusively in *D. ghatica* may indicate the presence of specific proteinaceous compounds, such as lectins, which have been reported from other medicinal plants and merit further biochemical characterization.

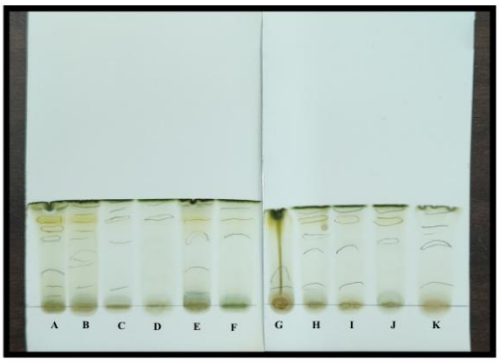
The absence of carbohydrates and lipids across all taxa should be interpreted cautiously, as qualitative tests provide only preliminary evidence and may fail to detect compounds present at low concentrations. Advanced chromatographic and spectroscopic analyses are therefore recommended to validate and refine the present findings.

Thin Layer Chromatography (TLC) Profiling

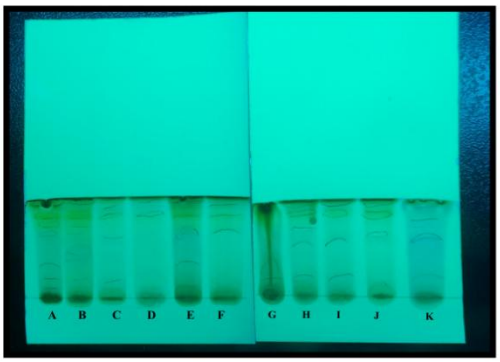
Figure 1 -TLC plate under various wavelengths such as short UV, Long UV, visible light and normal sunlight. Species are **A-***D. baphica* Nees. , **B-** *D. beddomei* C.B. Clarke. , **C-** *D. cuneata* Nees. , **D-** *D. foetida* (Forssk.) Blatt. , **E-** *D. ghatica* Santapau, **F-** *D. heterostegia* Nees. **G-** *D. leonotis* Dalzell ex C.B Clarke. , **H-** *D.nasikensis* Lakshmin. & B.D. Sharma, **I-** *D. paniculata* (Forssk.) I. Darbysh. **J-** D. srisailamica Rasingam, Nethaji & Susmitha **K-** *D. verticillata* (Forssk.) C. Chr.



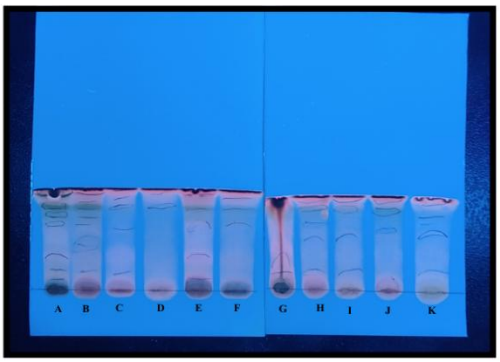
1.TLC plate in normal light



2.TLC plate under visible/white light



3.TLC plate under Short-wave UV



4.TLC plate under long wave UV

Table 3- Rf Values of TLC of eleven species of *Dicliptera* Juss. from Peninsular India

Sr. No.	Species	Distance travelled by solute / Distance travelled by solvent	Rf value
1.	*D. baphica* Nees	0.3 / 3.1 0.6 / 3.1 1.1 / 3.1 02 / 3.1 2.3 / 3.1 2.5 / 3.1 2.9 / 3.1	0.097 0.194 0.355 0.645 0.742 0.807 0.936
2.	*D. beddomei* C.B. Clarke	0.6 / 3.1 1.2 / 3.1 2.0 / 3.1 2.3 / 3.1 2.5 / 3.1 2.8 / 3.1 3.0 / 3.1	0.194 0.387 0.645 0.742 0.807 0.903 0.968

3.	*D. cuneata* Nees	0.5 / 3.1 1.9 / 3.1 2.6 / 3.1 2.9 / 3.1	0.161 0.613 0.839 0.936
4.	*D. foetida* (Forssk.) Blatt.	2.6 / 3.1 3.0 / 3.1	0.839 0.968
5.	*D. ghatica* Santapau	0.4 / 3.1 1.1 / 3.1 2.1 / 3.1 2.6 / 3.1 3.0 / 3.1	0.129 0.355 0.677 0.839 0.968
6.	*D. heterostegia* Nees	2.1 / 3.1 2.6 / 3.1 2.9 / 3.1	0.677 0.839 0.936
7.	*D. leonotis* Dalzell ex C.B Clarke	0.5 / 2.9 1.1 / 2.9	0.172 0.379
8.	*D.nasikensis* Lakshmin. & B.D. Sharma	0.5 / 2.9 1.7 / 2.9 2.0 / 2.9 2.5 / 2.9 2.7 / 2.9	0.172 0.586 0.690 0.862 0.931
9.	*D. paniculata* (Forssk.) I. Darbysh.	0.6 / 2.9 1.8 / 2.9 2.5 / 2.9 2.8 / 2.9	0.207 0.621 0.862 0.966
10.	*D. srisailamica* Rasingam, Nethaji & Susmitha	1.8 / 2.9 2.4 / 2.9 2.8 / 2.9	0.621 0.828 0.966
11.	*D. verticillata* (Forssk.) C. Chr.	0.7 / 2.9 1.8 / 2.9 2.2 / 2.9 2.8 / 2.9	0.241 0.621 0.759 0.966

Thin layer chromatographic profiling of methanolic extracts of eleven *Dicliptera* species using ethyl acetate:hexane (8:12) as the solvent system revealed multiple resolved bands with Rf values ranging from 0.097 to 0.968 **"Table. 3" "Fig. 1"**. The wide Rf range reflects the presence of compounds with varying polarity and supports the chemical heterogeneity observed in the qualitative screening. Lower Rf values were indicative of polar constituents such as glycosides and tannins, whereas intermediate Rf bands corresponded to moderately polar compounds, including flavonoids and alkaloids. Higher Rf values were associated with non-polar constituents such as triterpenoids, sterols, and saponins. These observations are in agreement with standard TLC behavior of plant secondary metabolites reported in earlier phytochemical studies (Azwanida, 2015; Gaikar et al., 2021). Among the investigated taxa, *D. baphica, D. beddomei*, and *D. nasikensis* exhibited the greatest diversity of Rf values, suggesting a comparatively richer array of phytoconstituents. Overall, the TLC profiles corroborated the results of the qualitative phytochemical screening and provided additional visual confirmation of interspecific variation in chemical composition.

IV. Conclusion

Taken together, the qualitative phytochemical screening and TLC profiling demonstrate that *Dicliptera* species from Peninsular India possess a diverse assemblage of secondary metabolites with both conserved and species-specific patterns. As a preliminary investigation, the present results establish a baseline phytochemical framework for the genus and provide a foundation for future quantitative, bioactivity-guided, and chemotaxonomic studies.

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