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PHARMACOGNOSTIC EVALUATION AND *IN-VITRO* ANTIOXIDANT ACTIVITY OF METHANOLIC LEAF EXTRACT OF *ERA NTHEMUM RETICULATUM*

Mrs. G. Jaya Syamala*, I. Charishma[1], M. Thanisha Chowdary[2], M. Madhu latha[3], G. Thanmaya swathi[4], B. Dharani[5], Dr. Y. A. Chowdary

NRI College of Pharmacy, Pothavarapadu Village, Agiripalli Mandal, Eluru District, Andhra Pradesh, PIN No. 521212.

Address for Correspondence: G. Jaya Syamala
E mail: ushamannam1984@gmail.com

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Abstract

This study was undertaken to establish comprehensive pharmacognostic, physicochemical, phytochemical, chromatographic, and antioxidant standards for the methanolic leaf extract of *Eranthemum reticulatum,* with the objective of providing scientific validation for its identification, authentication, quality control, and medicinal significance. Preliminary phytochemical screening revealed the presence of major bioactive constituents such as alkaloids, flavonoids, glycosides, tannins, phenolic compounds, proteins, and reducing sugars, Macroscopic evaluation showed characteristic features including a broad green lamina, entire margin, pointed apex, and reticulate venation, while microscopic studies revealed diagnostic markers such as a single-layered epidermis with cuticle, hypo stomatic leaf with diacytic stomata, multicellular glandular trichomes, differentiated palisade and spongy mesophyll, collateral vascular bundles, cystoliths, and calcium oxalate crystals. Quantitative microscopy parameters, including stomatal index, vein islet number, and palisade ratio, were found to be reliable identification constants. Physicochemical analysis indicated acceptable moisture content, low contamination, and the presence of water-soluble active constituents. Thin Layer Chromatography produced a characteristic fingerprint with distinct Rf values, and UV–Visible spectroscopy showed λmax at 418 nm, confirming chromophoric antioxidant compounds. The methanolic extract exhibited significant concentration-dependent antioxidant activity in the DPPH assay, with a lower IC_{50} value than ascorbic acid. These findings confirm the strong antioxidant potential and pharmacognostic importance of the plant, supporting its use as a natural therapeutic agent and recommending further studies for isolation and pharmacological evaluation of active compounds.

Keywords: Macroscopy and microscopy characteristics, Physico chemical Analysis, Anti oxidant Potential, Thin Layer Chromatography, DPPH Assay Method.

INTRODUCTION:

Eranthemum reticulatum is a medicinal plant from the Acanthaceae family that is known for its distinct pharmacognostic properties that aid in accurate identification and authentication. Macroscopically, the plant has broad green leaves with an entire margin, pointed apex, and prominent reticulate venation. Microscopic examination reveals important diagnostic features such as a single-layered epidermis with a cuticle, a hypostomatic leaf with diacytic stomata, multicellular glandular trichomes, and clearly differentiated palisade and spongy mesophyll tissues. The presence of collateral vascular bundles, cystoliths, and calcium oxalate crystals also aids in identification. Quantitative microscopic constants such as stomatal index, vein islet number, and palisade ratio are useful parameters for crude drug quality control and standardization.

The plant's therapeutic potential was demonstrated by its traditional use in folk medicine to treat infections, inflammatory conditions, and general health issues prior to the current study. Bioactive components like flavonoids, phenolic compounds, and other secondary metabolites were found in some early phytochemical investigations, indicating potential antibacterial and antioxidant qualities. Its biological activities had also been suggested by a few pharmacological screenings, but these investigations were disjointed and lacked systematic chromatographic fingerprinting, thorough pharmacognostic standardization, and thorough physicochemical profiling. Additionally, not enough information was available about its antioxidant potency using verified in vitro models. As a result, despite the plant's potential, a thorough scientific assessment and quality control system had not yet been established.

The current study aimed to close these gaps by developing detailed pharmacognostic standards, physicochemical constants, chromatographic fingerprints, and antioxidant validation for the methanolic leaf extract. By establishing these parameters, the study ensures accurate identification, authentication, and prevention of adulteration. The demonstrated antioxidant activity strengthens scientific evidence for its traditional medicinal use and confirms its potential as a natural source of bioactive compounds. Overall, the findings strengthen the plant's scientific foundation and pave the way for future isolation, characterization, and advanced pharmacological research.

PLANT PROFILE:

The biological source of *Eranthemum reticulatum* is the leaves, flowers, and whole plant of *Eranthemum reticulatum*. (synonym: Pseuderanthemum carruthersii var. reticulatum (W.Bull ex A.de Vos) Fosberg), belonging to the Acanthaceae family. Itis an evergreen tropical shrub in the Acanthaceae family known for its striking foliage of creamy-yellow leaves with green veins. It produces small white flowers with five petals and purple-pink spots at the base. It thrives in full sun to partial shade and grows to be 2-4 feet tall, making it ideal for hedges, containers, and shrub borders.

Common names include Yellow-Vein Eranthemum, Golden Pseuderanthemum, and Golden Net-Bush. The family is Acanthaceae. Polynesia is where this all began.

Fig No. 1: Plant Profile

Appearance: An erect, bushy shrub that typically grows to be 2-4 feet tall (but can reach 6 feet).

Foliage: Opposite, elliptic to ovate, ovate-lanceolate leaves, usually 2-5 inches long, with prominent, bright yellow veins.

Flowers: Small, white, 5-lobed flowers with deep magenta or purple-pink spots in the center/throat that bloom all season.

Hardiness: USDA Zones 10-11; frost-sensitive, but can be grown as an annual or container plant in colder areas.

Light: Full sun to partial shade. The bright yellow color of the leaves is enhanced by full sunlight.

Water and Soil: Needs moist, well-drained, fertile soil and frequent watering.

Use: Great for landscapes that are vibrant, tropical, and wildlife-friendly (butterflies are drawn to it). It is frequently used in foundation plantings, as a hedge, or as a screen.

Maintenance: To preserve shape, avoid legginess, and promote denser growth, prune frequently.

Botanical Description: It is an evergreen, upright shrub known for its variegated foliage (green-veined creamy yellow leaves) and small white flowers with purple-pink spots.

Origin: Native to Polynesia, specifically the Solomon Islands and Vanuatu.

Medicinal/Industrial Uses: The leaves and their juice are used in traditional medicine to treat headaches, fever, back pain, and colds. Additionally, the plant is used as a natural source of dye for cotton fabrics,

with the colorant identified as an anthocyanin pigment.

MATERIAL AND METHODS

Plant Material

The plant chosen for this study was Eranthemum reticulatum, which is a member of the Acanthaceae family. Yellow-vein eranthemum is the popular name for this plant, which is extensively grown as an attractive shrub in tropical and subtropical climates.

Collection of Plant Material

The indigenous region of Pothavarappadu, Andhra Pradesh, India, provided the fresh and healthy leaves for collection. To guarantee the highest possible phytochemical content, the plant was harvested in the right season. There was no pollution, illness, or insect infestation in the plant material that was gathered.

Authentication of Plant

Morphological traits such leaf form, vein pattern, color, texture, and arrangement were used for authentication.

Materials and Instruments Used:

Instruments: Soxhlet apparatus [Borosil Limited], UV-Visible spectrophotometer [Laurence & Mayo], Microscope [Microline], Muffle furnace [Aloe], Hot air oven [Kemi], Water bath [Guna Enterprises], Desiccator [spector litt], Centrifuge [Remi], TLC chamber [Supelco], Analytical balance [Wensar], Micropipette [Vertex].

Chemicals and Reagents:

All chemicals used were of analytical grade are: Methanol, Ethanol, DPPH reagent, Ascorbic acid, Sulfuric acid, Hydrochloric acid, Ferric chloride, Lead acetate, Benedict's reagent, Fehling's solution, Dragendorff's reagent, Mayer's reagent, Wagner's reagent, Magnesium turnings.

Preparation of Plant Material: To get rid of dust and contaminants, the gathered plant material was cleaned with distilled water. For 7–14 days, it was left at room temperature in the shade to avoid the phytoconstituents degrading.

After complete drying: A mechanical grinder was used to powder the material, Sieve No. 40 was used to filter the powder. The powder was kept in an airtight container.

Extraction Procedure:

Soxhlet Extraction Method

Principle: Phytoconstituents are extracted from plant material using the continuous hot extraction technique known as Soxhlet extraction.

Procedure: About 100 g of dried powder was taken and placed in a thimble, which was then inserted into the Soxhlet apparatus. Methanol was used as the solvent, and the extraction process was carried out for seventy-two hours. After extraction, the solvent was evaporated using a water bath, the extract was concentrated, and the dried extract was stored for further use.

Preliminary Phytochemical Screening

A qualitative analytical method called phytochemical screening is used to find out whether plant extracts contain physiologically active secondary metabolites. The therapeutic effectiveness of medicinal plants like *Pseuderanthemum reticulatum* is significantly influenced by these phytoconstituents. Based on particular chemical reactions between the phytochemicals and reagents that produce distinctive color changes, fluorescence, or precipitate formation, the qualitative tests are conducted. Drug identification, authenticity, and standardization all depend on these assays.

Detection of Carbohydrates

Carbohydrates are the main metabolites present in plants and act as energy sources. They can be identified using several qualitative tests.

Molisch's Test, Barfoed's Test, Seliwanoff's Test, Benedict's Test, Fehling's Test

Test for Proteins

Biuret Test, Xanthoproteic Test, Ninhydrin Test, Millon's Test, Detection of Alkaloids.

Detection of Alkaloids:

Alkaloids are fundamental chemical molecules that include nitrogen and have important pharmacological effects, including antioxidant, antibacterial, and analgesic effects. Alkaloids are identified using a variety of qualitative assays that are based on their capacity to combine with particular reagents to create complexes.

Dragendorff's Test, Mayer's Test, Wagner's Test, Hager's Test

Test for Glycosides

Many medicinal plants include glycosides, which are chemical compounds made up of a sugar moiety joined to a non-sugar component known as an aglycone. They are significant phytoconstituents that have a variety of pharmacological properties, including laxative, cardiotonic, anti-inflammatory, and antioxidant actions. Color reactions caused by the aglycone part are used in qualitative chemical assays to identify the presence of glycosides.

Keller–Kiliani Test (Test for Cardiac Glycosides), Borntrager's Test (Test for Anthraquinone Glycosides), Modified Borntrager's Test (For C-glycosides), Legal's Test (Test for Cardiac Glycosides), Baljet Test

Test for Volatile Oils:

Stain Test: Temporary stain on filter paper indicates volatile oils.
Sudan III Test: Red coloration confirms volatile oils.
Solubility Test: Solubility in alcohol indicates volatile oils.

Test for Terpenoids:
Salkowski Test, Liebermann-Burchard Test
Test for Tannins and Test for Phenolic Compounds

Thin Layer Chromatography (TLC):
Thin Layer Chromatography (TLC) is one of the most widely used techniques in preliminary phytochemical screening. Principle TLC is based on adsorption chromatography. Compounds are separated according to their relative affinity toward the stationary phase (usually silica gel or alumina) and the mobile phase (solvent system).
Stationary Phase: Silica gel G , Alumina.
Mobile Phase: Mixture of solvents (chloroform: methanol and toluene: ethyl acetate).
Procedure: Prepare herbal extract and concentrate. Spot a small quantity on the TLC plate. Develop the plate in a suitable solvent system. Remove and dry the plate. Visualize under UV light (254 nm / 366 nm) or spray with detecting reagents.
Rf Value: Rf = Distance travelled by solute / Distance travelled by solvent front.
Applications:
Identification of flavonoids, alkaloids, and glycosides; detection of adulteration; fingerprint profiling

Vein-Islet Number:
Vein-Islet Number Vein-islet number is the average number of small areas of mesophyll tissue enclosed by veins per square millimeter of the leaf surface. These small areas are called vein-islets. This constant is determined by clearing the leaf and observing under a microscope. It is a characteristic feature useful in identification and standardization.

Stomatal Index:
Stomatal Index Stomatal index is the percentage ratio of the number of stomata to the total number of epidermal cells, including stomata, present in a given area of the leaf. It is calculated using the formula:

$$\text{Stomatal Index (S.I.)} = S / (E + S) \times 100$$

Where: S = Number of stomata
E = Number of epidermal cells

Physicochemical Evaluation: This Evaluation is widely used in pharmacognosy, phytochemistry and is important for accurate determination of physicochemical parameters such as extractive value, ash value, and spectrophotometric analysis.

DPPH Free Radical Scavenging Antioxidant Assay:

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay is one of the most widely used and reliable in-vitro methods for evaluating the antioxidant activity of plant extracts. Antioxidants are compounds that can donate electrons or hydrogen atoms to neutralize free radicals, thereby preventing oxidative stress and cellular damage. Free radicals are unstable molecules that can cause damage to lipids, proteins, and DNA, leading to various diseases such as cancer, cardiovascular disorders, diabetes, and aging. The DPPH assay is simple, rapid, sensitive, and economical, and therefore it is commonly used in pharmacognostic and phytochemical studies to evaluate the antioxidant potential of herbal extracts such as methanolic leaf extract of *Eranthemum reticulatum.* This method helps in determining the free radical scavenging capacity of phytoconstituents such as flavonoids, phenols, tannins, and glycosides present in the plant extract. Principle The DPPH assay is based on the reduction of the DPPH free radical by antioxidants present in the plant extract. DPPH is a stable free radical having a deep violet color due to the presence of an unpaired electron. It shows maximum absorbance at 517 nm in a UV-Visible spectrophotometer. When an antioxidant donates a hydrogen atom or electron to DPPH, the DPPH radical is reduced to a stable non-radical form called diphenylpicrylhydrazine. As a result, the color changes from deep violet to light yellow. This decrease in color intensity corresponds to a decrease in absorbance, which indicates the scavenging activity of the antioxidant. The degree of discoloration reflects the antioxidant capacity of the extract. Higher discoloration indicates higher antioxidant activity.
Accurately weigh 1 mg of powdered plant extract and dissolve it in 25 ml of 99% methanol in a conical flask. The conical flask is then placed in a shaking water bath at room temperature and shaken at 100 rpm for 2 hours and 30 minutes to ensure proper extraction of phytoconstituents. After shaking, the solution is transferred into centrifuge tubes and centrifuged at 6000–8000 rpm for 15 minutes. Centrifugation helps to separate the solid particles from the liquid extract. After centrifugation, the supernatant is carefully collected and filtered through filter paper to obtain a clear extract solution. This extract is dried and preserved for further use. Preparation of Sample Stock Solution The extracted solution is dissolved in 10 ml of 99% methanol to prepare a stock solution of concentration 400 µg/ml. From this stock solution, serial dilutions are prepared to obtain different concentrations required for antioxidant activity testing. Preparation of DPPH Solution DPPH solution of 0.004% w/v concentration is prepared by dissolving 4 mg of DPPH in 100 ml of 99% methanol. The solution is mixed well until completely dissolved. This solution must be freshly prepared and stored in a dark place

because DPPH is sensitive to light and may degrade. The DPPH solution appears deep violet in color. Preparation of Standard Ascorbic Acid Solution Ascorbic acid is used as a standard antioxidant for comparison. Accurately weigh 2 mg of ascorbic acid and dissolve it in 2.5 ml of distilled water to prepare a stock solution of concentration 800 µg/ml. From this stock solution, serial dilutions are prepared to obtain different concentrations such as:12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml and 400 µg/ml respectively.

Preparation of Control : The control solution contains 3 ml of DPPH solution and methanol. This serves as a negative control. Methanol is used as blank. The control shows maximum absorbance. Procedure From the stock solution of standard ascorbic acid, serial dilutions are prepared in test tubes to obtain different concentrations such as 12.5 µg, 25 µg, 50 µg, 100 µg, 150 µg, and 200 µg. To each test tube, 4 ml of DPPH solution is added and mixed properly. The volume of each test tube is made up to 10 ml using methanol. Similarly, different concentrations of plant extract are prepared and treated in the same way. All the test tubes are incubated at room temperature for 30 minutes in a dark place to allow the reaction to occur. During incubation, the color changes from deep violet to light yellow depending on the antioxidant activity [154,155]. After incubation, the absorbance is measured at 517/ 520 nm using a UV Visible spectrophotometer against methanol as blank. Observation The antioxidant activity is indicated by a decrease in absorbance. Higher decrease in absorbance indicates higher antioxidant activity. Calculation of Percentage Inhibition The percentage inhibition of DPPH radical is calculated using the formula:

DPPH scavenging effect (%) = [(A0 – A1)/A0) × 100].

Where: A_0 = Absorbance of control

A_1 = Absorbance of samples and standard.

Determination of IC_{50} Value IC_{50} is defined as the concentration of extract required to inhibit 50% of DPPH free radicals. To determine IC_{50}: Different concentrations of extract are prepared. Percentage inhibition is calculated for each concentration.

A graph is plotted: X-axis = Concentration (µg/ml) Y-axis = Percentage inhibition.

From the graph, the concentration corresponding to 50% inhibition is determined as IC_{50}. It can also be calculated mathematically using the linear equation

$Y = mX + C$, where at $Y = 50\%$,

IC_{50} $(X) = (50 - C)/m$.

Where: Y = Percentage inhibition X = Concentration m = Slope C = Intercept

From the graph, the concentration corresponding to 50% scavenging activity was determined and recorded as the IC_{50} value. The lower IC_{50} value of the herbal extract compared to the standard indicates that the extract possesses significant antioxidant activity and has a stronger free radical scavenging potential. This antioxidant activity may be attributed to the presence of phytoconstituents such as flavonoids, phenolic compounds, and other secondary metabolites present in the plant. These results confirm that the herbal extract is a potent natural antioxidant and supports its potential use in the management of oxidative stress and related disorders. The IC_{50} value obtained from the DPPH assay serves as an important parameter for the evaluation and standardization of antioxidant activity of herbal drugs.

RESULTS AND DISCUSSION:

Table No. 1: Preliminary phytochemical analysis of MLEER
(Methanol Leaf Extract of Eranthemum Reticulatum)

S.No.	Test	Methanol Leaf Extract of Eranthemum Reticulatum
1	Reducing Sugars	Positive
2	Proteins	Positive
3	Alkaloids	Positive
4	Flavanoids	Positive
5	Terpenoids	Negative
6	Glycosides	positive
7	Tannins	Positive
8	Phenolic acids	Positive
9	Volatile oils	Negative

Table No. 2: Macroscopical Characteristics of Eranthemum Reticulatum Leaf

S. No	Character	Description
1	Size	Leaves are medium to large, measuring approximately 6–15 cm in length and 3–7 cm in width
2	Shape	Ovate to elliptic
3	Colour	Upper surface: Dark green; Lower surface: Light green
4	Surface	Upper surface smooth and glabrous, lower surface slightly rough
5	Texture	Soft, thin, and flexible
6	Arrangement	Opposite, decussate arrangement
7	Apex	Acute to acuminate
8	Base	Cuneate to attenuate
9	Margin	Entire (smooth margin without serrations)
10	Venation	Reticulate venation, prominent midrib with lateral veins forming network
11	Petiole	Present, short, slender, green, about 1–3 cm long
12	Odour	Characteristic, slightly herbal odour
13	Taste	Slightly bitter and astringent
14	Fracture	Short and soft
15	Leaf type	Simple leaf
16	Lamina	Broad, flat, and symmetrical
17	Midrib	Prominent on lower surface, less prominent on upper Surface

Table No. 3: Microscopic Characters of Eranthemum Reticulatum Leaf Powder

S. No	Character	Microscopic Description
1	Epidermis	Leaf shows single-layered upper and lower epidermis composed of compactly arranged, rectangular to polygonal cells covered with thin cuticle
2	Cuticle	Thin, smooth cuticle present on both surfaces, providing protection
3	Upper Epidermis	Consists of larger, compact, rectangular cells, usually without stomata
4	Lower Epidermis	Consists of smaller cells with numerous Stomata
5	Stomata	Diacytic type of stomata present predominantly on lower epidermis
6	Trichomes	Simple, multicellular covering trichomes present occasionally; glandular trichomes may also be present
7	Mesophyll	Mesophyll differentiated into palisade and spongy parenchyma
8	Palisade Parenchyma	Single layer of elongated, cylindrical, closely packed cells present below upper epidermis
9	Spongy Parenchyma	Consists of loosely arranged, irregular cells with intercellular spaces, present below palisade layer
10	Midrib	Midrib region is prominent and shows collenchymatous tissue below epidermis
11	Collenchyma	2–4 layers of collenchymatous cells present below both upper and lower epidermis in midrib region
12	Vascular Bundle	Single, centrally located, collateral vascular bundle present in midrib region
13	Xylem	Located towards inner side, composed of

		vessels, tracheids, and xylem parenchyma
14	Phloem	Located towards outer side, composed of sieve tubes, companion cells, and phloem parenchyma
15	Bundle Sheath	Parenchymatous bundle sheath surrounding vascular bundle
16	Calcium Oxalate Crystals	Calcium oxalate crystals present in mesophyll Region
17	Intercellular Spaces	Large intercellular spaces present in spongy parenchyma

Table No.4: Quantitative Microscopy characters of Eranthemum Reticulatum Leaf

Parameter	Value
Stomatal Number	210 / sq. mm
Stomatal Index	21%
Palisade Ratio	4.2
Vein Islet Number	19 / sq. mm

Table No. 5: Physico chemical evaluation of Eranthemum Reticulatum Leaf Extract

Parameter	Value (%W/W)	limit
Loss on drying	9.3	NMT 10
Total Ash value	11.7	NLT 10
Water soluble Extractive	2.83	
Acid insoluble ash	1.79	NMT 3

Table No. 6: TLC Profile of MLEER

Rf Value	Colour of the Spot
0.36	Yellow
0.46	Light Yellow
0.67	Green
0.79	Dark Yellow

Table No. 7: DPPH Assay of MLEER

Std. ASA & Sample dilutions	Wt (µg/ml)	A @ 520 nm (Std.)	A @ 520 nm (Sample)	% SCV of ASA	%SCV of sample
1	12.5	0.958	0.456	11.2963	22.580
2	25	0.905	0.401	16.203	31.918
3	50	0.808	0.340	25.185	42.275
4	100	0.495	0.274	54.166	53.480
5	200	0.324	0.109	70.00	81.494
6	400	0.251	0.079	76.759	86.587
Blank	0	1.08	0.589	0.0	0.0

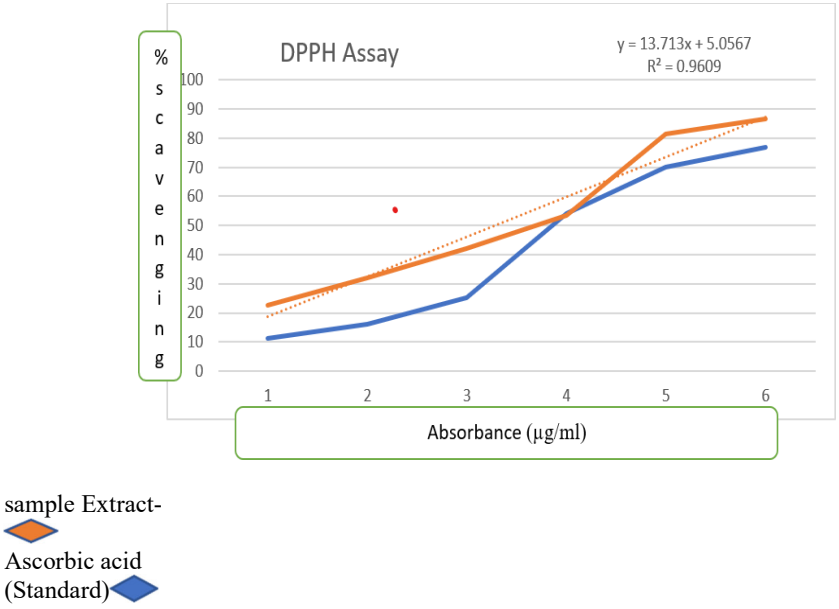

Fig No. 2: Calibration curve of DPPH radical scavenging activity for herbal extract and standard antioxidant

Table No. 8: IC50 Value Assay of MLEER in Invitro DPPH Method

IC50 Value Assay		
Assay Method	Sample Extract (µg/ml)	Ascorbic acid (µg/ml)
DPPH Scavenging Assay	112.29 µg/ml	177.45 µg/ml

CONCLUSION

For the methanolic leaf extract of *Eranthemum reticulatum,* the current study developed thorough pharmacognostic, physicochemical, phytochemical, chromatographic, and antioxidant standards. Important diagnostic characteristics such diacytic stomata, glandular trichomes, cystoliths, calcium oxalate crystals, and ordered mesophyll tissues were found by macroscopic and microscopic analyses, enabling precise identification and authentication. The plant material's stability, quality, and purity were verified by quantitative microscopy and physicochemical measurements. Flavonoids, phenolics, alkaloids, glycosides, and tannins were found through phytochemical screening, and its chemical profiling and antioxidant-related components were confirmed by TLC fingerprinting and UV-visible analysis. With a lower IC_{50} value than ascorbic acid and robust free radical scavenging activity, the extract showed better antioxidant capability. Eranthemum reticulatum exhibits great potential as a natural antioxidant. To validate its therapeutic potential, additional research on the isolation, characterisation, and in vivo assessment of active compounds is advised. The established standards serve as a foundation for quality control.

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