



International Journal of Research Publication and Reviews

Journal Homepage: www.ijrpr.com ISSN 2582-7421

Pharmacognostical, Phenetics and Preliminary Phytochemical Evaluation of the Leaves of *Cissus Rotundifolia* (FORSSK.) VAHL

Dr. A. Krishnaveni¹, D. Merlin Ilavarasi², M. Renuga², V. Nithya²

¹Assistant professor, ² II year M.Pharm,

Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai.

Affiliated to Tamil Nadu Dr. MGR Medical University, Chennai -600032.

Email: akrishnaveni72@rediffmail.com

ABSTRACT

Introduction: *Cissus rotundifolia* (Forssk.) Vahl belongs to Vitaceae and is a succulent medicinal plant traditionally used for its nutritional and therapeutic importance which includes anti-oxidant, anti-bacterial, anti-diabetic, anti-inflammatory, anti-fertility, anti-ulcer, anti-parasitic, and anti-malarial activity. The present study was undertaken to establish the pharmacognostical and phytochemical characteristics of the leaves of *Cissus rotundifolia* and to provide suitable parameters for its identification and quality evaluation.

Materials and Methods: Fresh leaves of *Cissus rotundifolia* were collected from Ari Nursery Garden, Madakkulam, Madurai District, Tamil Nadu. The leaves was identified and authenticated by Dr.Stephen, Retired Assistant Professor, Department of Botany, American College, Madurai-625002. Morphological, microscopical, powder microscopical, quantitative microscopical and histochemical studies were carried out. Physicochemical parameters, fluorescence behaviour, preliminary phytochemical screening and quantitative estimation of total phenolic, tannin and flavonoid contents were performed using standard methods. Limit of detection (LOD) and limit of quantitation (LOQ) were also determined.

Results and Discussion: The leaves were characterized by a simple, circular to ovate, thick and succulent lamina with a cordate base, obtuse to rounded apex, crenate to serrate margin and reticulate venation. Microscopical examination revealed a dorsiventral leaf with distinct palisade and spongy tissues, mucilage cells, raphides, prismatic calcium oxalate crystals and conjoint collateral vascular bundles. Powder microscopy showed anomocytic stomata, palisade cells, raphide bundles, reticulate and spiral vessels, mucilage cells and acicular crystals. Histochemical studies confirmed lignin, starch, tannins, fixed oils and mucilage. The total ash, ethanol extractive and aqueous extractive values were $12.53 \pm 0.10\%$, $22 \pm 0.40\%$ and $10.8 \pm 0.20\%$ w/w, respectively. Phytochemical screening indicated the presence of alkaloids, carbohydrates, tannins, flavonoids, saponins, mucilage, steroids, terpenoids and coumarins. The total gallic acid equivalent, tannic acid equivalent and quercetin equivalent were found to be 438 mg GAE/g, 206 mg TAE/g and 655.4 mg QE/g, respectively. The LOD and LOQ were found to be 1.98 and 5.99 µg/ml.

Conclusion: The present study establishes distinctive morphological, microscopical, physicochemical and phytochemical characteristics of *Cissus rotundifolia* leaves. These findings provide useful diagnostic parameters for the proper identification, authentication and quality control of the plant material and may serve as a reference for further pharmacological and phytochemical investigations.

Keywords: *Cissus rotundifolia*, histochemistry, pharmacognostical evaluation, phenetics, physicochemical parameters, phytochemical screening, powder microscopy, qualitative and quantitative analysis of phytoconstituents, Vitaceae.

INTRODUCTION

Cissus rotundifolia (Forssk.) Vahl. belonging to the Vitaceae, is a wild edible medicinal plant widely distributed across several regions of East and Central Africa, Zimbabwe, Mozambique, Egypt, Yemen, Saudi Arabia, and South Africa. The plant has been reported to possess diverse pharmacological activities, including anti-oxidant, anti-bacterial, anti-diabetic, anti-inflammatory, anti-fertility, anti-ulcer, anti-parasitic and anti-malarial activity. Earlier phytochemical investigations of *Cissus rotundifolia* have led to the identification and characterization of various bioactive constituents, including triterpenes, flavonoids, steroidal saponins, cissuxinoside, and cissoic acid. The cooked leaves of *Cissus rotundifolia* are commonly consumed in rural communities of South Africa and other regions due to their nutritional and therapeutic value. The plant is also utilized as an economical natural thickening agent. Studies conducted on the leaves and fruits collected from various regions of Saudi Arabia and Yemen have demonstrated the presence of essential nutrients such as carbohydrates, proteins, lipids, vitamins, minerals, and amino acids. [1-10]

An investigation was performed to identify the Pharmacognostical parameters and preliminary phytochemical screening and phenetics for this medicinal plant.

MATERIALS AND METHODS

Collection of leaf and authentication

Leaves were collected from Ari Nursery Garden, Madakkulam, Madurai district, Tamil Nadu in the month of February 2026. The leaves were identified and authenticated by Dr. Stephen, Retired Assistant Professor, Department of Botany, 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 *Cissus rotundifolia* were studied. [11]

Microscopical study

The microscopical studies of the petiole and leaf of *Cissus rotundifolia* were carried out at the Department of Pharmacognosy, Siddha Central Research Institute (CCRS), Ministry of Ayush, Government of India, Chennai. The samples were preserved in FAA fixative for more than 48 h, followed by preparation of thin transverse sections using a sharp blade. The sections were stained with 0.8% safranin and 0.5% Astra blue and examined under a trinocular microscope. Photomicrographs of the transverse sections were captured under bright-field illumination with appropriate scale bars. Powder microscopy was performed by mounting the powdered leaf sample on microscopic slides using 50% glycerol after clearing with a saturated solution of chloral hydrate. The powder was also treated with iodine solution for confirmation of starch grains. Diagnostic microscopical characters were observed and documented using a trinocular microscope under bright-field illumination.

Quantitative microscopy

It was performed using square pieces of leaf taken from the region between the midrib and the margin. These samples were decolourised and examined under a microscope to assess vein islet number, vein termination, and palisade ratio. For stomatal index analysis, the upper and lower epidermal layers were carefully peeled from separate leaf pieces, mounted in glycerin water on a slide, and observed microscopically to determine stomatal index and stomatal type [12]

Powder microscopy

Powder microscopy of the leaves of *Cissus rotundifolia* was carried out at the Department of Pharmacognosy, Siddha Central Research Institute (CCRS), Ministry of Ayush, Government of India, Chennai. The powdered leaf sample was cleared with a saturated solution of chloral hydrate and mounted on a microscopic slide using 50% glycerol. The sample was treated with iodine solution for confirmation of starch grains. The diagnostic microscopical characters were examined under a trinocular microscope using bright-field illumination, and photomicrographs were captured and documented.

Behaviour of Powder with various chemical reagents

A small quantity of leaf powder was transferred into a test tube, to which 1–2 drops of freshly prepared reagents were added. The resulting colour changes were observed under visible light as well as under UV at 254 nm and 366 nm, following standard procedure.[13]

Physicochemical parameter

The powder was subjected to physicochemical 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[14]. The ash was subjected to inorganic elements identification as per Atherden[15] and heavy metals analysis of lead were performed as per I.P 1985[16].

Preparation of fresh juice of *Cissus rotundifolia* (*C.rotundifolia*frj)

Fresh leaves of 25g were weighed. It was washed with water and 30 ml of water is added and made into a fresh juice of 25.5ml.

Preliminary phytochemical screening

Preliminary phytochemical screening were carried out by using different reagents for identification the presence of phytoconstituents as per standard procedures [17].

Quantitative estimation of phytoconstituents

Limit of Detection (LOD)

LOD of an individual analytical procedure is the lowest amount of an analyte in a sample that can be detected but not necessarily quantified

Limit of Quantitation (LOQ) of *C.rotundifolia*frj

LOQ of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected and quantified

Procedure

*C.rotundifolia*frj were prepared and scanned under UV range of (200-700nm). *C.rotundifolia*frj were analyzed to identify the presence of peak and the corresponding wavelength was recorded. Subsequently, *C.rotundifolia*frj at concentrations of (0 to 10 µg/ml) were prepared and examined under UV spectroscopy at the wavelength identified from the *C.rotundifolia*frj. The LOD and LOQ values were calculated based on the standard deviation of the response and the slope derived from the *C.rotundifolia*frj [18,19].

Determination of gallic acid equivalent in *C.rotundifolia*frj

Procedure

The method was adopted as per Singleton V.L, *C.rotundifolia*frj of 20,40,60,80 and 100µg/ml is taken. To each of this solution add 5ml of distilled water and 0.5ml of folin ciocalteu's reagent is added, mixed and shaken. After 5 minutes, 1ml of 10% sodium carbonate solution is added and the volume is made upto 10ml 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 was recorded at 725nm and compared with gallic acid as standard[20].

Determination of tannic acid equivalent in *C.rotundifolia*frj

Procedure

The method was adopted as per Boham AB, Various concentrations of (0 to 100µg/ml) *C.rotundifolia*frj were prepared by dissolving 0.5ml of *C.rotundifolia*frj and add 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 distilled water. From this 20,40,60,80 and 100µg/ml is prepared and compared with Tannic acid as standard. Then the tubes were shaken and the absorbance was recorded at 700nm after 30min [21].

Determination of quercetin equivalent in *C.rotundifolia*frj

Procedure

The total flavonoid content of *Cissus rotundifolia* leaf extract was determined by the aluminium chloride colorimetric method using quercetin as the standard. Various concentrations of *C.rotundifolia* were prepared (0 to 100µg/ml). Standard quercetin solutions of 20, 40, 60, 80 and 100 µg/ml were prepared in methanol. An aliquot of 1 ml of each standard solution was transferred into a 10 ml volumetric flask, followed by the addition of 4 ml of distilled water. Then, 0.3 ml of 5% sodium nitrite (NaNO₂) solution was added and mixed thoroughly. After 5 minutes, 0.3 ml of 10% aluminium chloride (AlCl₃) solution was added. After a further 5 minutes, 2 ml of 1 M sodium hydroxide (NaOH) was added and the volume was made up to 10 ml with distilled water. The solution was mixed thoroughly and the absorbance was measured at 510 nm against a reagent blank using a UV-Visible spectrophotometer [22].

Phenetics

Numerical taxonomy of plant derived by using six characters such as leaf, blade, venation, apex base and margin[23]

RESULTS AND DISCUSSION

Morphological study

Cissus rotundifolia (Forssk.) Vahl is a succulent and vigorous climber belonging to Vitaceae. The leaves are simple, alternate, circular to ovate, thick and fleshy, with a glabrous to slightly pubescent surface. The leaves possess a cordate base and an obtuse apex with a crenate margin. The petiole is short, glabrous or pubescent, and measures up to 1 cm in length. The leaf lamina may attain up to 8 × 8 cm in size. The leaves are green in colour and have a succulent texture. The venation is reticulate. The plant bears tendrils and has characteristic succulent, often angular stems.[24]

Table 1: Morphological characters of *Cissus rotundifolia* leaves

S.No.	Parameters	Observation
1.	Colour	Green
2.	Odour	Characteristic odour
3.	Taste	Slightly bitter
4.	Leaf type	Simple

S.No.	Parameters	Observation
5.	Shape	Circular to ovate
6.	Arrangement	Alternate
7.	Apex	Obtuse to rounded
8.	Base	Cordate
9.	Petiole	Short
10.	Margin	Crenate to serrate
11.	Venation	Reticulate
12.	Texture	Thick and fleshy / succulent
13.	Length	5 to 8 cm
14.	Width	5 to 7 cm
15.	Petiole length	0.8-1.2 cm

Fig 1: Habitat of *C. rotundifolia*Fig 2: Twig of *Cissus rotundifolia*

Fig 3: Ventral view of leaf



Fig 4: Dorsal view of leaf

Microscopical study

T.S of leaf

Microscopic examination of *Cissus rotundifolia* leaf showed the following characteristics using 0.8% safranin as dye and 0.5% Astra blue and viewed under microscope of lens 45x

Epidermis

Lamina region showed a single-layered upper epidermis composed of rectangular to polygonal cells covered with a thick cuticle. The lower epidermis was also single-layered and composed of comparatively smaller cells. Trichomes were absent on both the upper and lower epidermis.

Mesophyll

Mesophyll was distinctly differentiated into palisade and spongy parenchyma, indicating the dorsiventral nature of the leaf. The palisade parenchyma occurred below the upper epidermis and consisted of 1–2 layers of elongated, compactly arranged cells. The spongy parenchyma was present towards the lower epidermis and consisted of loosely arranged cells with prominent intercellular spaces. Numerous mucilage cells and raphides were observed in the mesophyll, along with a few prismatic calcium oxalate crystals.

Vascular bundles

Vascular bundles were conjoint and collateral in nature. Three to four vascular bundles were observed in the midrib region, with the xylem oriented towards the outer side and the phloem towards the inner side. Veins were also observed traversing through the mesophyll tissue.

T.S of midrib

Midrib region was prominent and consisted of an outer epidermal layer followed by broad ground tissue. The outer ground tissue was composed of 2–4 layers of collenchymatous cells, followed by a parenchymatous zone. Numerous large mucilaginous cavities and raphides were observed in the parenchymatous region. Three to four conjoint, collateral vascular bundles were present in the midrib region, with xylem facing towards the outer side and phloem towards the inner side.

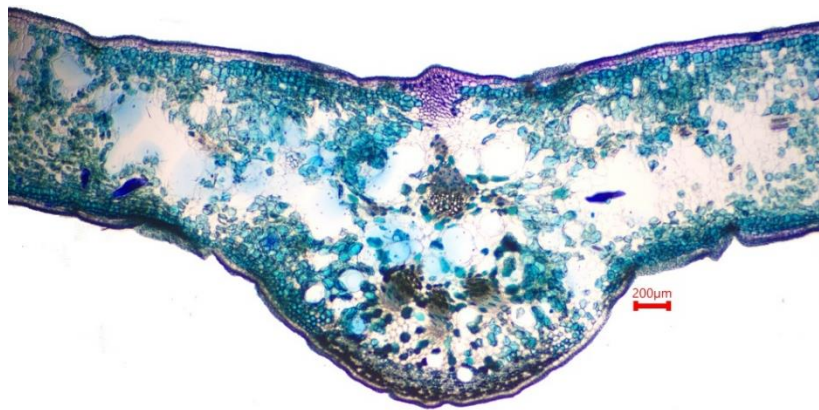


Fig 5: Microscopy of leaf- TS of the lamina passing through midrib

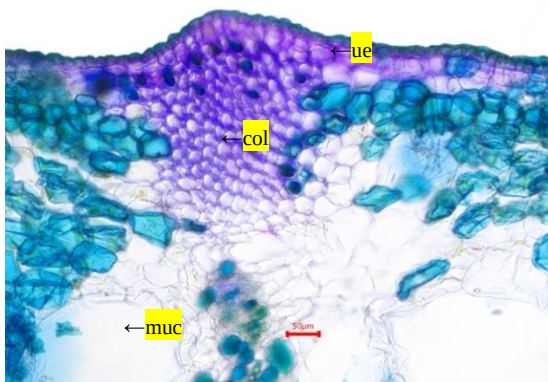


Fig 6 :Outer portion enlarged

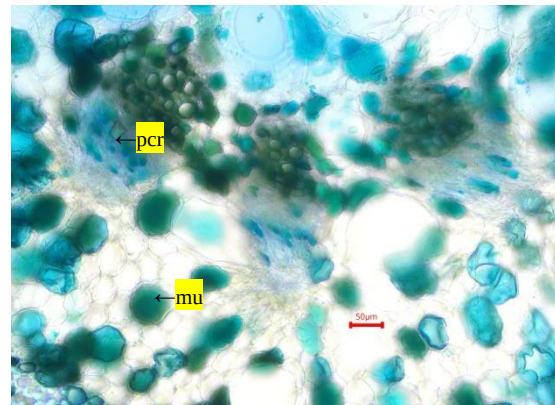


Fig 7: Middle portion enlarged

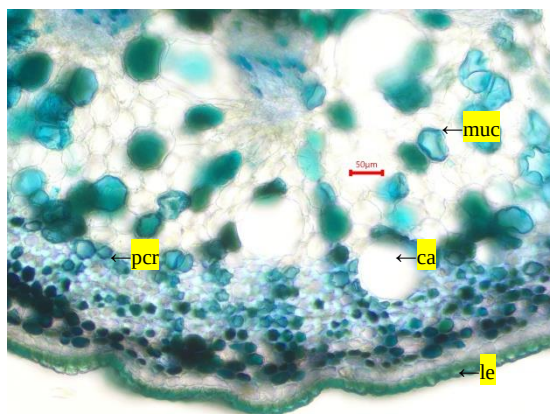


Fig 8: Lower portion enlarged



Fig 9: TS of lamina

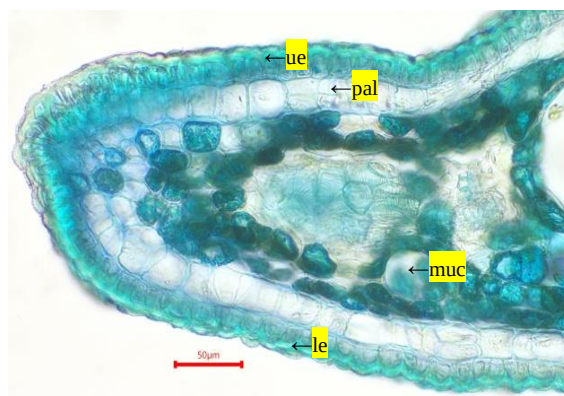
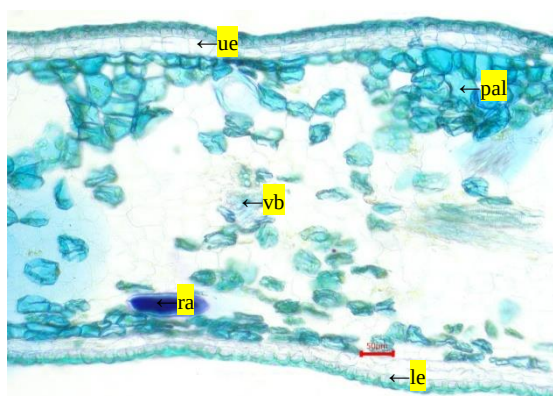


Fig: 10 and Fig 11: Enlarged view of lamina

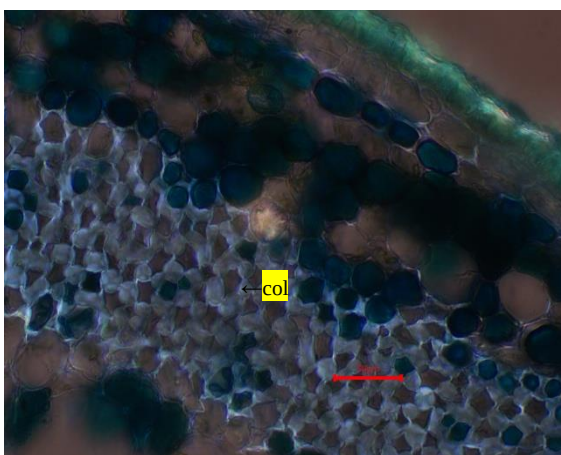
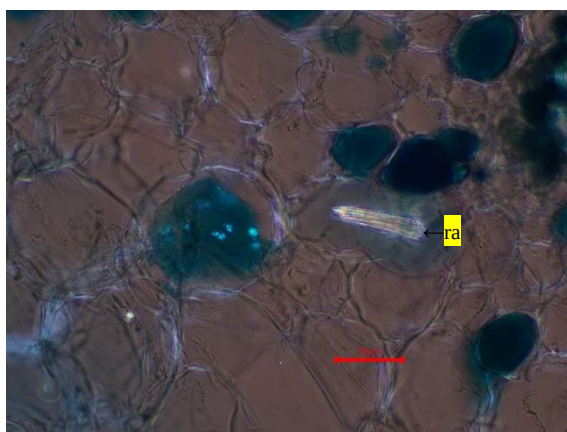


Fig 12 and 13: Crystals under a polariser

col - collenchyma; **cot** - cortex; **cu** - cuticle; **e** - epidermis; **hyp** - hypodermis; **muc** - mucilage cell; **pa** - parenchyma; **ph** - phloem; **ra** - raphide; **vb** - vascular bundle

The transverse section of the leaf showed a dorsiventral nature, with a single-layered upper and lower epidermis and a thick cuticle. The mesophyll was differentiated into palisade and spongy parenchyma. The presence of mucilage cells, raphides and calcium oxalate crystals, along with conjoint collateral vascular bundles, provides useful anatomical characters for the identification and standardization of *Cissus rotundifolia* leaves

T.S of petiole

Transverse section of the petiole was circular to slightly undulating in outline. The epidermis was single-layered, compact and covered with a thick cuticle; trichomes were absent. It was followed by 2–5 layers of collenchymatous hypodermis. The cortex consisted of outer collenchymatous and inner parenchymatous zones containing mucilage cells, raphides and few chlorenchymatous cells. Vascular bundles were arranged in a ring and were collateral and open, with xylem towards the inner side and phloem towards the outer side, accompanied by sclerenchymatous caps. The broad central pith consisted of thin-walled parenchymatous cells containing starch grains and calcium oxalate crystals, with few large mucilaginous cavities.

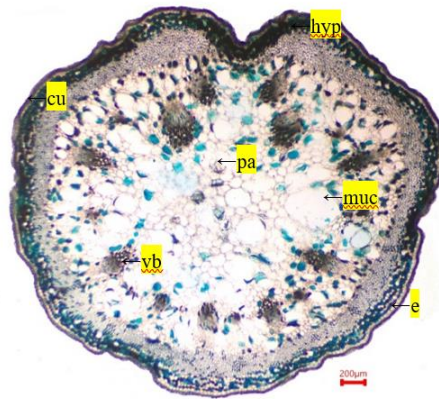


Fig 14: Microscopy of petiole- TS of petiole

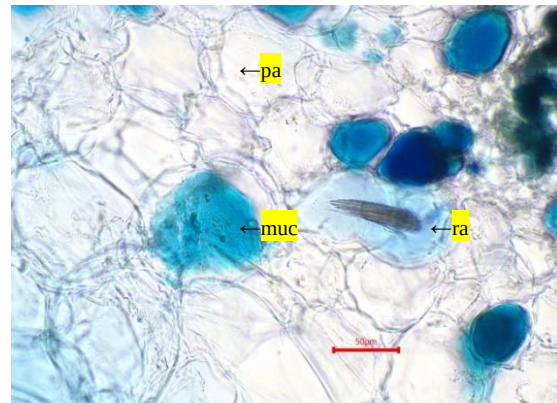
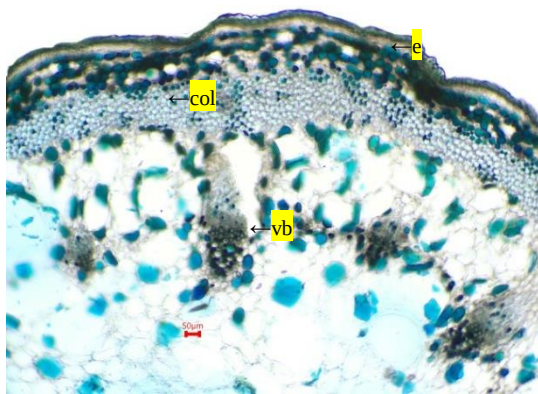


Fig 15 and 16: Enlarged portion of petiole

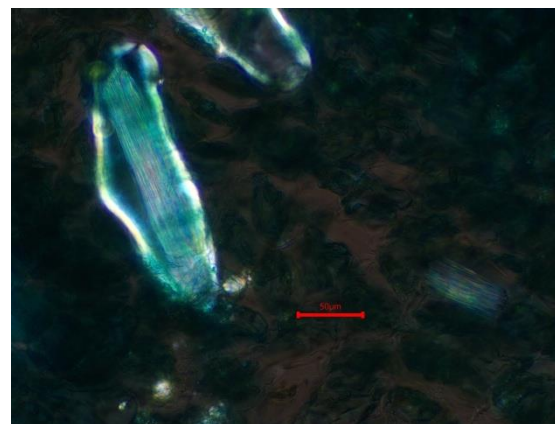
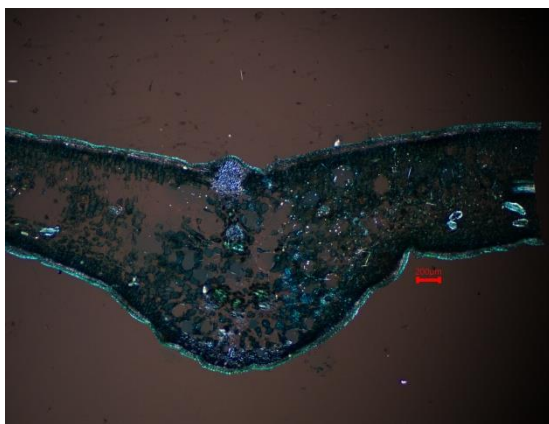


Fig 17: Crystals under a polarizer

col - collenchyma; cu - cuticle; le - lower epidermis; mes - mesophyll; muc - mucilage cavity; pa - parenchyma; pal - palisade cells; pcr - prismatic crystal; ra - raphides; sp - spongy parenchyma; ue - upper epidermis; vb - vascular bundle;

The transverse section of the petiole showed a circular to slightly undulating outline with a single-layered epidermis and thick cuticle. The cortex contained collenchymatous and parenchymatous zones with mucilage cells and raphides. The vascular bundles were arranged in a ring, while the central pith contained starch grains, calcium oxalate crystals and mucilaginous cavities. These anatomical features serve as important diagnostic characters for authentication of the plant material

HISTOCHEMICAL STUDIES

The section of the leaves of *Cissus rotundifolia* (Forssk) Vahl., were stained by using specific reagents.

Table 2: Histochemical studies of *Cissus rotundifolia* leaf

S.No	Reagent	Test for	Observation	Identification
1.	Phloroglucinol + HCl	Lignin	Pink colour	Xylem vessels
2.	Iodine solution	Starch	Blue colour	Starch grains
3.	Ferric chloride solution	Tannins	Bluish green colour	Tannin containing cells
4.	Sudan III	Fixed oils	Orange-red colour	Oil droplets
5.	Ruthenium red	Mucilage	Pink colour	Mucilage containing cells

The histochemical study of *Cissus rotundifolia* leaves confirmed the presence and localization of important cellular constituents, including lignin, starch, tannins, fixed oils and mucilage. The characteristic colour reactions observed with specific reagents supported the anatomical identification and provided useful diagnostic characters for the authentication and standardization of the leaf drug.

Quantitative microscopy of *Cissus rotundifolia*

Determination of phytoconstants

Stomata: Anomocytic Stomata is present on both surface.

Vein islet number and Veinlet termination number

Leaf surface showed the presence of veins and vein islets and vein termination.

Table 3 : Quantitative microscopy of *Cissus rotundifolia*

S.No.	Parameters	Cells /mm ²
1.	Epidermal number	185
2.	Vein islets number	9
3.	Vein termination	11
4.	Stomatal number	30
5.	Stomatal Index	14
6.	Palisade ratio	4-6

Leaf surface showed the presence of veins, vein islets, vein termination, epidermal number, stomatal number, stomatal index and palisade ratio were determined in upper epidermis and given in Table 3.

Powder Microscopy

Powdered sample was examined for its external and organoleptic characteristics. The powder was cleared with saturated chloral hydrate solution and mounted on a microscopic slide using 50% glycerol. The sample was treated with iodine solution to confirm the presence of starch grains.

The powder is greyish-green in colour, with a characteristic odour and mucilaginous taste. It shows fragments of epidermis with anomocytic stomata, palisade cells in surface view, cells with raphide bundles, reticulate and spiral vessels and mucilage cells (Fig. 18-24)

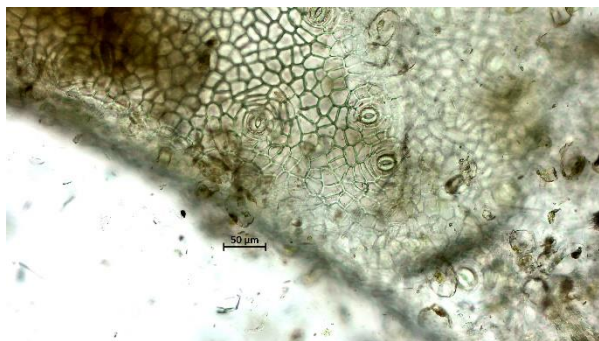


Fig 18: Surface view of epidermal cells with stomata

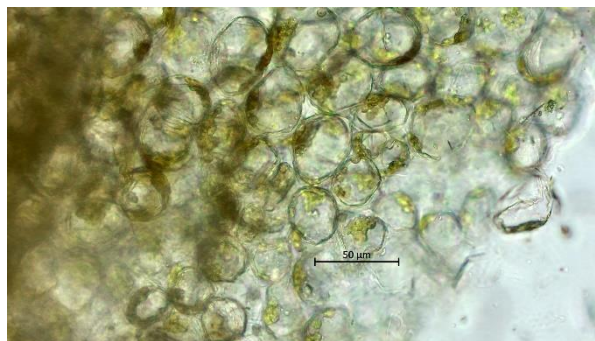


Fig 19: Palisade cells in surface view



Fig 20: Reticulate vessel



Fig 21: Spiral vessel



Fig 22:



Cells with raphide bundles

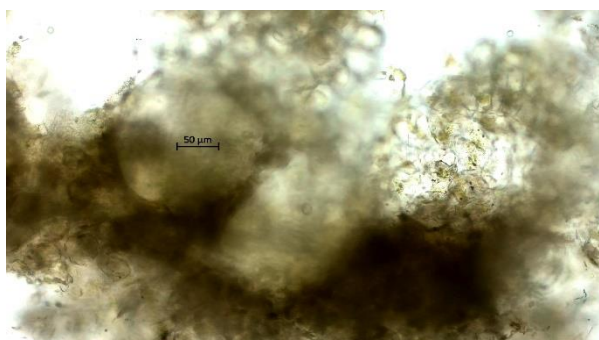


Fig 23: Mucilage cell



Fig 24: Isolated acicular crystals

It shows fragments of epidermis with anomocytic stomata, palisade cells in surface view, cells with raphide bundles, reticulate and spiral vessels and mucilage cells

Behaviour of powder with various chemical reagents

Powdered drug of plant gave different fluorescence under visible and ultraviolet (UV) radiation (254nm& 366nm) when treated with various reagents. The colour observed in different radiations were recorded. It is used for the identification of plant and powdered drug.

Table 4: Behaviour of powder with various chemical reagents of *Cissus rotundifolia* leaves

S No.	Reagent	Observation		
		Visible (<400 nm)	UV (254nm)	UV (366 nm)
1.	Powder+ H ₂ O	Greenish brown	Brown	Pale green
2.	Powder+ conc.HCl	Brown	Black	Pale brown
3.	Powder+ conc. H ₂ SO ₄	Dark brown	Black	Dark brown
4.	Powder+ conc. HNO ₃	Yellow	Yellow	Pale green
5.	Powder+ CH ₃ COOH	Light brown	Dull orange	Dull orange
6.	Powder+ HCl+ water	Brown	Brown	Pale brown
7.	Powder+ H ₂ SO ₄ + water	Dark brown	Black	Dark brown
8.	Powder+ HNO ₃ + water	Orange yellow	Orange	Pale green
9.	Powder+ CH ₃ COOH+ water	Brown	Brown	Pale brown
10.	Powder+ aq.KOH	Brown	Brown	Greenish
11.	Powder+ alcoholic KOH	Brown	Brown	Brown
12.	Powder+ aq.FeCl ₃	Dark brown	Brown	Dark green
13.	Powder+ alcoholic FeCl ₃	Brown	Brown	Dark green

The powdered leaves of *Cissus rotundifolia* showed characteristic colour changes and fluorescence reactions when treated with various chemical reagents under visible and UV light. These reactions indicate the presence of different phytoconstituents and provide useful diagnostic characters for the identification, authentication and standardization of the powdered leaf drug

Determination of physicochemical parameters & inorganic element analysis

The physicochemical parameters of the plant drug were estimated using standard procedures which showed the loss on drying and total solid content was found to be 14 ± 0.20 % w/w and 85 ± 0.50 % w/w & total ash of 12.53 ± 0.10 % w/w, water soluble ash 3.96 ± 0.10 %w/w, acid insoluble ash 2.14 ± 0.10 %w/w .The percentage extractive value of Petroleum ether, Ethyl acetate, Ethanol, Aqueous was found to be 10.6 ± 0.20 %w/w, 19.7 ± 0.30 %w/w, 22 ± 0.40 %w/w, 10.8 ± 0.20 %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.

Table 5: Physiochemical parameters of *Cissus rotundifolia*

S.No.	Physiochemical Parameters	Results
1.	Foreign matter	Nil
2.	Loss on drying	14 ± 0.20 % w/w
3.	Total solid	85 ± 0.50 % w/w
4.	Petroleum ether extractive	10.6 ± 0.20 % w/w
5.	Ethyl acetate extractive	19.7 ± 0.30 % w/w
6.	Ethanol extractive	22 ± 0.40 % w/w
7.	Aqueous extractive	10.8 ± 0.20 % w/w
8.	Total ash	12.53 ± 0.10 % w/w
9.	Water soluble ash	3.96 ± 0.10 % w/w
10.	Acid insoluble ash	2.14 ± 0.10 % w/w

S.No.	Physiochemical Parameters	Results
11.	Presence of inorganic elements	Nil
12.	Heavy metals – Lead	Absent

The physicochemical evaluation of *Cissus rotundifolia* leaves showed nil foreign matter, indicating good purity of the crude drug. The loss on drying was $14 \pm 0.20\%$ w/w, while the total solid content was $85 \pm 0.50\%$ w/w. Among the extractive values, ethanol showed the highest extractive value ($22 \pm 0.40\%$ w/w), indicating the presence of appreciable amounts of ethanol-soluble constituents. The total ash, water-soluble ash and acid-insoluble ash were $12.53 \pm 0.10\%$, $3.96 \pm 0.10\%$ and $2.14 \pm 0.10\%$ w/w, respectively. The low acid-insoluble ash indicates limited extraneous siliceous matter. Inorganic elements were not detected, and lead was absent, supporting the purity and safety of the plant material. Overall, these parameters can serve as useful standards for the quality control and standardization of *C. rotundifolia* leaves.

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, coumarins, steroids, phenol, tannins, terpenoids, mucilage,saponins and flavonoid

Table 6: Qualitative phytochemical analysis of *C.rotundifolia*

S.No	Phytochemical analysis	Observation
1.	Test for alkaloids	+
2.	Test for carbohydrates	+
3.	Test for proteins	-
4.	Test for tannins	+
5.	Test for flavonoids	+
6.	Test for saponin	+
7.	Test for mucilage	+
8.	Test for gum	-
9.	Test for amino acid	-
10.	Test for steroids	+
11.	Test for anthraquinone glycosides	-
12.	Test for terpenoids	+
13.	Test for coumarin	+
14.	Test for cardiac glycosides	-

The preliminary phytochemical screening of *Cissus rotundifolia* leaf extract revealed the presence of alkaloids, carbohydrates, tannins, flavonoids, saponins, mucilage, steroids/sterols, terpenoids and coumarins. Proteins, amino acids, gums, anthraquinone glycosides and cardiac glycosides were found to be absent. The presence of these bioactive phytoconstituents indicates the pharmacological potential of *C. rotundifolia* and may contribute to its reported antioxidant and gastroprotective activities.

Quantitative estimation of phytoconstituents

Quantitative analysis such as total gallic acid equivalent, total tannic acid equivalent and total quercetin equivalent were estimated for the *C.rotundifolia*frj.

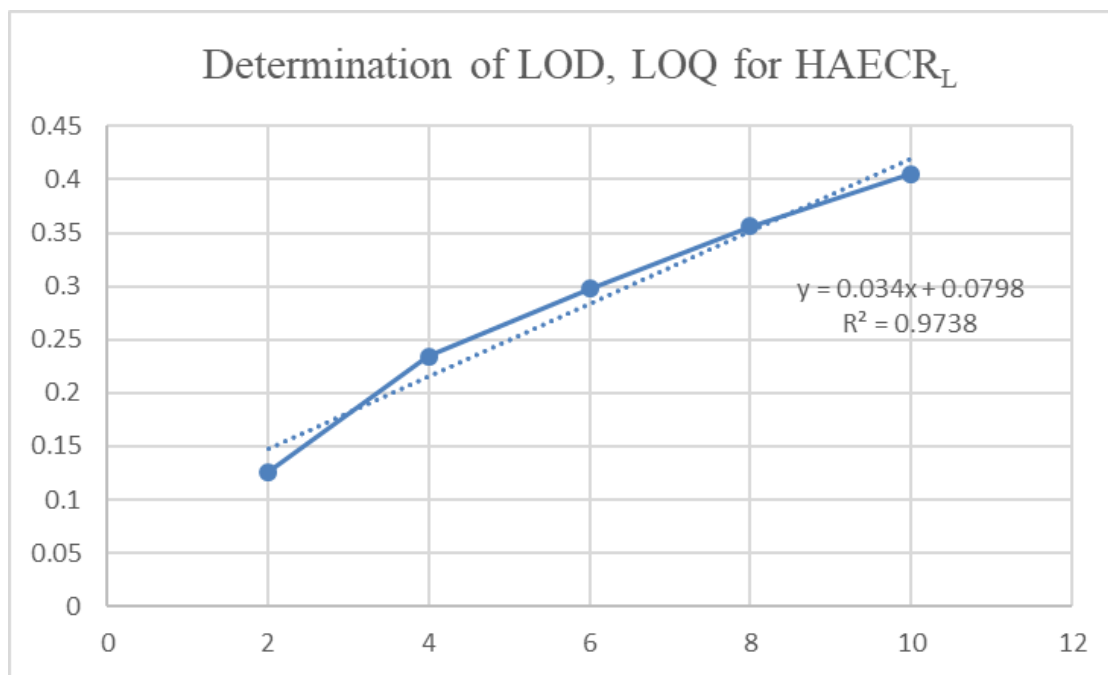
Limit of Detection (LOD) and Limit of Quantitation (LOQ) of *C.rotundifolia*frj

Various concentrations (0 to 10 µg/ml) of *Cissus rotundifolia*frj were prepared and observed under UV at λ_{max} 340 nm. Absorbance was displayed in table 7.

Table 7 : Determination of LOD and LOQ of *Cissus rotundifolia*

S. No	Concentration (µg/ml)	Absorbance
1	2	0.126
2	4	0.234

S. No	Concentration (µg/ml)	Absorbance
3	6	0.298
4	8	0.356
5	10	0.405
Limit of detection (LOD)		1.98
Limit of quantity (LOQ)		5.99

Figure 25: Determination of LOD and LOQ of *Cissus rotundifolia*

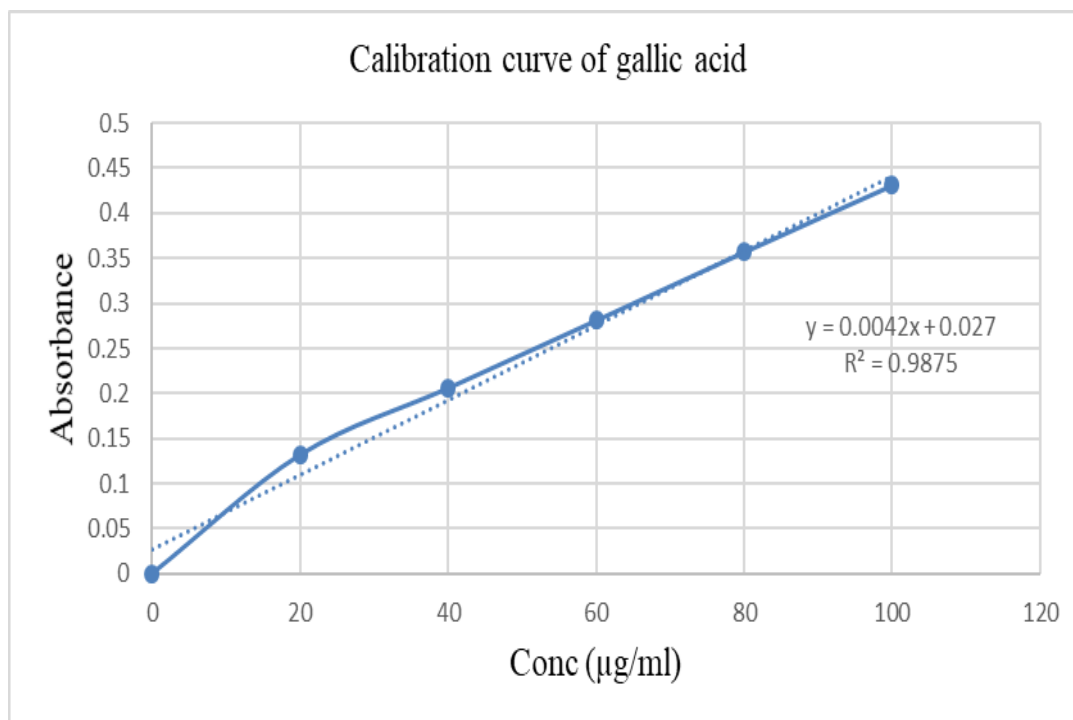
The quantitative estimation LOD and LOQ of *C. rotundifolia* frj was found to be **1.98** and **5.99 µg/ml**.

Determination of gallic acid equivalent in *C. rotundifolia* frj

Various concentrations (0 to 100 µg/ml) of *C. rotundifolia* frj leaf were treated by colorimetric method, with Folin- cio calteu, which produce blue colour and the absorbance was observed and displayed in table 8 and fig 26

Table 8: Determination of gallic acid equivalent in *C. rotundifolia*

S.No.	Concentration (µg/ml)	Absorbance of gallic acid	Absorbance of <i>C. rotundifolia</i>
1	0	0	0
2	20	0.132	0.039
3	40	0.206	0.061
4	60	0.281	0.100
5	80	0.357	0.106
6	100	0.431	0.211
		GAE	438 mg GAE/g

Figure 26: Calibration curve of gallic acid

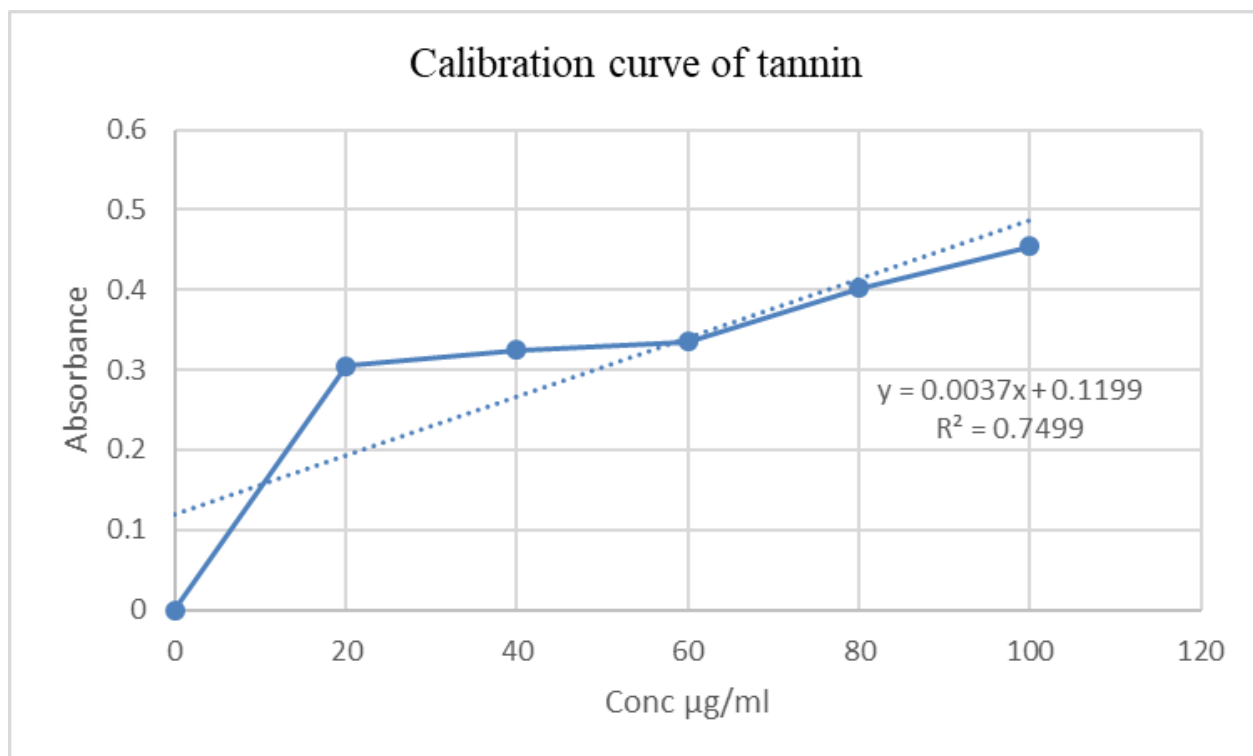
Quantitative estimation of phenolic was done by Folin- cio calteu method using gallic acid as standard. The total gallic acid equivalent in *C.rotundifolia*frj was found to be **438 mg GAE/g** (Fig 8)

Determination of tannic acid equivalent in *C.rotundifolia*frj

Various concentrations (0 to 100 µg/ml) of *C.rotundifolia*frj leaf were treated with Folin Denis, which produce blue colour and the absosrbance was observed and displayed in table 9 and fig 27.

Table 9: Determination of Tannic acid equivalent in *Cissus rotundifolia*

S.No.	Concentration (µg/ml)	Absorbance of tannic acid	Absorbance of <i>C.rotundifolia</i>
1	0	0	0
2	20	0.305	0.108
3	40	0.325	0.132
4	60	0.335	0.156
5	80	0.402	0.181
6	100	0.454	0.196
		TAE	206 mg TAE/g

Fig 27: Calibration curve of tannic acid

Quantitative estimation of tannin was done by Folin denis assay using tannic acid as standard. The total tannic acid equivalent in *C.rotundifolia*frj was found to be **206 mg TAE/g (Fig 9)**

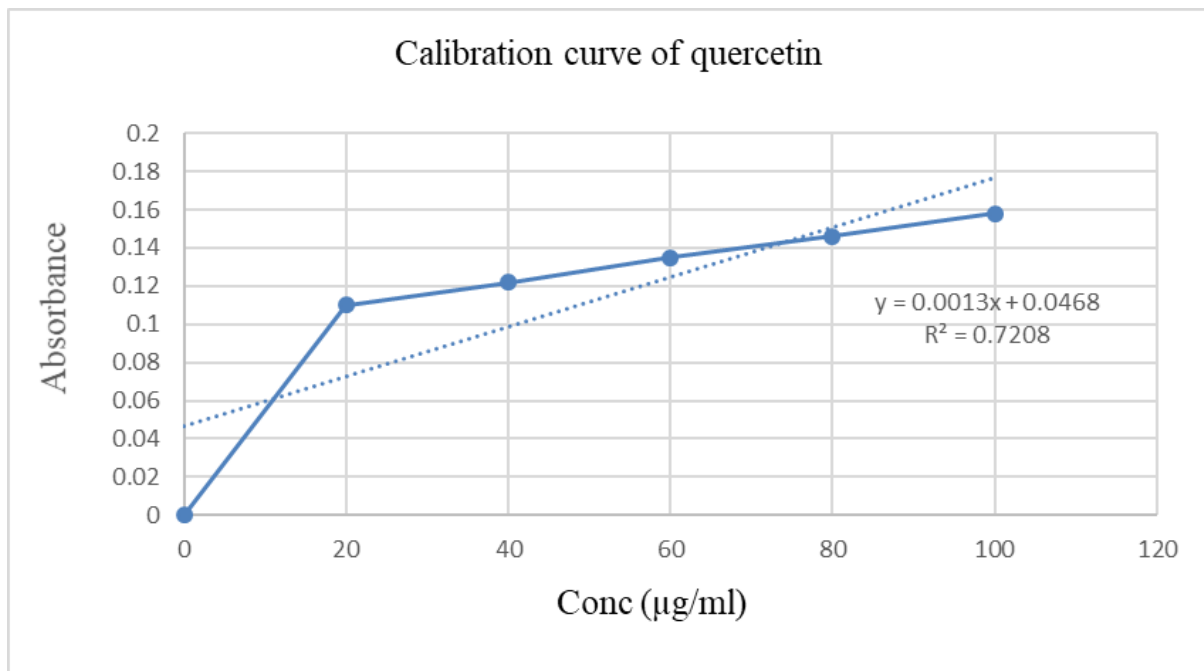
Determination of quercetin equivalent in *C.rotundifolia*frj

Various concentrations (0 to 100 µg/ml) of *C.rotundifolia*frj leaf were treated with aluminium chloride colorimetric method using quercetin as the standard, which produce yellow colour and the absorbance was observed and displayed in table 10 and fig 28

Table 10 : Determination of Quercetin equivalent in *Cissus rotundifolia*

S.No.	Concentration (µg/ml)	Absorbance of Quercetin	Absorbance of <i>C.rotundifolia</i>
1	0	0	0
2	20	0.110	0.099
3	40	0.122	0.109
4	60	0.135	0.118
5	80	0.146	0.157
6	100	0.158	0.177
		QE	655.4 mg QE/g

Figure 28: Calibration curve of Quercetin



Quantitative estimation of flavonoid was done by aluminium chloride method using quercetin as standard. The total tannic acid equivalent in *C.rotundifolia* fr was found to be **655.4 mg QE/g** (Fig 9)

Phenetics

There are two primary accepted species of succulent *Cissus* extensively cited in regional ethnomedicinal texts. These are taken as the botanical sources of Arabian Wax Leaf / Arabian Grape Ivy in many texts of desert flora and traditional medicinal plants source.

- *Cissus quadrangularis* L
- *Cissus rotundifolia* (Forssk.) Vahl

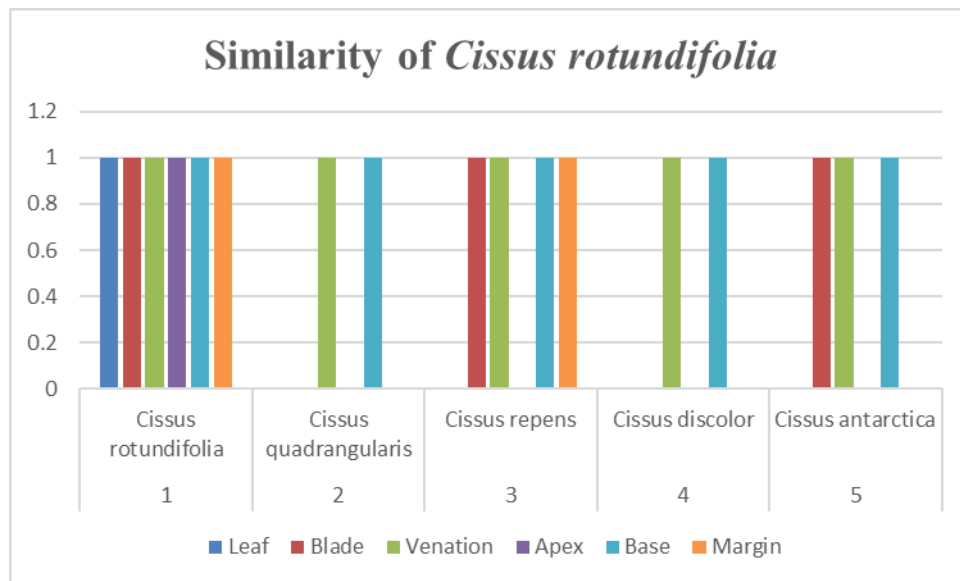
Review from 11 floras shows that *Cissus rotundifolia* and *Cissus quadrangularis* are maximum mentioned botanical sources among succulent Vitaceae. Still, an additional third variety has been mentioned by some regional African and Arabian Peninsula floras.

- *Cissus repens*
- *Cissus cactiformis*

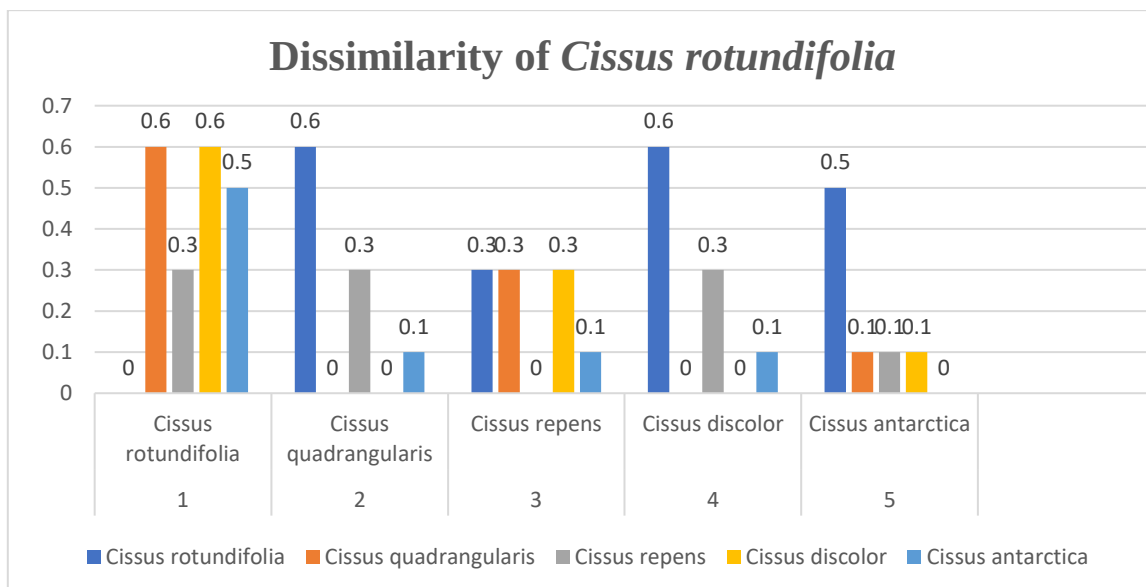
Table 11: Table of similar *Cissus rotundifolia* characters with other four species of *Cissus*

S.No	Species	Leaf	Blade	Venation	Apex	Base	Margin
1.	<i>Cissus rotundifolia</i>	1	1	1	1	1	1
2.	<i>Cissus quadrangularis</i>	0	0	1	0	1	0
3.	<i>Cissus repens</i>	0	1	1	0	1	1
4.	<i>Cissus discolor</i>	0	0	1	0	1	0
5.	<i>Cissus antarctica</i>	0	1	1	0	1	0

1-Present, 0-Absent

Fig 29: Chart of similarity characters of *Cissus rotundifolia* leavesTable 12: Table of dissimilar *Cissus rotundifolia* characters with other four species of *Cissus*

S.No	Species	<i>Cissus rotundifolia</i>	<i>Cissus quadrangularis</i>	<i>Cissus repens</i>	<i>Cissus discolor</i>	<i>Cissus antarctica</i>
1	<i>Cissus rotundifolia</i>	0	0.6	0.3	0.6	0.5
2	<i>Cissus quadrangularis</i>	0.6	0	0.3	0	0.1
3	<i>Cissus repens</i>	0.3	0.3	0	0.3	0.1
4	<i>Cissus discolor</i>	0.6	0	0.3	0	0.1
5	<i>Cissus antarctica</i>	0.5	0.1	0.1	0.1	0

Fig 30 : Chart of Dissimilarity characters of *Cissus rotundifolia* leaves

Sample matching coefficient $SSM = NS / (NS + ND) \times 100$

Where,

NS= Number of similarity characters

ND= Number of dissimilarity characters

NS=11; ND=13

The matching coefficient of *Cissus rotundifolia* with respect to other species was found to be **46%**.

The numerical taxonomic analysis of the genus *Cissus* evaluated six primary vegetative characters—leaf presence, blade form, venation pattern, apex architecture, base structure, and margin type—across five selected species: *Cissus rotundifolia*, *Cissus quadrangularis*, *Cissus repens*, *Cissus discolor*, and *Cissus antarctica*. The objective was to delineate morphological boundaries and quantify phenotypic relationships among succulent Vitaceae members frequently cited in regional ethnomedicinal texts. The resulting matching coefficient of 46% demonstrates moderate overall phenetic convergence among these *Cissus* species.

CONCLUSION

The present study established the pharmacognostical and phenetic characteristics of *Cissus rotundifolia* leaves, representing the first scientific report of its kind. The morphological, microscopical, histochemical, fluorescence, phenetic, quantitative, and physicochemical studies revealed distinctive diagnostic features useful for the identification and quality evaluation of the plant material. Phytochemical screening and quantitative estimation confirmed the presence of important bioactive constituents, particularly phenolics, tannins, and flavonoids. Overall, the findings provide valuable parameters for the authentication and standardization of *C. rotundifolia* leaves and support further phytochemical and pharmacological investigations.

ACKNOWLEDGEMENT

The authors sincerely express their gratitude to Dr. Venkata Rathina Kumar, M.Pharm., Ph.D., Principal, and Prof. Dr. L. Arul Sundares Kumar, M.S. (ENT), Dean, for their support, guidance, and encouragement throughout this research work.

REFERENCE

1. Alqahtani J, Formisano C, Chianese G, Luciano P, Stornaiuolo M, Perveen S, et al. Glycosylated phenols and an unprecedented diacid from the Saudi plant *Cissus rotundifolia*. *J Nat Prod*. 2020;83(11):3298-3304. doi:10.1021/acs.jnatprod.0c00597.
2. Onyechi UA, Ibeanu VN. Effects of diets containing *Cissus rotundifolia* flour on lipid profile of rats and postprandial glucose levels of normoglycemic human adults. *Afr J Biotechnol*. 2016;15(14):557-564. doi:10.5897/AJB2015.14677.
3. Al-Fatimi M, Wurster M, Schröder G, Lindequist U. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *J Ethnopharmacol*. 2007;111(3):657-666. doi:10.1016/j.jep.2007.01.018.
4. Alshawsh MA, Mothana RA, Al-Shamahy HA, Alsillami SF, Lindequist U. Assessment of antimalarial activity against *Plasmodium falciparum* and phytochemical screening of some Yemeni medicinal plants. *Evid Based Complement Alternat Med*. 2009;6(4):453-456. doi:10.1093/ecam/nem148.
5. Mziray AM, Maina CI, Kaingu CK. Anti-fertility properties of *Cissus rotundifolia* (Forssk.) Vahl extract using female Wistar rats. *Discovery Phytomedicine*. 2020;7(2):58-64. doi:10.15562/phytomedicine.2020.120.
6. Said A, Aboutabl EA, Melek FR, Abdel Jaleel Raheem Abdel Jaleel G, Raslan M. Phytoconstituents profiling of *Cissus rotundifolia* (Forssk.) Vahl by HPLC-MS/MS, and evaluation of its free radical scavenging activity (DPPH) and cytotoxicity. *Trends Phytochem Res*. 2018;2(2):65-74.
7. Al-Bukhaiti WQ, Noman A, Mahdi AA, Ali AH, Abed SM, Rashed MM, et al. Profiling of phenolic compounds and antioxidant activities of *Cissus rotundifolia* (Forssk.) as influenced by ultrasonic-assisted extraction conditions. *J Food Meas Charact*. 2019;13(1):634-647. doi:10.1007/s11694-018-9976-0.
8. Korish M. Nutritional evaluation of wild plant *Cissus rotundifolia*. *Ital J Food Sci*. 2016;28(1):43-49. doi:10.14674/1120-1770/ijfs.v456.
9. Al-Bukhaiti WQ, Noman A, Mahdi AA, Abed SM, Ali AH, Mohamed JK, et al. Proximate composition, nutritional evaluation and functional properties of a promising food: Arabian wax *Cissus* (*Cissus rotundifolia* Forssk.) leaves. *J Food Sci Technol*. 2019;56(11):4844-4854. doi:10.1007/s13197-019-03947-8.
10. Hegazy AK, Mohamed AA, Ali SI, Alghamdi NM, Abdel-Rahman AM, Al-Sobeai S. Chemical ingredients and antioxidant activities of underutilized wild fruits. *Heliyon*. 2019;5(6):e01874. doi:10.1016/j.heliyon.2019.e01874.
11. Ramachandran VS, Baluprakash T, Udhayavani C, Kannan M, Gopinath K. *Cissus rotundifolia* (Forsk.) Vahl (Vitaceae) – a potential ornamental climber. *J Hortic*. 2017;105:161-164.
12. Anonymous authors, Quality control methods for medicinal plant materials. 1998, World Health Organization Geneva. pp. 910-20 16.
13. Kokoshi CJ and Kokoshi RJ, Sharma PJ. Fluorescence of powder vegetables drugs under UV radiation. *J Am. Pharm. Assoc*. 47,1958,715-717.

14. Anonymous authors, The Ayurvedic pharmacopoeia of India. Edn 1, vol. 2, Government of India, New Delhi, 1986, pp. 189-196.
15. Bentley and driver's Text book of pharmaceutical chemistry. Edn. 8, Revised by L.M. Atherden, 1961, pp. 174-176.
16. The Pharmacopoeia of India. Edn 3, 1985, 245.
17. J.B. Harbone. Phytochemical methods A guide to modern techniques of plant analysis. Edn 3, 1998.
18. Seo, Ji-Hye, Jung-Eun Kim, Jung-Hyun Shim, Goo Yoon, Mi-Ae Bang, Chun-Sik Bae, Kyung-Jin Lee, Dae-Hun Park, and Seung-Sik Cho. HPLC Analysis, Optimization of Extraction Conditions and Biological Evaluation of *Corylopsis coreana* Uyeki Flos. *Molecules*. 2016; 21(1): 94.
19. [(accessed on 21 December 2020)]; Available https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf.
20. Singleton VL, Orthofer R, Lamuela-Raventos R.M. Analysis of Total Phenols and other Oxidation Substrates and Anti-oxidants by means of Folin-Ciocalteu reagent Methods. *Enzymol.* 1999; 152-178.
21. Boham AB, Kocipai AC. Flavonoids and condensed tannin from leaves of Hawaiian *vaccinium vaticulum* & *Viocalycinium*. *Pacific science*. 48, 1974, 458-463.
22. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal.* 2002; 10(3): 178-182.
23. Ashwini Kumar and Baidyanath Kumar 2016. Biosystematic Studies in *Heliotropium indicum*, *Trichodesma indicum* and *T. zeylanicum* of Boraginaceae. *Int.J.Curr.Microbiol.App.Sci.* 5(4), 2016, 720
24. Ramachandran VS, Baluprakash T, Udhayavani C, Kannan M, Gopinath K. *Cissus rotundifolia* (Forsk.) Vahl (Vitaceae) – a potential ornamental climber. *J Hort.* 2017; 105: 161-164.