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Research

Pharmacognostical, preliminary phytochemical probe and phenetics of *Heliotropium indicum* Linn leaves

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Abstract

Heliotropium indicum Linn belongs to Boraginaceae, is an annual herb commonly known as Indian Heliotrope. *H.indicum* is native to Asia, Tamil Nadu and is widely used in traditional medicine. In Kancheepuram district of Tamilnadu, India, tribal communities used this plant to treat skin infections, poisonous animal bites, Stomach related problems and nervous diseases. Phytochemical report on *H.indicum* revealed the presence of alkaloids, triterpenes, sterols, amines and volatile oils. The plant is reported to possess antibacterial, antitumor, wound healing, anti-inflammatory, anti-nociceptive and diuretic activity. The fresh leaves of *Heliotropium indicum* were authenticated, collected, shade dried, coarsely powdered and extracted with hydroalcohol (70%). The extract was concentrated and stored in air tight container for further use. The present study was aimed to investigate the Pharmacognostical, Physico-chemical parameters, Qualitative and Quantitative analysis of phytoconstituents present in *Heliotropium indicum* leaf along with phenetics are studied.

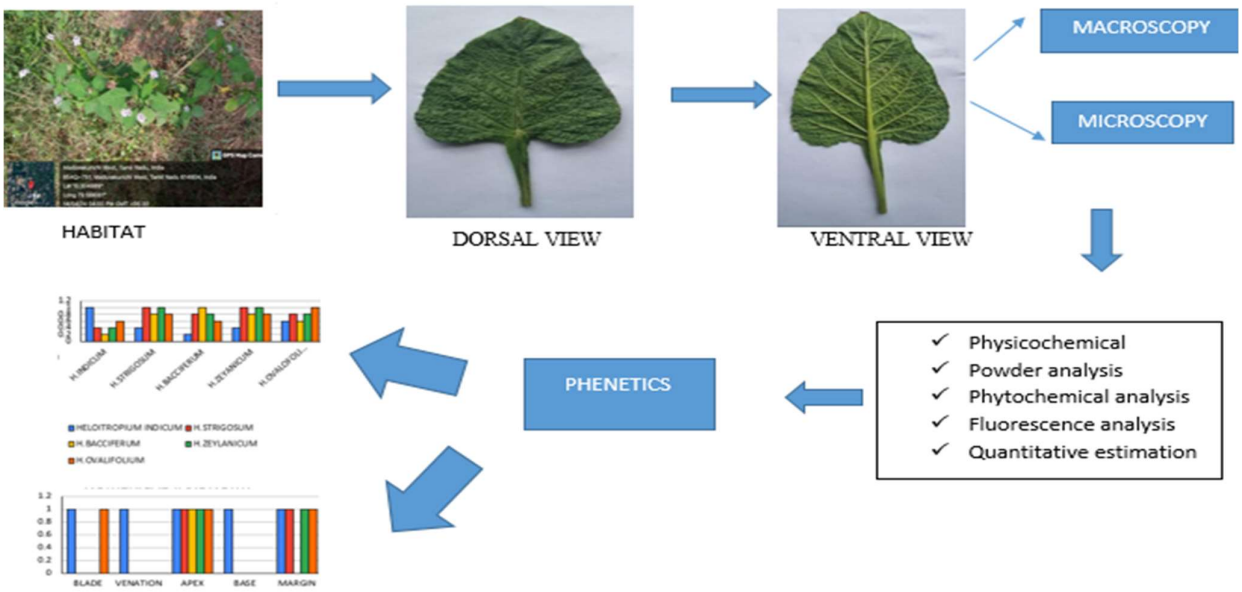
Keywords: *Heliotropium indicum* Linn Boraginaceae, Pharmacognostical, physico- chemical, Qualitative and Quantitative analysis of phytoconstituents, phenetics.

INTRODUCTION

Heliotropium indicum Linn belongs to Boraginaceae, is an annual herb commonly known as Indian Heliotrope[1]. *H.indicum* is native to Asia and India, tribal communities of Kancheepuram district, Tamilnadu, used the plant preparation to treat skin infections, poisonous animal bites, stomach related problems and nervous diseases [2]. Malasar tribal communities of Tamilnadu used leaves sap preparations to treat dandruff [3]. At Cachar district of Assam, India folkore claim reveals that root sap is used to treat eye diseases, fresh leaf paste is applied externally to cure wounds [4]. In Southern parts of India leaf extract is used externally to prevent rheumatoid arthritis [5]. In African countries tribal people use *H.indicum* to treat inflammation and tumors [6]. In Jamaica traditional system of medicine, infusion of the whole plant is used to treat fever stomach infections, cold and cough [7]. In West Indies traditional system of medicine decoction of the flower and buds is taken orally by the women as an emmenagogue and as abortifacient [8].The phytochemical review showed the presence of pyrrolizidine alkaloids indicine, indicine-N-oxide, acetyl indicine, heliotrine N-oxide, spermidine,

spermine, putrescine, β-amyrin, lupeol, β-sitosterol, stigmasterol, phytol,1-dodecanol and β-linalool[9]. Pharmacological survey revealed antimicrobial, anti-inflammatory, antitussive, anti-ulcer, anti-fertility, antihyperglycemic, antipyretic, histo-gastroprotective, antitumor, antitubercular, to treat cataract, analgesic and wound healing [10-20]. It is essential to explore the pharmacognostical parameters of the leaf. Consequently the present investigation include macroscopical, microscopical evaluation, determination of physico-chemical constants including inorganic elements, qualitative and quantitative analysis of phytoconstituents present in leaves of *Heliotropium indicum* and phenetics are also studied.

Graphical abstract



MATERIALS AND METHODS

Collection of leaf and authentication

Plant collected from the domestic garden Mavadukurichi village,Thanjavur district,Tamil Nadu in the month of April 2024. It was identified and authenticated by DR.Stephen, Professor, Department of Botany The American College,Madurai-625002.The herbarium of this specimen was kept in the department for further reference.

Pharmacognostical evaluation

Morphology

Morphological characters such as size, shape, apex, margin, venation, base, petiole, surface and colour of leaves of *Heliotropium indicum* were studied [21&22].

Microscopical study

Thin free hand transverse sections of the leaf (petiole and midrib)of *Heliotropium indicum* were taken. Sections were stained with phloroglucinol along with conc. hydrochloric acid and mounted with dil.hcl and was observed under microscope[23].

Quantitative Microscopy

Square Pieces of leaf was taken between midrib and margin was decolourised and viewed under a microscope for its vein islet number, vein termination and palisade ratio of the leaf. In case of stomatal index the upper and lower epidermis ,the leaf piece were peeled out separately and kept on the slide to mount in the glycerin water and were observed under a microscope for the determination of stomatal index and type[24].

Powder microscopy

The coarse powder was treated with routine reagent to identify the diagnostic features of the plant [25].

Fluorescence analysis

A small quantity of leaf powdered was transfered to test tube and 1-2 drops of freshly prepared various solution was added and the colour was observed under visible, UV 254 nm & 365nm as per procedure [26].

Physico-chemical parameter

The powder was subjected to physiochemical parameters such as loss on drying, ash value, and extractive value with different solvents in increasing order of polarity as per standard procedures recommended by Ayurvedic pharmacopoeia of India [27]. The ash was subjected to inorganic elements identification as per Atherden [28].

Preparation of hydroalcoholic extract of *Heliotropium indicum* (HAEHI)

The collected leaves were washed with water, shade dried and powdered, extracted with hydroalcohol (70%) by maceration technique until the complete extraction of material and filtered. The extract was concentrated under reduced pressure to obtain a residue.

Preliminary phytochemical screening

Preliminary phytochemical screening were carried out by using different reagents for identification the presence of phytoconstituents as per standard procedures [29].

Quantitative estimation of phytoconstituents

- Determination of Gallic acid equivalent in HAEHI.
- Determination of Quercetin equivalent in HAEHI.
- Determination of Tannic acid equivalent in HAEHI.

Determination of Gallic acid equivalent in HAEHI

A series of calibrated 10ml volumetric flask is taken and standard solution (gallic acid) and HAEHI solution of various concentrations (5µg/ml, 10 µg/ml, 15 µg/ml and 20 µg/ml) is taken. To each of this solution add 5ml of distilled water and 0.5ml of Folin's Ciocalteu's reagent is added, mixed and shaken. After 5 minutes, 1ml of 10% sodium carbonate solution is added and the volume is made up to 10 ml with distilled water. It is allowed to incubate for 2 hours at room temperature. Intense blue colour is developed. The reaction mixture without sample is used as blank. After incubation, absorbance is measured at 725nm using UV spectrophotometer and the mean values will be recorded.The calibration curve will be plotted using standard gallic acid. Total phenolic content of HAEHIextract is expressed in terms of mg of Gallic acid equivalent per gm of extract (mg GAE/g) [30].

Determination of Quercetin equivalent in HAEHI

Total flavonoid content was measured with the aluminium chloride colorimetric assay. A series of calibrated 10ml volumetric flask were taken and standard solution (Quercetin) and HAEHI solution of various concentrations (10µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml) were taken. To each of these solution add 4ml of water and 0.3ml of 5% sodium nitrite solution is added. After 5 minutes, 0.3ml of 10% aluminium chloride is added. At 6th minute, 2ml of 1M sodium hydroxide is added. Finally, volume is make up to 10ml with distilled water and mix well. Orange yellowish colour is developed. The absorbance is measured at 510 nm spectrophotometer using UV-visible spectrophotometer and the mean values will be noted. The blank is performed using distilled water. The calibration curve is plotted using standard Quercetin. The total flavonoid content in the extract is expressed as milligrams of Quercetin equivalent per gram of extract [30].

Determination of Tannic acid equivalent in HAEHI

Prepare various concentration of HAEHI into test tubes. To this, 0.5ml of Folin-Denis reagent and 0.8mL of distilled water was added. The tubes were kept aside for 15min. To this, 1mL of sodium carbonate solution was added and the remaining volume was made up with 7.5mL of distilled water. Then the tubes were shaken and the absorbance was recorded at 700nm after 30min. Tannicacid, used as a standard was taken at different concentration 5,10,15,20mcg/ml in different test tubes and the procedure adopted above was followed. The calibration curve for tannic acid was plotted using concentration versus absorbance. A linear regression equation was calculated and the equation was used to calculate the amount of total tannins as tannic acid equivalent. The amount of tannin content is expressed in mg/g of extract [31].

Phenetics

Numerical taxonomy of plant derived by using five characters such as leaf blade, venation, apex base and margin [32].

Morphological study

Leaves of *H.indicum* showed green colour in ventral view,dark green in dorsal view with characteristic odour and bitter taste,ovate –deltoid shape, length 5-10cm long, width 4.4-5 cm, petiole 5-9 cm, sub –opposite decussate arrangement ,acute apex with serrulate venation, sparsely hairy.(Tab 1&fig 1)

Table1: Morphological characters of leaves of *H.indicum*

S. NO	Parameters	Observation
1	Colour/surface Dorsal Ventral	Dark green Green
2	Odour	Characteristic odour
3	Taste	Bitter
4	Leaf Type	Simple
5	Shape	Ovate- deltoid
6	Arrangement	Sub –opposite decussate
7	Apex	Acute
8	Base	Flattened
9	Margin	Undulate
10	Venation	Serrulate
11	Texture	Slightly coriaceous
12	Length	5 - 10 cm
13	Width	4.4-5 cm
14	Petiole length	5 - 9 cm long

Fig 1: Dorsal view of leaf

Fig 1.1: Ventral view of leaf

Fig 1.2: Habitat

Microscopical study

T.S. of Petiole

T.S. of petiole shows an epidermis consisting of thick walled rectangular cells interrupted at places by unicellular warty trichomes &glandular trichomes with unicellular head and parenchyma cells are present (Fig 2).

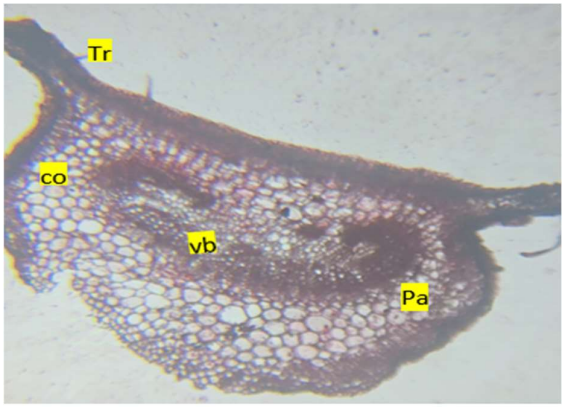


Fig 2: T.S. of Petiole (10X)

Tr – Trichome; Pa – Parenchyma;Co – Collenchyma; vb-vascular bundle

T.S. of leaf

Epidermis: Leaf dorsiventral, upper epidermis cuticularized, barrel shaped cells with anomocytic stomata. It consists of single layer upper and lower epidermis. Upper epidermal cells are thick walled, irregular in shape with non –undulated outline, whereas lower epidermal cells have undulated outline.

Stomata: Stomata are present on both surfaces with anomocytic type of stomata.

Trichomes: Glandular and non- glandular types of trichomes are present. surfaces are calcified, in some cases silicified. Trichomes are short unicellular with a more or less bulbous stalk and an acute end, thickening like that of long unicellular type.Prominent markings present on the surface.

Collenchyma: Lacunar collenchyma cells are round to hexagonal, more on the abaxial and lateral cells.

Parenchyma: Cortical parenchyma cells are larger somewhat isodiametric to hexagonal. Spongy parenchyma cells are roundish, chlorophyllous, loosely arranged.

Vascular bundle: Cells with brownish content are present around the vascular bundle and occasionally in the mesophyll cells.Single layered starch sheath consisting of irregular shaped cells occurs at the innermost cortical layer, only at the abaxial side of the vascular bundle.

Phloem: The phloem consists of sieve tube, companion cells and some narrower cells with a brownish content. Phloem ray cells are isodiametric, somewhat roundish in appearance.

Xylem: The xylem arch consists of radial rows of trachery elements. cells immediately inner to the xylem arch are smaller in comparision to the cortical cells and resembles the pith. An isolated chlorophyllous zone occurs just beneath the epidermis. Besides, some isolated chloroplast grains are scattered in the cortex.

Mesophyll: Ground tissue forming the mesophyll is differentiated into palisade and spongy parenchyma cells.

Palisade cell: Palisade cells are columnar, chlorophyllous, arranged in a single row with scanty intercellular spaces.(Fig 3)

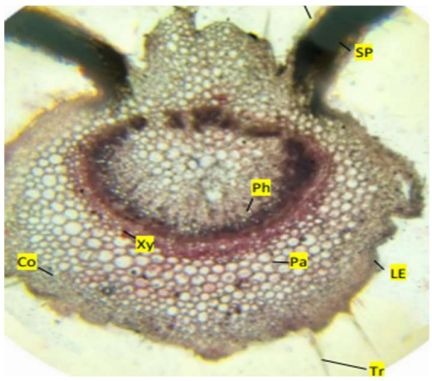


Fig 3: T.S. of Midrib (10x)

Co – Collenchyma; LE-Lower epidermis; SP – Spongy parenchyma; Ph-Phloem; UE – Upper epidermis; Xy – Xylem; Tr – Trichome; Pa – Parenchyma

Histochemical studies

The section of the leaves of *Heliotropium indicum* Linn., were stained by using specific reagents such as N/50 Iodine, sulphuric acid, Phloroglucinol and Conc. Hydrochloric acid, KoH .to observe and locate lignin,cellulose, suberin, calcium oxalate respectively as per the protocol.(Fig 4.1-4.4, Table 2)

Table 2: Histochemical studies of *Heliotropium indicum* leaf

S. no	Reagents	Test for	Nature of change	Histology
1	Phloroglucinol + HCL	Lignin	Pink	Vessels
2	Iodine solution followed by Sulphuric acid	Cellulose	Yellow	Parenchyma Cells
3	Heating with KOH	Suberin	Yellow	Trichomes Cortex Stomata
4	Hcl	Calcium Oxalate	Dark yellow	Parenchyma cells

Fig 4.1: Stomata

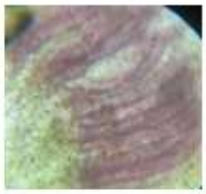


Fig 4.2: vessels

Fig 4.3: Parenchyma cells

Fig 4.4: Trichomes

Quantitative microscopy of *Heliotropium indicum*

Stomata

The stomata present in both surface it composed of ranunculaceous type. The stomata surrounded by varying number of subsidiary cells.

Veinislet number and Veinlet termination number

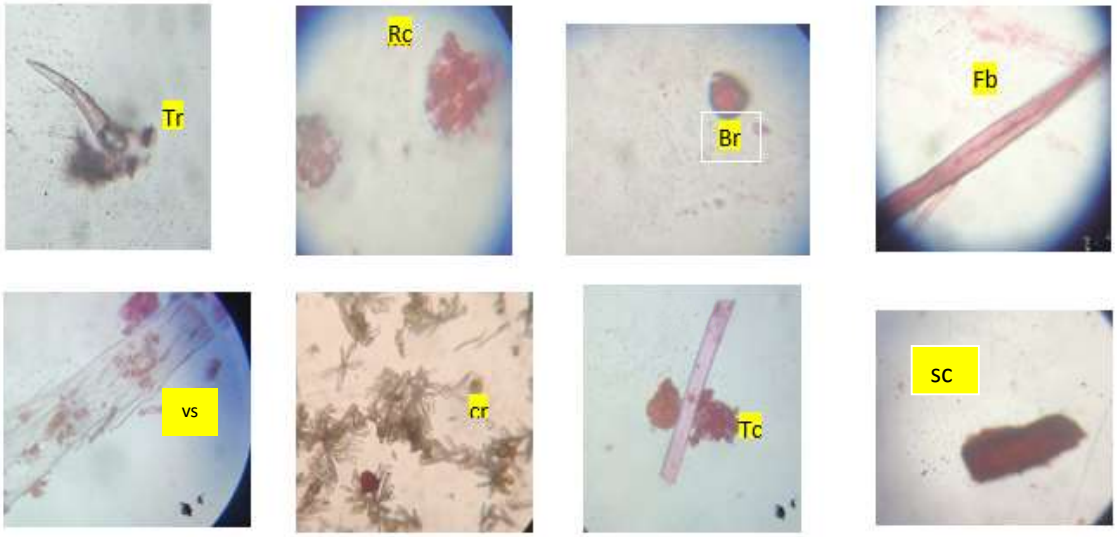
Leaf surface showed the presence of veins and veinislets and vein termination. Each vein end consists of two spirally coiled tracheids attached side by side.(Table 3)

Table 3: Quantitative microscopy of *Heliotropium indicum*

S.no	Parameters	Cells /mm^2
1	Epidermal number	14.25
2	Vein islets number	23.25
3	Vein termination	43.25
4	Stomatal number	
	Upper epidermis	15
	Lower epidermis	11
5	Stomatal Index	
	Upper epidermis	12
	Lower epidermis	10
6	Palisade ratio	4-6

Powder microscopy

The shade dried, powdered plant material was used for powder microscopic analysis. The organoleptic characters were observed and to identify the different characteristic features various staining reagents are used. It showed epidermal cells, stomata of normocytic type, parenchyma cells, spirally thickened vessel members and tracheids, long narrow pitted fibres with pointed ends, glandular as well as non – glandular trichomes.(fig 5)



Tr- Trichomes; Rc- Rosette crystals; Br- Brown content ; Fb- Fibre; Vs- Vessels ; Cr- Crystals
Tc- Tracheids; Sc- sclerenchyma cells

Fig 5: Powder microscopy of *Heliotropium indicum* Linn leaves

Fluorescence analysis

Powdered drug of plant gave different fluorescence under visible and ultraviolet (UV)radiation (254nm& 365nm) when treated with various reagents.The colour observed in different radiations were recorded.It is used for the identification of plant and powdered drug.(Tab 4)

Table 4: Fluorescence analysis of *Heliotropium indicum* Linn leaves

REAGENT	OBSERVATION		
	Visible (<400 nm)	UV (254nm)	UV (365 nm)
Powder + HCl	Green	Black	Green
Powder + HCl+ H_2O	Green	Black	Green
Powder HNO_3	Brown	Black	Green
Powder + HNO_3+H_2O	Orange	Brown	Green
Powder+ H_2SO_4	Dark green	Black	Green
Powder+ H_2SO+ H_2O	Brown	Black	Green
Powder + Acetic acid	Green	Black	Green
Powder+NaOH	Green	Black	Green
Powder+AlcoholicNaOH	Dark Yellow	Black	Green
Powder+Picricacid	Orange	Brown	Green
Powder+ Fe3Cl4	Red	Black	Green
Powder+ Iodine	Brown	Black	Green
Powder+Ammonia	Brown	Black	Green

Determination of physicochemical parameters & inorganic element analysis

The physicochemical parameters of the plant drug were estimated using standard procedures which showed the loss on drug and total solid content was found to be 6.276±0.811%w/w &, total ash of 15.085±0.0583%w/w,water soluble ash0.587±0.00120%w/w,acid insoluble ash3.833±0.8572%w/w .The percentage extractive value of Petroleum ether, Ethyl acetate, Ethanol, Aqueous was found to be 23.3±0.33%w/w, 29.4±0.077%w/w, 32.1±0.065%w/w, 20.4±0.100%w/w respectively.To the ash of the leaves was treated with 50%v/v hydrochloric acid and kept for 1 hr .It was filtered ,filtrate was used for inorganic analysis.(Tab 5)

Table 5: Physiochemical parameters of *Heliotropium indicum* Linn

S.no	Physiochemical parameters	Results
1	Foreign matter	Nil
2	Loss on drying	6.276±0.811%w/w
3	Total solid	93.274%w/w
4	Petroleum ether extractive	23.3±0.33%w/w
5	Ethylacetate extractive	29.4±0.077%w/w
6	Ethanol extractive	32.1±0.065%w/w
7	Aqueous extractive	20.4±0.100%w/w
8	Total ash	15.085±0.0583%w/w
9	Water soluble ash	0.587±0.00120%w/w
10	Acid insoluble ash	3.833±0.8572%w/w
11	Presence of inorganic elements	Chloride,Sulphate
12	Heavy metals	Absence

Qualitative phytochemical analysis

The extract were subjected to preliminary phytochemical screening to determine the presence of various phytoconstituents. Its showed the presence of alkaloid,carbohydrates, glycosides, phytosterol, phenol, tannins, saponins , flavonoid and proteins.(Tab 6)

Table 6: Qualitative phytochemical analysis of *Heliotropium indicum* Linn

S.no	Phytochemical analysis	Observation
1	Test for Alkaloids-Pyrrolizidine	+
2	Test for Carbohydrates	+
3	Test for Glycosides	+
4	Test for Phytosterols	+
5	Test for Phenolic compound	+
6	Test for Tannins	+
7	Test for Saponins	+
8	Test for Flavonoids	+
9	Test for Proteins	+
10	Test for Coumarin	+
11	Test for Quinone	+
12	Test for Gums & mucilage	+
13	Test for Anthocyanin	-

+ presence, - absence

Quantitative estimation of phytoconstituents

Quantitative analysis such as total tannin content, total phenolic content and total flavonoid content were estimated for the HAEHI

Determination of Gallic acid equivalent in HAEHI

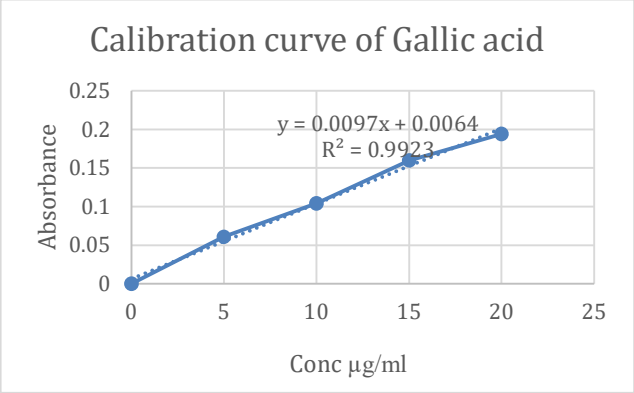


Fig 6: calibration curve of Gallic acid

Table 7: Determination of Gallic acid equivalent in HAEHI

S.no	Concentration	Absorbance	
	Gallic acid & HAEHI (µg/ml)	Gallic acid Mean± SEM	HAEHI Mean± SEM
1	0	0	0
2	5	0.061333±0.000882	0.040667±0.000333
3	10	0.104±0.001	0.093±0.003
4	15	0.16±0.002887	0.143667±0.000333
5	20	0.193667±0.001202	0.178333±0.000333
		GAE	**88.95mg/g**

Quantitative estimation of Phenol was done by Folin- cio calteu method using Gallic acid as standard. The total phenolic content in HAEHI was found to be **89 GAE/g** (Fig 6 &Tab 7).

Determination of Quercetin equivalent in HAEHI

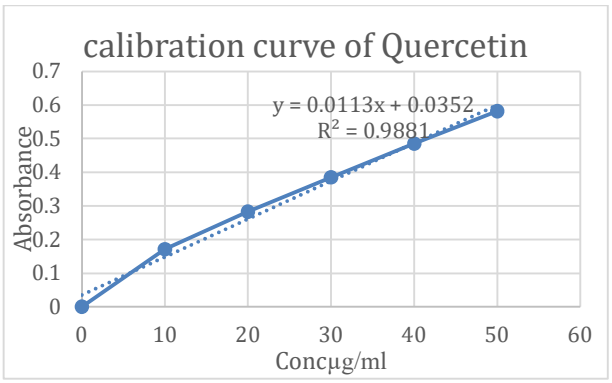


Fig 7: calibration curve of Quercetin

Table 8: Determination of Quercetin equivalent in HAEHI

S.no	Concentration	Absorbance	
	Quercetin & HAEHI (µg/ml)	Quercetin Mean± SEM	HAEHI Mean± SEM
sss1	0	0	0
2	10	0.171333± 0.000882	0.156±0.000577
3	20	0.282667±0.000333	0.207667±0.000333
4	30	0.384667±0.000333	0.302333±0.000333
5	40	0.484667±0.000667	0.45±0.005774
6	50	0.581667±0.000333	0.495±0.000577
		QE	**81.38mg/g**

Quantitative estimation of Flavonoid was done by Aluminium chloride colorimetric assay using Quercetin as standard. The total Flavonoid content in HAEHI was found to be **81.38 QE/g** (Fig7&Tab 8)

Determination of tannic acid equivalent in HAEHI

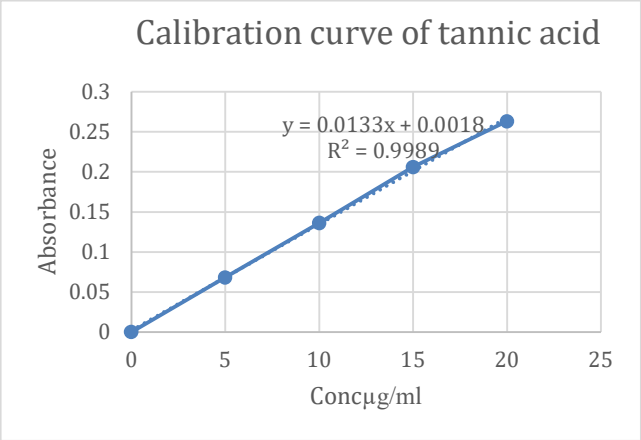


Fig 8: Calibration curve of Tannic acid

Table 9: Determination of Tannic acid equivalent in HAEHI

S.no	Concentration	Absorbance	
	Tannic acid & HAEHI (µg/ml)	Tannic acid Mean± SEM	HAEHI Mean±SEM
1	0	0	0
2	5	0.068 ± 0.001155	0.055333±0.000333
3	10	0.135667±0.000882	0.105667±0.000333
4	15	0.205667±0.00033	0.186±0.000577
5	20	0.262667±0.00033	0.219667±0.000333
		TAE	**81.65mg/g**

Quantitative estimation of Tannic acid was done by Folin denis assay by using tannic acid as a standard. The total tannic acid content in HAEHI was found to be 82 TAE/g (Fig 8 & Tab 9)

Phenetics

There are around 250 accepted species of *Heliotropium indicum* around the world.Among those five species are available in south India.(Table 10&11.Fig 9&10)

Heloitropium indicum
H.strigosum
H.bacciferum
H.zeylanicum
H.ovalifolium

The characters such as leaf blade, leaf venation, apex, base, margin
Leaf blade-Present -1,Absent-0
Leaf venation- Present -1,Absent-0
Leaf apex- Present -1,Absent-0
Leaf base- Present -1,Absent-0
Leaf margin- Present -1,Absent-0

Table 10: Table of similar *Heliotropium indicum* characters with other four species of *Heliotropium*

SPECIES	BLADE	VENATION	APEX	BASE	MARGIN
Heloitropium indicum	1	1	1	1	1
H.strigosum	0	0	1	0	1
H.bacciferum	0	0	1	0	0
H.zeylanicum	0	0	1	0	1
H.ovalifolium	1	0	1	0	1

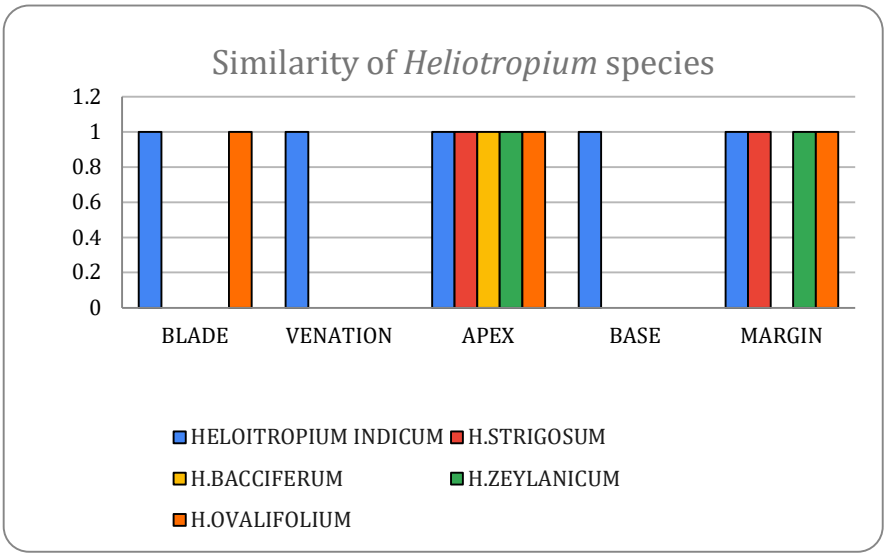


Fig 9: Chart of similarity characters of *Heliotropium indicum* Linn Leaves

Table 11: Table of dissimilar *Heliotropium indicum* characters with other four species of *Heliotropium*

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SPECIES	*Heloitropium indicum*	*H.strigosum*	*H.bacciferum*	*H.zeylanicum*	*H.ovalifolium*
Heloitropium indicum	1	0.4	0.2	0.4	0.6
H.strigosum	0.4	1	0.8	1	0.8
H.bacciferum	0.2	0.8	1	0.8	0.6
H.zeylanicum	0.4	1	0.8	1	0.8
H.ovalifolium	0.6	0.8	0.6	0.8	1

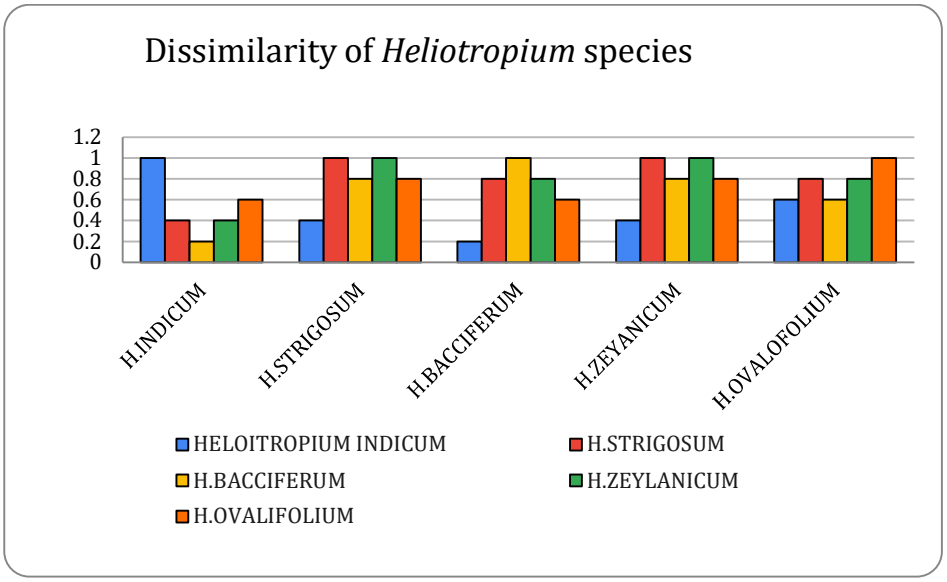

Fig 10: chart of Disimilarity characters of *Heliotropium indicum* Linn Leaves

Sample matching coefficient
SSM= NS/NS+ND*100
Where,
NS=Number of similarity characters-7
ND=Number of dissimilarity characters-18
N=Number of samples
SSM of *HI*=28%
The matching coefficient of *Heliotropium indicum* with respect to other species was found to be 28%.

CONCLUSION

Pharmacognostical and physiochemical evaluation provided specific parameters that will helpful in proper identification and standardisation of the observations can be considered for further researcher .Phenetical study imparts taxonomical enlightenment of this *Heliotropium indicum* can be made to differentiate from other species of *Heliotropium.*

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