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Research

Pharmacognostical, preliminary phytochemical probe and Phenetics of *Hibiscus vitifolius* Linn Leaves.

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Abstract

Hibiscus vitifolius also known as tropical rose mallow and grape leaved mallow, belongs to Malvaceae. It is found in India and in United States of America. In India, *Hibiscus vitifolius* is traditionally used for the treatment of jaundice, diabetics, inflammation, urease activity. The literature look over revealed the presence of alkaloids, flavones, gossypin, carotenoids, atropine, promethazine, aldose, galactose, bioflavonoids, morphine and pentahydroxy glycosyl flavones. The plant exhibited antibacterial, antimicrobial, antioxidant, antihepatotoxic, anticancer, diuretic, anti-inflammatory and antiepilepsy effect. The fresh leaves of *Hibiscus vitifolius* Linn were authenticated, collected, shade dried and coarsely powdered, was extracted with hydroalcohol (70%). The extract was concentrated and stored in air tight container for further use. The aim of the present research study was to carry with pharmacognostical, physico chemical parameters and qualitative and quantitative analysis of phytoconstituents present in *Hibiscus vitifolius* leaf, along with phenetics are also studied.

Keywords: *Hibiscus vitifolius* (L), Malvaceae, Pharmacognostical, Physico chemical, qualitative and quantitative analysis of phytoconstituents, phenetics.

INTRODUCTION

Hibiscus vitifolius Linn is medicinal herb, belongs to Malvaceae, also known as tropical rose mallow and grape leaved mallow [1]. It is a common found in India and United state of America, widely distributed in tropical Asia, Africa, Australia and it is also naturalized in West Indian Islands, Cuba, Hispaniola, Jamaica and Central America [2]. In India traditional system of *Hibiscus vitifolius* is used for the treatment of jaundice, diabetics, inflammation, urease activity [3]. Ethnomedicinal claim of Chittur Taluk in Palakkad District, Kerala, leaf powder

is used to treat inflammation. In Tiruppur district, and Tamilnadu, leaf juice is used to treat cuts and wounds, Sivagangai district and Tirunelveli district, Tamilnadu, used leaf preparations to control diarrhoea [4-7]. The phytochemical survey revealed the presence of alkaloids, flavones, gossypin, carotenoids, atropine, promethazine, aldose, galactose, bioflavonoids, morphine, and pentahydroxy glycosyl flavones [8]. The pharmacological survey showed various activities such as antibacterial, antioxidant, anti-inflammatory, hepatoprotective activity, antiepilepsy, anti-cancer activity and anti-microbial [9-15]. It is immediate and essential to explore pharmacognostical parameters of this medicinal plant. Consequently the present investigation include macroscopical, microscopical evaluation, determination of physico-chemical constants including inorganic elements, qualitative and quantitative analysis of phytoconstituents and phenetics are determined.

MATERIALS AND METHODS

Authentication and Collection

The leaf was identified and authenticated by a botanist DR.Stephen, Professor, Department of Botany, The American College, Madurai-625002. The herbarium of this specimen was kept in the department for further reference. Leaves were collected from the cultivable fields of Rayagiri village, Sivagiri Taluk, Tenkasi Dist, Tamil Nadu in the month of Mar 2024.

Pharmacognostical evaluation

Fresh leaves were subjected to pharmacognostical studies. Organoleptic, macroscopy and microscopy of the leaves of *Hibiscus vitifolius* (L) were studied.

Organoleptic evaluation

Fresh leaves are collected and checked for their colour, odour and taste by sensory characters.

Macroscopical evaluation

It includes length, width, base, apex, arrangement and venation of the leaf was identified. (figure 1&2 and table 1).

Microscopy evaluation

Free handmade sections were taken, stained with routine staining reagents and were observed under microscope as per Wallis[16] . Sections were first observed in distilled water, then stained with safranin examined to assess different cellular structures and contents. The samples were observed under a compound microscope. (figure 3&4).

Histochemical studies

The section of the leaves of *Hibiscus vitifolius* Linn.,were stained by using specific reagents such as N/50 iodine, phloroglucinol and con.hydrochloric acid, picric acid and KOH to observe and locate lignin, starch, alkaloids, tannins respectively as per the protocols presented in (fig 5.1 to 5.4 and table 2).

Determination of leaf constants

Fresh leaves were peeled to observe the stomata, epidermal number, stomatal number and stomatal index were determined as per WHO guidelines reported in table 3.[17]

Preparation of powder

Leaves were collected, washed, shade dried, coarsely powdered and was passed through sieve no 40.

Powder microscopy

A small amount of the powdered sample was mounted on a microscopic slide with routine reagents as per [18]. Photomicrographs of diagnostic characters were detected displayed and documented in fig6.

Fluorescence analysis

A small quantity of leaf powder was transferred to test tube and 1-2 drops of freshly prepared various solution was added and the colour was observed under visible, UV-254 and 365 nm is presented in table 4[19].

Determination of Physico-chemical parameters

The leaf powder was analysed by various physio-chemical parameters such as foreign matters, loss on drying, total solids, ash values and extractive values using various solvents were determined by Ayurvedic Pharmacopoeia and is given in table 5 [20]. The ash was subjected to inorganic elements identification as per Atherden .[21]

Preliminary phytochemical screening

Preliminary phytochemical screening were carried out by using different reagents through standard procedure for identification the presence of phytoconstituents as per standard procedures [22] and is tabulated in table 6.

Quantitative estimation of phytoconstituents

- ✓ Determination of Gallic acid equivalent in HAEHV.
- ✓ Determination of Tannic acid equivalent in HAEHV
- ✓ Determination of Quercetin equivalent in HAEHV.

Determination of gallic acid equivalent in HAEHV

A series of calibrated 10ml volumetric flask is taken and standard solution (gallic acid) and HAEHV 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 HAEHV extract is expressed in terms of mg of Gallic acid equivalent per gm of extract (mg GAE/g) and is presented in figure 7 &table7.

Determination of Tannic acid equivalent in HAEHV

Prepare various concentration of HAEH 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. Tannic acid, used as a standard was taken at different concentration 5,10,15,20 mcg/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 and is presented in figure 8 &table8.

Determination of Quercetin equivalent in HAEHV

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 HAEHV 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 [23&24] and is presented in figure 9 &table9.

Phenetics

Numerical taxonomy is derived by using 5 characters such as leaf stipulses, margin, texture, venation, shape. [25] and is presented in figure 10&11, table10&11.

RESULTS AND DISCUSSIONS

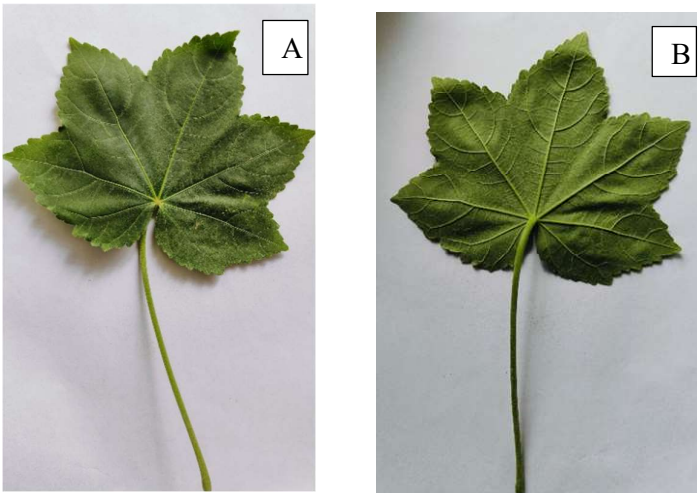
Leaves of *Hibiscus vitifolius* showed dark green colour in dorsal view and light green colour in ventral view, characterstic odour, Mucilage taste, simple type, 2 to 10 cm Length and 2 to 8.5 cm width, palmate-lobed shape, actinodromous venation, subcordate rounded leaf base, acute apex, alternative arrangement and densly pubescent surface.

Table 1: Macroscopic studies of *Hibiscus vitifolius*

S.No	Character	Observation
1	Colour	Dorsal-green Ventral-light green
2	Odour	Characteristic odour
3	Taste	Mucilage taste
4	Type	Simple
5	Length	2 to10cm
6	Width	2 to 8.5 cm
7	Shape	Palmate-lobed shape
8	Venation	Actinodromous- Three or more primary veins diverge radially from a single point and the secondary veins are generally lacking in the admedial sides of the first two primaries
9	Base	Subcordate rounded

10	Apex	Acute but not lobed shallowly
11	Arrangement	Alternate
12	Surface	Densly pubescent on both surface

Fig 1: Habitat of *Hibiscus vitifolius*



A-Upper surface **B-Lower surface**

Fig 2: Dorsal and ventral view entire leaves of *Hibiscus vitifolius*

MICROSCOPY

Transverse section of *Hibiscus vitifolius* showed following characters

The leaf shows both upper and lower epidermis. The epidermis consists of polygonal tubular cells, lignified glandular, stellate trichomes and unlignified covering trichomes are present. Starch grains are present in the mesophyll region.

Epidermis

The upper and lower surface have a single layer of epidermal cells which may contain stomata. Thin cuticle is present.

Palisade paranchyma

Located just below the upper epidermis, rectangular shape consists of tightly packed chlorenchyma cells.

Spongy paranchyma

Below the palisade layer, is loosely arranged cells with intercellular spaces.

Vascular bundle

The bundle are arranged in a scattered pattern within the mesophyll, consisting of xylem (typically on the upper side) and phloem (on the lower side) sphaero-crystals are also presented in vascular bundle.

xylem vessels are larger and often appear as hollow spaces,

Phloem

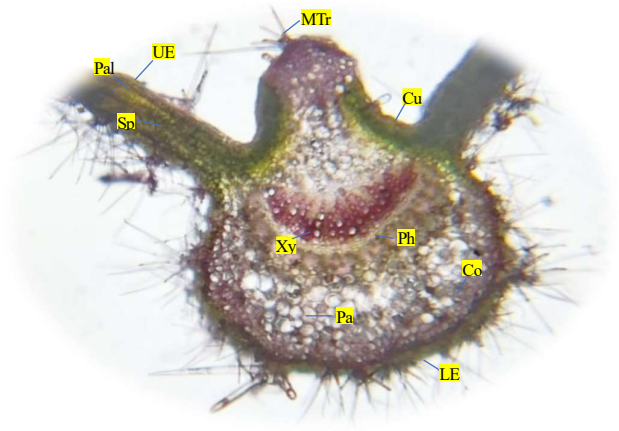
It is composed of sieve tubes and companion cells lignified walls, often with perforation plates at the ends.

Collenchyma

cells are elongated and polygonal in cross section. Collenchyma cells are arranged in strands or layers. The cell walls are unevenly thickened, primarily at the corner.

Multicellular trichomes

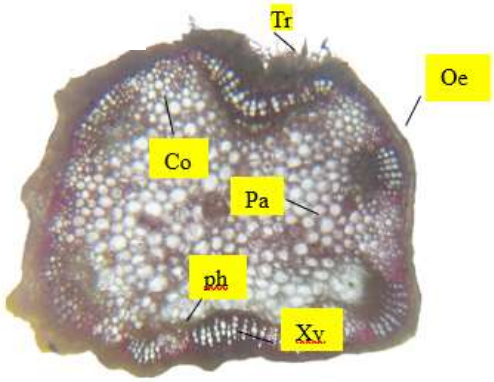
Multicellurar trichomes are primarily found on the surface of the leaf epidermis. They can be filamentous or hair -like structure. It is also known as stellate trichome.



Co– Collenchyma; LE – Lower epidermis; Pal – Palisade parenchyma; Ph – Phloem; UE – Upper epidermis; Xy – Xylem; M.Tr – Multicellular Trichome; Sp- Spongy Parenchyma Cu- cuticle

Fig 3: T.S. *Hibiscus vitifolius* (5x)

Transverse section of *Hibiscus vitifolius* petiole



Tr- Trichome, Co-collenchyma, Pa-paranchyma, Ph-phloem, Xy-xylem,Oe-outer epidermis

Fig 4: T.S *Hibiscus vitifolius* (petiole)

Vascular bundle

The petiole contains vascular bundles arranged in a ring or scattered throughout the section. These bundles include xylem and phloem. The xylem tends to be larger and more prominent than the phloem.

Collenchyma

Beneath the epidermis, you might find layers of collenchyma cells, which provide flexible support. These cells are typically elongated and may be arranged in strands or cylinders.

Pith

In the center of the petiole, there is usually a pith region made up of parenchyma cells.

Trichome

Trichomes can vary in size and shape, generally appearing as small, hair-like projections. Glandular trichomes may have a bulbous tip.

Histochemical analysis of *Hibiscus vitifolius*

Table 2: Histochemical studies of *Hibiscus vitifolius* leaf

S.no	Reagents	Test	Nature of change	Observation
1	Phloroglucinol+HCL	Lignin	Pink	vessels
2	Iodine solution followed by sulphuric acid	Cellulose	Brown	Paranchyma cells
3	Picric acid	Alkaloids	Yellow	Trichomes
4	Heating with KOH	Suberin	Green	Trichomes and palisade cells

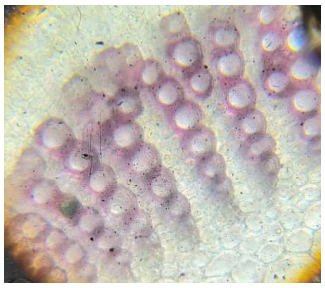


Fig 5.1 Vb

Fig 5.2 Pa

Fig 5.3 Tr

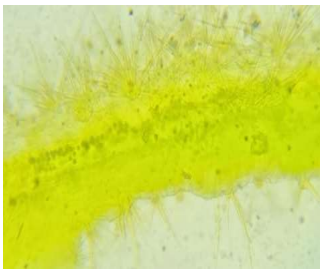


Fig 5.4 Pc&Tr

Vb-vascular bundle, Pa- paranchyma cells, Tr-Trichomes, Pc-palisade cells

Determination of leaf constant of *Hibiscus vitifolius*

Quantitative microscopic parameters such as epidermal cells, stomatal number, stomatal index and palisade ratio were analysed.

Table 3: Determination of leaf constants of *Hibiscus vitifolius*

Parameters	Upper epidermis (cells/mm2)	Lower epidermis (cells/mm2)
Epidermal cells	13	18
Stomatal number	5	8
Stomatal index	28	30
Palisade ratio	4	4

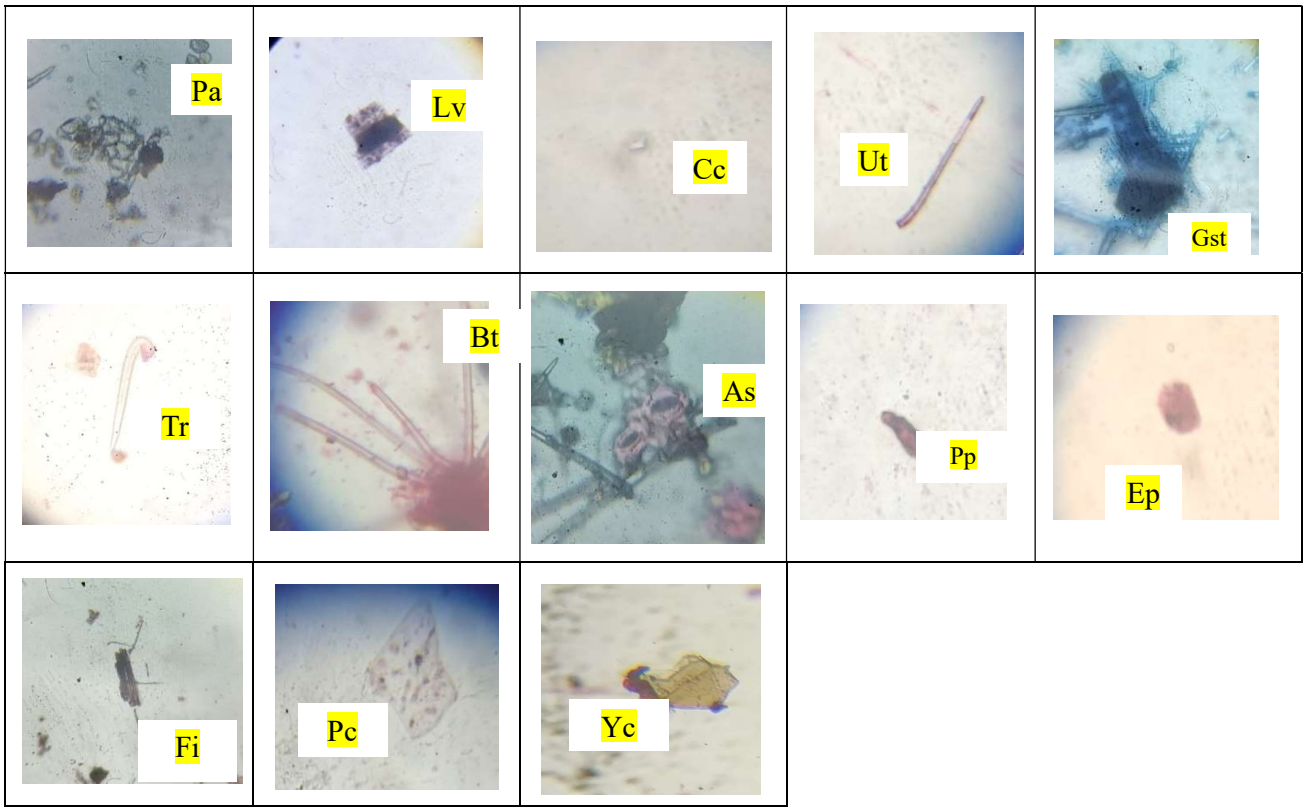


Fig 6: powder microscopy of *Hibiscus vitifolius* leaf

Powder microscopy of *Hibiscus vitifolius* leaf

The powder light green in colour with no characterstic odour and mucilage taste showed paranchyma cells,lignified spiral vessels, calcium oxalate crystals,Unicellular trichomes, Trachiods, Bundle of trichome, fibers, anisocytic trichomes, Glub-shaped body spatulate trichomes, palisade cells, epidermis, palisade paranchyma cells, yellow content.

Pa-paranchyma cells, Lv-Lignified spiral vessels, Cc-calcium oxalate crystals, Ut-Unicelluar trichomes, Gst- Glub shaped and body saptulate trichomes, Tr-Trachiods, Bt-Bundle of trichomes, As- Anisocytic stomata, Pp-palisade paranchyma cells, Ep-Epidermis, Fi-Fibers, Pc-palisade cells, Yc-yellow content.

Determination of fluorescence analysis of *Hibiscus vitifolius* (powder)

Powdered drug of *Hibiscus vitifolius* Linn plant gave different fluorescence under visible and ultraviolet (UV) radiation. (254nm and 365 nm), when treated with various reagents. Therefore, fluorescence evaluation is used for the identification of powdered drug. Some crude drugs are often assessed qualitatively in this way, and it is an important parameter of pharmacognostic evaluation.

Table 4: **Determination of flourescence analysis of *Hibiscus vitifolius***

Sample + Reagent	Visible (<400 nm)	UV short wave (254 nm)	UV long wave (365 nm)
Powder + HCl	Green	Black	Green
Powder + HCl + H_2O	Green	Black	Green
Powder +H_2SO_4	Brown	Dark Green	Green
Powder +H_2SO_4 + H20	Brown	Dark Green	Green
Powder + HNO_3	Yellow	Black	Green
Powder + HNO_3+ H_2O	Yellow	Dark Green	Green
Powder + 20% NaOH	Light Green	Brown	Fluorescent Green
Powder + Alcoholic NaOH	Light Green	Yellow	Fluorescent Green
Powder + Acetic acid	Yellow	Brown	Green
Powder + Fe_3Cl_4	Dark Green	Black	Green
Powder + Iodine	Red	Brown	Green
Powder + Picric Acid	Yellow	Dark Green	Fluorescent Green
Powder+ammonia	Dark green	Brown	Green
Powder+water	Dark Green	Black	Green

Determination of Physiochemical parameters and inorganic elements and heavy metal analysis of the *Hibiscus vitifolius*

The ash values of the plant were estimated using standard procedures which showed a total ash of 0.283±0.002% w/w, water soluble ash 0.4±0.057 %w/w and acid insoluble ash of 0.9±0.419 w/w. Loss on drying and total solid value of the powder was determined as 7.19±0.072 & 92.81± 0.072%w/w respectively. Petroleum ether extractive, Ethyl acetate extractive, Ethanol extractive, Methanol extractive, Hexane extractive, water extractive the percentage yield of the extractive was found to be 18.6±0.92%, 15.8±3.50%, 19.6±9.105%, 22.266±6.354%, 21.67±7.51%, 22.2±3.453 % w/w respectively. To the ash of *Hibiscus vitifolius* Linn., leaves was treated with 50%v/v hydrochloric acid and kept for 1 hour. It was filtered, filtrate was used for inorganic and heavy metal analysis using various reagents.

Table 5: Determination of Physiochemical parameters and inorganic elements analysis of the *Hibiscus vitifolius*

S.no	Physio-chemical parameters	Results
1	Foreign matter	Nil
2	Loss on drying	7.19±0.072%w/w
3	Total solid	92.81±0.072%w/w
4	Petroleum ether extractive	18.6±0.92%w/w
5	Ethyl acetate extractive	15.8±3.50%w/w
6	Ethanol extractive	19.6±9.105%w/w
7	Methanol extractive	22.266±6.354%w/w
8	Hexane extractive	21.67±7.51%w/w
9	Water extractive	22.2±3.453%w/w
10	Total Ash	0.283±0.002%w/w
11	Water soluble ash	0.4±0.057%w/w
12	Acid insoluble ash	0.9±0.419%w/w
13.	Inorganic mineral analysis	Presence of chloride, iron and sulphate
14	Heavy metals	Absence

Determination of the phytochemical analysis of the *Hibiscus vitifolius*

Table 6: Determination of the phytochemical analysis *Hibiscus vitifolius* Linn

S.no	Phytochemical Analysis	Observation
1	Test for Carbohydrate	+
2	Test for Flavonoids	+
3	Test for Alkaloids	+
4	Test for Saponins	+

5	Test for Tannins	+
6	Test for Coumarin	+
7	Test for Quinone	+
8	Test for Glycoside	+
9	Test for Protein	-
10	Test for Gum and mucilage	+
11	Test for Phenolic compound	+
12	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 HAEHV.

Determination of Gallic acid equivalent in HAEHV

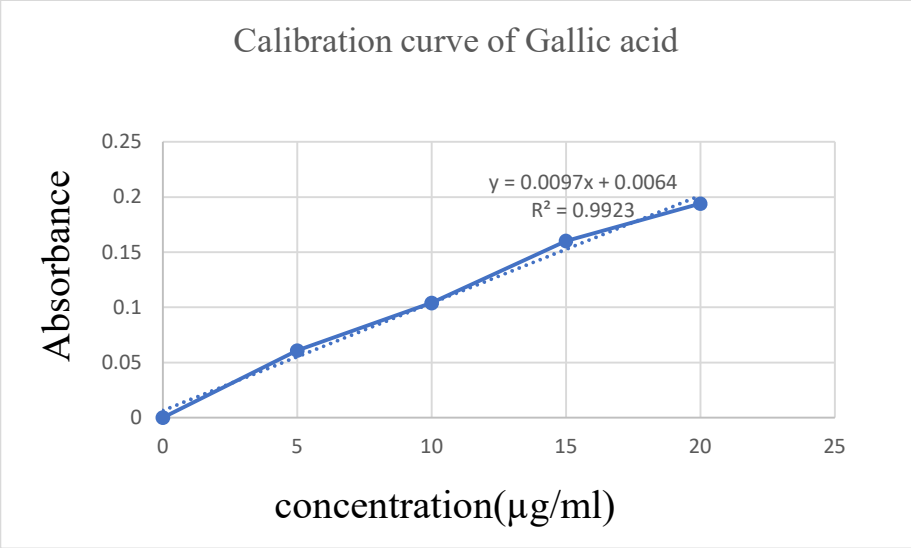


Fig 7: Calibration curve of Gallic acid

Table 7: Determination of gallic acid equivalent in HAEHV

S.no	Concentration	Absorbance	
	Gallic acid & HAEHV (µg/ml)	Gallic acid Mean ± SEM	HAEHV Mean ± SEM
1	0	0	0
2	5	0.061±0.0008	0.033±0.0003
3	10	0.104±0.001	0.131±0.0006
4	15	0.16±0.0028	0.158±0.0014
5	20	0.194±0.0012	0.181±0.0008
		GAE	**90mg/gm**

Quantitative estimation of phenol was done by folin- ciocalteu method using gallic acid as standard. The total phenolic content in HAEHV was found to be **90GAE/gm.**

Determination of Tannic acid Equivalent in HAEHV

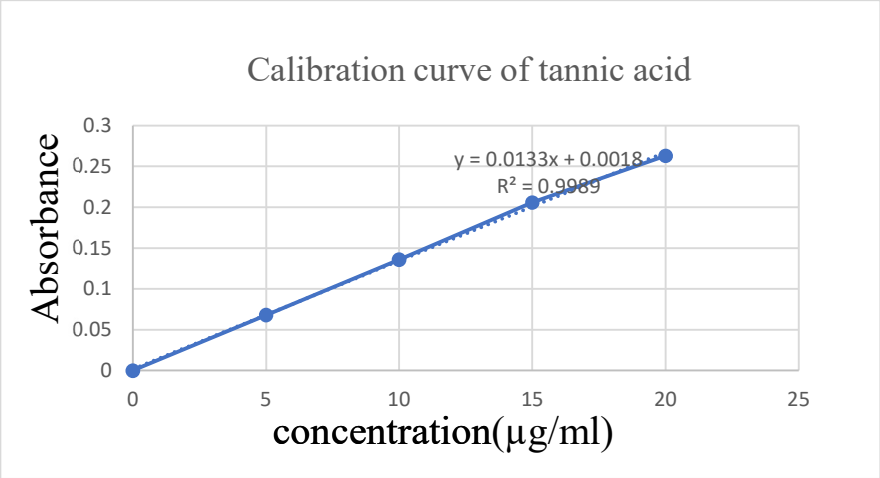


Fig 8: Calibration curve of tannic acid

Table 8: Determination of Tannic acid equivalent in HAEHV

S.no	Concentration	Absorbance	
	Tannic acid & HAEHV (µg/ml)	Tannic acid Mean ± SEM	HAEHV Mean ± SEM
1	0	0	0
2	5	0.068±0.0115	0.094±0.0012
3	10	0.136±0.0008	0.098±0.0011
4	15	0.206±0.0003	0.15±0.0011
5	20	0.263±0.0003	0.238±0.0003
		TAE	**82.70 mg/gm**

Quantitative estimation of Tannin was done by folin-denis method using Tannic acid as standard. The total Tannin content in HAEHV was found to be **82.70 TAE/gm.**

Determination of Quercetin Equivalent in HAEHV

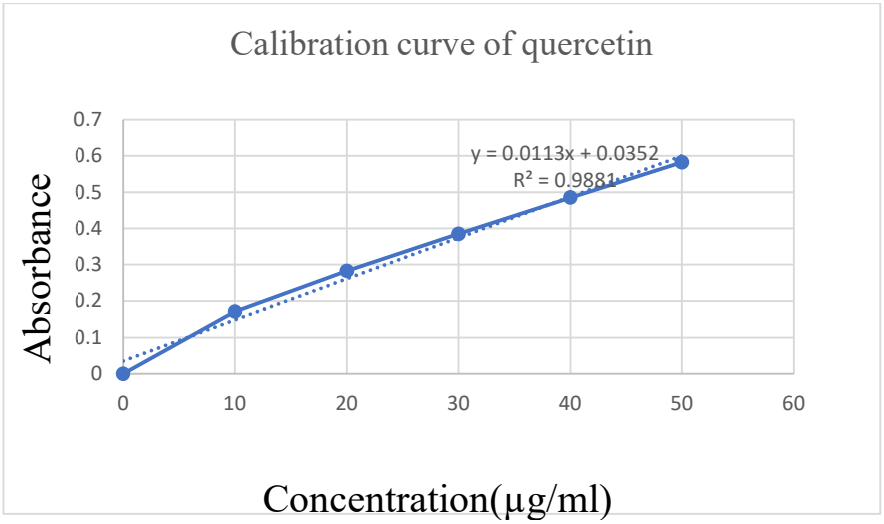


Fig 9: Calibration curve of Quercetin

Table 9: Determination of Quercetin equivalent in HAEHV

S.no	Concentration	Absorbance	
	Quercetin& HAEHV (µg/ml)	Quercetin Mean ± SEM	HAEHV Mean ± SEM
1	10	0.119±0.0008	0.119±0.0003
2	20	0.283±0.0003	0.283±0.0003
3	30	0.360±0.0003	0.36±0.0003
4	40	0.440±0.0006	0.44±0.0003
5	50	0.562±0.0003	0.562±0.0006
		QE	**93.23mg/gm**

Quantitative estimation of flavonoids was done by aluminium chloride colorimetric assay using quercetin as standard. The total flavonoids content in HAEHV was found to be **93.23 QE/gm**.

Phenetics

There are around 20 species of hibiscus present in south India temperate, subtropical,tropical region . Among those species, five species are available in South India,

- *Hibiscus vitifolius,*
- *Hibiscus sabdaffia,*
- *Hibiscus rosa-sinesis,*
- *Hibiscus syriacus,*
- *Hibiscus hirtus.*

The characters such as leaf stipulses, venation, texture, margin, surface are considered.
Leaf stipulses - Linear to filiform; present-1, absent-0.
Leaf venation- Actinodromous; Present- 1 Absent-0.
Leaf texture- Densely pubescent on both surface; Present-1 Absent- 0.
Leaf margin- crenate-denate; Present-1 Absent-0.
Leaf surface- Broadly ovate to orbicular,3 to 7 lobed; Present -1 absent-0.

Table 10: Table of similarity for *Hibiscus vitifolius* with its four other species

Species	Stipules	Venation	Texture	Margin	surface
Hibiscus vitifolius	1	1	1	1	1
Hibiscus sabdaffia	1	0	1	1	0
Hibiscus rosa-sinesis	1	0	0	1	0
Hibiscus syriacus	1	0	0	0	1
Hibiscus hirtus	1	0	0	1	0

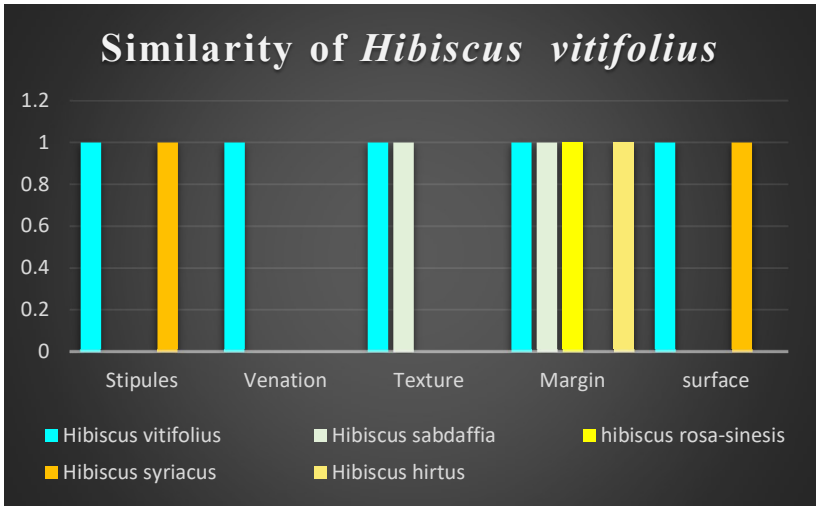


Fig 10: Chart of similarity characters of *Hibiscus vitifolius* Linn Leaves

Table 11: Table of Disimilarity for *Hibiscus vitifolius* with its four other species

SPECIES	*Hibiscus vitifolius*	*Hibiscus Sabdaffia*	*Hibiscus Rosa-sinesis*	*Hibiscus syriacus*	*Hibiscus hirtus*
Hibiscus vitifolius	1	0.6	0.4	0.4	0.4
Hibiscus sabdaffia	0.6	1	0.8	0.4	0.8
Hibiscus Rosa-sinesis	0.4	0.4	1	0.4	0.8
Hibiscus syriacus	0.4	0.4	0.4	1	0.6
Hibiscus hirtus	0.4	0.8	0.6	0.6	1

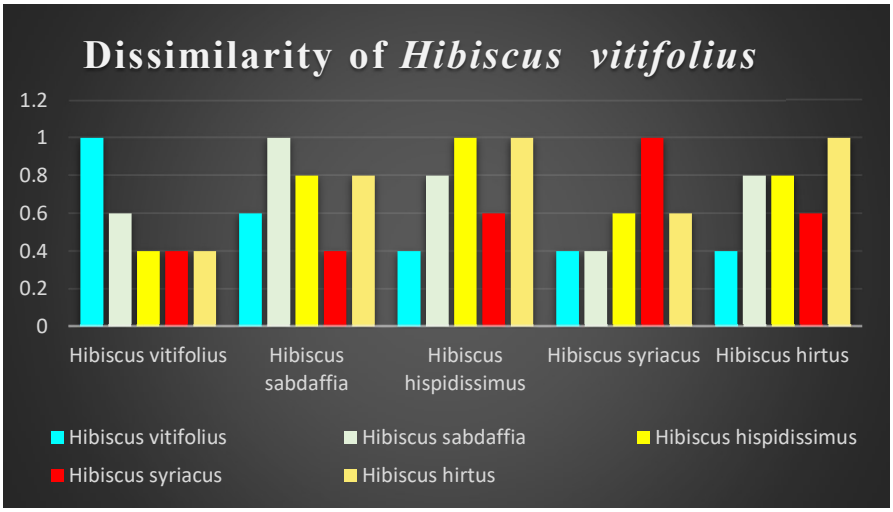


Fig 11: Chart of Disimilarity characters of *Hibiscus vitifolius* Linn Leaves

Sample matching coefficient

SSM=NS/NS+ND * 100

where, NS - Number of similiarity characters,ND-Number of dissimiliarity characters

N-Number of samples

Number of similiarity character =11

Number of dissimilarity character =14

=11/11+14*100=44%

The matching coefficient of *Hibiscus vitifolius* with respect to other species was found to be 44%

CONCLUSION

Macroscopical and microscopical findings help to identify the plant in whole and powder form. Powder analysis, physicochemical analysis, fluorescence analysis and phenetics helps to prove the authenticity of the plant. Hence the pharmacognostical and phenetics study adds taxonomical information to this plant. Physico-chemical constants, leaf constants add benefits in the identification of this plant. Phytochemical screening helps to identify the phytoconstituents present in hydroalcoholic extract. These parameters can serve as standards so as to draw the pharmacopoeial monographs. Phenetical study imparts taxonomical enlightement of this *Hibiscus vitifolius* can be made to differentiate from other species of Hibiscus.

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