Frontiers
2025, Vol. 5, No. 4, pp. 176–182
https://doi.org/10.11648/j.frontiers.20250504.13

Research Article

Morphological and Biochemical Study of Four Species of the Genus *Heliotropium* L. (Boraginaceae) from the State of Punjab, India

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Abstract

Four *Heliotropium* species *viz. H.bacciferum*, *H.currasavicum*, *H.ellipticum* and *H.strigosum* were documented from Malwa region of Punjab, India during the years 2019-2021. Different morphological parameters were examined for identification. For screening of Phytochemicals of four species of the genus *Heliotropium,* two parts of plant (inflorescence and rest of plant) were selected. Extracts of each plant parts were prepared in water, ethanol and hexane and examined twenty one phytochemicals (Alkaloids, Amino acids, Anthocyanin, Betaxanthin, Carbohydrates, Coumarins, Flavonoids, Glycosides, Gums and mucilage, Oxalate, Phenols, Phlobatannins, Proteins, Quinones, Reducing sugars, Resin, Saponins, Starch, Steroids, Tannins and Terpenoids) were studied. Present study will be useful for taxonomists, botanists, ethnobotanists *etc*. in identification of *Heliotropium* species and information about important biochemicals will be useful for pharmaceutical departments.

Keywords

Inflorescence, Flowers, Leaf, Morphology, Punjab, Taxonomy, Weed

1. Introduction

Genus *Heliotropium* (family boraginaceae) represented with about 300 species in the world [3, 15]. Plants of this genera is generally annual to perennial; prostrate to erect; herb to undershrubs; hairy or glabrous; alternate leaves; terminal or axillary, long or medium scorpiod cyme inflorescence; small flowers; five lobed and tubular corolla, style erect, elongated to conical stigma; fruit with nutlets [33].

Identification of any plant species based on morphological parameters is easy and cost effective method [41]. According to [40] morphological features such as stem, leaf, flower, fruit, seed *etc*. plays a vital role in differentiation of species.

Every plant species synthesize some chemicals which are called biochemicals, phytochemicals or botanochemicals. Alkaloids, flavonoids, glycosides, reducing sugar, saponins, steriods, terpenoids, tannins *etc*. act as a potential source of useful drugs [31, 39].

Reports such as [25, 34, 36-38, 42, 43] available about floristics of the state of Punjab, India. But updated information about occurrence of species of genus *Heliotropium* is incomplete. Keeping this in view, present study was carried out for morphological and biochemical study of four species of the genus *Heliotropium*.

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Received: 24 September 2025; **Accepted:** 25 October 2025; **Published:** 11 December 2025

2. Materials and Methods

2.1. Study Area

Punjab state covers an area of 50362 Km^2 and divided into three regions i.e., Majha, Malwa and Doaba. Present study was carried out in major kharif crops of district Faridkot, Fazilka, Ferozpur, Bathinda, Mukatsar and Moga (Malwa region) in the years 2019 and 2021. Out of three zones, Malwa is the largest zone and total land area covered by agriculture. Temperature of this zone is 3°C to 47°C in different seasons and average rainfall ranges from 480 mm to 960 mm.

2.2. Morphological Study

For morphological study, about 10-15 samples has been analyzed. Morphological features such as habit, leaf (arrangement, shape, type, color and edge), stem (pubescent, trailing, erect, hairy, glabrous), flower (color, shape, size), sepals (shape, number, color, hairy, glabrous), petals (shape, number, color, nature), inflorescence, stamens (number and nature), stigma (number, nature), fruit (shape, color, glabrous, hairy), seed (shape, color, nature) *etc*. have been considered for characterization of species. The collected plant specimens were identified on the basis of available literature [5, 8, 9, 20, 26, 35, 45] and by consulting the Herbaria, Department of Botany, Panjab University, Chandigarh and Punjabi University Patiala.

2.3. Biochemical study

For biochemical study, the plant materials were collected from different sites of the study area. The collected material was washed under running tap water and drying at room temperature. The dried plant materials were powdered using an electric grinder. The plant powder was mixed in water, ethanol and hexane for preparation of plant extracts (Table 1).

Table 1. *Methods of preparation of plant extracts in water, ethanol and hexane.*

Aqueous Extracts	Ethanol Extracts	Hexane Extracts
20 gm powdered plant material was added to 100 ml of distilled water in a conical flask. The mouth of flask was covered with cotton plug and kept on orbital rotary shaker for 24 hours at room temperature. After 24 hours, the mixtures were filtered through muslin cloth and then passed through Whatman No.1 filter paper. After proper filtration, the filtrate was stored in vials and kept in a refrigerator at 4°C.	10 gm of the powdered plant material was added to 130 ml of ethanol in round bottom flask and extracted by using Soxhlet apparatus at temperature 60°C. The extraction was done for 24 hours. After proper extraction, the solvents were transferred to beakers and covered with silver paper and made some small holes for evaporation to reach upto $1/3^{rd}$ of the original volume. Then extracts were stored in vials and kept in refrigerator at 4°C for phytochemical analysis.	All the process was done as earlier for preparation of ethanol extract but during extraction by Soxhlet apparatus, temperature was fixed at 35°C.

2.4. Biochemical Analysis

The biochemical such as alkaloids, carbohydrates, flavonoids, glycosides, phenols, proteins, reducing sugar, saponins, steriods, terpenoids, tannins *etc*. were analyzed using standard procedures (Table 2).

Table 2. *Procedures of screening of different biochemicals.*

S.No.	Biochemical	Procedure	Observation	References
1.	Alkaloids (Mayer's test).	3 ml of plant extract + heat + 1 ml of 1% HCl for 20 minutes + cooled + filtered + 2 drops of Mayer's reagent.	White creamy precipitate	[6, 21, 28]
2.	Amino acids	2 ml of plant extract + boiled + 2 ml of Ninhydrin's reagent	Blue coloration	[12, 16, 46]
3.	Anthocyanin	2 ml of plant extract + 2 ml (2N HCl) + 22 drops of NH_3.	Pink red color then turning into blue violet	[16]
4.	Betaxanthin	1 ml plant extract + heat + 0.5 ml of 2N NaOH for 5 minutes.	Yellow coloration	[18]

S.No.	Biochemical	Procedure	Observation	References
5.	Carbohydrates	2 ml of test solution + 2 drops of Molisch's reagent + shaken well + few drops of concentrated Sulphuric acid along the sides of test tube.	Violet ring at the junction of two liquids.	[6, 22, 46, 47]
6.	Coumarins	2 ml of plant extract + 3 ml of 10% NaOH (aqueous).	Yellow coloration	[16]
7.	Flavonoids	a) NaOH test 3 ml plant extract + 1 ml 10% NaOH solution. b) H_2SO_4 test Procedure- 1 ml of test solution + few drops of Conc. H_2SO_4 in	a) Yellow coloration and disappeared by addition of dilute HCl. b) Yellow coloration.	[19, 21, 47]
8.	Glycosides	2.5 ml plant extract + boiled + 5 ml Conc. H_2SO_4 for 15 minutes in water bath + cooled + neutralized with KOH + 3 drops of $FeCl_3$ solution.	Green to black colored precipitates.	[44]
9.	Gums and Mucilage	2.5 ml ethanol in 1 ml test solution + continue stirring.	White to creamy whitish colored precipitates	[44, 48, 49]
10.	Oxalate	3 ml plant extract + few drops of Glacial Acetic Acid.	Greenish black color	[49]
11.	Phenols	2 ml plant extract + few drops of 10% $FeCl_3$ solution	Bluish green color	[4, 48]
12.	Phlobatannins	1 ml test solution + boiled + few drops of 2% HCl for 2-3 minutes.	Reddish colored precipitates	[2]
13.	Proteins (Xantho-proteic test)	1 ml test solution + few drops of Conc. HNO_3.	Yellow precipitates	[49]
14.	Reducing Sugars	1 ml Fehling's solution A + 1 ml Fehling's solution B + mixed + heated + cooled+ 1 ml plant extract + boiled for 10 minutes.	Brownish red colored precipitates.	[13]
15.	Resin	1 pellet of NaOH+ 1 ml test solution + mixed gently.	Reddish coloration	[11]
16.	Quinones	1 ml plant extract + few drops of conc. HCl.	Yellowish color	[50]
17.	Saponins	2 ml plant extract + shacked vigorously in a test tube.	Formation of froth.	[4, 10, 12, 21, 46, 50]
18.	Starch (Iodine test)	1 ml plant extract + few drops of iodine solution.	Bluish-violet coloration was appeared	[29]
19.	Steroids	1 ml plant extract + 6-7 drops of Conc. H_2SO_4.	Reddish coloration.	[23]
20.	Tannins	a) $FeCl_3$ test 1 ml test solution 1-2 + drops of $FeCl_3$ solution. b) KOH test 1 ml plant extract + 1 ml freshly prepared 10% KOH solution.	a) Dark green coloration. b) White precipitates	[4, 13, 44]

S.No.	Biochemical	Procedure	Observation	References
21.	Terpenoids	1 ml plant extract +2 ml chloroform+ mixed + 3 ml Conc. H_2SO_4 along the sides of test tubes.	Reddish-brown coloration was appeared at interface.	[10, 12, 44, 47, 50]

3. Results and Discussion

During present investigation four *Heliotropium* species *viz.H.bacciferum*, *H.currasavicum*, *H.ellipticum* and *H.strigosum*were documentedfromMalwa region of Punjab, India during the years 2019-2021. These species were identified on the basis of various morphological features.

3.1. Morphological Features

H. *bacciferum* Forsk.

It is an annual to perennial, decumbent to erect herb. Stem is hairy, branched, herbaceous to woody, thick, greenish brown in color. Leaves are lanceolate, linear, sessile or small petiole, hairy, thick and green in color. Flowers borne in terminal spike. Flowers arranged in one rank. Flowers bisexual, complete; sepals-5, green, hairy; corolla white with 5 petals; stamens, 5 epipetalous; stigma-1, broad conical with reduced style. Nuts are smooth, trigonous and black.

H. *curassavicum* L.

It is commonly called salt heliotroph because it is commonly found in salt-loamy soil. It is, decumbent to erect, perennial herb. Stem is glabrous, branched, fleshy, hebaceous and green to violet shaded. Leaves are glabrous, linear to lanceolate, fleshy, sessile, whorled and bluishgreen appearance. Inflorescence is terminal, long with many flowers arranged in two ranks. Flowers are complete, bisexual; sepals-5, smooth, bluish-green; petals-5, white on upper side and yellow on lowers side; stamens-5, epipetalous, dithecous, stigma-1, conical with small style. Nutlets, smooth, shining, trigonous and black.

H. *ellipticum* Ledeb.

It erect, rough, greyish-white, woolly herb, branching from a woody base. Leaves are obovate, elliptic- oblong, glabrous. Inflorescence long, terminal in position with many two ranked flowers. Flowers complete, bisexual, actinomorphic; sepals-5, green, hairy; petals-5, white; stamens-5, epipetalous in condition, stigma-1, conical with small style. Nutlets four in each fruit, trigonous, black.

H. *strigosum* Willd.

A prostrate or often procumbent much branched, annual, deep rooted herb. Leaves are small, linear to linear-lanceolate, acute apex and green. Inflorescence small with many one ranked flowers. Flowers are bisexual, complete with equal number of sepals and petals (5). Stamens-5, epipetalous, bithecous. Stigma-1, conical with long style. Fruits globose, depreesed 4- lobed with long sepals. Nuts are trigonous, black and smooth.

3.2. Biochemical Profile

In species of genus *Heliotropium* (inflorescence and rest of plant) two parts of plant were selected for screening of phytochemicals or biochemicals. Extracts of each plant parts were prepared in water, ethanol and hexane and examined twenty one phytochemicals (Alkaloids, Amino acids, Anthocyanin, Betaxanthin, Carbohydrates, Coumarins, Flavonoids, Glycosides, Gums and mucilage, Oxalate, Phenols, Phlobatannins, Proteins, Quinones, Reducing sugars, Resin, Saponins, Starch, Steroids, Tannins and Terpenoids). *Heliotropium* species shown variable concentrations of phytochemicals listed in Table 3.

Phytochemical profile of stem, leaf and root of *Heliotropium bacciferum* were studied [1] from Pakistan. They prepared extract of plant parts in water, ethyl acetate, n- butanoland n- hexane solvents. Alkaloids were reported in all the extracts of three parts of the plant. According to [14] *Heliotropium* genus are rich in Alkaloids (pyrrolizidine). [7] Investigated phytoconstituents of aerial parts of *H. strigosum* from Bahawalpur, Pakistan. They used 70% methanol for preparation of plant extract and examined seven biochemicals (alkaloids, saponins, flavonoids, anthraquinones, tannins, glycosides and coumarins). Out of seven phytochemicals four (alkaloids, saponins, tannins and coumarins) were shown positive result. They suggested these phytochemicals are responsible for antihyperglycemic activity of the plant. [32] examined eight phytochemicals (alkaloids, glycosides, flavonoids, saponins, steroids, tannins, terpenoids and quinones) in 70% methanol extract of whole plant of *H. strigosum* from Pakistan. They suggested *H. strigosum* have antidiarrheal potential due to these phytoconstituents. [27] Studied phytochemical profile of leaves and stem of *H. bacciferum* from Soba region, southwest Khartoum (Sudan). They suggested these phytochemicals are responsible for antimicrobial activity against infectious microorganisms.

Biochemicals such as alkaloids, amino acids, glycosides, reducing sugars, saponins, starch, steroids, tannins and terpenoids were examined by [24] in *Heliotropiumzeylanicum* from Tamil Nadu. Phytochemicals of whole plant of *H. curassavicum* were screened by [17] from Covelong, India. They suggested *H. curassavicum* possess antibacterial potential due to presence of these phytoconstituents. [30] Suggested *H. bacciferum*, *H. curassavicum*, *H. ovalifolium* and *H. su-*

pinum have vital importance in folk medicine and treatment of malaria, abdominal pain, fever, diarrhea, urticarial, menstrual disorders *etc*. due to presence of rich amount of phytochemicals such as Lupeol and beta-sitosterol. Due to incomplete knowledge about biochemical profile of *Heliotropium* species from the state of Punjab therefore present study was planned for examination of biochemicals of whole plant as well as Inflorescence in different solvents.

Table 3. *List of various phytochemicals found in Heliotropium species.*

S.No.	Botanical Name	PP	Ex	Phytochemicals																					
				A	B	C	D	E	F	G_1	G_2	H	I	J	K	L	M	N	O	P	Q	R	S	T_1	T_2	U
1.	*Heliotropium bacciferum*Forssk.	I	Aq	±	±	±	±	+	±	+	±	±	±	+	±	+	±	±	±	+	±	+	±	±	+	+
			Et	+	±	±	+	+	+	+	-	+	-	+	+	-	+	+	-	±	-	±	-	±	+	+
			He	±	-	-	±	±	+	-	-	±	-	±	±	-	-	±	±	-	-	+	-	-	-	±
		ROP	Aq	+	±	+	+	+	+	+	+	+	±	+	+	+	+	±	+	+	+	+	+	+	+	+
			Et	+	±	+	+	+	+	+	-	+	-	+	+	-	+	+	-	-	-	+	+	±	±	+
			He	±	-	-	+	+	+	-	-	-	-	±	+	-	-	-	-	-	-	±	-	±	±	+
2.	*Heliotropiumcurassavicum*L.	In	Aq	±	±	±	+	+	+	+	+	±	±	±	+	+	+	±	-	±	+	+	+	+	+	+
			Et	+	±	±	+	+	+	+	±	±	±	+	+	-	±	±	±	±	-	±	±	±	±	±
			He	-	-	-	+	+	+	+	-	+	-	-	-	±	-	±	-	-	+	+	-	-	+	+
		ROP	Aq	±	±	±	+	+	+	+	+	±	±	+	+	+	+	±	-	±	+	+	+	+	+	+
			Et	+	±	±	+	+	+	+	±	±	±	+	+	±	±	±	±	+	±	±	±	+	+	±
			He	-	-	-	+	+	+	+	-	+	-	±	±	-	-	±	±	-	+	+	+	-	+	+
3.	*Heliotropiumellipticum*Ledeb.	In	Aq	+	±	±	+	+	+	+	+	+	±	±	+	±	+	±	±	±	±	+	+	+	+	+
			Et	±	+	±	+	+	+	+	+	+	-	±	+	±	+	±	-	±	-	±	±	±	±	+
			He	±	-	-	±	+	±	+	+	+	-	-	±	-	-	±	-	-	±	+	+	±	+	+
		ROP	Aq	+	±	±	+	+	+	+	+	+	±	±	+	±	+	±	+	±	±	+	+	+	+	+
			Et	+	+	-	+	+	+	+	+	+	±	+	+	-	+	±	-	-	-	+	-	+	+	+
			He	±	-	-	+	+	+	±	-	-	-	±	+	-	-	-	-	-	-	±	-	±	±	+
4.	*Heliotropiumstrigosum*Willd.	In	Aq	+	±	±	±	+	±	±	±	±	±	±	+	+	+	±	±	±	±	+	±	+	+	+
			Et	±	±	+	±	±	±	±	-	+	±	±	±	-	±	-	-	±	-	+	-	±	-	±
			He	-	-	-	±	+	±	±	-	±	±	+	+	-	-	-	-	-	-	±	-	±	+	+
		ROP	Aq	+	±	±	±	+	+	±	+	+	±	+	+	+	+	±	+	±	±	+	+	+	+	+
			Et	±	±	+	+	+	+	+	-	+	+	+	+	-	±	-	-	+	±	+	-	+	-	±
			He	-	-	-	+	+	+	+	-	+	±	+	+	-	-	±	-	-	-	+	-	+	+	+

Phytochemicals- A= Alkaloids; B=Amino Acids; C= Anthocyanin; D= Betaxanthin; E= Carbohydrates; F= Coumarins; G (G_1& G_2) = Flavonoids; H= Glycosides; I= Gums and mucilage; J= Oxalate; K= Phenols; L= Phlobatannins; M= Proteins; N= Quinones; O= Reducing sugars; P= Resin; Q= Saponins; R= Starch; S= Steroids; T (T_1 & T_2)= Tannins; U= Terpenoids. PP= Plant Part; In= Inflorescence; ROP= Rest of Plant; Ex= Extracts; Aq= Aqueous; Et= Ethanol; He= Hexane; + = Present; - = Absent; ± = Traces.

4. Conclusion

Present study provides an updated information about occurrence of *Heliotropium* species from the state of Punjab, India. Morphological details of each species will be useful for researchers, scientists, botanists etc. for accurate identification. Biochemical profile throws a light on medicinally im-

portant phytochemicals of *Heliotropium* species.

Acknowledgments

Author is thankful to the chairperson department of botany, Panjab University Chandigarh for providing necessary facilities for this research work. Author is also grateful to Prof. (Dr.) Navdeep Shekhar former Professor and head Botany Department, Govt. Brijindra College Faridkot, Prof. (Dr.) M I S Saggoo former Professor and Head Botany Department Punjabi University Patiala and Dr. Yadvinder Singh Assistant Professor department of Botany, Central University of Punjab, Bathinda for encouragement and moral support during this research work.

Author Contributions

Rai Singh Dhillon is the sole author. The author read and approved the final manuscript.

Conflicts of Interest

The author declares no conflicts of interest.

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