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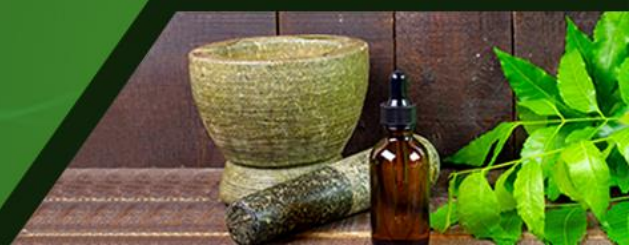

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Assessing the impact of solvent polarity on the phytochemical profile of *Hygrophila auriculata* leaf extracts

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Abstract
The research explores the phytochemical profile of *Hygrophila auriculata* leaf extract through the application of solvents with differing polarities, aiming to uncover biologically active constituents with therapeutic potential. Leaves of *Hygrophila auriculata* were subjected to extraction using solvents ranging from non-polar to highly polar namely petroleum ether (low polarity), chloroform, ethyl acetate, ethanol, and water (high polarity). Phytochemical screening identified a diverse array of bioactive compounds namely alkaloids, flavonoids, saponins, phenolics, tannins, glycosides, and terpenoids whose relative abundance varied depending on the solvent used during extraction. The ethanol extract demonstrated the most comprehensive array of phytochemicals, particularly rich in phenolic compounds, tannins, alkaloids, carbohydrates, terpenoids, and flavonoids compounds widely recognized for their antioxidant capabilities. These findings affirm that *Hygrophila auriculata* harbors a diverse set of bioactive agents potentially beneficial for managing various health conditions, including infertility. Continued investigation, particularly focusing on the isolation and structural elucidation of individual compounds, is recommended to further evaluate their pharmacological relevance.

Keywords: *Hygrophila auriculata*, phytochemicals, solvents, polarity

Introduction
Indian medicinal plants have long sparked academic interest, as reflected in the growing volume of research publications. However, a significant challenge remains: the precise mechanisms by which these plants exert their therapeutic effects are often not clearly understood, leading to scientific skepticism [1]. Many medicinal plants help mitigate the harmful effects of oxidative stress through the activity of key compounds known as antioxidants. The disease-preventive potential of these plants is largely attributed to the antioxidant properties of their bioactive constituents [2]. Consequently, there has been a substantial increase in efforts to isolate natural antioxidants, particularly those derived from plants, over the past decade. Notably, the mechanisms by which these plants act vary widely depending on the species [3].

Hygrophila auriculata (Buch. Ham.) is a thorn-armed sub-shrub in the Acanthaceae family that thrives in moist settings, particularly along marshy edges such as ditches and paddy fields. In Ayurvedic literature, the plant is known by several names, including *Ikshura*, *Ikshugandha*, *Kokilaksha*, and *Indian Cuckoo*. Within the Ayurvedic system of medicine, it is further characterized by its properties, being referred to as *Seethaveryam* and *Mathuravipaka* [4].

Distribution: Found across the world in regions such as Sri Lanka, Myanmar, Indonesia, Malaysia, and throughout the plains of India, this species typically inhabits moist environments like marshy canal edges, and is also present in the tropical Himalayas [5].

Taxonomy: [6]
- Kingdom: Plantae
- Class: Magnoliopsida
- Subkingdom: Viridiplantae

- Superorder Asteranae
- Infrakingdom: Streptophyta
- Order Lamiales
- Superdivision: Embryophyta
- Family: Acanthaceae
- Division: Tracheophyta
- Genus: *Hygrophila* R. Br.
- Subdivision: Spermatophyta
- Species: *auriculate*

Fig 1: *Hygrophila auriculata*

Leaf of *Hygrophila auriculata:* [7]

Table 1: Leaf of *Hygrophila auriculata*

Feature	Description
Leaf Type	Dorsiventral and glabrous, with a noticeably raised midrib.
Midrib Shape (Cross-section)	Plano-bowed; adaxial side flush, abaxial side broad and crescent-shaped
Midrib Size	750 µm (median perpendicular plane), 1 µm (parallel plane)
Upper Epidermis	Prominent; square cells with a well-developed cuticle
Collenchyma (Adaxial side)	About 3 layers of small collenchyma cells beneath the epidermis
Parenchyma (Adaxial side)	Beneath the collenchyma lies 4–5 layers of broad, thin-walled parenchyma cells.
Lower Epidermis (Abaxial)	Similar to adaxial epidermis
Collenchyma (Abaxial side)	Just beneath the abaxial epidermis, there are typically one to two layers of collenchyma cells.
Ground Tissue	"Composed of compact, wide, thin-walled parenchyma cells.
Vascular Bundle	Single, elliptical in cross-section; 350 µm (horizontal), 150 µm (vertical)
Xylem	8–10 parallel rows; angular, thin-walled, narrow elements
Phloem	A thin parenchymatous sheath occurs along the abaxial (lower) side of the xylem.
Accessory Strands	Two small, circular vascular strands on the upper (adaxial) leaf surface, each comprising a cluster of xylem vessels and a small phloem nest

Materials and methods

Chemicals

In this study, all the chemicals purchased from Naresh Scientific Company, Puducherry- 605010. The chemicals used were analytical grade.

Collection and identification of plant material

The *Hygrophyla auriculata* leaves were collected by Tirunelveli region and the plant was authenticated by Dr. M. Syed Ali Fathima, Assistant Professor and Head, Sadakathullah Appa Arts and Science College, Tirunelveli.

Fig 2: Collection and powdered *Hygrophila auriculata* leaf

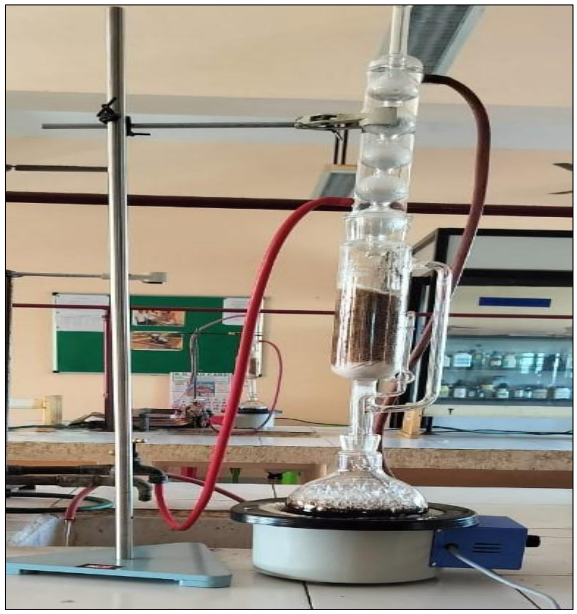

Fig 3: Extraction of leaf material

Extraction of plant material

The collected leaves were air-dried at ambient temperature until fully desiccated. Subsequently, the dried leaves were finely powdered using a mechanical grinder to increase the surface area for extraction.

The powdered material was subjected to sequential extraction using a Soxhlet apparatus, employing solvents of varying polarity to ensure comprehensive extraction of bioactive compounds. The solvents utilized, in order of increasing polarity, were petroleum ether, chloroform, ethyl acetate, ethanol, and water. Each extraction was conducted until the solvent in the siphon tube appeared clear, indicating the exhaustion of the plant material. The extracts obtained were then concentrated under reduced pressure using a rotary evaporator to remove the solvents, yielding crude extracts for subsequent analysis.

This method ensures the efficient extraction of a wide range of phytochemicals, facilitating comprehensive phytochemical analysis.

Preliminary phytochemical analysis

The extracts were treated with the specific reagents to find out the presence of various phytoconstituents [8-40].

Table 2: Preliminary phytochemical analysis

Phytoconstituent	Test	Procedure	Observation
Alkaloids	Dragendroff's test	Extract + Dragendroff's reagent	A precipitate red colour.
	Hager's test	Extract + Hager's reagent	White colour
	Mayer's test	Extract + Mayer's reagent	A creamy white/yellow precipitate
	Wagner's test	Extract + Wagner's reagent	A brown/reddish precipitate
	Picric acid test	Extract + 2% picric acid solution	An orange-coloured precipitate
	Iodine Test	Extract + iodine solution	Formation of blue colour
	Bouchardat's test	Extract + Bouchardat's reagent	Formation of brick red colour.
	Tannic acid test	Extract +10% tannic acid solution	A buff colour precipitate
Carbohydrates	Barfoed's test	Extract is heated with Barfoed's reagent	Form red precipitate to confirm the monosaccharides.
	Molish's test	Extract + Molish's reagent and con. Sulphuric acid along the side of the test tube	Voilet ring appears
	Seliwanoff's test	Extract is heated with seliwanoff's reagent	Appearance of rose red colour for the confirmation of ketoses.
	Resorcinol test	Heat the Extract+ Resorcinol + Con.HCl	A rose red colour
	Test for Pentoses	Heated in amixture of Extract + Con. HCl + Phloroglucinol	A red colour
	Test for starch	Extract + 5% KOH solution	A cinary colouration
Reducing sugars	Benedict's test	Extract + Benedict's reagent and heated	Green/ Yellow/ Red colour
	Fehling's test	Extract + Fehling's solution A and B and heated	A red precipitate
	Borntrager's test	Extract + Chloroform and 10% ammonia solution	A pink colour solution
	Modified Borntrager's test	Extract+ Ferric chloride and boil for few minutes + Benzene + Ammonia	Blood red coloured solution
	Legal's test	Extract + pyridine + Sodium nitroprusside + 10% Sodium hydroxide	A pink coloured solution
	10% NaOH test	Extract + dil. H2SO4 boiled for 15min and neutralize with 10% NaOH + Fehling's solution A & B	A brick red precipitate
	Aqueous NaOH test	Extract + aqueous NaOH solution	A yellow colour
	Concentrated H2SO4 test	Extract + glacial acetic acid + a drop of 5% FeCL3 + conc. H2SO4	A brown ring
	Raymond's test	Extract + dinitrobenzene in hot methanolic alkali	A violet colour
Cardiac glycosides	Keller-Killani test	Extract + glacial acetic acid + 5% ferric chloride + conc. H2SO4	A blue coloured solution
	Kedee's test	Extract + methanol + alcoholic KOH + 1% alcoholic 3,5 dinitrobenzene and heated	A disappearing violet colour
	Test for Cardenolides	Extract + pyridine + Sodium nitroprusside + 20% NaOH	A red colour, fades to brownish yellow
	Bromine water test	Extract + bromine water	A yellow precipitate
	Baljet test	Extract + Baljet's reagent	A yellow-orange colour
Proteins and Amino acids	Biuret test	Extract + 2% copper sulphate + 95% ethanol + KOH pellets	A pink coloured solution
	Millon's test	Extract + few drops of Millon's reagent	A white precipitate
	Ninhydrin test	Extract + Ninhydrin solution	A purple-coloured solution {Amino acids}
	Xanthoprotein test	Extract + Few drops of conc. Nitric acid	A yellow -coloured solution (Aromatic amino acids)
Flavonoids	Alkaline reagent test	Extract + 2% NaOH solution	An intense yellow colour
	Ammonium hydroxide test	Extract + 10% ammonium hydroxide solution	A yellow fluorescence
	Lead acetate test	Extract + 10% lead acetate solution	A yellow precipitate
	Shinoda's test	Extract + 5mL alcohol + Fragments of magnesium ribbon + few drops of conc. HCl	A pink to crimson coloured solution
	Shibata's reaction	Extract + 50% methanol by heating + metal magnesium + 5-6 drops of conc. HCl	A red colour {flavonols}, orange colour {flavones}

	Ferric chloride test	Extract + 10% ferric chloride solution	A green precipitate
	Pew's test	Extract + 0.1gm metallic zinc + conc. H2SO4	A red colour {flavonols}
	Zinc-hydrochloride reduction test	Extract + pinch of zinc dust + conc. HCl along the side of test tube	Magenta colour appears
	Ammonia test	Filtrate + dil. Ammonia solution + conc. H2SO4	A yellow colour
	Conc. H2SO4 test	Extract + conc. H2SO4	An orange colour
Phenolic compounds	Iodine test	Extract + few drops of dil. Iodine solution	A transient red colour
	Ferric chloride test	Extract + few drops 5% ferric chloride	Dark green/bluish black colour
	Gelatin test	Extract + 1% gelatin solution + 10% NaCl	A white precipitate
	Lead acetate test	Extract + 10% lead acetate solution	A white precipitate
	Ellagic Acid Test	Extract + 5% glacial acetic acid + 5% sodium nitrite solution	Solution turns muddy / Niger brown precipitate
	Potassium dichromate test	Extract + few drops of potassium dichromate solution	A dark colour
	Hot water test	Warm water in beaker + mature plant part is dipped + warmed for a min.	Black or brown colour ring at the junction of dipping
	Test for Cartenoids	Extract + chloroform, (vigorously shaken and filtered). Filtrate + conc. H2SO4	A blue colour at the interface
Tannins	Gelatin test	Extract +1% gelatin solution + 10% NaCl	A white precipitate
	Braymer's test	Extract+ distilled water + 10% Ferric chloride solution	Blue-green colour
	10% NaOH test	Extract + 10% NaOH + shaken well	Formation of emulsion {Hydrolysable tannins}
	Bromine water test	bromine water + plant extract	Decoloration of bromine
	Lead sub acetate test	Extract + lead sub acetate solution	A creamy gelatinous precipitate
	Phenazone test	Extract + sodium acid phosphate, heated, allowed to cool + filtered); filtrate + 2% solution of phenazone	Precipitation formation
	Mitchell's test	Extract solution + iron + sodium tartarate (+ ammonium acetate solution)	A water-soluble iron-tannin complex, which is insoluble in solution of ammonium acetate
Phlobatannins	HCl test	Extract + 1% HCl (boiled)	A red precipitate
Saponins	Foam test	Plant extract + 2mL water (vigorously shaken)	Persistent foam
	NaHCO3 test	Plant extract + sodium bicarbonate solution + distilled water (vigorously shaken)	Stable honeycomb like froth
	Olive oil test	Extract + distilled water; shaken vigorously + few drops of olive oil + shaken vigorously	Appearance of foam
	Haemolysis test	Drop of fresh blood on glass slide + plant extract	Zone of hemolysis
Phytosterols	Salkowski's test	Extract + few drops of conc. H2SO4 (Shaken well and allowed to stand)	Red colour (in lower layer)
	Libermann-Burchard's test	Extract + acetic anhydride + 1-2 drops of conc. H2SO4 (along the side of test tube)	An array of colour change
	Acetic anhydride test	Plant extract + acetic anhydride + conc. H2SO4	Change in colour from violet to blue/green
	Hesse's response	Extract + chloroform + conc. H2SO4	Pink ring / Red colour (in lower chloroform layer)
	Sulphur test	Extract solution + pinch of sulphur powder	Sulphur sinks to the bottom
Cholesterol		Extract + chloroform + acetic anhydride + conc. H2SO4	A red-rose colour
Terpinoides		Chloroform + plant extract + conc. H2SO4 (boiled on water bath)	A grey coloured solution
Triterpinoides	Salkowski's test	Extract+ conc. H2SO4 (Shaken well and allowed to stand)	Golden yellow layer (at the bottom)
Diterpenes	Copper acetate test	Plant extract + copper acetate solution	Emerald green colour
Lignins	Labat test	Extract solution + gallic acid	An olive -green colour
	Furfuraldehyde test	Extract solution + 2% furfuraldehyde solution	A red colour
Carotenoids	Carr-Price reaction	Extract + saturated solution of antimony trichloride in chloroform	A blue-green colour eventually changing to red
Quinones	Alcoholic KOH test	Plant extract + alcoholic potassium hydroxide	Red to blue colour
	Conc. HCl test	Plant extract + conc. HCl	A green colour
	Sulphuric acid test	Extract + isopropyl alcohol + a drop of conc. H2SO4	A red colour
Anthraquinones	Borntrager's test	10% ammonia solution + plant extract (shaken vigorously for 30 sec.)	A pink, violet, or red coloured solution
	Ammonium hydroxide test	Extract + isopropyl alcohol + conc. ammonium hydroxide solution	Formation of red colour after 2 min.
Anthocyanins	HCl test	Extract +2N HCl	Pink-red sol. which turns blue-violet after addition of ammonia
Leuconthocyanins	Isoamyl alcohol test	Extract + isoamyl alcohol	Upper layer appears red
Carboxylic acid	Effervescence test	Extract + sodium bicarbonate solution	Appearance of Effervescence
Coumarins	NaOH paper test	Moistened extract is taken in test tube, mouth of test tube is covered with 1N NaOH treated filter paper, heated for few min. in water bath	Yellow fluorescence from paper under the UV light

	NaOH test	Plant extract + 10% NaOH + Chloroform	A yellow colour
Emodins		Plant extract + NH4OH + benzene	A red colour
Gums and Mucilages	Alcohol test	Extract + distilled water + absolute alcohol (constant stirring)	White or cloudy precipitate
Resins	Acetic anhydride test	Extract + Acetic anhydride solution + conc H2SO4	Orange to yellow
	Turbidity test	Extract dissolved in acetone, poured in distilled water	Turbidity
Fixed Oils and Fat	Spot test/ Stain test	Little quantity of plant extract is pressed in between to filter papers	Oil stain on the paper
	Saponification test	Extract + few drops of 0.5N alcoholic KOH + A drop of phenolphthalein (Heated for 2hr.)	Soap formation or partial neutralization of alkali
Volatile Oils	Fluorescence test	Extract, filtered till saturation, exposed to UV light	Bright pinkish fluorescence

Results

Table 3: Results of the phytochemical analysis

Phytoconstituent	Test	Extract				
		Pet. Ether	Chloroform	Ethyl acetate	Ethanol	Water
Alkaloids	Dragendroff's test	+	+	+	+	+
	Hager's test	+	+	+	+	+
	Mayer's test	+	+	+	+	+
	Wagner's test	+	+	+	+	+
	Picric acid test	+	+	+	+	+
	Iodine Test	+	+	+	+	-
	Bouchardat's test	-	-	-	+	-
	Tannic acid test	-	+	-	+	-
Carbohydrates	Barfoed's test	+	+	+	+	+
	Molish's test	+	+	+	+	+
	Seliwanoff's Test	+	+	-	+	+
	Resorcinol test	+	+	+	+	+
	Test for pentoses	-	-	-	+	-
	Test for starch	-	-	-	+	-
Reducing sugars	Benedict's test	-	+	-	+	+
	Fehling's test	-	+	-	+	+
Glycosides	Borntrager's test	+	+	+	+	+
	Modified Borntrager's test	+	+	+	+	+
	Legal's test	+	+	-	-	-
	10% NaOH test	+	+	+	-	-
	Aqueous NaOH test	-	+	-	+	+
	Con. H2SO4 test	-	+	-	+	-
	Raymond's test	-	-	-	-	-
Cardiac Glycosides	Keller-Killani test	+	+	+	+	+
	Kedee's test	-	+	-	-	+
	Test for Cardenolides	-	-	-	+	-
	Bromine water test	+	+	+	+	+
	Baljet test	-	+	-	-	-
Proteins and Amino acids	Biuret test	+	-	-	-	-
	Millon's test	+	-	-	-	-
	Ninhydrin test	-	-	-	-	-
	Xanthoproteic test	-	-	-	-	-
Flavonoids	Alkaline reagent test	+	-	+	+	+
	Lead acetate test	+	-	+	+	+
	Shinoda's test	+	-	+	+	+
	Shibata's reaction	+	-	-	-	+
	Ferric chloride test	+	-	-	+	+
	Pew's test	-	-	-	+	+
	Zinc-hydrochloride reduction test	-	-	-	-	-
	Ammonia test	+	-	+	+	+
	Conc. H2SO4 test	+	-	-	+	-
Phenolic compounds	Iodine test	-	-	-	+	-
	Ferric chloride test	+	-	-	-	+
	Gelatin test	-	-	+	+	+
	Lead acetate test	-	+	+	+	+
	Ellagic Acid Test	-	-	+	-	+
	Potassium dichromate test	-	+	+	+	+
	Hot water test	-	-	-	+	+
	Test for Cartenoids	-	-	-	+	-
Tannins	Gelatin test	-	-	+	+	+
	Braymer's test	-	-	+	+	+

	10% NaOH test	-	-	+	+	-
	Bromine water test	-	-	+	+	+
	Lead sub acetate test	-	-	-	-	-
	Phenazone test	-	-	+	-	-
	Mitchell's test	-	-	-	+	-
Phlobatannins	HCl test	-	-	-	-	-
Saponins	Foam test	-	-	-	+	+
	NaHCO3 test	-	-	-	+	+
	Olive oil test	-	-	-	+	-
	Haemolysis test	-	-	-	+	+
Phytosterols	Salkowski's test	+	-	+	+	+
	Libermann-Burchard's test	+	-	+	+	+
	Acetic anhydride test	+	-	+	+	+
	Hesse's response	-	-	-	-	-
	Sulphur test	-	+	+	-	+
Cholesterol	Test :1	+	-	+	-	-
Terpenoids	Test :2	+	-	+	+	+
Triterpenoids	Salkowski's test	+	-	+	+	+
Diterpenes	Copper acetate test	-	-	-	+	-
Lignins	Labat test	-	+	-	-	-
	Furfuraldehyde test	-	-	-	-	-
Carotenoids	Carr-Price reaction	-	-	-	+	-
Quinones	Alcoholic KOH test	+	+	+	+	+
	Conc. HCl test	+	+	+	+	+
	Sulphuric acid test	+	+	+	+	+
Anthraquinones	Borntrager's test	+	+	+	+	+
	Ammonium hydroxide test	+	+	+	+	+
Anthocyanins	HCl test	-	-	-	-	-
Leuconthocyanins	Isoamyl alcohol test	-	-	-	-	-
Carboxylic acid	Effervescence test	-	-	-	-	-
Coumarins	NaOH paper test	-	-	-	-	-
	NaOH test	-	-	-	-	-
Emodins	Test:1	-	-	-	-	-
Gums and Mucilages	Alcohol test	-	-	-	-	-
Resins	Acetic anhydride test	-	-	-	+	-
	Turbidity test	-	-	-	-	-
Fixed Oils and Fat	Spot test	-	-	-	-	-
	Saponification test	-	-	-	-	-
Volatile Oils	Fluorescence test	+	-	-	+	-

(+) indicates the compound which is present, (-) indicates the compound which is absent

Discussion

The research indicates that the leaves of *Hygrophila auriculata* harbor a variety of bioactive compounds, which may offer health benefits for humans. These compounds could possess therapeutic properties, particularly in addressing infertility issues. Their presence aligns with the traditional use of the entire plant in treating various health conditions. This underscores the notion that natural plant-based substances often contain elements that contemporary science is beginning to explore and validate. Future research will likely concentrate on isolating and purifying the specific bioactive compounds responsible for the observed medicinal effects. Techniques such as chromatography (e.g., HPLC or TLC) may be employed to separate and identify these compounds' structures.

Conclusion

The phytochemical analysis of *Hygrophila polysperma* has identified a diverse array of bioactive compounds, including alkaloids, flavonoids, phenolic acids, glycosides, and other secondary metabolites. These compounds are believed to contribute to the plant's therapeutic properties, particularly in treating conditions like infertility. This aligns with the traditional use of the whole plant in various medicinal practices. The presence of these bioactive substances supports the notion that natural plant products often contain compounds that modern science is beginning to understand and validate. Future research will likely focus on isolating and purifying these specific bioactive compounds to fully comprehend their molecular structures, potency, and mechanisms of action. Techniques such as chromatography (e.g., HPLC or TLC) may be employed for this purpose. Subsequent in vitro and *in vivo* studies will be essential to evaluate the effectiveness and safety of these compounds in human health applications.

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