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Morphology, Comparative Phytochemical, Chromatographic, and Fluorescence Studies of Three Acanthaceae Members

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Abstract

The present study provides a comparative evaluation of morphological characteristics, phytochemical composition, quantitative estimation of major secondary metabolites, and chromatographic profiling of three ethnomedicinal members of the family Acanthaceae, namely *Rungia repens* (L.) Nees, *Hygrophila auriculata* (Schumach.) Heine, and *Peristrophe paniculata* (Forssk.) Brummitt. Detailed morphological investigations revealed distinct diagnostic features related to growth habit, stem structure, leaf morphology, inflorescence type, floral organization, and fruit and seed characters, supporting accurate identification and taxonomic differentiation of the selected species.

Preliminary phytochemical screening of leaf and stem powders using aqueous, methanolic, ethanolic, and chloroform extracts indicated the presence of diverse secondary metabolites, including alkaloids, cardiac glycosides, terpenoids, flavonoids, phenolics, saponins, reducing sugars, and steroids. Methanolic and ethanolic extracts exhibited a broader range of phytoconstituents compared to aqueous and chloroform extracts, highlighting their superior extraction efficiency. Quantitative analysis revealed that phenolic content was significantly higher than flavonoid content in all three species, with leaf samples consistently showing greater accumulation than stems. Among the studied plants, *H. auriculata* recorded the highest phenolic content, followed by *R. repens* and *P. paniculata*.

Thin Layer Chromatography (TLC) profiling of methanolic and ethanolic extracts demonstrated the presence of multiple phytochemical constituents, with leaf extracts showing a greater number of spots than stem extracts. The occurrence of common Rf values among the species suggests the presence of shared bioactive compounds within the family. The integration of morphological, phytochemical, quantitative, and chromatographic analyses provides valuable baseline data for pharmacognostic standardization and supports the medicinal potential

of the selected Acanthaceae species.

Key words : *Rungia repens*; *Hygrophila auriculata*; *Peristrophe paniculata*; Morphological characterization; Phytochemical screening; Thin Layer Chromatography (TLC)

The family Acanthaceae comprises more than 250 genera and nearly 4,000 species, many of which are widely distributed in tropical and subtropical regions and are well known for their medicinal, ecological, and taxonomic significance. Members of this family exhibit considerable morphological diversity and are rich sources of biologically active secondary metabolites, including alkaloids, flavonoids, phenolics, terpenoids, and glycosides, which account for their extensive use in traditional systems of medicine such as Ayurveda, Siddha, and folk remedies across India and other parts of Asia[5,11].

Rungia repens (L.) Nees, *Hygrophila auriculata* (Schumach.) Heine, and *Peristrophe paniculata* (Forssk.) Brummitt. are three ethnomedicinally important members of Acanthaceae that are commonly encountered in moist, marshy, or semi-shaded habitats. These species are traditionally used for the treatment of ailments such as inflammation, urinary disorders, liver complaints, wound healing, and microbial infections, suggesting the presence of diverse pharmacologically active constituents[14,18]. Despite their medicinal relevance, comparative studies integrating detailed morphological characterization with phytochemical profiling of these species remain limited.

Morphological and anatomical studies continue to play a crucial role in accurate identification, authentication, and differentiation of medicinal plants, particularly in families like Acanthaceae where closely related taxa often show overlapping vegetative features. Diagnostic traits related to stem habit, leaf arrangement, inflorescence structure, floral morphology, and fruit characteristics are essential for taxonomic validation and quality control of raw herbal materials[17,19]. Detailed morphological documentation also supports pharmacognostic standardization, which is a prerequisite for ensuring the safety and efficacy of herbal drugs.

In addition to morphology, phytochemical investigation provides valuable insight into the therapeutic potential of medicinal plants. Preliminary phytochemical screening serves as an important step in detecting the presence of major classes of secondary metabolites and guiding further isolation and characterization of bioactive compounds[8, 31]. Solvent polarity plays a significant role in the extraction efficiency of phytoconstituents, with methanol and ethanol often reported as superior solvents for extracting phenolics, flavonoids, and glycosides from plant materials[26].

Among secondary metabolites, phenolic compounds and flavonoids are of particular interest due to their strong antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective properties. Quantitative estimation of these compounds is widely employed to evaluate the medicinal value of plant species and to compare the bioactive potential of different plant parts

such as leaves and stems[4, 27]. Generally, leaves tend to accumulate higher levels of phenolics and flavonoids owing to their active role in photosynthesis and defense against environmental stressors.

Furthermore, Thin Layer Chromatography (TLC) is a rapid, cost-effective, and reliable technique for profiling phytochemicals and establishing characteristic fingerprints of medicinal plants. TLC analysis aids in the detection of multiple compounds, comparison of chemical diversity among species and plant parts, and preliminary assessment of extract complexity, thereby contributing to quality control and standardization of herbal drugs[30, 32].

In this context, the present study aims to provide a comprehensive comparative account of morphological features, preliminary and quantitative phytochemical composition, and TLC profiling of *Rungia repens*, *Hygrophila auriculata*, and *Peristrophe paniculata*. The integration of classical taxonomy with phytochemical and chromatographic analyses is expected to enhance the understanding of their medicinal potential and provide baseline data useful for pharmacognostic standardization, ethnopharmacological validation, and future drug discovery research.

Plant Material Collection and Identification :

Fresh and healthy plant materials of *Rungia repens* (L.) Nees, *Hygrophila auriculata* (Schumach.) Heine, and *Peristrophe paniculata* (Forssk.) Brummitt. were collected from their natural habitats during the active vegetative and flowering season. The collected specimens were cleaned to remove adhering soil and debris and were authenticated based on morphological characters using standard regional floras[11, 20]. Voucher specimens were prepared, pressed, dried, and deposited in the herbarium for future reference[5,14].

Morphological Study :

Morphological characterization was carried out on fresh and preserved specimens following standard taxonomic procedures. Observations were recorded for habit, stem, leaf arrangement and morphology, inflorescence, floral structure, androecium, gynoecium, fruit, and seed characters. Diagnostic features were examined using a hand lens and simple dissection tools. Terminology used for description follows standard botanical literature[17,21].

Preparation of Plant Powder :

Leaves and stems of each plant species were separated, washed thoroughly with tap water followed by distilled water, and shade-dried at room temperature (25–30°C) until a constant weight was achieved. The dried materials were pulverized using a mechanical grinder to obtain a coarse powder and passed through a 60-mesh sieve. The powdered samples were stored in airtight containers for further analysis[32].

Preparation of Extracts :

Approximately 10 g of powdered leaf and stem material from each species was extracted separately using four different solvents of increasing polarity: distilled water (aqueous extract), methanol, ethanol, and chloroform. Extraction was carried out by cold maceration for 24–48 h with intermittent shaking. The extracts were filtered through

Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure or air-dried at room temperature. The dried extracts were stored at 4 °C until further use[8,27].

Preliminary Phytochemical Screening :

Preliminary phytochemical screening of leaf and stem extracts was performed to detect the presence of major secondary metabolites such as alkaloids, cardiac glycosides, terpenoids, saponins, tannins, flavonoids, phenolics, steroids, reducing sugars, carbohydrates, and proteins and amino acids. Standard qualitative tests were employed for each class of compounds following established protocols described by Harborne[5] and Trease and Evans[32]. The intensity of reactions was recorded as strong (++), moderate (+), or absent (–).

Quantitative Estimation of Phytochemicals :

Estimation of Total Phenolic Content :

Total phenolic content was estimated using the Folin–Ciocalteu method. Approximately 1 ml of extract was mixed with Folin–Ciocalteu reagent followed by sodium carbonate solution. The mixture was incubated at room temperature, and absorbance was measured at 765 nm using a UV–Visible spectrophotometer. Gallic acid was used as the standard, and results were expressed as mg of gallic acid equivalent (GAE) per gram of dry sample[30].

Estimation of Total Flavonoid Content :

Total flavonoid content was determined by the aluminium chloride colorimetric method. The extract was mixed with aluminium chloride solution and incubated for the specified time, and absorbance was recorded at 415 nm. Quercetin was used as the standard, and flavonoid content was expressed as mg of quercetin equivalent (QE) per gram of dry sample[4].

All estimations were carried out in triplicate, and results were expressed as mean ± standard deviation.

Thin Layer Chromatography (TLC) Analysis :

TLC analysis was performed to profile the phytochemical constituents present in methanolic and ethanolic extracts of leaf and stem powders. Pre-coated silica gel 60 F_{254} plates were used as the stationary phase. The mobile phase consisted of petroleum ether : acetone : formic acid (70 : 20 : 10). Extract samples were spotted on the plates using capillary tubes and developed in a TLC chamber saturated with solvent vapors. After development, the plates were air-dried and visualized under UV light at 254 nm and 365 nm. The retention factor (Rf) values were calculated using the formula:

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

The number of spots and their corresponding Rf values were recorded and compared among species and plant parts[31,33].

Statistical Analysis :

All quantitative experiments were conducted in triplicate. Data were expressed as mean ± standard deviation (SD). Comparative interpretations were made based

on observed variations among species and between leaf and stem samples.

Morphological study :

Rungia repens (L.) Nees, belonging to the family Acanthaceae, is a perennial, prostrate to creeping herb commonly occurring in moist and shaded habitats. The plant exhibits a mat-forming growth habit due to its ability to root at the nodes. The stems are slender, soft, decumbent to creeping in nature, green to slightly purplish in colour, and generally glabrous or occasionally sparsely pubescent.

The leaves are simple, opposite and decussate, borne on short petioles. The lamina is ovate to elliptic-ovate with an entire margin, acute to obtuse apex, and a cuneate base. The leaf surface is smooth and green, with a prominent midrib and lateral venation clearly visible.

The inflorescence is axillary or terminal, forming compact and short spikes. The bracts are green, ovate to lanceolate, imbricate, and possess entire margins. Flowers are bisexual and zygomorphic, exhibiting bluish-violet to pale purple coloration. The calyx is five-lobed, with narrow lobes that are equal or subequal in size. The corolla is tubular and distinctly bilabiate, with a smaller upper lip and a larger, spreading lower lip.

The androecium consists of two fertile, didynamous stamens bearing two-celled anthers. The gynoecium is bicarpellary and syncarpous, with a superior ovary that is bilocular and contains two ovules per locule. The style is slender and terminates in a bifid stigma. The fruit is an oblong-ellipsoid capsule containing two seeds. The seeds are small, discoid in shape, and equipped with retinacula (jaculators), which aid in seed dispersal.

Hygrophila auriculata (Schumach.) Heine (syn. *Asteracantha longifolia* Nees), a member of the family Acanthaceae, is a robust, erect, annual to perennial herb commonly distributed in marshy areas, riverbanks, and seasonally waterlogged habitats. The plant attains a height of about 1–2 m and exhibits a strong, well-developed taproot system. The stem is erect, stout, quadrangular, and much branched, often bearing nodes with prominent whorls of sharp spines. The stem surface is green to purplish-green and may be sparsely pubescent. Leaves are simple, sessile to shortly petiolate, and arranged in opposite or pseudo-whorled fashion, particularly at the nodes. The lamina is linear-lanceolate to oblong-lanceolate, with an entire to slightly undulate margin, an acute to acuminate apex, and a narrowed or attenuate base. The leaf surface is rough to slightly hairy, with a prominent midrib and conspicuous lateral veins. The inflorescence consists of dense, axillary, sessile whorls of flowers borne at the nodes, forming characteristic spiny heads. Bracts are rigid, lanceolate, and modified into sharp spines, which are a distinctive diagnostic feature of the species. Flowers are bisexual, zygomorphic, and bluish-purple to violet in colour. The calyx is deeply five-lobed, with linear-lanceolate, unequal lobes. The corolla is tubular and bilabiate, with the upper lip reduced and the lower lip larger and three-lobed. The androecium comprises four didynamous stamens, with two longer and two shorter fertile stamens bearing two-celled anthers. The gynoecium is bicarpellary and syncarpous, with a superior, bilocular ovary containing two

ovules in each locule. The style is slender and ends in a bifid stigma. The fruit is an oblong to ovoid capsule, typically containing four seeds. Seeds are small, compressed, and provided with retinacula, facilitating effective seed dispersal.

Peristrophe paniculata (Forssk.) Brummitt., is an erect to sub-erect, much-branched perennial herb or undershrub, attaining a height of approximately 60–120 cm. The stem is cylindrical, green to purplish, glabrous to sparsely pubescent, distinctly nodose, with opposite branching.

Leaves are simple, opposite, decussate, and petiolate; petioles measure 1.5–4.0 cm in length. Leaf lamina is ovate to elliptic-ovate, measuring 6–15 cm long and 3–8 cm wide, with an acute to acuminate apex and a cuneate to rounded base. Margins are entire, surface glabrous or sparsely pubescent, dark green adaxially and lighter abaxially. Venation is reticulate with prominent midrib and lateral veins. Inflorescence is a terminal or axillary, lax to dense panicle, often elongated, bearing numerous flowers. Bracts are conspicuous, foliaceous, ovate-lanceolate, green to purplish, and persistent. Flowers are zygomorphic, bisexual, and pedicellate. Calyx is 5-lobed, deeply divided, with narrow, linear to lanceolate lobes, persistent and green. Corolla is tubular, bilabiate, 3–5 cm long, pink to purplish-violet with darker markings; the upper lip is smaller and bifid, while the lower lip is trilobed and spreading. Androecium consists of two fertile stamens, epipetalous, with dithecous anthers; staminodes are absent or rudimentary. Gynoecium is bicarpellary, syncarpous; ovary superior, bilocular with two ovules per locule; style slender with a bifid stigma. Flowering from September to January, depending on climate. Fruit is a compressed, oblong to ellipsoid capsule, approximately 1.5–2.5 cm long, containing 2–4 seeds. Seeds are discoid, brown, smooth to slightly roughened, and provided with hygroscopic retinacula.

The morphological features recorded in this study closely align with earlier floristic and taxonomic descriptions of the respective species, confirming their correct identification. *Rungia repens* exhibited a prostrate habit with nodal rooting and compact inflorescences, a growth strategy considered adaptive for moist and shaded microhabitats[3,5]. Such vegetative plasticity is common among creeping Acanthaceae members and contributes to their ecological success.

Hygrophila auriculata showed robust erect growth, quadrangular spiny stems, and axillary whorls of flowers forming characteristic spiny heads. These traits are well recognized as diagnostic characters and are associated with protection against herbivory in wetland environments. Similarly, *Peristrophe paniculata* was distinguished by its erect branched habit, foliaceous bracts, and elongated panicles, which are consistent with recent taxonomic treatments of the genus[15]. Accurate morphological documentation remains fundamental for preventing substitution and adulteration of herbal raw materials, which continues to be a major challenge in herbal drug trade[10].

Preliminary phytochemical analysis :

Preliminary phytochemical analysis of leaf powder extract :

The preliminary phytochemical

screening of powdered leaf and stem samples of the three selected ethnomedicinal plants—*Rungia repens* (L.) Nees., *Hygrophila auriculata* (Schumach.) Heine and *Peristrophe paniculata* (Forssk.) Brummitt. was carried out using four different solvent extracts: aqueous (Aq. E), methanol (ME), ethanol (ET), and chloroform (CL). The results of the phytochemical analysis are summarized in Table- 1.

The findings revealed that the selected species are rich in secondary metabolites, particularly alkaloids, terpenoids, phenolics, flavonoids, cardiac glycosides, reducing sugars, and steroids. These phytoconstituents were detected more consistently in the methanolic and ethanolic extracts compared to aqueous and chloroform extracts. Notably, carbohydrates and proteins and amino acids were absent in all solvent extracts across all species, with the exception of a few isolated observations.

For *Rungia repens*, the methanolic extract demonstrated strong positivity for alkaloids, cardiac glycosides, reducing sugars, saponins, tannins, flavonoids, phenolics, and steroids. The ethanolic extract of the leaf showed the presence of alkaloids, cardiac glycosides, reducing sugars, tannins, and phenolics. In the stem, most phytochemicals were present, except for reducing sugars, saponins, and flavonoids. The chloroform extract of the leaf revealed the presence of cardiac glycosides, terpenoids, reducing sugars, saponins, flavonoids, and steroids.

In *Hygrophila auriculata*, the aqueous extract showed the presence of terpenoids, saponins, flavonoids, steroids, cardiac glycosides, and reducing sugars. The methanolic and ethanolic extracts exhibited similar phytochemical profiles, including cardiac glycosides, terpenoids, reducing sugars, saponins, tannins, and steroids. The chloroform extract tested positive for terpenoids, reducing sugars, saponins, tannins, flavonoids, phenolics, and steroids.

In *Peristrophe paniculata*, the aqueous extract showed the presence of alkaloids, cardiac glycosides, reducing sugars, terpenoids, saponins, and steroids. The methanolic extract was positive for cardiac glycosides, reducing sugars, terpenoids, saponins, tannins, phenolics, and steroids. The ethanolic extract showed the presence of alkaloids, cardiac glycosides, reducing sugars, and flavonoids, whereas the chloroform extract tested positive for cardiac glycosides, reducing sugars, tannins, phenolics, flavonoids, and carbohydrates.

Preliminary phytochemical analysis of stem powder extract :

The preliminary phytochemical analysis of the stem powdered material of the selected ethnomedicinal plants—*Rungia repens, Hygrophila auriculata,* and *Peristrophe paniculata*—was conducted using four solvent systems: aqueous (Aq. E), methanol (ME), ethanol (ET), and chloroform (CL). The results are presented in Table-2.

The phytochemical screening revealed that all the selected plant species tested positive for terpenoids, reducing sugars (strong reaction), flavonoids, and steroids in all four extracts. Aqueous and ethanol extracts demonstrated a broader spectrum of phyto-chemical constituents, followed closely by methanol extracts, which were also rich in

several bioactive compounds. Flavonoids were consistently detected in the aqueous, methanolic, and ethanolic extracts of all species. Steroids were present in all solvent extracts across all species. In contrast, tannins, carbohydrates, and proteins and amino acids were absent in all solvent extracts of all selected plants. Phenolics were detected only in the aqueous and methanolic extracts of *Rungia repens*. For *Rungia repens*, most phytoconstituents were detected except tannins, phenolics, carbohydrates, and proteins and amino acids. The methanolic extract revealed the presence of cardiac glycosides, terpenoids, reducing sugars, phenolics, and steroids. The ethanolic extract showed positivity for alkaloids, cardiac glycosides, terpenoids, reducing sugars, flavonoids, and steroids. The chloroform extract was positive for terpenoids, reducing sugars, saponins, and steroids.

In *Hygrophila auriculata*, the aqueous extract tested positive for terpenoids, saponins, flavonoids, and steroids. The methanolic extract showed the presence of alkaloids, cardiac glycosides, terpenoids, flavonoids (strong), and steroids. The ethanolic extract was positive for all phytochemicals except tannins and phenolics. The chloroform extract tested positive for terpenoids, reducing sugars, saponins, and steroids.

In *Peristrophe paniculata*, the aqueous extract tested positive only for alkaloids, saponins, and flavonoids. The methanolic extract showed the presence of alkaloids, cardiac glycosides, terpenoids, flavonoids, and steroids. The ethanolic extract tested positive for alkaloids, cardiac glycosides, terpenoids, reducing sugars, saponins, flavonoids, and steroids. The chloroform extract showed a limited profile, with positivity only for cardiac glycosides and reducing sugars.

It was noted that terpenoids, reducing sugars, flavonoids, and steroids emerged as the most consistently detected phytochemicals across all stem extracts of the selected species. Methanolic and ethanolic solvents demonstrated higher extraction efficiency for multiple phytochemicals, whereas chloroform and aqueous extracts exhibited selective extraction patterns. The absence of tannins, carbohydrates, and proteins and amino acids in all extracts highlights a common chemical profile among the studied Acanthaceae species.

Preliminary phytochemical screening revealed a broad spectrum of bioactive secondary metabolites across all three species. The consistent presence of terpenoids, flavonoids, steroids, and cardiac glycosides supports earlier reports that Acanthaceae members are chemically rich and pharmacologically promising[26]. Methanolic and ethanolic extracts showed superior extraction efficiency compared to aqueous and chloroform extracts, which is in agreement with recent studies emphasizing the effectiveness of polar solvents in extracting phenolics and flavonoids[2,6].

The observed similarity in phytochemical profiles among the studied species suggests a degree of chemotaxonomic coherence within Acanthaceae. At the same time, interspecific variation in the presence and intensity of certain compounds reflects metabolic specialization, which may account for differences in ethnomedicinal applications. Such patterns have

Table 1 Preliminary phytochemical analysis of leaf powder extract from selected medicinal plants.

Sr. No	Plant Name	Solvent	Alkaloids	Cardiac Glycosides	Terpenoids	Reducing Sugar	Saponins	Tannins	Flavonoids	Phenolics	Steroids	Carbohydrates	Protein and Amino Acids
1.	*Rungia repens*	D.W	+	++	-	++	+	+	-	-	-	-	-
		Methanol	+	++	+	+	+	+	+	+	+	-	-
		Ethanol	+	++	-	+	-	+	-	+	-	-	-
		Chloroform	-	+	+	++	+	-	+	-	+	-	-
2.	*Hygrophila auriculata*	D.W	-	++	+	++	+	-	+	-	+	-	-
		Methanol	-	+	++	+	+	+	-	-	++	-	-
		Ethanol	+	++	++	++	-	-	-	-	++	-	-
		Chloroform	-	-	+	+	+	+	+	+	+	-	-
3.	*Peristrophe paniculata*	D.W	+	+	+	+	+	-	-	-	+	-	-
		Methanol	-	++	+	+	+	+	-	+	+	-	-
		Ethanol	+	++	-	++	-	-	+	-	-	-	-
		Chloroform	-	++	-	++	-	+	+	+	-	+	-

(Aq. E= Aqueous extract, ME=Methanolic extract, ET= Ethanolic extract, CL= Chloroform).

been increasingly highlighted in recent chemotaxonomic investigations of medicinal plant families[21].

Quantitative analysis of major phytochemicals:

The crude content of major phytochemical compounds in *Rungia repens* Nees., *Hygrophila auriculata* (Schumach.) and *Peristrophe paniculata* was determine using standard protocols. It has been found that among all four tested phytochemicals, selected plant showed higher level of phenolics followed by flavonoids. The observation found that among all three plants leaf extract showed higher content than stem in phenolics though same trend followed in stem powder.

The content level of phenolics was significantly higher in leaf powder of H. auriculata (leaf 94.5 ± 1.41), followed by R. repens (leaf 84.5 ± 1.3), and P. paniculata (leaf 82.3 ± 1.16). The while the stem extract showed the same trend (Table-3). As we see flavonoids content in leaf extract Rungia repens (1.33 ± 0.32) showed higher level followed by H. auriculate (1.26 ± 0.34), and very less amount in P. paniculata (0.74 ± 0.63) The comparative account of quantitative analysis of these five plants is presented in (Table-3).

Quantitative analysis demonstrated that phenolic compounds were present in significantly higher concentrations than flavonoids in all three species, with leaf samples consistently outperforming stem

Table-2. Preliminary phytochemical analysis of stem powder extract from selected medicinal plants.

Sr. No.	Plant Name	Solvent	Alkaloids	Cardiac Glycosides	Terpenoids	Reducing Sugar	Saponins	Tannins	Flavonoids	Phenolics	Steroids	Carbohy-drates	Protein and Amino Acids
1.	*Rungia repens*	D.W	+	+	+	+	+	-	+	-	+	-	-
		Methanol	-	++	+	+	-	-	-	+	+	-	-
		Ethanol	+	+	+	+	-	-	+	-	+	-	-
		Chloroform	-	-	+	+	+	-	-	-	+	-	-
2.	*Hygrophila auriculata*	D.W	-	-	+	-	+	-	+	-	+	-	-
		Methanol	+	++	+	-	-	-	++	-	+	-	-
		Ethanol	+	+	+	+	+	-	+	-	+	-	-
		Chloroform	-	-	+	+	+	-	-	-	+	-	-
3.	*Peristrophe paniculata*	D.W	+	-	-	-	+	-	+	-	-	-	-
		Methanol	+	+	+	-	-	-	+	-	+	-	-
		Ethanol	+	++	+	+	+	-	+	-	+	-	-
		Chloroform	-	+	-	++	-	-	-	-	-	-	-

(Aq. E= Aqueous extract, ME=Methanolic extract, ET= Ethanolic extract, CL= Chloroform).

samples. This observation is in line with contemporary findings that leaves act as primary sites for phenolic biosynthesis due to their role in photosynthesis and defense mechanisms[16,28].

Hygrophila auriculata exhibited the highest phenolic content among the studied species, supporting its well-documented antioxidant, hepatoprotective, and anti-inflammatory properties in traditional and experimental studies[23,24]. The comparatively higher flavonoid content observed in *Rungia repens* suggests its potential contribution to antimicrobial and anti-inflammatory activities, as flavonoids are known modulators of oxidative stress and immune responses[1,19].

The marked difference between leaf and stem phytochemical content highlights the importance of plant-part selection in herbal formulations, an aspect that is often overlooked but increasingly emphasized in pharmacognostic standardization guidelines[33].

TLC analysis of the crude powder extracts :

The TLC (Thin Layer Chromatography) analysis was performed to identify the number of compounds extracted from the powder samples in their methanolic and ethanolic extract. The mobile phase used was Petroleum ether: Acetone: Formic acid (70:20:10).

The methanolic extract of *Rungia repens* leaf, stem showed 5 and 7 spots respectively while *Hygrophila auriculata* leaf and stem showed 7 and 3 resp. whereas *Peristrophe paniculata* leaf and stem showed

Table-3. Quantitative phytochemical analysis of powdered samples of selected 3 plants (crude content in mg/g of dry sample)

Sr.No.	Phytochemicals / Plant Name		Phenolics (mg/g)	Flavonoids (mg/g)
1	Rungia repens	Leaf	84.5 ± 1.3	1.33 ± 0.32
		Stem	73.7 ± 1.2	0.94 ± 0.25
2	Hygrophila auriculata	Leaf	94.5 ± 1.41	1.26 ± 0.34
		Stem	77.2 ± 1.22	1.09 ± 0.16
3	Peristrophe paniculata	Leaf	82.3 ± 1.16	0.92 ± 0.60
		Stem	69.6 ± 0.9	0.78 ± 0.52

(Note: Results are average of triplicate analysis)

5, 3, 6 and 5 spots respectively. In selected three plants *i.e. Rungia repens, Hygrophila auriculata* and *Peristrophe paniculata* leaf showed more spots than stem. The leaf of given plants share three compounds are common of Rf value 0.13, 0.11 and 0.07, The comparative account of TLC analysis of methanolic extract of selected plants are given below in (Table-4).

The ethanolic extract of all plants showed more spots as compare to methanolic extract. *Rungia repens* leaf, stem showed 7 and 6 spots respectively while *Hygrophila auriculata* leaf and stem showed 5 and 4 respectively whereas *Peristrophe paniculata* leaf and stem showed 6, 1, 7 and 5 spots respectively. In selected three plants i.e Rungia repens, *Hygrophila auriculata* and *Peristrophe paniculata* leaf showed more spots than stem. The leaf of selected plants shares two common compounds having RF value 0.28, 0.10, where the stem share only one compound common having RF value 0.12. The comparative account of TLC analysis of ethanolic extract of five plants are given below in (Table- 4).

TLC profiling of methanolic and ethanolic extracts revealed multiple distinct spots with reproducible Rf values, indicating considerable chemical diversity in the selected species. Leaf extracts consistently produced a greater number of spots than stem extracts, suggesting higher metabolic complexity and secondary metabolite accumulation in foliar tissues. Similar trends have been reported in recent TLC-based profiling studies of medicinal plants[22,28].

The presence of shared Rf values among species indicates the occurrence of common phytochemical constituents, which may explain their overlapping ethnomedicinal uses. Conversely, species-specific TLC fingerprints can serve as reliable tools for authentication and quality control of crude drug materials. Recent studies have emphasized TLC fingerprinting as a rapid and cost-effective approach for herbal drug standardization, particularly in resource-limited settings[13].

The integration of morphological, phytochemical, quantitative, and chromatographic data in the present study provides a robust framework for the pharmacognostic standardization of *R. repens*, *H. auriculata*, and *P. paniculata*. The documented abundance of

phenolics, flavonoids, terpenoids, and glycosides supports their traditional medicinal relevance and underscores their potential as sources of bioactive compounds for further pharmacological exploration. In the context of growing global interest in plant-based therapeutics, such baseline studies are crucial for bridging traditional knowledge with modern scientific validation. Future work should focus on advanced chromatographic and spectroscopic techniques (HPTLC, HPLC, LC–MS/MS) coupled with bioactivity-guided fractionation to identify marker compounds and substantiate therapeutic claims[7,9].

Table- 4. RF values obtained from TLC analysis of Powdered Samples (Methanol extract)

Rungia repens				*Hygrophila auriculata*				*Peristrophe paniculata*			
Leaf		Stem		Leaf		Stem		Leaf		Stem	
No. of spot	RF value	No. of spot	RF Value	No. of spot	RF value	No. of spot	RF value	No. of spot	RF value	No. of spot	RF value
1	0.19	1	0.24	1	0.95	1	0.16	1	0.95	1	0.93
2	0.14	2	0.21	2	0.13	2	0.13	2	0.13	2	0.21
3	0.11	3	0.17	3	0.11	3	0.11	3	0.10	3	0.16
4	0.10	4	0.15	4	0.08			4	0.08	4	0.15
5	0.07	5	0.12	5	0.07			5	0.07	5	0.13
6		6	0.10	6	0.06			6	0.03		
7		7	0.07								

Table-5. RF values obtained from TLC analysis of Powdered Samples (Ethanol Extract)

Rungia repens				*Hygrophila auriculata*				*Peristrophe paniculata*			
Leaf		Stem		Leaf		Stem		Leaf		Stem	
No. of spot	RF value	No. of spot	RF Value	No. of spot	RF value	No. of spot	RF value	No. of spot	RF value	No. of spot	RF value
1	0.92	1	0.92	1	0.92	1	0.42	1	0.92	1	0.32
2	0.42	2	0.38	2	0.28	2	0.28	2	0.41	2	0.28
3	0.35	3	0.30	3	0.17	3	0.15	3	0.28	3	0.20
4	0.28	4	0.15	4	0.12	4	0.11	4	0.14	4	0.12
5	0.18	5	0.12	5	0.08	5		5	0.10	5	0.08
6	0.12	6	0.10	6		6		6	0.08		
7	0.10					7		7	0.05		

The present study offers an integrated evaluation of morphological traits, phyto-chemical composition, quantitative estimation of key secondary metabolites, and chromato-graphic profiling of three ethnomedicinal species of Acanthaceae, namely *Rungia repens*, *Hygrophila auriculata*, and *Peristrophe paniculata*. Such multidisciplinary approaches are increasingly emphasized in medicinal plant research to ensure correct botanical authentication, chemical standardization, and validation of traditional claims[7,9].

Inference :

The study presents a comparative evaluation of morphological characters, phytochemical composition, quantitative estimation, and TLC profiling of *Rungia repens*, *Hygrophila auriculata*, and *Peristrophe paniculata*. Distinct morphological features supported accurate taxonomic identification of the selected Acanthaceae species. Phytochemical screening revealed the presence of important secondary metabolites, with methanolic and ethanolic extracts showing higher extraction efficiency. Quantitative analysis indicated that phenolic content was higher than flavonoids, particularly in leaf samples, with *H. auriculata* showing maximum phenolic concentration. TLC analysis demonstrated greater chemical diversity in leaves compared to stems and revealed both common and species-specific compounds. Overall, the findings provide essential baseline data for pharmacognostic standardization and support the medicinal potential of the studied species.

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