Pharmacognostical and phytochemical analysis and identification of bioactive compounds using GC-MS Techniques of Ruellia tuberosa

Dr. K. L. Senthil kumar[1], Dr. P. D. Gokulan[2] , Mr. S. Venkateshwaran[3], G. Praveen Kumar[4], S. Priyadharshini[5], M. Punith Kumar[6]

[1] Principal, Sri Vijay Vidyalaya College of Pharmacy, Dharmapuri, Tamilnadu, India
[2] Head of the Department of Pharma ceutical Chemistry, Sri Vijay Vidyalaya College of Pharmacy, Dharmapuri, Tamil nadu, India)
[3] Associate professor, Sri Vijay Vidyalaya College of Pharmacy, Dharmapuri, Tamil Nadu (India)*
*[*4, 5, 6] B. Pharm Students, Sri Vijay Vidyalaya College of Pharmacy, Dharmapuri, Tamil Nadu (India)*

---***---

Abstract- ***Aim:*** *The aim of this study was to analyze the different bioactive compounds found in the leaves of Ruellia tuberosa collected from the Dharmapuri district. The study focused on identifying the bioactive constituents present in the chloroform extract and ethanol extracts of the Ruellia tuberosa leaves using the GC-MS technique along with phytochemical screening.*

Materials and methods: *The collected leaves of Ruellia tuberosa were thoroughly washed, air-dried, and ground into a coarse powder. The leaf extract was prepared by using the Soxhlet extraction method. The selection of solvents is based on their polarity.*

Result: *The phytochemical screening and GC-MS analysis confirmed the presence of various bioactive compounds in Ruellia tuberosa leaves. The study revealed several active chemical constituents, such as alkaloid, glycosides, flavonoids, proteins, tannins, terpenoids, etc.*

Conclusion: *The present study shows that Ruellia tuberosa leaves contain a rich source of secondary metabolites.*

Key words: Ruellia tuberosa, extraction, pharma cogenetic studies, phytochemical analysis, and GC-MS technique.

Introduction

Ruellia tuberosa is a medicinal plant that has been traditionally used since ancient times. It is an erect, perennial herb belonging to the family Acantharean. The plant is known by various names, such as Minnie root, fever root, snapdragon root, and sheep potato. It is commonly referred to as the "cracker plant." The plant grows near the roadside in grasslands, cultivated fields, and even in a dry(xerophilic)area. It is a short-lived perennial plant with striking funnel-shaped violet bracteates flowers on dichotomous cymes. The fruit is a sub-cylindrical puberulent capsule having more or less 20 seeds per locule. The plant also has thick, fusiform tuberous roots that grow in clusters. Ruellia tuberosa L. is a tropical plant that is commonly found in many parts of Asia, including Indonesia, Malaysia, and India. The plant flowers and produces fruits from June to February. It can be easily recognized by its attractive, trumpet-shaped, violet-colored flowers with a five-lobed corolla. The plant is mainly grown in moist and shady environments. The plant attracts many pollinators, especially butterflies and other insects. The flowers appear light bluish-purple due to the presence of anthocyanin, a flavonoid pigment that is also used as a natural dye in industries. The plant contains several important phytochemical constituents, such as alkaloids, glycosides, flavonoids, tannins, proteins, terpenoids, diterpenes, sterols, etc. This study aims to investigate the extraction of bioactive compounds from Ruellia tuberosa using different solvents and evaluate the phytochemical content using Gas chromatography-mass spectrometry analysis

FIG no : 01 Ruellia tuberosa

Traditionally, the plant is used for anti-diabetic, anti-inflammatory, ant nociceptive, antipyretic, analgesic, antioxidant, anti-hypertensive, insecticidal, anticancer, and antidotal toxic agents.

The leaf extract of Ruellia tuberosa L. has anti-diuretic, anti-cancer, and anti-oxidant activity.

Ruellia tuberosa root extract is used for anti-diuretic activity, reducing blood sugar levels and kidney disease.

It is used in ancient days for many traditional purposes.

Vernacular name:

- ✓ Assamese: Chapatti
- ✓ Bangladesh: Potpati
- ✓ Marathi :Ruwel
- ✓ Sri Lankan: Heenamukkara
- ✓ Tamil: Kiranthinayakam, Pattaskai
- ✓ Telugu: Jurbulagadda

Taxonomic Hierarchy:

- ✓ Kingdom: Plantae
- ✓ Subkingdom: Viridiplantae
- ✓ Infrakingdom: Streptophyta
- ✓ Superdivision: Embryophyta
- ✓ Class : Magnoliopsida
- ✓ Superorder: Asternae
- ✓ Order : Lamiales
- ✓ Family : Acanthaceae
- ✓ Genus : Ruellia L.
- ✓ Species: Ruellia tuberosa L.

Pharmacognostical Studies:

Methodology

1. Macro soppy(SOP No. PCOG-004-SOP): The external feature of the test sample was documented using a Nikon D-5600 digital camera.

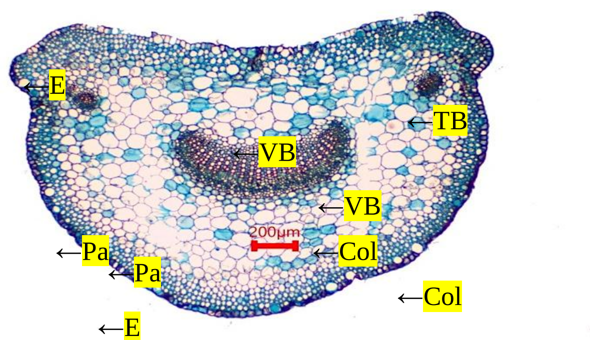
2. Micro scopy(SOP No. PCOG-005-SOP): Thesample was preserved in fixative FAA for more than 48 h. The preserved specimens were cut into thin transverse sections using a sharp blade, and the sections were stained with 0.8% safranin and 0.5% Astra blue. Transverse sections were photographed using an Axiolab5 trinocular microscope attached to a Zeiss Axiocam208 color digital camera under bright field light. Magnifications were indicated by a scale bar.

Result:

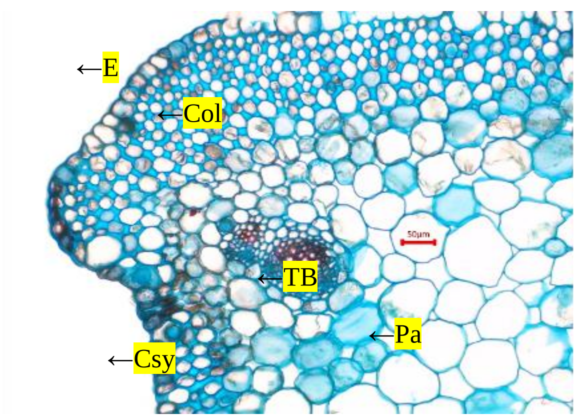
Microscopy:

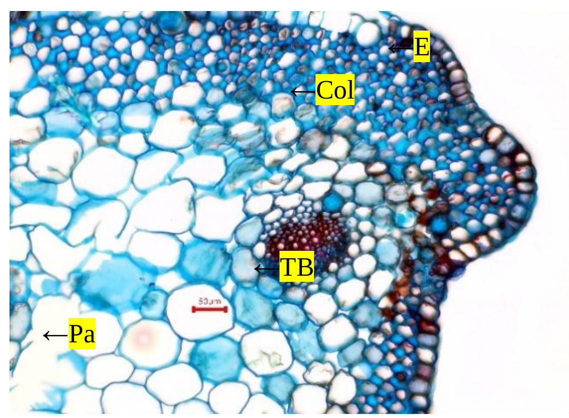
Petiole

The transverse section of the petiole is urn-shaped. The lower surface is convex, while the upper surface is slightly concave and bears lateral wings. The outermost layer is a single-layered epidermis covered with a cuticle. Beneath it, 4-5 layers of collenchymatous tissue are present, which continue with the parenchymatous ground tissue. At the center, an arc-shaped vascular bundle is seen. The vascular bundles are conjoint, collateral, closed, and composed of normal vascular tissues. Xylem is oriented towards the upper side and
Phloem towards the lower side. Two trace bundles can be observed on each upper side below the wing region. Cystoliths are found in epidermal cells, and starch grains are distributed in the inner parenchyma cells.

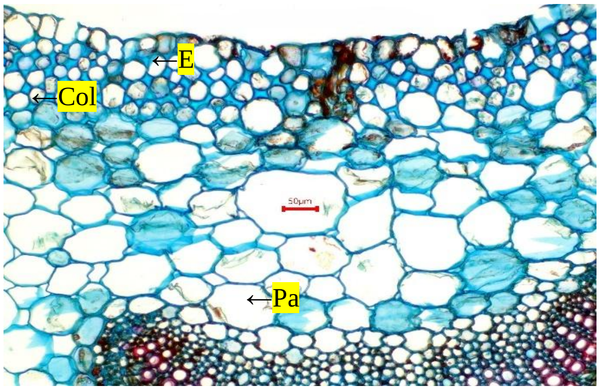


TS of petiole

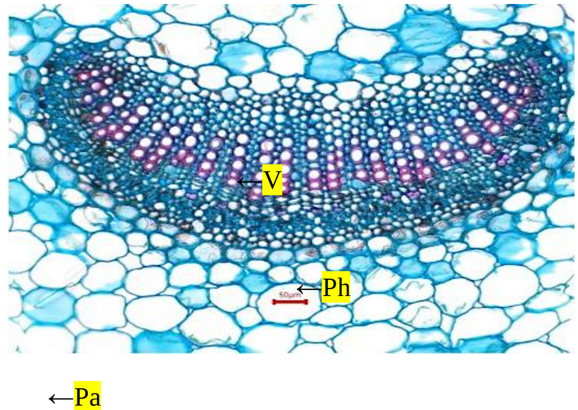




Enlarged view of wing region

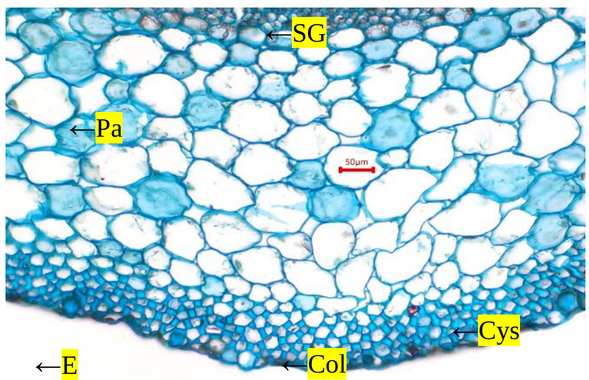


Upper portion enlarged



Enlarged view of vascular region

Figure no: 02 Micro scopy of Ruellia tuberosa petiole



Enlarged view of lower portion

Col - collenchyma; **Cys -** cystolith; **E -** epidermis; **Pa -** parenchyma; **Ph -** phloem; **SG -** starch grain; **TB -** trace bundle; **V -** vessel; **VB -** vascular bun

Leaf

The TS of the leaf is dorsiventral with a flat lower surface and a slightly elevated upper surface and shows ribbon-like lateral laminar extensions.

Midrib

TS of the midrib shows an outer single-layered upper and lower epidermis covered by cuticle and bears several covering trichomes; 3 to 4 layers of collenchymatous hypodermal layer are present, followed by parenchymatous ground tissue embedded with a crescent-shaped conjoint, collateral, and closed vascular bundle at the center; xylem is placed towards the upper side and phloem towards the lower side; xylem is formed of vessels, tracheids,fibers, and parenchyma, whereas phloem is made up of sieve elements and phloem parenchyma; two or three trace bundles can be seen on the upper side facing towards the lamina.

Lamina

The transverse section of the lamina shows that both the upper and lower epidermis are single-layered and covered with a cuticle. These layers also bear covering trichomes. The mesophyll tissue is well differentiated into an upper, tightly packed single layer of palisade cells, followed by four to five layers of loosely arranged spongy parenchyma cells. Veins run through the mesophyll tissue. Cystoliths are found in specialized epidermal cells on both surfaces of the lamina, while oil globules are scattered throughout the lamina. A few collenchyma cells are also present near the leaf margin.

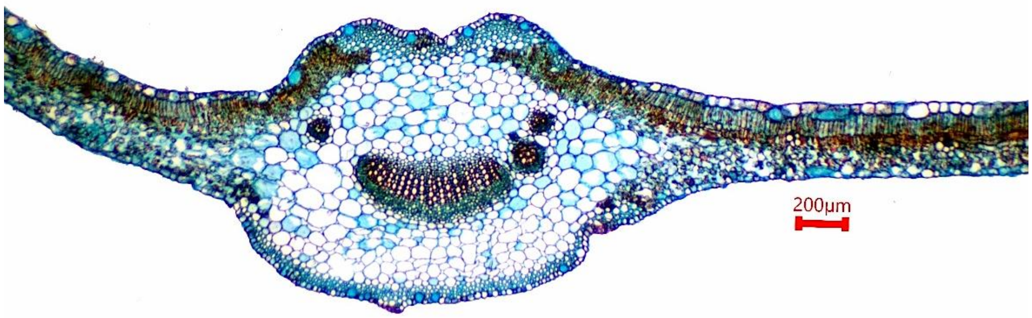
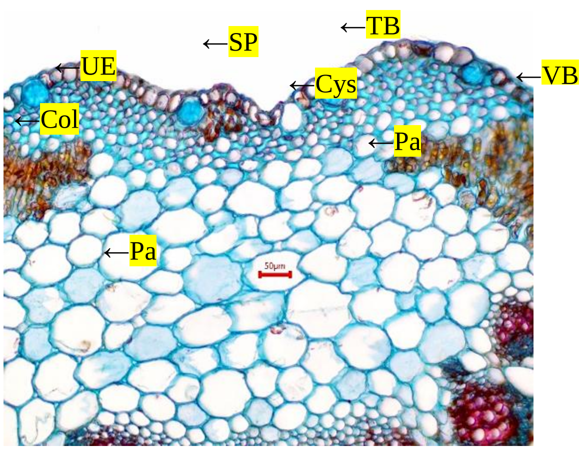
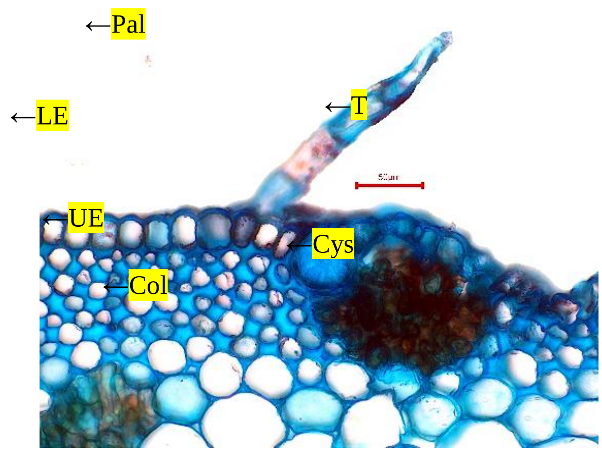


Figure no: 03 Microscopy of Ruellia tuberosa leaf passing through midrib

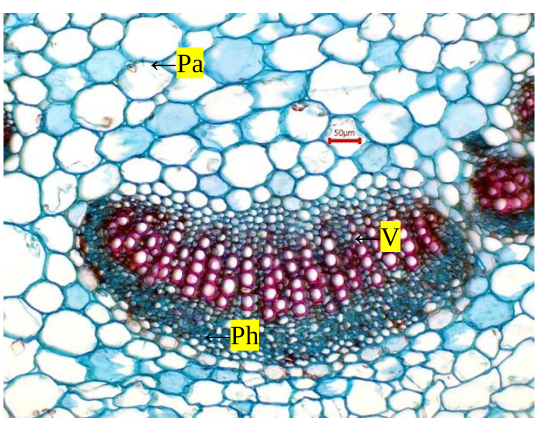
←UE

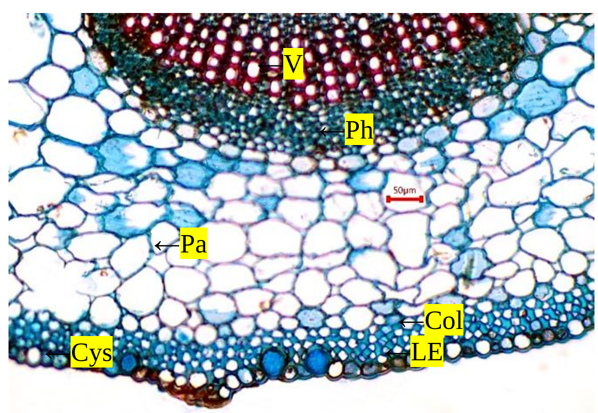
TS of leaf



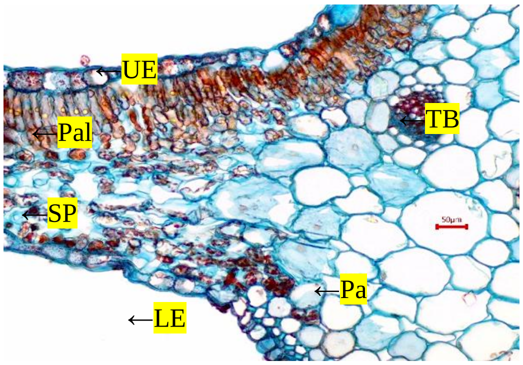


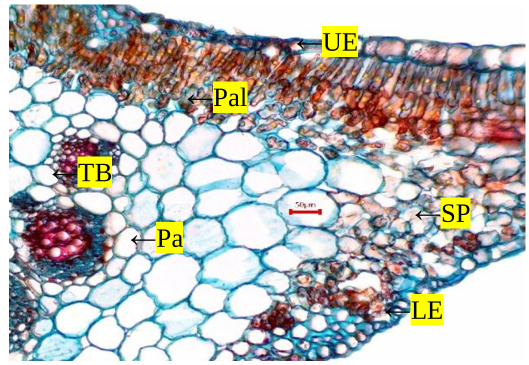
Midrib upper portion enlarged



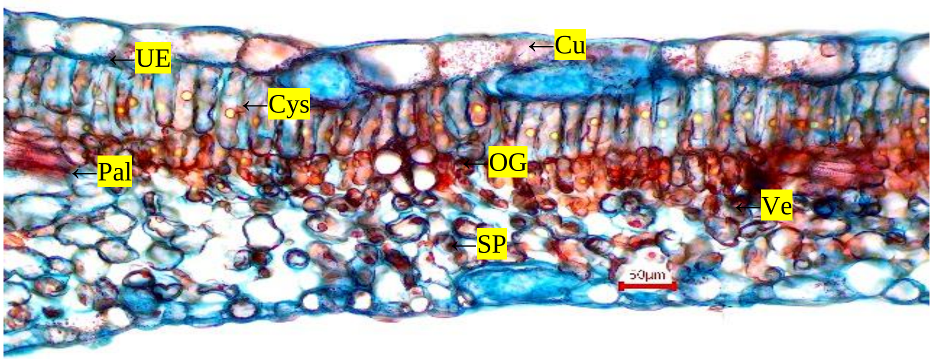


Enlarged vascular portion **Midrib lower portion enlarged**

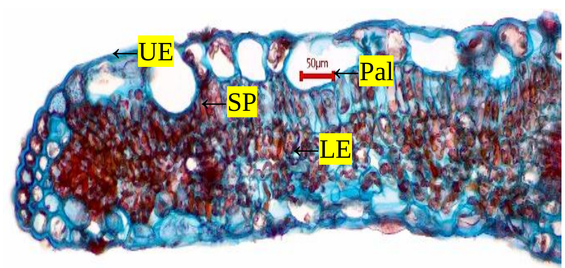


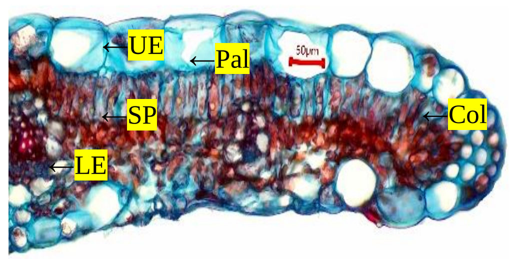


Enlarged wing portion

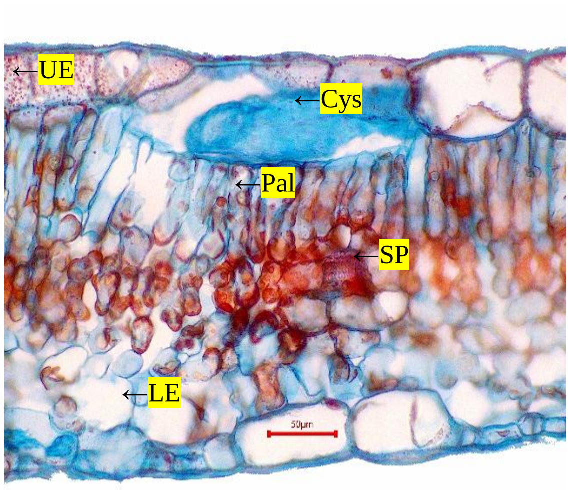


Ts of lamina

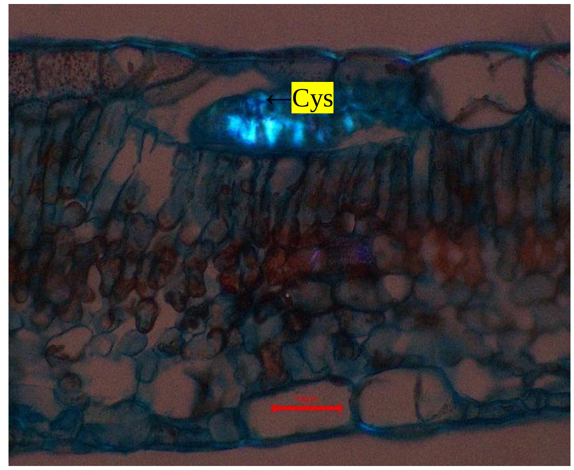




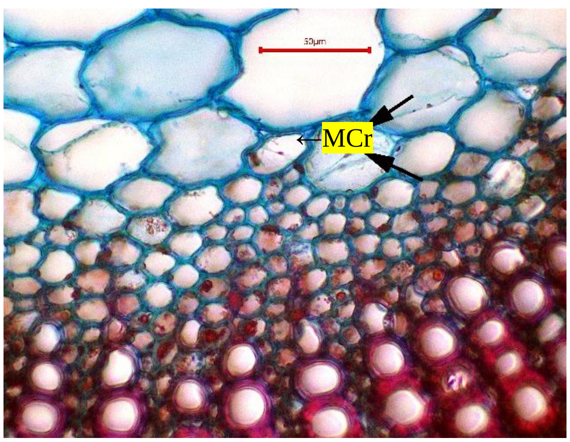
TS of leaf margin



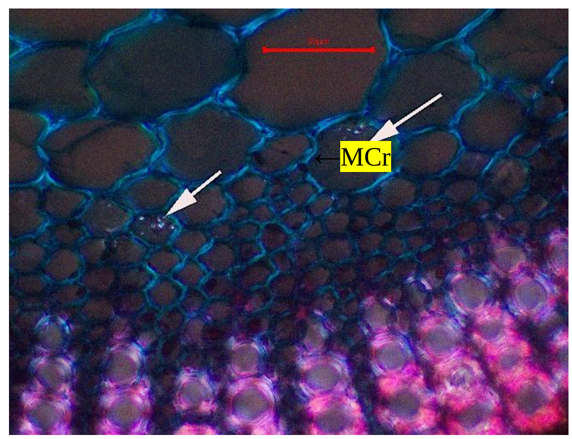
Lamina with cystolith



Under a polarizedfield



Microsphenoidal crystals



Crystals under polarized field

Col - collenchyma; **Cu** - cuticle; **Cys** - cystolith; **LE** - lower epidermis; **MCr**- micro sphenoidal crystals; **OG** - oil globule; **Pa** - parenchyma; **Pal** - palisade; **Ph** - phloem'**s** - spongy parenchyma - trichome; **TB** - trace bundle; **UE** - upper epidermis; **V** - vessel; **VB** - vascular bundle; **Ve** – vein

PHYTOCHEMICAL STUDIES:

The obtained extracts were subjected to phytochemical analysis. This qualitative study was carried out to identify the presence of secondary metabolites such as alkaloids, glycosides, flavonoids, terpenoids, diterpenes, proteins, tannins, sterols, etc.

1. Test for Alkaloids

Dragendorff's test

To 1 ml of extract, add 1 ml of Dragendorff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

Mayers Test

To 1 ml of extract, add 1 ml of Mayer's reagent (potassium mercuric iodide solution). Whitish yellow or cream-colored precipitate indicates the presence of alkaloids.

2. Test for Glycosides

Test solution: It was prepared by dissolving the sample in alcohol.

Keller-Killiani Test

To a test solution, a few drops of ferric chloride solution and concentrated sulphuric acid were added; the formation of two layers occurred,a lower layer of reddish-brown color and an upper layer of bluish-green color simultaneously.

3. Test for Triterpenoids

Test solution: It was prepared by dissolving the extracts in chloroform.

Salkowski test

A few drops of concentrated sulfuric acid were added to the solution and allowed to stand for some time. The lack of formation of yellow color in the lower layer indicates the absence of triter penoids.

4. Test for Carbohydrate

The Molisch testto 2-3ml of the test solution, added few drop Molish reagent solution and was shaken. Concentrated sulphuric acid was added from the side of the test tube. A violet ring was formed at the junction of two lipids.

5. Test for Protein

Test solution: It was prepared by dissolving extract in water and making aqueous extract.

Biuret test

To about 3 ml of the extract,40% sodium hydroxide solution and a few drops of 1% copper solution were added.White produces blue color.

6. Test for Anthraquinone

Borntrager's Test: Add 10 ml of 10% ammonia solution with a few ml of 3 ml of Aq. The extract is shaken with ml of benzene and filtered (shaken vigorously 30 mins) appears as a pink, violet, or red-colored solution, it shows the presence of anthraquinones.

7. Test for Flavonoids

Test solution:A small amount of extract and 2M HCL was added and heated for about 30-40 mins. The extract was cooled down and again extracted with ethyl acetate, which was further concentrated to dryness and ready to be used as a test sample.

Lead acetate test: To a small quantity of extract, lead acetate was added. Formation of yellow-colored precipitate shows the presence of flavonoids.

8. Test for Quinone

Conc. HCl test

Plant extract with conc. HCl appearsgreen incolor; it shows the presence of quinones.

9. Test for Tannins

Test solution: The test solution was prepared by dissolving the extract in water and alcohol.

Ferric chloride solution test

To 1 ml of the extract, ferric chloride was added. The formation of a dark blur or greenish-blackcolor shown in the presence indicates the tannins.

10. Test for Cholesterol

Add 2 ml of chloroform with 10 drops of acetic anhydride and add 2-3 drops of conc. H_2SO_4. The appearance of a red-rose color shows the absence of cholesterol.

11. Test for Diterpenes

Plant extract is dissolved in distilled water, and 3-4 drops of copper acetate solution are added. It appears emerald green incolor; it shows the presence of diterpenes.

12. Test for Terpenoids

Add 2 ml of chloroform with 5 ml of plant extract and add 3 ml of conc. H_2SO_4 (boiled in a water bath). Do not appear ina grey-coloredsolution in the absence of terpenoids.

13. Test for Saponins

Test solution: It was prepared by dissolving extract in water and making aqueous extract.

Foam Test

The drug extract was vigorously shaken with water. Persistent foam formation does not indicate the absence of saponins.

14. Test for Fat

Solubility test

Fats are insoluble in water and ethanol. But soluble in chloroform, benzene, and ether. It shows the presence of Fat.

15. Test for sterols

Liberman-Burchard Test

1 gm of the test substance was dissolved in a few drops of chloroform, 3 ml of acetic anhydride and 3 ml of glacial acetic acid were added, and it was warmed and cooled under the tap, and drops of conc. H_2SO_4were added along the side of the test tube. The non-appearance of a bluish-greencolor shows the absence of sterols.

Table 1: Phytochemical analysis of Ruellia tuberosa

S.NO	PHYTOCHEMICALS	CHLOROFORM	ETHANOL	WATER
1	Alkaloids	+	+	+
2	Glycosides	+	–	–
3	Triterpenoids	–	–	+
4	Carbohydrate	–	–	–

5	Protein	+	+	+
6	Anthraquinone	–	–	–
7	Flavonoids	+	–	–
8	Quinones	–	+	+
9	Tannins	–	+	+
10	Cholesterol	+	+	+
11	Diterpenes	+	+	+
12	Terpenoids	+	+	+
13	Saponins	–	+	+
14	Sterols	+	–	+
15	Fat	+	+	+

PHYTOCHEMICAL SCREENING: (+) Presence (-)/Absence

The extract of Ruelliatuberosa L. was subjected to phytochemical analysis. The selection of solvent is based on their polarity. The solvents used for the extraction were chloroform, ethanol, and water. The phytochemical screening of secondary metabolites such as alkaloids, glycosides, flavonoids, triterpenoids, diterpenes, carbohydrates,proteins, tannins, cholesterol, and sterols was determined.

GAS CHROMATOGRAPHY AND MASS SPECTROPHOTOMETRY

The Ruellia tuberosa leaves contain a rich source of secondary metabolites. The extraction and characterization of biologically active components from medicinal plants have resulted in the treatment for various diseases. The bioactive compound present in the Ruellia tuberosa was separated by using gas chromatography and mass spectrometry.

GC-MS analysis of ruellia tuberosa

PROCEDURE

Sample preparation:

Weigh 1 g of homogenized sample (chloroform and ethanol) into a 15 ml plastic tube. Add 1 ml of ethyl acetate and vortex for 5 minutes.

Then transfer to a 1 ml vial for injection of µl of the sample into GC-MS

INSTRUMENT CONDITIONS:

C–MS analysis was performed using a Shimadzu QP2020 gas chromatograph–mass spectrometer equipped with a split/splitless injector and an electron impact ionization source operating in EI^+ mode. Separation was achieved on an HP-5MS capillary column (30 m × 0.32 mm, film thickness 0.25 µm). An injection volume of 1.0 µL was employed, with the injector temperature maintained at 285°C.

Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature program was set with an initial temperature of 50°C, which was gradually increased to 290°C and held for 10 minutes to ensure complete elution of all components. The split valve was opened 3 minutes after injection to purge the injector. All injections were carried out using a 10 µL microsyringe.

Peak	Retention time	Area	Area %	Compound name
1.	4.162	871923	0.84	Cyclotrisiloxane, hexamethyl
2.	7.114	350497	0.34	Silane, methyldiethoxyisopropoxy
3.	8.047	140835	0.14	2-Furancarboxaldehyde, 5-methyl
4.	8.864	244782	0.24	Cyclotetrasiloxane, octamethyl
5.	9.125	759887	0.74	Benzyl chloride
6.	11.398	417116	0.40	2(3H)-Furanone, 5-acetyldihydro
7.	12.860	517851	0.50	N-Aminopyrrolidine
8.	14.349	529195	0.51	Phthalic anhydride
9.	15.630	44644	0.04	(E)-1-(2,3,6-trimethylphenyl)buta-1,3-diene
10	16.219	1087884	1.05	Dimethyl phthalate
11	17.313	15184054	14.69	1,2-Benzenedicarboxylic acid, ethyl methyl ester
12	18.587	82727469	80.02	Diethyl Phthalate
13	19.076	545537	0.49	Tetrahydrofuran-2-one, 5-(1-hydroxyhexyl)

Table 2: Phytochemical screening by using chloroform extract

Peak	Retention time	Area	Area %	Compound Name
1.	16.492	453736	1.99	1-Doodecanol
2.	28.066	1023909	4.50	Dibutyl phthalate
3.	39.970	451333	1.98	Eicosane
4.	41.606	532830	2.34	Dotriacontane
5.	41.830	654348	2.88	Squalene
6.	42.233	738698	3.25	Tetracontane
7.	43.892	1847483	8.12	Cis-1-Chloro-9-octadecene
8.	44.326	382940	1.68	Cholesterol
9.	44.964	3088226	13.57	4-pentenoic acid, 2,2-diethyl-3-oxo-5-phenyl
10.	45.518	458902	2.02	Campesterol
11.	46.691	6959349	30.58	Gamma-sitosterol
12.	47.563	1281577	5.63	Alpha-Amyrone
13.	47.952	413497	1.82	Alpha-amyrin

Conclusion:

The extraction of *Ruellia tuberosa* using solvents of varying polarity resulted in different percentage yields. Phytochemical screening was carried out to identify the presence of bioactive constituents in each extract. The detection of these phytochemicals through preliminary analysis substantiates the medicinal significance of the plant.

The plant extract was subjected to gas chromatography–mass spectrometry (GC–MS) analysis under standardized experimental conditions. The separation of compounds was achieved based on their retention time and relative peak area, and detection was performed using a suitable detector such as a flame ionization detector (FID). The resulting chromatogram exhibited multiple peaks corresponding to diverse bioactive constituents. GC analysis further confirmed the chemical heterogeneity of the extract by effectively resolving volatile compounds. The identified constituents may contribute to the therapeutic potential of *Ruellia tuberosa*.

REFERENCE

[1]Juswardi, Nur Salamah, Nina Tanzerina, Kamila Alawiyah, Syafrina Lamin, and Mustafa Kamal.Metabolite profiling of Minnieroot (Ruellia tuberosa L.) leaves using gas chromatography-mass spectrometry (GC-MS), 2025

[2] Sandhya Sharma, Jeline Rani J, Sohan Lal, Harpal Singh and Sugimani MarndiRuellia tuberosa L.: a medicinal plant of India, 2024

[3] Gokulakrishnan S, Dharanya M, and Dr. K. B. Ilango. STANDARDIZATION AND SOLVENT COMPARATIVE STUDY OF RUELLIA TUBEROSA L., 2023

[4] Thakur Adhika Govindsinh, M. H. Ghante, N. B. Ghiware, S. K. Sarje. Pharmacological evaluation of Ruellia tuberosa stem bark on antianxiety activity, 2020

[5] M.N.L.C. Harika and P. Radhika, Department of Biochemistry.Phytochemical Analysis of Ruellia tuberosaTuber Ethanolic Extract Using UV-VIS, FTIR, and GC-MS Techniques, 2019

[6] Ghaidaa Jihadi Md., Aseel Mohammad Omran, and HaiderM.H., Antibacterial and Phytochemical Analysis of Piper nigrum using Gas Chromatography-Mass spectrum and Fourier transform infrared dchromato graphy, 2016

[7] Srinivasan K., Sivasubramanian S., Kumaravel S., Phytochemical profiling and GCMS study Adhathoda vasica leaves, 2014

[8] Senthilkumar P,Sambath R, and Vasantharaj. Antimicrobial Potential and Screening of Antimicrobial compounds of Ruellia tuberoseUsing GC-MS, 2013

[9] Arirudran, A. Saraswathy, and Vijayalakshmi Krishnamurthy. Antimicrobial activity of Ruellia tuberosa L. 2011