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Pharmacognostical, Phenetics, Preliminary Phytochemical Investigation and DNA Barcode of Clerodendrum Inerme.

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ABSTRACT

Background

Clerodendrum inerme, belongs to Lamiaceae. It is traditionally refers to Sangakuppi & Anjali by Tamilnadu peoples. The plant native to India, China, Bangladesh, Taiwan, Myanmar and Africa. Tribal communities of Anantapur district, Andhrapradesh used the plant to treat the skin disease, atopic rhinitis and oedema.

Materials and methods

Leaves were collected from Ari nursery garden, Madakkulam, Madurai district, Tamilnadu in the month of February 2026. It was identified and authenticated by Dr. Stephen Assistant professor (Retd), Dept of Botany, American college, Madurai. The leaves were collected and Pharmacognostical studies includes Macroscopical, Microscopical, Powder analysis and DNA Barcoding were determined. Leaves were collected, washed with water, shade dried, powdered, extracted with hydro alcohol, extract was concentrated and was subjected to preliminary phytochemical screening, Limit of quantification (LOQ), Limit of detection (LOD), Hydroalcoholic extract of *Clerodendrum inerme* leaf (HAECI_L) was used for evaluation of In-Vitro Anti-oxidant studies.

Results and discussion

Pharmacognostical Macroscopical studies showed that leaves were arranged in opposite, acute apex, short, sturdy petiole, entire margin with reticular venation. Microscopical studies of leaf showed single layered upper and lower epidermis with barrel shaped cells, few rosette and prismatic crystals were present. Petiole appeared with plano-convex shape, non-glandular trichomes, Calcium oxalate crystals were present. Quantitative estimation of microscopy reported for leaf such as epidermal number(108cells/mm²), stomatal number(12 cells/mm²), stomatal index(10 cells/mm²), vein islet(cells/mm²), vein-termination (6 cells/mm²) and palisade ratio(15 cells/mm²). Powder microscopy showed covering of trichome, petiole, anomocytic stomata, reticular vessels, prismatic and rosette crystals. Physiochemical parameters such as foreign organic matter, loss on drying, total solid content, extractive values with petroleum ether, ethyl acetate, chloroform, ethanol and water showed results of 20.9%w/w, 28%w/w, 29.25%w/w, 40.9%w/w and 32.7%w/w respectively. Ash values includes total ash, acid insoluble ash and water soluble ash and showed values of 11.20%w/w, 8.75%w/w and 4.05%w/w respectively. Foaming index was found to be 1000. DNA barcoding was determined showed the sequences of nucleotides that complies within the acceptable limits 71 out of 80. Phenetics were done to find similarities and dissimilarities, Sample matching Coefficient proved 48%.

HAECI_L was prepared by maceration technique and percentage was found to be 25.75%w/v. Phytochemical screening were done by HAECI_L and showed the presence of flavonoids, phenols, tannins, sterols, triterpenoids, reducing sugar, quinone and coumarins. LOD & LOQ were performed and the result was observed as 36.3 µg/ml & 110µg/ml respectively. HAECI_L checked for quantitative estimation of flavonoid, phenol and tannin equivalent and was estimated as 590QE/g, 509GAE/g & 633TAE/g respectively.

Conclusion

The first article reported the Phenetics, DNA barcode were collected from Madurai district, Tamilnadu. The pharmacognostical, phytochemical and quantitative estimation showed the plant characteristics and support for future reference article.

INTRODUCTION

Clerodendrum inerme (Linn)GAERTN belongs to *Lamiaceae.*, is widely distributed in tropical regions of India, Bangladesh, Southeast asia, Srilanka and Mediterranean. Certain Communities of Anantapur district of Andhrapradesh used the leaves for treating elephantiasis and atopic rhinitis (1).In Peoples of Taiwan leaves were utilised for treating skin disease (2).

People of China consume decoction from leaves to reduce beriberi and skin disease (3). Few Communities of African countries utilised the leaf for mitigation of skin disease (4). Inhabitants of Myanmar used leaves and roots preparations to manage fever, asthma and venereal infections (5). Natives of Bangladesh administer the plants leaves and flowers formulation to treat night blindness, pneumonia and rheumatoid arthritis (6). The phytochemical literature revealed the presence of flavonoids, terpenoids, phenolics and iridoid glycosides(7). *Clerodendrum inerme* leaves showed immunomodulatory effect, anti-pyretic, anti-bacterial, anti-viral, anti-fungal, anti-helminthic, anti-hypertensive, hepatoprotective, anti-diabetic(8-16).The present study investigate the pharmacognositcal paramters of leaf such as macroscopical, microscopical, powder analysis, DNA barcode, phenetic studies involves similarities and dissimilarities of *Clerodendrum inerme*. Preliminary phytochemical analysis involves physio-chemical qualitative analysis. Quantitative estimation of phytoconstituents.

MATERIALS & METHODS

1. Plant Collection And Authentication

Leaves were collected from Ari nursery garden, Madakulam Madurai district, Tamil Nadu in the month of January 2026. The leaves was identified and authenticated by Dr.Stephen, Assistant Professor(Retd), Department of Botany, American medical college, Madurai-625 002. The herbarium of this specimen was kept in the department for further reference.

Fresh leaves were subjected to pharmacognostical studies includes organoleptic and morphological studies.

2. Macroscopical Studies

Leaves were studied separately for its morphological characters by organoleptic test includes size, shape, texture, colour(17,18)

3. Microscopical Studies

Sample was preserved in fixative FAA for more than 48 h. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with 0.8% Safranin and 0.5% Astra blue. Transverse sections were photographed using Axiolab5 trinocular microscope attached with Zeiss Axiocam208 color digital camera under bright field light. Magnifications were indicated by scale bar(17,18).

Quantitative Microscopy

Determination Of Phyto -Constants

The important identifying characteristic of leaf constants like Stomatal number, Stomatal Index, Vein-islet number, Vein termination number, palisade ratio were found out.[Ayurvedic Pharmacopoeia.,2004]

Preparation of Leaf Powder

The leaves were collected, shade dried and coarsely powdered was sieved and stored in a well closed container.

Powder Analysis

A pinch of the powdered sample was mounted on a microscopic slide with a drop of 50% glycerol after clearing with saturated solution of chloral hydrate. Sample was treated with iodine solution to confirm the presence of starch grains. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss ERc5s digital camera under bright field light. Photomicrographs of diagnostic characters 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 365 nm, following standard procedure(19)

Physio-chemical parameters

The powder was subjected to physiochemical parameters such as foreign organic matter, loss on drying, ash value, and extractive value with different solvents in increasing order of polarity.[WHO guidelines.,2004](20)

Determination of Foreign Matter

A thin layer of precisely weighed 10g of air-dried coarse drug was spread out. The sample drug was examined using a 10x lens or the unaided eye, and the foreign organic matter was manually separated as thoroughly as possible before being weighed. The percentage of foreign organic matter was computed using the drug's weight.

Determination of Foaming Index

Foaming index determined by 1g of coarse powder taken in conical flask, add 100ml of water, then boiling for 30mins. Cool and filter it, then dilute upto 100ml using water. Transfer this decoction into 10 test tube from 1ml to 10ml, then dilute it upto 10ml using water. Stopper the tube, then shake for 15seconds, 2 shakes per seconds. Keep it aside for 15mins and measure the height of foam.

$$\text{Foaming index} = 1000/a$$

Where a = volume of ml of decoction used for preparing the dilution in tube where foaming height is 1cm observed(21).

DNA Barcoding

Genomic DNA Isolation

About 100 mg of plant tissue was ground with liquid nitrogen to a fine powder using a mortar and pestle. 1 ml of preheated (65°C) CTAB extraction buffer and 20 µl of β-mercaptoethanol were added to the mortar, and the mixture was finely ground. The contents were transferred into a 2 ml centrifuge tube and incubated for 20 to 30 min at 65°C in a water bath. After centrifugation at 12,000 rpm for 10 min in a refrigerated centrifuge (Eppendorf 5418R), the transparent supernatant was transferred to a fresh centrifuge tube, and an equal volume of chloroform: isoamyl alcohol (24:1) was added. The mixture was gently inverted until an emulsion formed. The tubes were centrifuged at 13,000 rpm for 12 min. The clear aqueous phase was transferred to fresh centrifuge tubes, and an equal volume of ice-cold isopropanol was added. The sample is incubated for 30–60 minutes (or overnight for maximum yield) and then centrifuged at 12000 rpm for 3 min. The supernatant was discarded, and the pellet was washed with 70% ethanol. DNA pellets were air-dried at room temperature by leaving the centrifuge tubes uncovered. The pellet of genomic DNA was suspended in 20 to 30 µl of Tris-EDTA (T10E1) buffer.

Qualitative and Quantitative analysis of DNA

The quality and concentration of genomic DNA was checked by electrophoresing the DNA sample on 1% agarose gel. The DNA concentrations were rechecked by visual assessment of band intensity under a UV transilluminator (Biorad, GelDoc Go, USA). The concentration of DNA in 1 µl of aliquot was checked using Nanodrop (Thermo Scientific, Nanodrop One, USA).

PCR Amplification

The DNA barcode candidate ITS was used for PCR amplification. The isolated DNA was used as a template for the PCR reaction in a thermocycler (Applied Biosystem, Veriti™, USA). The PCR products were then loaded onto 1% agarose gel, and the amplification was confirmed.

Sequence analysis and submission to NCBI

The nucleotides sequence in FASTA format was copied from the chromatogram using Finch TV (v1.4.0). The FASTA was fed into the Basic Local Alignment Search Tool (BLAST) algorithm of NCBI to identify the closest matching sequence in GenBank's nucleotide database. After confirmation of the species, the sequence was submitted to NCBI with the necessary details to obtain a GenBank ID. The sequence was converted to a Barcode using the Bio-Rad barcode generator (22-24)

PHENETICS STUDIES

Phenetics is a method of biological classification that groups organisms based on their overall similarities and differences. It is also known as **numerical taxonomy** because mathematical and statistical methods are used to compare organisms. Unlike phylogenetic classification, phenetics does not primarily consider evolutionary relationships or common ancestry. Instead, it evaluates a large number of observable characteristics, including morphological, anatomical, physiological, biochemical, and molecular features. Each character is assigned a numerical value, and the data are analyzed using computers or statistical techniques. Organisms showing a high degree of similarity are placed together in the same group. Similarity coefficients and clustering methods are commonly used to construct phenograms, which represent the degree of similarity among organisms. Phenetics provides an objective and systematic approach to classification and reduces dependence on individual taxonomic judgment. It has been particularly useful in microbial taxonomy and comparative studies. However, its major limitation is that overall similarity may not always accurately represent evolutionary relationships(25).

PHYTOCHEMICAL STUDIES

Preparation of extracts

Preparation of Hydroalcoholic extract of *Clerodendrum inerme*. collect leave part of *Clerodendrum inerme* (L). dried and make a coarse powder. The powder will soak into ethanol and water, kept aside for 72 hrs by maceration technique. then filter the solution and make concentrate of extract.

Qualitative estimation

Preliminary phytochemical screening for HAECI_L

Hydro-alcoholic extract subjected to qualitative chemical analysis. The various chemical tests were performed on this extract for the identification of phenol, tannin, sterols, and saponin by using the standard procedure(26).

Quantitative estimation for HAECI_L

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) .

The lowest analyte concentration in a sample that may be identified and measured is known as the limit of quantification (LOQ) of a particular analytical method. C.inerme were produced and scanned in the 200–700 nm UV range. C.inerme were examined to determine whether a peak was present, and the associated wavelength was noted. C.inerme was then generated at concentrations ranging from 0 to 10 µg/ml and analysed using a Shimadzu UV-1900i UV-Vis spectrophotometer at the wavelength determined by the C.inerme.

The response's standard deviation and the slope obtained from the C.inerme were used to get the LOD and LOQ values(27).

Quantitative estimation of HAECI_L

Determination of Gallic acid (Phenol) equivalent for HAECI_L

Principle

Total phenolics content of the various concentrations of extract was determined by Folin ciocalteu reagent method. The hydroxyl group (OH) of phenolic compounds reduces the phosphomolybdic acid to molybdenum blue in the presence of alkaline medium (present in Folin reagent). The blue coloured complex was then spectrophotometrically measured at 760nm. Instrument UV Visible spectrophotometer. Shimadzu (Model 1800.) Reagents required - Folin-Ciocalteu Reagent (IN), Sodium carbonate solution (10%) Standard Gallic acid solution.

Procedure

About 1 mL (1mg/ml) of extract, 0.5mL of Folin-ciocalteu reagent (IN) were added and allowed to stand for 15 minutes. Then 1 ml of 10% sodium carbonate solution was added to the above solution. Finally the mixtures were made up to 10 ml with distilled water and allowed to stand for 30 minutes at room temperature and total phenolic content was determined spectrophotometrically at 760nm wavelength. The calibration curve was generated by sample was used as blank. Total phenolic content of extract is expressed in terms of mg of Gallic acid equivalent per gm of extract (mg GAE/g)(28).

Determination of Tannic acid (tannin) equivalent for HAECI_L

Principle

Total tannin content of extract was determined by Folin Denis reagent method. Tannin like compounds reduces phosphor tungsto molybdic acid in alkaline solution to produce a highly coloured blue solution. The intensity of which is proportional to the amount of tannins and it was estimated by spectrophotometer at 760 nm. Instrument UV-Visible spectrophotometer. Shimadzu (Model 1800). Reagents required - Folin Denis Reagent, Sodium carbonate solution (10%), Standard tannic acid solution.

Procedure

0.2 mL of (1 mg/ml) extract, was made up to 1 mL with distilled water. Then add 0.5 mL. of Folin Denis reagent and allowed to stand for 15 mins, then 1 mL of sodium carbonate solution was added to the mixture and it was made up to 10 mL. with distilled water. The mixture was allowed to stand for 30 mins at room temperature and the tannin content was determined spectrophotometrically at 760 nm. The calibration curve was generated by Preparing tannic acid at different concentration (2, 4, 6, 8µg/mL). The reaction mixture without sample was used as blank. The total tannin content in the leaf extract was expressed as milligrams of tannic acid equivalent per gm of extract(28).

Determination of Quercetin (Flavonoid) equivalent for HAECI_L

Principle

Flavonoids present in the extract form, a charge transfer complex with several heavy metals to give a characteristic color. In this reaction, the high electron positive nature of aluminium attracts the atomic nuclei of the aromatic rings in the flavonoids. Then it will react with potassium acetate in alkaline medium to form a pink coloured complex that is measured spectrophotometrically at 460 nm. Instrument UV Visible spectrophotometer, Shimadzu (Model 1800) Reagents required - 10% Aluminium chloride, 1M Potassium acetate, Standard Rutin.

Procedure

1ml of extract, 0.1 ml of aluminium chloride solution, 0.1 ml. of potassium acetate solution and 2.8 ml. of ethanol were added and the final volume was then made up to 5 ml. with distilled water. After 20 min the absorbance was measured at 415 nm. A calibration curve was constructed by plotting absorbance reading of Rutin at different concentrations (10,15,20µg/mL). The sample without aluminium chloride was used as a blank. The total flavonoid content in the extract was expressed as milligrams of rutin equivalent per g of extract(29)

RESULTS AND DISCUSSION

PHARMACOGNOSTICAL STUDIES

Macroscopical evaluation

Clerodendrum inerme leaves has the simple leaf type, obovate leaflet shape, acute base, entire margin, reticular venation, short and sturdy petiole. The leaf's length was around 2-7cm and width around 0.5-5cm.

Table 1 – Macroscopical Characters of *Clerodendrum inerme* leaf

s.no	characters	Observation
1.	colour	Green
2.	Odour	Unpleasant
3.	Taste	bitter
4.	type	Simple
5.	shape	Obovate
6.	arrangement	Opposite
7.	apex	Obtuse to mucronate
8.	base	Acute
9.	petiole	Short and sturdy
10.	margin	Entire
11.	venation	Reticular
12.	texture	Smooth
13.	Dorsal surface	Smooth, dark green
14.	Ventral surface	Smooth, pale green
15.	petiole	1-2cm
16.	length	2-7 cm
17.	width	0.5-5 cm



Figure 1 – habitat of *C. inerme*

Figure 2- Dorsal view of *C.inerme*

Figure 3 – Ventral view of *C.inerme*

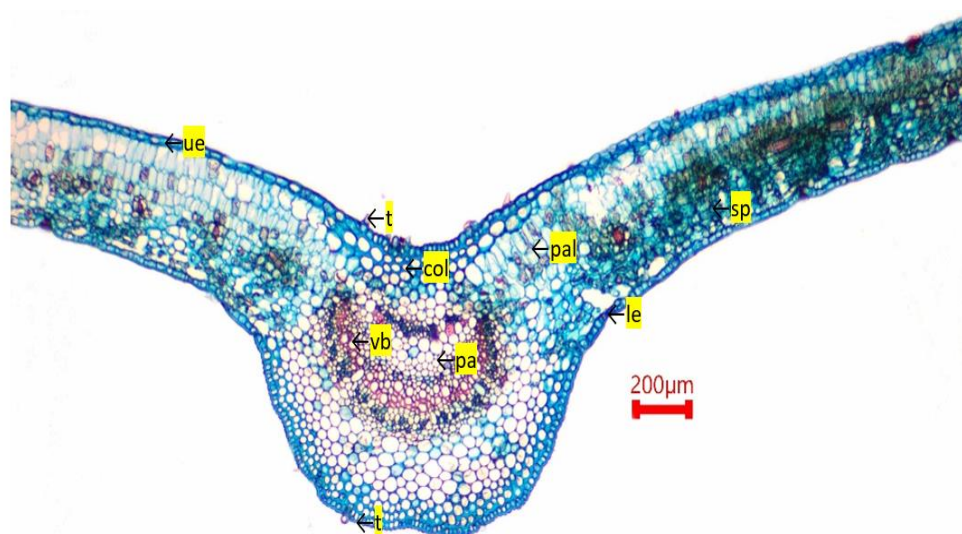
Microscopical Evaluation

Leaf

The leaf is dorsiventral and differentiated into a prominent midrib region and two lateral laminar wings. The midrib projects conspicuously towards the abaxial side while appearing slightly depressed on the adaxial side.

The upper and lower epidermis are single-layered and composed of compactly arranged, rectangular to barrel-shaped cells covered by a thin cuticle; a few covering trichomes are also present; subepidermal collenchyma cells, in 3 to 4 layers, are prominent in the midrib region, followed by parenchymatous ground tissue; a few cells are larger and vacuolated; a single prominent vascular bundle is present in the centre of the midrib, which is collateral, conjoint and arc-shaped, with xylem towards the inner side and phloem towards the outer side; xylem consists of vessels, tracheids, fibres and parenchyma; phloem consists of sieve elements, companion cells and parenchyma; the vascular bundle is surrounded by a parenchymatous bundle sheath; in the lamina region below the upper epidermis, two layers of elongated, cylindrical palisade parenchyma cells are present, followed by loosely arranged spongy parenchyma cells; veins traverse the mesophyll, and a few rosette and prismatic crystals are also present (Fig. 4).

Figure 4 . Microscopy of *Clerodendrum inerme* leaf

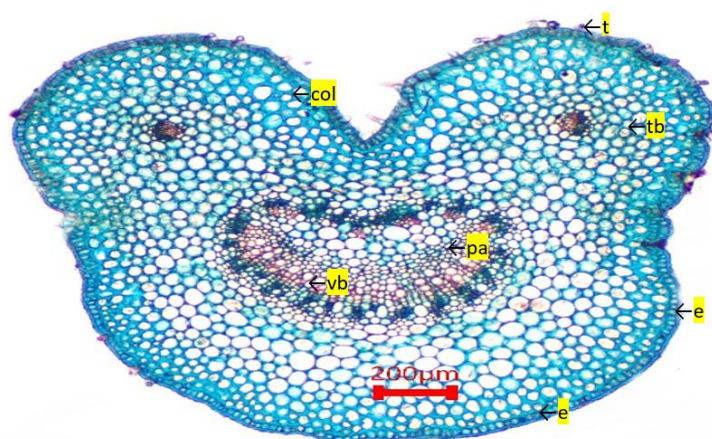


Margin portion enlarged view

bs - bundle sheath; col - collenchyma; cu - cuticle; gt - glandular trichome; le - lower epidermis; mes - mesophyll; og - oil globule; pa - parenchyma; pal - palisade cells; pcr - prismatic crystal; ph - phloem; rcr - rosette crystal; sp - spongy parenchyma; ue - upper epidermis; ve - vein; vb - vascular bundle; xy - xylem

Petiole

TS is broadly plano-convex to semicircular in outline, with a slightly flattened adaxial surface and a prominently convex abaxial surface and differentiated into epidermis, cortex, vascular tissue, and central ground tissue. The epidermis is uniseriate and composed of compactly arranged, rectangular to tangentially elongated cells covered by a distinct cuticle; numerous non-glandular trichomes are present; beneath the epidermis, the hypodermis consists of 2 to 4 layers of collenchymatous cells, especially prominent at the ridges and margins; the remaining cortical region is composed of several layers of thin-walled, polygonal to rounded parenchymatous cells with distinct intercellular spaces; central region of the petiole is composed of large ground tissue composed of thin-walled parenchyma cells; vascular system consists of a prominent central collateral vascular bundle and one smaller trace bundles located towards the lateral wings; bundles are arranged in an arc-shaped or crescentic manner and a parenchymatous bundle sheath surrounds each vascular bundle; phloem is composed of sieve elements, companion cells, and phloem parenchyma and occurs on the outer side of the vascular bundle; xylem occupies the inner side and consists of vessels, tracheids, xylem fibres, and xylem parenchyma; xylem elements are arranged radially, with the larger vessels situated towards the centre of the bundle; calcium oxalate crystals in the form of prism are distributed in the cortical and ground tissues; starch grains occur abundantly in the parenchymatous cells (Fig.5).

Figure 5- Microscopy of Clerodendrum inerme petiole**TS of petiole****Determination of Quantitative microscopy**

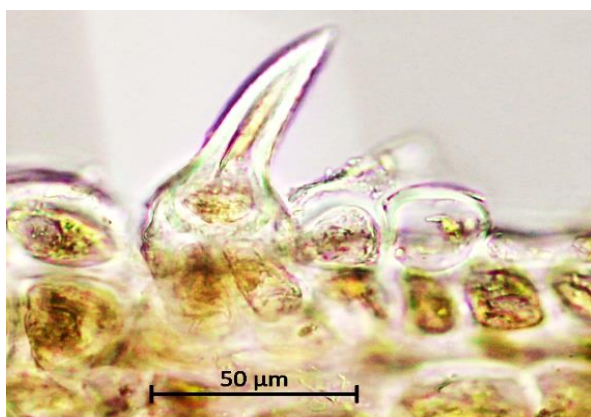
The quantitative parameters obtained during microscopic observation of epidermal peelings of leaf were recorded and the leaf with anomocytic stomata

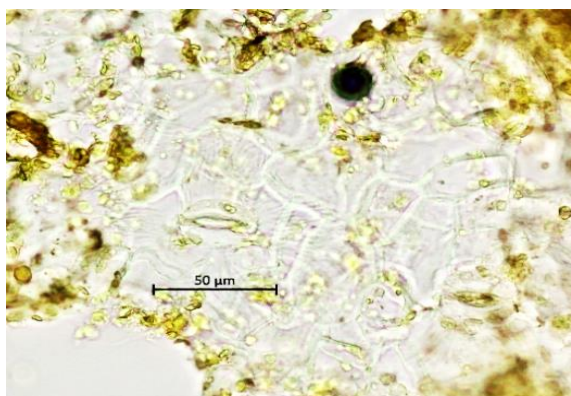
Table 2 - quantitative microscopy of Clerodendrum inerme leaf

parameters	Upper epidermis (cells/ mm ²)	Lower epidermis (cells/ mm ²)
Epidermal number	108-153	160-230
Stomatal number	12-17	16-23
Stomatal index	10-12	9-11
Palisade ratio	14-17	28-32
Vein islets number	3-7	4-9
Vein termination number	5-10	6-11

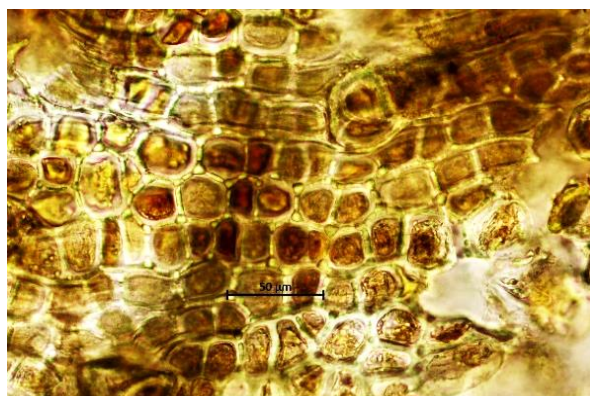
Powder analysis**Powder Microscopy**

The powder is greyish green in colour, with a characteristic odour and taste and shows the presence of covering trichomes, petiole and leaf epidermis with anomocytic stomata; spiral and reticulate vessels, prismatic and rosette crystals and oil globules (Fig. 6).

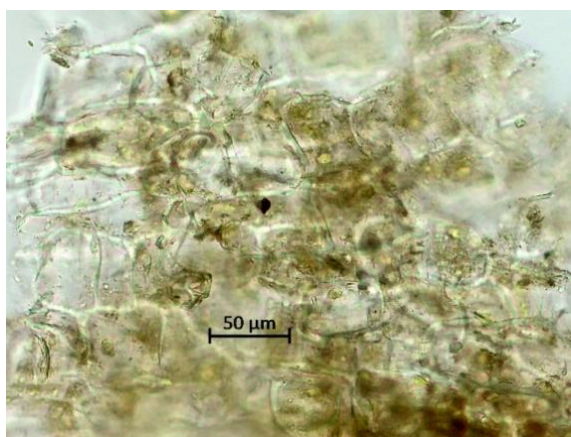
Figure 6- Powder microscopy of Clerodendrum inerme leaf**Trichome**



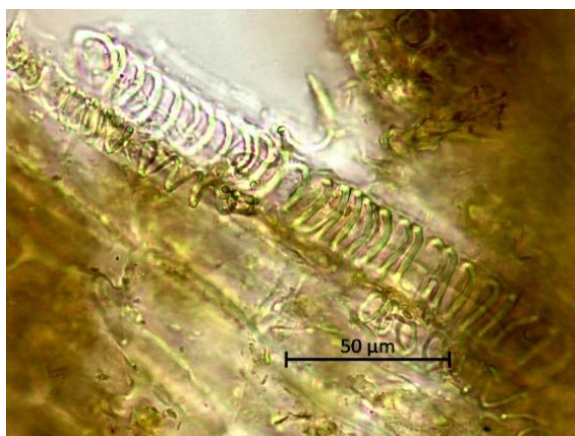
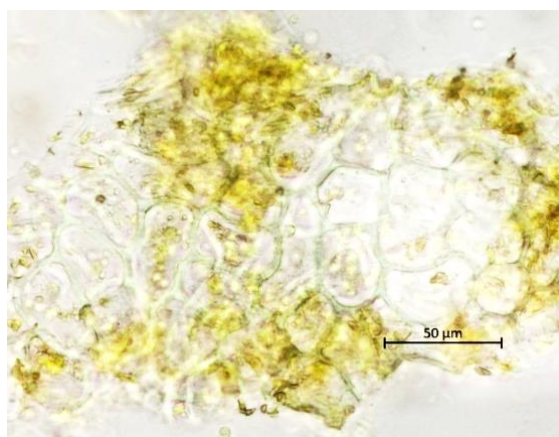
Petiole epidermis anomocytic stomata



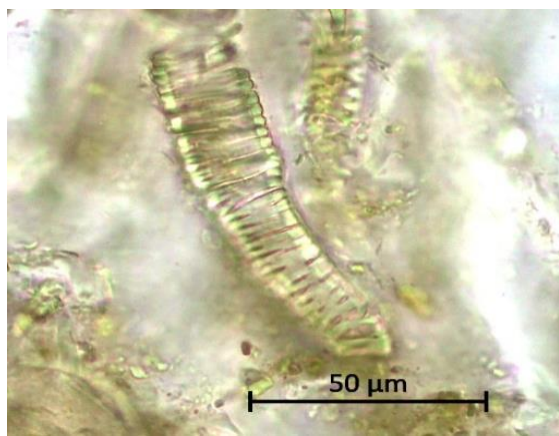
Epidermis is surface view



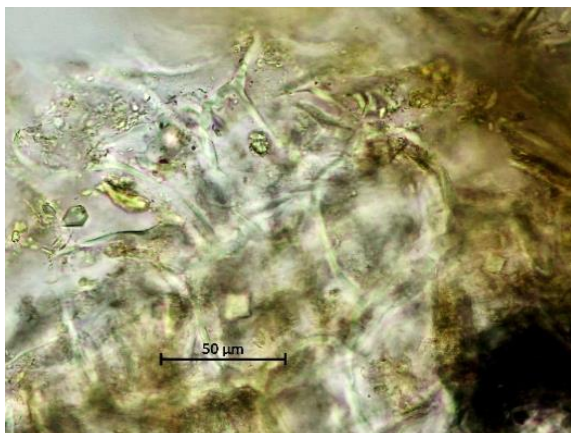
Leaf epidermis



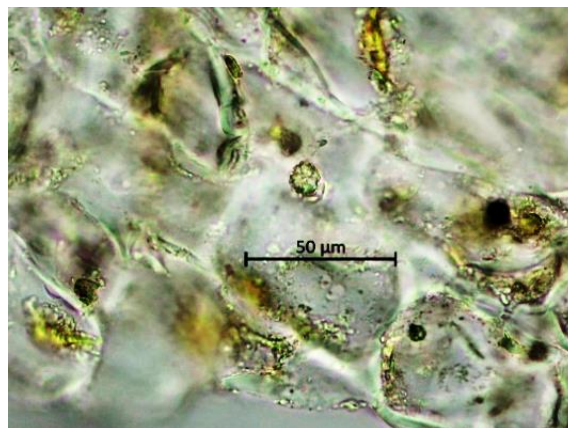
Spiral vessel



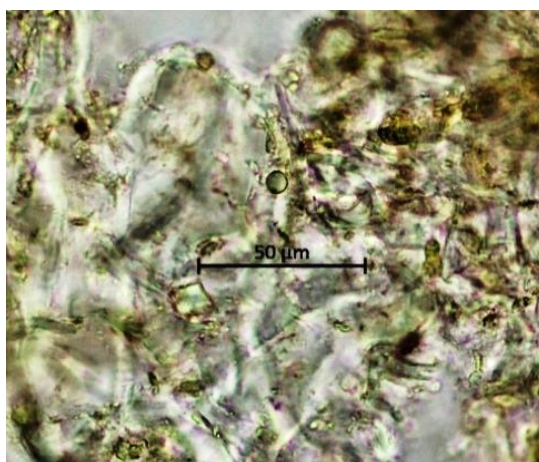
Reticulate vessel



Prismatic crystal



Rosette crystal



Oil globule

Behaviour of powder with various chemical reagents

The powdered leaf of *Clerodendrum inerme* was treated with various chemical reagents and viewed visible and UV (254nm and 365nm) with different fluorescnece the results were depicted in table 8.

Table 3- Behaviour of *Clerodendrum inerme* leaf powder with various chemical reagents

s.no	Sample and reagent	visible	UV light(254nm)	UV light(365nm)
1.	Powder + water	Brown	Black	brown
2.	Powder + con.HCl	Dark brown	Black	brown
3.	Powder + con.H ₂ SO ₄	Orange	Yellow	Grey
4.	Powder + HNO ₃	Orange	Yellow	Grey
5.	Powder + CH ₃ COOH	Brown	Black	brown
6.	Powder + con.HCl + Water	Brown	Black	brown
7.	Powder + con.H ₂ SO ₄ +water	Brown	Red	Green
8.	Powder + HNO ₃ + water	Yellow	Pink	Green
9.	Powder + CH ₃ COOH +water	Brown	Red	Pale green
10.	Powder + Aqueous KOH	Brown	Black	brown
11.	Powder + alcoholic KOH	Dark green	Red	Brown
12.	Powder + Aqueous FeCl ₃	Dark Brown	Red	Green

13.	Powder + Alcoholic FeCl ₃	black	Brick red	brown
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Determination of Physiochemical analysis of *Clerodendrum inerme* leaf powder

The physiochemical analysis was evaluated for *Clerodendrum inerme* leaf powder, and report is mentioned in table 4.

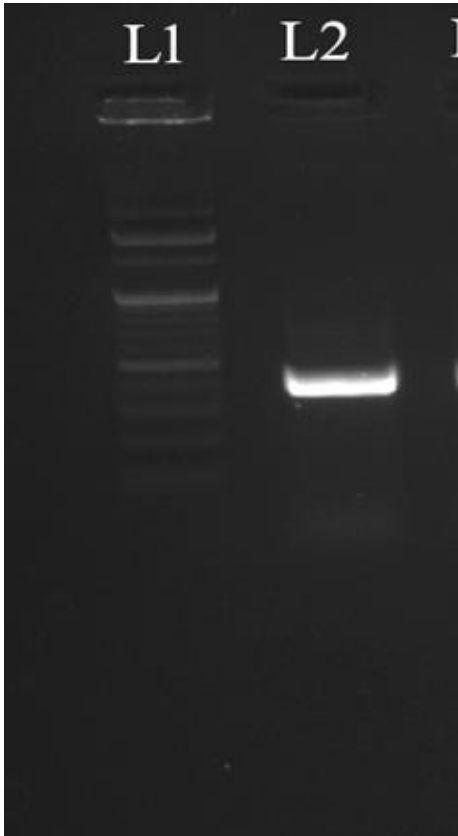
Table 4- determination of physiochemical parameters of *Clerodendrum inerme* leaf

s.no	Physiochemical parameters	Result
1.	Foreign matter	Nil
2.	Loss on drying	2.10±1.23%w/w
3.	Total solid	97.90 ±98.77%w/w
4.	Ethanol soluble extractive	40.9±2.4%w/w
5.	Ethyl acetate soluble extractive	28±1.56%w/w
6.	Petroleum ether soluble extractive	20.9±0.5%w/w
7.	Chloroform soluble extractive	29.25±2.56%w/w
8.	Water soluble extractive	32.7±1.9%w/w
9.	Total ash	11.20± 1.26 %w/w
10.	Acid insoluble ash	8.75 ± 1.03% w/w
11.	Water soluble ash	4.05±2.55 %w/w
12.	Inorganic elements	nil
13.	Foaming index	1000

DNA Barcoding

Genomic DNA was isolated from the authenticated sample, and its quality was assessed spectrophotometrically and by agarose gel electrophoresis. The genomic DNA concentration was 154.5, with an absorbance (A₂₆₀/A₂₈₀) ratio of 2.04, indicating insignificant levels of contaminating proteins and polysaccharides (Table 10). The PCR-amplified product, after electrophoresis, was subjected to gel documentation alongside a 100 bp DNA ladder (Fig.7). The sequence obtained is shown in Figs. 8; the sequence was converted to a barcode (Table 5), the BLAST hit is presented in Fig. 9, and the GenBank sequence submission details are given in Table 6.

Fig 7. Gel image of PCR amplified products



L1- 10kb ladder; L2 – *Clerodendrum inerme* leaf

Table 5. Quality check and quantification of DNA

Sample	Concentration in ng/μl	A _{260/280}	A _{230/260}
1660.04032601	154.5	2.04	1.48

71 nucleotide out of 80 are being within the acceptable limits, obtained sequence was determined as *Clerodendrum inerme*.

Figure 8. Chromatogram of 1660.04032601

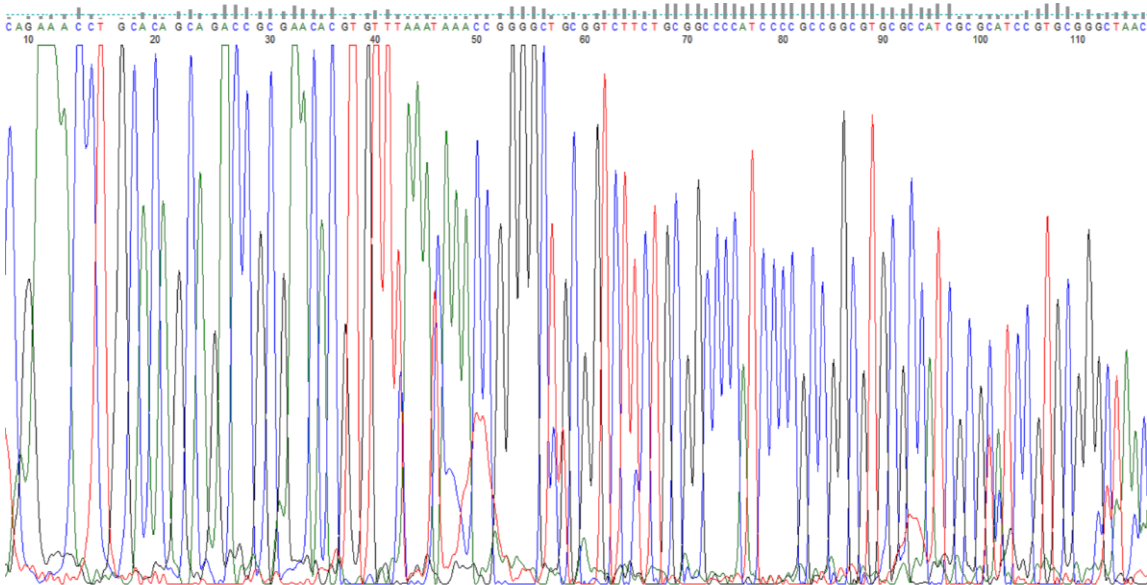


Figure 9. Blast Hit of 1660.04032601

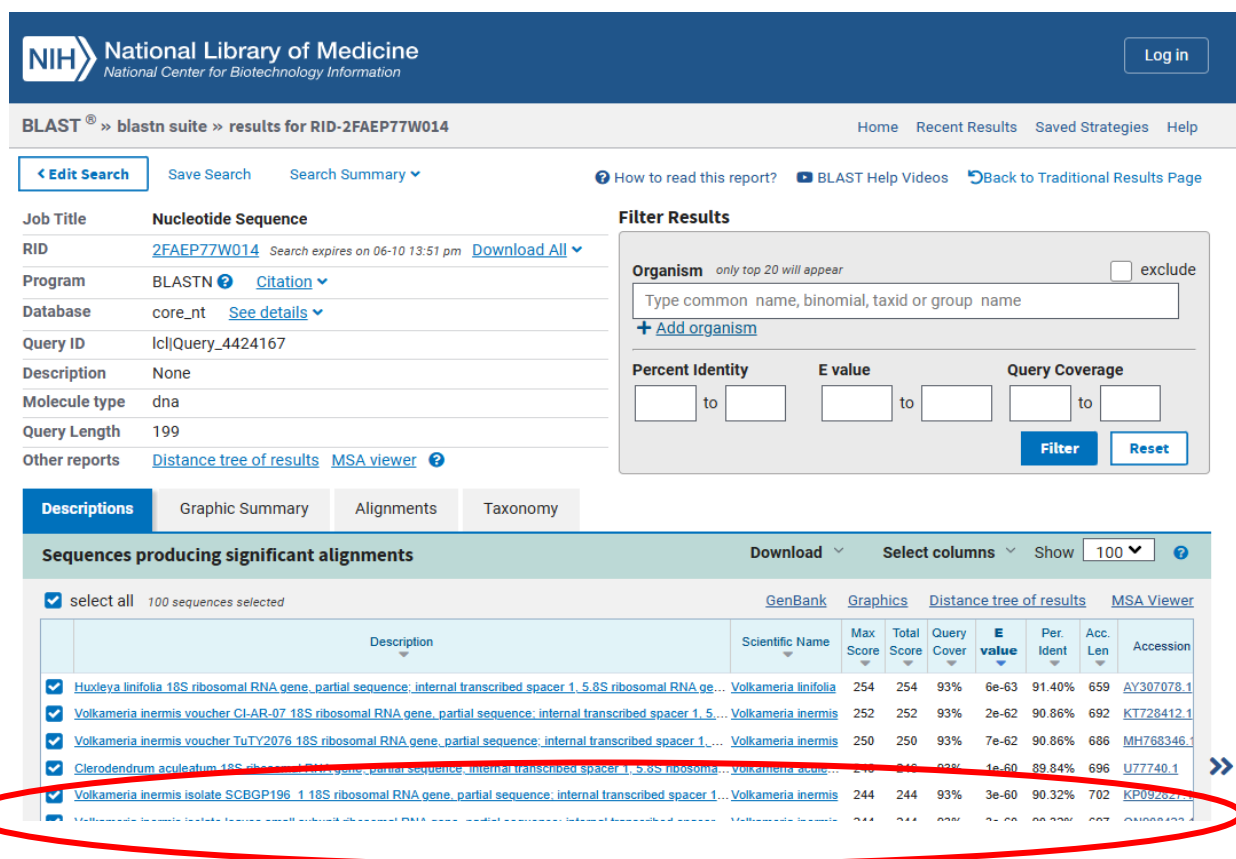


Table 6. Sequence obtained

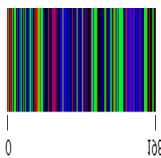
Sample	Sequence with Barcode
1660.04032601	ttagtgcagaaacctgcacagcagaccgcgaacacgtgtttaataaacggggctcgggtcttctcgggcccatccccgcggcgtgcgccatcgcgcatccgt gcgggctaacaataatcgggcggaatcgcgcaagggaatacacaacacgggtccctccctcgccccgtccgcgaaatcgtgggaaggt 

Table 7. GenBank Submission details

Submission number	Plant name	Voucher no.	Acce.No	Authors
SUB16225023	<i>Volkameria inermis</i>	SCRIPCOGVI109	PZ477855	Volkameria inermis voucher SCRIPCOGVI109 internal transcribed spacer r - Nucleotide - NCBI

PHENETICS

Taxometric analysis

The genus of *Clerodendrum* comprises approximately 6 accepted species, primarily distributed across India near sea. The recognized species include

The characters such as shape, venation, margin, apex, base are considered.

Leaf shape - obovate

Leaf venation -reticular

Leaf margin -entire

Leaf apex -obtuse to mucronate

Leaf base - acute to attenuate

Table 8- determination of similar character matrix of *Clerodendrum inerme*

species	shape	venation	margin	apex	base
<i>Clerodendrum inerme</i>	1	1	1	1	1
<i>Clerodendrum infortunatum</i>	1	1	0	0	0
<i>Clerodendrum buxifolium</i>	1	0	0	1	1
<i>Clerodendrum trichotomum</i>	1	0	1	1	0
<i>Clerodendrum japonicum</i>	1	0	0	1	0
<i>Clerodendrum erratamioids</i>	1	0	1	0	0

Present-1, absent-0

Fig 10- Chart for determination of similarities character of *Clerodendrum inerme*

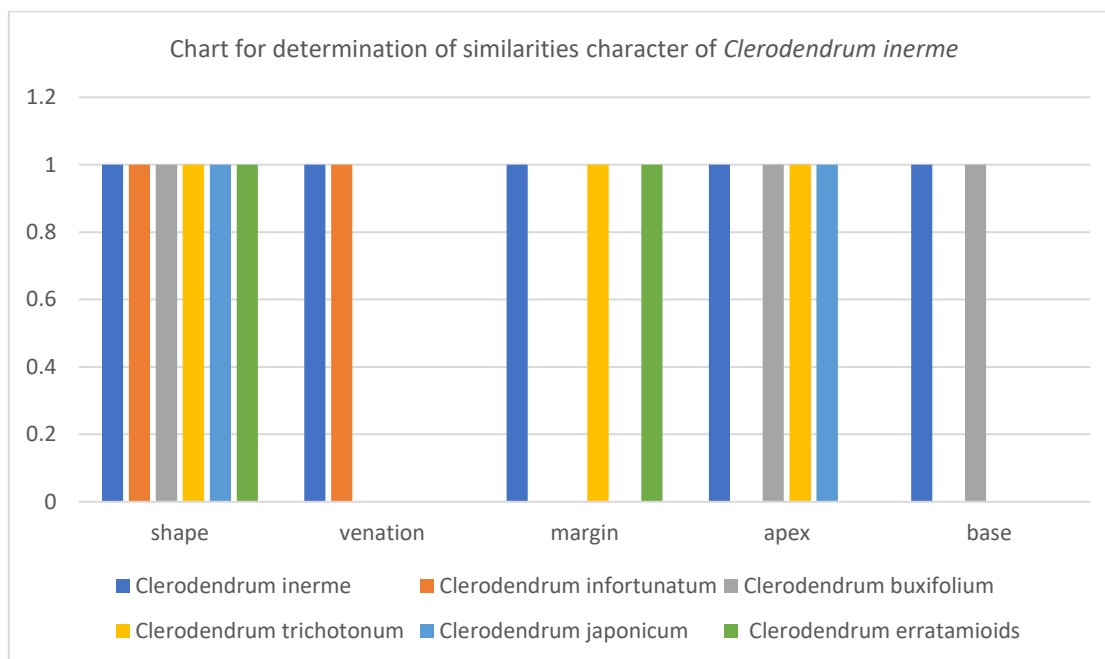
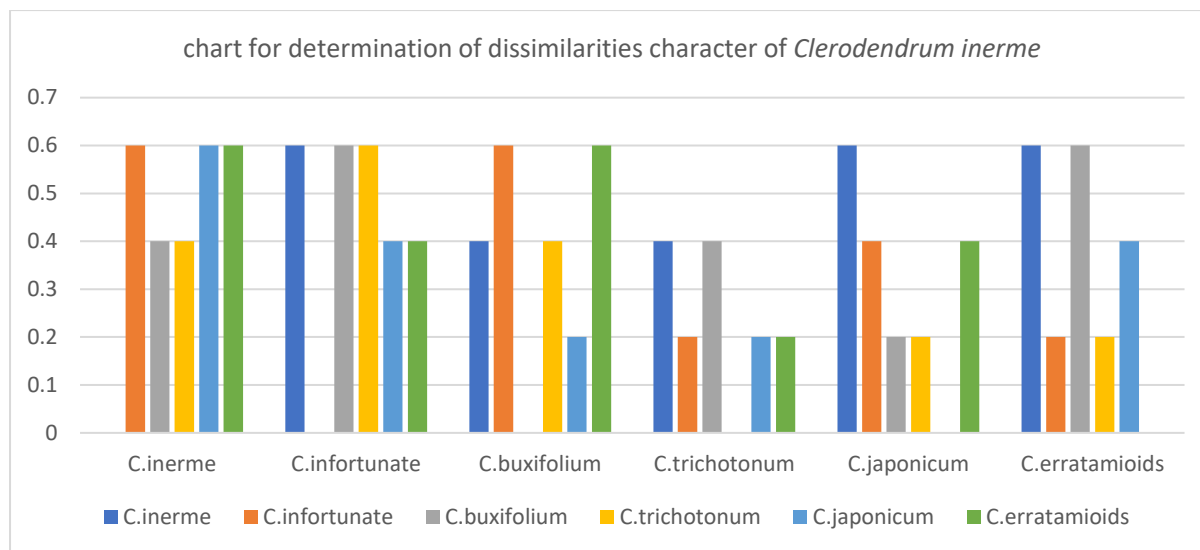


Table 9 – determination of dissimilar character matrix of *Clerodendrum inerme*

species	<i>C. inerme</i>	<i>C. infortunatum</i>	<i>C. buxifolium</i>	<i>C. trichotomum</i>	<i>C. japonicum</i>	<i>C. erratamioids</i>
<i>C. inerme</i>	0	0.6	0.4	0.4	0.6	0.6
<i>C. infortunatum</i>	0.6	0	0.6	0.6	0.4	0.4
<i>C. buxifolium</i>	0.4	0.6	0	0.4	0.2	0.6
<i>C. trichotomum</i>	0.4	0.2	0.4	0	0.2	0.2
<i>C. japonicum</i>	0.6	0.4	0.2	0.2	0	0.4
<i>C. erratamioids</i>	0.6	0.2	0.6	0.2	0.4	0

Fig 11- chart for determination of dissimilarities character of *Clerodendrum inerme***Sample matching coefficient**

$$SSM = NS/NS + ND * 100$$

NS-Number of similarity characters

ND- number of dissimilar characters

NS=12, ND=13

The matching coefficient of *Clerodendrum inerme* with respect to other species was found to be **48%**

Preparation of hydroalcoholic extract of *Clerodendrum inerme* Leaf (HAECI_L)

The collected leaves were washed with water, shade dried and powdered. 10grams of power was extracted with hydroalcohol by maceration technique until the complete extraction of material and filtered. The filtrate was concentrated to dryness and percentage was found to be **25.75%w/v**. The pH was obtained as **5.8**.The preliminary test was carried from this extract.

Determination of Preliminary Phytochemical Screening of HAECI_L

The preliminary phytochemical screening for HAECI_L was evaluated and determine the presence of steroids, tannins, phenols, saponin, glycosides, triterpenoids and result given in table-10

Table 10- Determination of Preliminary phytochemical analysis of HAECI_L

s.no	Phytochemical analysis	Observation
1.	Test for reducing sugar	+
	Benedicts test	+
	fehlings test	+
2.	Test for flavonoids	+
	Shinoda test	+
	Lead acetate test	+
3.	Test for terpenoids	+
	Salkowski test	+
4.	Test for triterpenoids	+
	Tschugajeu test	+
5.	Test for saponin	+

s.no	Phytochemical analysis	Observation
	Foam test	+
6.	Test for tannin	+
	With aqueous FeCl ₃	+
7.	Test for sterol	+
	Salkowski test	+
	Liebermann burchard test	+
8.	Test for phenolic compound	+
	With alcoholic FeCl ₃	+
9.	Test for coumarins	+
	With KOH & 0.5NHCl	+
10.	Test for quinones	+
	Bamberger test	+
11.	Test for diterpenes	-
	Copper acetate test	-
12.	Test for cardiac glycosides	-
	Killer kellani test	-
	Legals test	-
	Baljet's test	-
13.	Test for beta carboline alkaloids	-
14.	Test for alkaloid	-
	Mayer's test	-
	Dragendroff's test	-
	Wagner's test	-
	Hager's test	-
15.	Test for protein	-
	Biuret test	-
16.	Test for anthraquinone	-
	Borntrager's test	-
	Modified borntrager's test	-
17.	Test for aminoacids	-
	Millon's test	-
	Ninhydrin test	-
19.	Test for gums	-
20.	Test for mucilage	-
	With ruthenium red	-
21.	Test for iridoid glucosides	-

s.no	Phytochemical analysis	Observation
	Trim -hill test	-
22.	Test for starch	-
23.	Test for phlobatannin	-
	With HCl	-
24.	Test for anthocyanin	-

+ indicates presence, - indicates absence

Determination of Quantitative estimation of Phytoconstituents of HAECI_L.

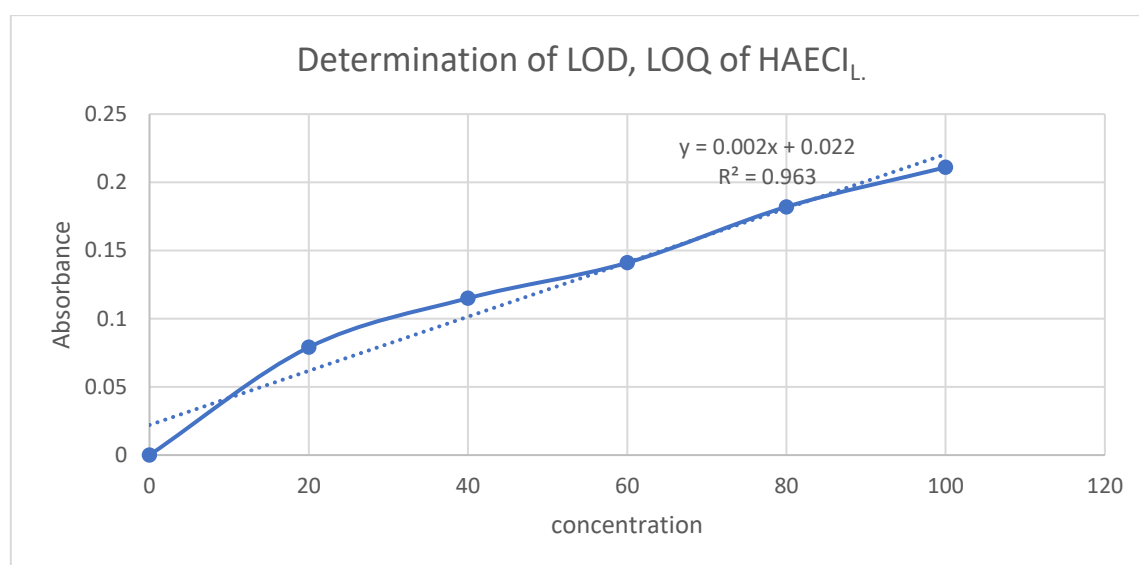
Various concentration (0 to 100 µg/ml) of HAECI_L was treated with water and absorbance was observed under UV and values displayed in table 11 and fig-12.

Determination of LOD and LOQ of HAECI_L.

Table 11 - Determination of LOD and LOQ of HAECI_L.

Concentration(µg/ml)	Absorbance of HAECI _L
0	0
20	0.079
40	0.115
60	0.141
80	0.182
100	0.211
LOD	36.3µg/ml
LOQ	110µg/ml

Fig 12- Determination of LOD and LOQ of HAECI_L.



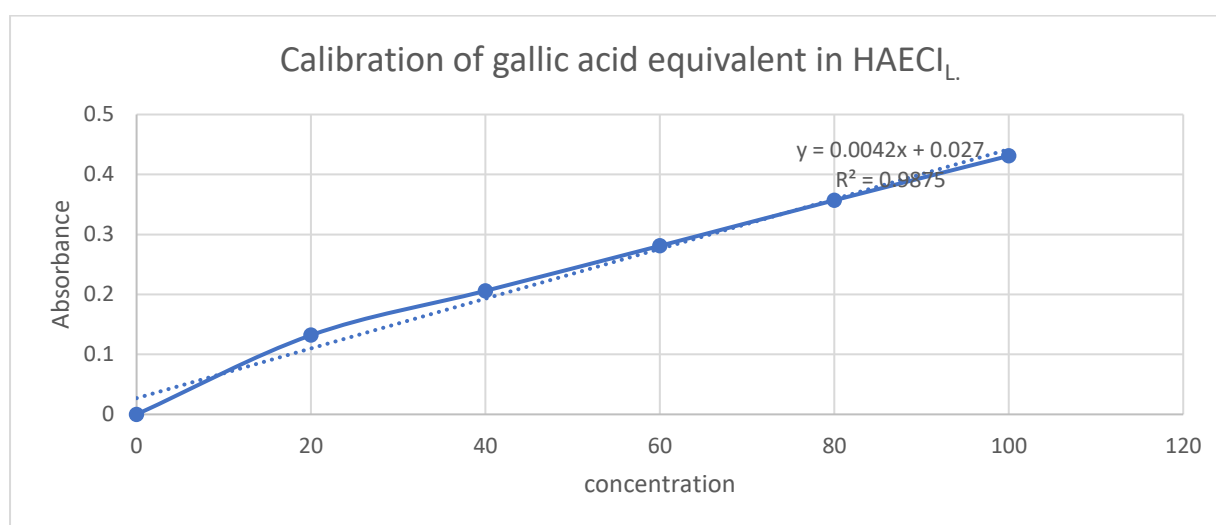
Quantitative estimation

Determination of gallic acid equivalent in HAECI_L.

Various concentration (0 to 100 µg/ml) of HAECI_L was treated with folin-ciocalteu which produce blue colour and the absorbance was observed and displayed in table 12 and fig-13.

Table 12- determination of gallic acid equivalent in HAECI_L.

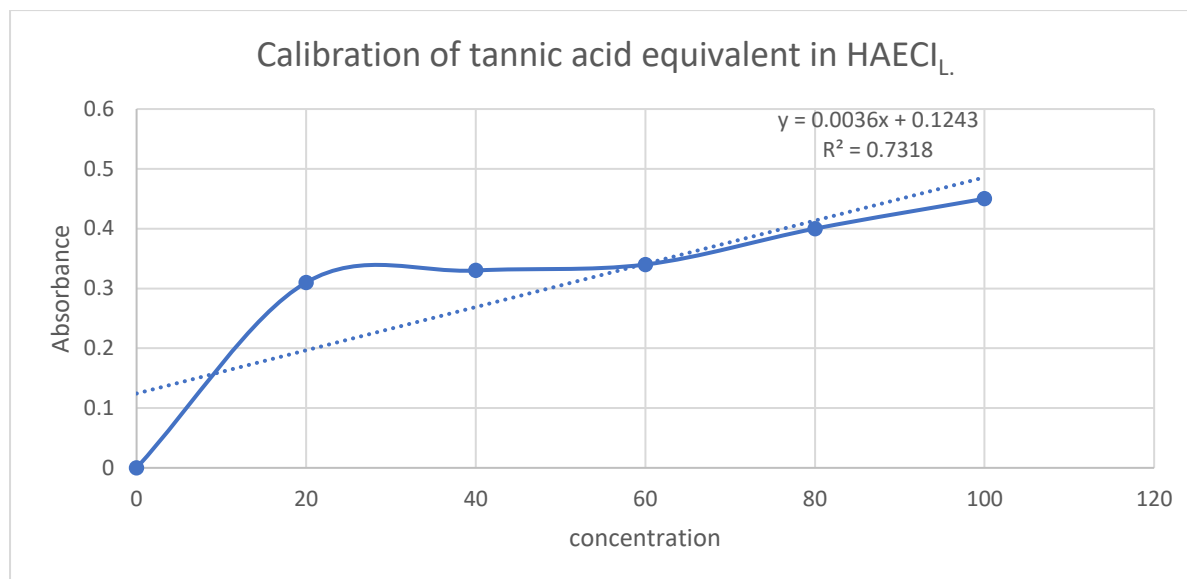
s.no	Concentration (µg/ml)	Absorbance of gallic acid	Absorbance of HAECI _L
1.	0	0	0
2.	20	0.132	0.213
3.	40	0.206	0.216
4.	60	0.281	0.227
5.	80	0.357	0.237
6.	100	0.431	0.241
TOTAL PHENOLIC CONTENT – 509GAE/g			

Fig 13- Calibration curve for gallic acid equivalent in HAECI_L.**Determination of tannic acid equivalent in HAECI_L.**

Various concentration (0 to 100 µg/ml) of HAECI_L was treated with folin-denis which produce blue colour and the absorbance was observed and displayed in table 13 and fig-14.

Table 13- determination of tannin acid equivalent in HAECI_L.

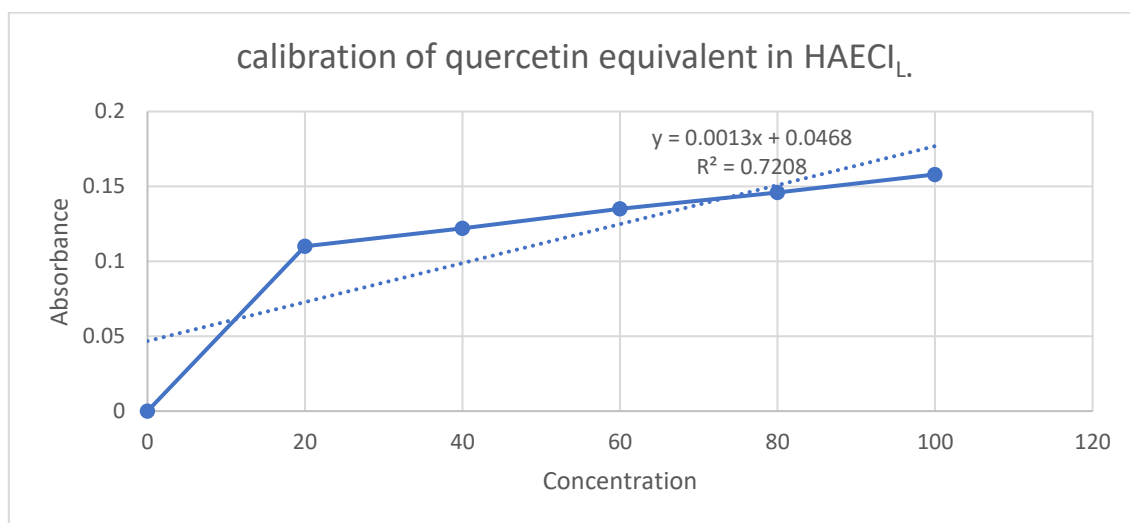
s.no	Concentration (µg/ml)	Absorbance of tannic acid	Absorbance of HAECI _L
1.	0	0	0
2.	20	0.305	0.252
3.	40	0.325	0.265
4.	60	0.335	0.274
5.	80	0.402	0.301
6.	100	0.454	0.325
TOTAL TANNIN CONTENT - 633 TAE/g			

Fig 14- Calibration curve for tannic acid equivalent in HAECI_L.**Determination of quercetin equivalent in HAECI_L.**

Various concentration (0 to 100 μg/ml) of HAECI_L leaf was treated with which produce orange yellowish colour and the absorbance was observed and displayed in table-14 and fig-15.

Table 14- determination of quercetin equivalent in HAECI_L.

s.no	Concentration (μg/ml)	Absorbance of quercetin	Absorbance of HAECI _L
1.	0	0	0
2.	20	0.110	0.056
3.	40	0.122	0.067
4.	60	0.135	0.075
5.	80	0.146	0.0977
6.	100	0.158	0.123
TOTAL FLAVONOID CONTENT - 590 QE/g			

Fig 15- Calibration curve for Quercetin equivalent in HAECI_L.

CONCLUSION

This is the first analytical report with Phenetics and DNA barcode for this medicinal plant *Clerodendrum inerme* collected in the area of Madurai, Tamilnadu. The Pharmacognostical, phenetic, phytochemical and DNA barcode revealed the plant characteristics and support future research and development.

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