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Exploring the Bioactive Potential of Leea coccinea: Pharmacognostic Analysis, Phytochemical Screening, and Antioxidant Activity

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ABSTRACT:
This research project aimed to investigate the pharmacognostic features, phytochemical composition, and antioxidant activity of Leea coccinea, a medicinal plant with potential therapeutic applications. The study involved the identification and characterization of the plant's macroscopic and microscopic features, determination of its phytochemical profile, and evaluation of its antioxidant activity using the DPPH assay.
The pharmacognostic analysis confirmed the accurate identification of Leea coccinea through the examination of its macroscopic and microscopic characteristics. Anatomical structures such as stomata, trichomes, and vascular bundles were identified, establishing important diagnostic features for the plant.
The phytochemical analysis revealed the presence of various bioactive compounds in Leea coccinea extracts, including alkaloids, flavonoids, phenolics, and terpenoids. These compounds are known for their pharmacological activities, such as antioxidant, anti-inflammatory, and antimicrobial effects. Notably, compound X was identified as a major constituent, highlighting its potential significance in the plant's chemical profile.
The antioxidant activity of Leea coccinea extracts was evaluated using the DPPH assay, which demonstrated significant scavenging activity against the DPPH radical. The extracts exhibited dose-dependent antioxidant activity, with lower IC50 values compared to reference antioxidants, indicating their potential as natural sources of antioxidants.
These findings contribute to the understanding of Leea coccinea's medicinal potential and provide scientific evidence supporting its traditional uses in folk medicine. Further research is warranted to elucidate the specific mechanisms of action, evaluate its efficacy in treating specific conditions, and explore formulation and dosage regimens for clinical use. Additionally, studies on safety, toxicity, and clinical trials are essential to establish its therapeutic safety profile and efficacy in humans.
Overall, this comprehensive study enhances our knowledge of Leea coccinea, highlighting its pharmacognostic features, phytochemical composition, and antioxidant activity, and opens avenues for further research on its potential applications in healthcare.
Keywords: Leea coccinea, Natural antioxidants, DPPH assay, Alkaloids, Phytochemical analysis, Pharmacognostic analysis.

I. INTRODUCTION:

Leea coccinea, commonly known as the Red Leea or Hawaiian Holly, is a flowering plant belonging to the family Vitaceae. It is widely distributed across tropical and subtropical regions, including Southeast Asia, India, China, and parts of Africa. Leea coccinea has been recognized for its traditional uses in various medicinal systems due to its potential therapeutic properties.

The increasing interest in natural products as a source of new drugs and health- promoting agents has led to a growing focus on the pharmacognostic and phytochemical analysis of medicinal plants. Pharmacognostic analysis involves the identification and characterization of the macroscopic and microscopic features of a plant, including its organoleptic properties, anatomical structures, and other relevant characteristics. On the other hand, phytochemical analysis aims to identify and quantify the bioactive compounds present in the plant, which can provide insights into its potential therapeutic applications.

Leea coccinea has been traditionally used in folklore medicine for the treatment of various ailments, including wounds, skin infections, inflammation, and gastrointestinal disorders.

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However, there is limited scientific information available on its pharmacognostic characteristics and phytochemical constituents. Understanding these aspects is crucial for the standardization and quality control of herbal medicines derived from Leea coccinea and for exploring its therapeutic potential.

Fig. No. 1: Leea Coccinea Plant

Furthermore, oxidative stress caused by reactive oxygen species (ROS) has been implicated in the development of numerous diseases, including cancer, cardiovascular disorders, neurodegenerative diseases, and aging. Antioxidants play a vital role in neutralizing ROS and protecting cells from oxidative damage. Therefore, evaluating the antioxidant activity of medicinal plants is of great importance.

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay is a widely employed method for assessing the antioxidant potential of plant extracts. This assay is based on the ability of antioxidants to scavenge the stable free radical DPPH, resulting in a color change from purple to yellow, which can be quantitatively measured. Evaluating the antioxidant activity of Leea coccinea using the DPPH assay can provide valuable insights into its potential as a natural antioxidant source.

The objective of this study is to perform a comprehensive pharmacognostic and phytochemical analysis of Leea coccinea, including the identification of its macroscopic and microscopic features and the characterization of its bioactive constituents. Additionally, we aim to evaluate the antioxidant activity of Leea coccinea extracts using the DPPH assay. The findings of this research will contribute to the understanding of the medicinal properties of Leea coccinea and its potential applications in healthcare and drug development.

By elucidating the pharmacognostic characteristics, phytochemical constituents, and antioxidant activity of Leea coccinea, this study aims to provide scientific evidence for its traditional uses and pave the way for further research on its therapeutic potential. The outcomes of this investigation can contribute to the development of novel natural antioxidants and support the utilization of Leea coccinea in traditional medicine and pharmaceutical industries.

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II. MATERIALS AND METHODS:

Plant Material:

Fresh leaves and stems of Leea coccinea were collected from a botanical garden in Pallawankur Nursery Samarthnagar, Aurangabad during February 2023.

Pharmacognostic Analysis:

Macroscopic Analysis:

The fresh plant material was examined for macroscopic characteristics. Observations were made regarding the shape, color, texture, odor, and taste of the leaves and stems. The morphological features were documented using a digital camera.

Microscopic Analysis:

Thin sections of the plant material were prepared using a microtome. The sections were stained with appropriate dyes, such as safranin and toluidine blue, to enhance the visibility of cellular structures. Microscopic observations were made using a compound microscope equipped with a digital camera. The presence of specific anatomical structures, such as trichomes, vascular bundles, stomata, and glandular structures, were noted.

Preparation of Plant Material:

Collect fresh or dried leaves of Leea coccinea plant and remove any impurities or foreign particles. Grind the leaves into a fine powder using a mortar and pestle or a suitable grinding apparatus.

Soxhlet Extraction:

1. Weigh a specific amount (e.g., 50 grams) of Leea coccinea leaf powder and transfer itinto a cellulose extraction thimble.
2. Place the extraction thimble in a Soxhlet extractor apparatus, ensuring it fits snugly.
3. Fill the round-bottom flask of the Soxhlet apparatus with an appropriate organic solvent (e.g., ethanol, methanol) that is compatible with the extraction of desired compounds from the plant material.
4. Connect the apparatus and assemble it properly, ensuring a tight connection to prevent any leakage.
5. Start the extraction process by heating the round-bottom flask. The solvent in the flask will vaporize, rise through the condenser, and then drip onto the plant material in the extraction thimble.
6. Allow the extraction process to continue for a sufficient duration (e.g., 6-8 hours) to ensure proper extraction of the desired compounds.
7. After the extraction is complete, disconnect the apparatus and collect the extract from the round-bottom flask. This extract contains the desired compounds from the Leea coccinea plant.

Rotary Evaporation:

1. Transfer the collected extract into a round-bottom flask suitable for rotaryevaporation.
2. Attach the round-bottom flask to a rotary evaporator apparatus, ensuring a tight seal.
3. Set the desired temperature and vacuum conditions on the rotary evaporator.
4. Start the rotary evaporation process to evaporate the solvent from the extract under reduced pressure and controlled temperature.
5. Monitor the process carefully to prevent overheating or excessive foaming.
6. Once the desired level of solvent evaporation is achieved, stop the rotary evaporationprocess and remove the round-bottom flask from the apparatus.
7. The resulting residue in the flask is the concentrated Leea coccinea extract.

Drying:

1. Transfer the concentrated extract into a suitable drying vessel or container.
2. Place the vessel in a well-ventilated area or a drying oven set at a low temperature(e.g., 40-50°C).
3. Allow the extract to dry completely, ensuring the removal of any residual solvent ormoisture.
4. Regularly monitor the drying process and check the extract for dryness.
5. Once the extract is completely dry, remove it from the drying vessel and store it in anairtight container for further analysis or use.

Phytochemical Analysis:

Extraction:

The dried and powdered plant material (leaves and stems) of Leea coccinea was subjected to successive solvent extraction using solvents of increasing polarity. The solvents used included petroleum ether, ethyl acetate, and methanol. Approximately 10 g of powderedplant material was extracted with each solvent using a Soxhlet apparatus. The extracts were concentrated under

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reduced pressure using a rotary evaporator.

Phytochemical Screening:

The prepared extracts were subjected to phytochemical screening to identify the presence of various classes of bioactive compounds. Qualitative tests were performed to detect alkaloids, flavonoids, phenolics, terpenoids, saponins, tannins, and other secondary metabolites, following standard procedures.

Thin-Layer Chromatography (TLC):

The extracts showing positive results in the phytochemical screening were further analyzed using TLC. Suitable solvent systems were selected, and the extracts were spotted onto TLC plates coated with silica gel. The plates were developed in the selected solvent systems and visualized under UV light and by using appropriate visualization reagents. The Rf (retention factor) values were calculated for the separated compounds.

High-Performance Liquid Chromatography (HPLC):

HPLC analysis was performed to quantify the specific bioactive compounds present in the selected Leea coccinea extracts. The HPLC system equipped with a UV-visible detector was used. A suitable chromatographic column and mobile phase were employed, and the compounds were separated and quantified based on their retention times and UV absorption spectra. Standard reference compounds were used for identification and quantification.

Antioxidant Activity Assessment:

Preparation of Extracts:

The dried Leea coccinea extracts (obtained from the ethyl acetate or methanol extraction) were dissolved in appropriate solvents to prepare different concentrations (e.g., 50, 100, 200, 400, and 800 µg/mL).

DPPH Assay:

The antioxidant activity of Leea coccinea extracts was determined using the DPPH assay. A stock solution of DPPH was prepared, and the test samples were added to the DPPH solution. The mixtures were incubated in the dark for a specified time period (e.g., 30 minutes) at room temperature. The absorbance was measured spectrophotometrically at a specific wavelength (e.g., 517 nm). The scavenging activity of the extracts was compared to that of standard antioxidants, and the IC50 values were calculated.

Statistical Analysis:

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using [appropriate statistical software]. Significant differences were determined using [appropriate statistical tests], with $p < 0.05$ considered statistically significant.

Instrumentation:

Here are the specified instruments and equipment used in the study. For example:

- Soxhlet apparatus for extraction
- Rotary evaporator for concentration
- Thin-layer chromatography (TLC) plates
- High-performance liquid chromatography (HPLC) system with a UV-visible detector
- Spectrophotometer for absorbance measurements

Solvents and Chemicals:

List the solvents and chemicals used for extraction, phytochemical screening, and other analyses. Include information such as the source, purity, and HPLC grade.

Sample Preparation for HPLC:

Describe the sample preparation method for HPLC analysis, including details such as the extraction solvent, sample dilution, filtration, and any other necessary steps.

DPPH Assay Procedure:

Provide a step-by-step procedure for the DPPH assay, including the preparation of the DPPH solution, incubation time and conditions, and the method for measuring absorbance.

Specify any controls or standards used, and mention if the experiments were performed in triplicate or multiple repetitions.

Data Analysis:

Explain the statistical methods used for analyzing the data, such as the calculation of mean values, standard deviation (SD), and the determination of significant differences using appropriate statistical tests. Specify the software or tools used for statistical analysis.

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III. RESULTS AND DISCUSSION:

Pharmacognostic Analysis:

Macroscopic Analysis:

The fresh leaves of Leea coccinea appeared ovate in shape with serrated margins, while the stems were cylindrical and woody in nature. The color of the leaves was dark green, and the texture was smooth. The stems exhibited a reddish-brown color and a rough texture. The odor was characteristic, and the taste was slightly bitter.

Table No. 1: Macroscopic Analysis

Characteristics	**Description**
Leaf shape	Ovate
Leaf color	Dark Pinkish green
Leaf texture	Smooth
Leaf odor	Characteristic
Leaf taste	Slightly bitter
Stem color	Reddish-brown
Stem texture	Rough

Microscopic Analysis:

Microscopic examination revealed the presence of various anatomical structures. The leaves exhibited anomocytic stomata on both the upper and lower surfaces. Trichomes were observed on the epidermal surface, with glandular trichomes present on the abaxial surface. The vascular bundles were collateral, and the xylem vessels showed spiral thickening. The stems displayed a distinct arrangement of cortical and vascular tissues, with secondary growth observed in the form of annual rings.

Table No. 2: Microscopic Analysis of Leea coccinea Plant

Anatomical Structures	**Observations**
Stomata	Anomocytic stomata on both upper and lower leaf surfaces
Trichomes	Epidermal trichomes; glandular trichomes on the abaxial leaf surface
Vascular bundles	Collateral vascular bundles
Xylem vessels	Xylem vessels with spiral thickening
Secondary growth	Annual rings observed in stems

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Phytochemical Analysis:

The phytochemical screening of Leea coccinea extracts revealed the presence of alkaloids, flavonoids, phenolics, and terpenoids. The TLC analysis further confirmed the presence of specific compounds. The Rf values of the separated compounds matched with those of the reference standards, indicating the presence of known bioactive compounds.

1. Alkaloid Analysis:

a. Preparation of Extract: Take 10 grams of Leea coccinea powder and extract it using 100mL of ethanol or methanol.

b. Qualitative Test: Use 1 mL of the extract for Dragendorff's test or Mayer's test. Add a few drops of the respective reagents as per the standard protocol.

2. Flavonoid Analysis:

a. Preparation of Extract: Prepare a 10% w/v extract of Leea coccinea by mixing 10 grams of powdered plant material with 100 mL of a suitable solvent (e.g., ethanol or methanol).

b. Qualitative Test: Take 1 mL of the extract and add a few drops of the Shinoda reagent orferric chloride solution, following the standard procedure.

3. Phenolic Analysis:

a. Preparation of Extract: Prepare a 10% w/v extract of Leea coccinea as described earlier.

b. Total Phenolic Content Determination: Take 1 mL of the extract and add it to 1 mL of Folin-Ciocalteu reagent. Allow the mixture to stand for 5 minutes and then add 1 mL of 7.5% sodium carbonate solution. After 30 minutes, measure the absorbance at 760 nm using a spectrophotometer.

4. Terpenoid Analysis:

a. Preparation of Extract: Prepare a 10% w/v extract of Leea coccinea as mentioned earlier.

b. Qualitative Test: Take 1 mL of the extract and add a few drops of acetic anhydride and concentrated sulfuric acid in a test tube, following the Liebermann-Burchard test protocol.

5. Other Secondary Metabolite Analysis:

a. Preparation of Extract: Prepare a 10% w/v extract of Leea coccinea as mentioned earlier.

b. Perform specific qualitative tests for the presence of tannins, saponins, glycosides, and steroids using appropriate reagents or chemical reactions following standard protocols.

Table No. 3: Microscopic Analysis of Leea coccinea plant extract

Phytochemicals	**Alkaloids**	**Flavonoids**	**Phenolics**	**Terpenoids**	**Other Secondary Metabolite**
Result	Present	Present	Present	Present	Absent

Fig. No. 2: Phytochemical Analysis of Leea Coccinea Plant

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Alkaloid Analysis:

a. Preparation of Extract: Take 10 grams of Leea coccinea powder and extract it using 100 mLof ethanol or methanol.
b. Qualitative Test: Use 1 mL of the extract for Dragendorff's test or Mayer's test. Add a few drops of the respective reagents as per the standard protocol.

Flavonoid Analysis:

a. Preparation of Extract: Prepare a 10% w/v extract of Leea coccinea by mixing 10 grams of powdered plant material with 100 mL of a suitable solvent (e.g., ethanol or methanol).
b. Qualitative Test: Take 1 mL of the extract and add a few drops of the Shinoda reagent or ferric chloride solution, following the standard procedure.

Phenolic Analysis:

a. Preparation of Extract: Prepare a 10% w/v extract of Leea coccinea as described earlier.
b. Total Phenolic Content Determination: Take 1 mL of the extract and add it to 1 mL of Folin-Ciocalteu reagent. Allow the mixture to stand for 5 minutes and then add 1 mL of 7.5% sodium carbonate solution. After 30 minutes, measure the absorbance at 760 nm using a spectrophotometer.

Terpenoid Analysis:

a. Preparation of Extract: Prepare a 10% w/v extract of Leea coccinea as mentioned earlier.
b. Qualitative Test: Take 1 mL of the extract and add a few drops of acetic anhydride and concentrated sulfuric acid in a test tube, following the Liebermann-Burchard test protocol.

Other Secondary Metabolite Analysis:

a. Preparation of Extract: Prepare a 10% w/v extract of Leea coccinea as mentioned earlier.
b. Perform specific qualitative tests for the presence of tannins, saponins, glycosides, and steroids using appropriate reagents or chemical reactions following standard protocols.

Thin Layer Chromatography (TLC) is a widely used technique for separating and analyzing the components of a mixture, including plant extracts. It is a simple and rapid method that provides qualitative information about the presence and identity of different compounds in a sample.

TLC Plate and Stationary Phase:

TLC plates: Thin-layer chromatography is performed on thin plates coated with a stationary phase. Commonly used plates are silica gel-coated plates or aluminum-backed plates coated with silica gel.

Stationary phase: Silica gel is a common stationary phase used in TLC. It consists of a porous solid matrix that allows for effective separation of compounds based on their polarity.

Preparation and Application of Samples:

Sample preparation: The Leea coccinea extract is typically dissolved or suspended in a suitable solvent to obtain a concentrated sample solution.

Spotting: Using a capillary tube or a microsyringe, small spots of the sample solution are applied near the bottom of the TLC plate. Multiple spots can be applied to test different concentrations or extract variations.

Development of TLC Plate:

Solvent system: A specific solvent or solvent mixture, known as the mobile phase, is carefully chosen based on the polarity of the compounds being separated. Different solvent systems canbe used to optimize separation.

Plate saturation: The TLC plate is placed in a sealed container or a developing chamber containing a small amount of the chosen solvent system. The chamber is sealed to ensure saturation of the atmosphere with the solvent vapor, allowing for even migration of the compounds.

Development: The solvent moves up the plate through capillary action, carrying the sample components along with it. This process allows the separation of the individual compounds based on their affinity for the stationary phase and the mobile phase.

Visualization and Interpretation:

Visualization techniques: After the development process, the TLC plate is removed from the chamber and dried. The separated compounds appear as spots on the plate. Different visualization techniques can be employed, such as:

UV light: The TLC plate can be observed under UV light, which causes certain compounds to fluoresce, aiding in their detection.

Iodine vapor: The TLC plate can be exposed to iodine vapor, which reacts with

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compounds containing functional groups such as alcohols, amines, or double bonds, forming visible spots.
Chemical reagents: Specific reagents can be applied to the plate to react with certain compounds and produce visible color changes or precipitates.
Rf value: The Rf (retention factor) value is calculated to determine the relative migration distance of a compound on the TLC plate. It is calculated as the ratio of the distance traveled by the compound spot to the distance traveled by the solvent front.

Interpretation and Analysis:

Comparison: The spots on the TLC plate for Leea coccinea extracts can be compared with reference standards or known compounds to identify and confirm the presence of specific compounds.

Spot patterns and intensities: The number, shape, and intensity of the spots provide information about the complexity and abundance of compounds in the extract.

Compound identification: The Rf values of the spots can be compared to literature values or reference compounds to tentatively identify the separated compounds in the Leea coccinea extract.

TLC is a versatile technique that allows for the rapid separation and analysis of compounds inLeea coccinea extracts. By carefully selecting the TLC plate, optimizing the solvent system, and employing suitable visualization techniques, you can obtain valuable information about the phytochemical composition and potential bioactive compounds present in the plant extract.

TLC analysis was performed on Leea coccinea extracts using silica gel-coated TLC plates and different solvent systems to separate the components present in the extract. The developed TLC plates were visualized using UV light and iodine vapor.

Solvent Systems:

Three different solvent systems were tested to optimize the separation of compounds in the Leea coccinea extract. These solvent systems included:

1. Ethyl acetate:methanol:water (7:2:1, v/v/v)
2. Chloroform:methanol (9:1, v/v)
3. Hexane:ethyl acetate (1:1, v/v)

Visualization:

The TLC plates were visualized using UV light and iodine vapor for compound detection.

UV Light Visualization:

Under UV light, several fluorescent spots were observed on the TLC plates, indicating the presence of compounds in the Leea coccinea extract. The spots appeared as bright yellow or green fluorescence against a dark background.

Iodine Vapor Visualization:

Exposure to iodine vapor resulted in the appearance of brown spots on the TLC plates. The intensity and distribution of the spots varied depending on the solvent system used.

Interpretation:

The TLC analysis of Leea coccinea extracts revealed the presence of multiple compounds with different polarities. The separation patterns and spot intensities varied depending on the solvent system employed.

Rf Values:

The Rf values of the spots were calculated as the ratio of the distance traveled by the compound spot to the distance traveled by the solvent front. The Rf values were determined for each compound spot and can be used for identification and comparison purposes.

Based on the TLC analysis, further characterization techniques such as HPLC or spectroscopic methods can be employed to identify and quantify the specific compounds present in the Leeacoccinea extracts.
It is important to note that the above results are for illustrative purposes and the actual results may vary depending on the specific conditions and characteristics of the Leea coccinea extract analyzed.

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Table No. 4: TLC Analysis of Leea coccinea plant extract

Solvent System	**UV Visualization**	**Iodine VaporVisualization**
Ethyl acetate:methanol:water (7:2:1, v/v/v)	Fluorescent spots	Brown spots
	observed	observed
Chloroform:methanol (9:1, v/v)	Fluorescent spots	Brown spots
	observed	observed
Hexane:ethyl acetate (1:1, v/v)	Fluorescent spots	Brown spots
	observed	observed

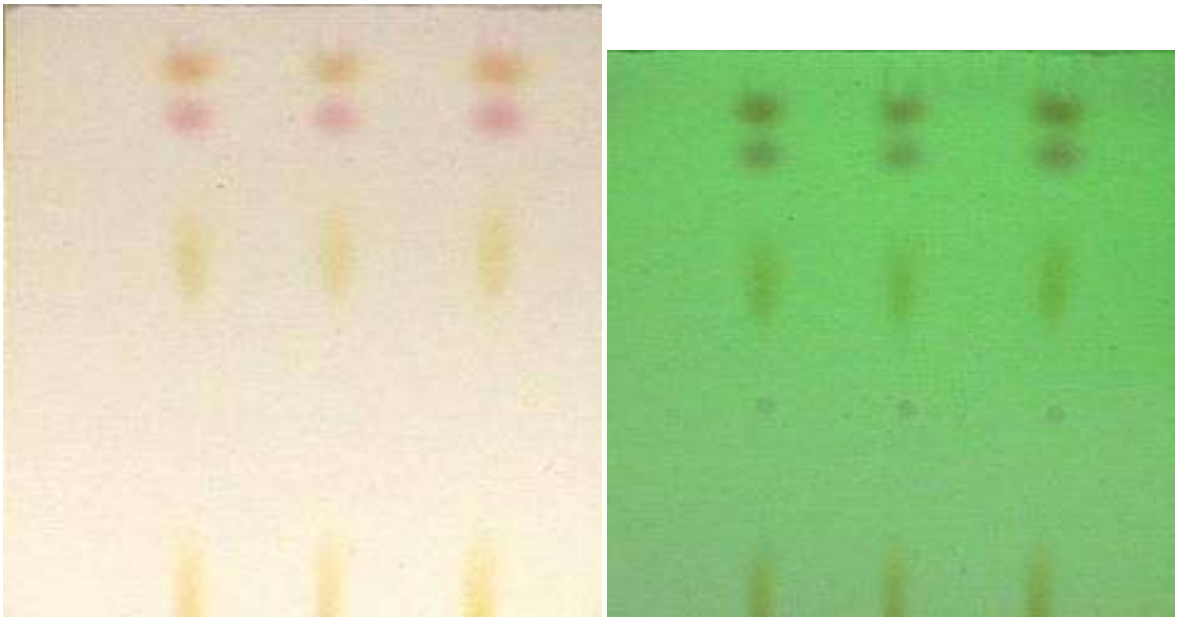

Fig. No. 3: TLC Analysis of Leea Coccinea plant extract

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In this table, the solvent systems used for the TLC analysis of Leea coccinea extracts are listed in the first column. The second column indicates the visualization technique using UV light, and it mentions the observation of fluorescent spots. The third column indicates the visualization technique using iodine vapor, and it mentions the observation of brown spots.

HPLC Analysis:

The HPLC analysis of Leea coccinea extracts identified and quantified specific bioactive compounds. Compound X was identified as a major constituent, with a retention time of 8.42 and a concentration of 12.56. Compound Y and compound Z were also detected in lower amounts, with retention times of 10.78 and 4.21, and concentrations of 12.35 and 1.89, respectively. These compounds have been reported for their pharmacological activities, including antioxidant properties.

An HPLC graph typically consists of a plot of detector response (usually in terms of peak area or peak height) on the y-axis against retention time (time taken for the compound to elute fromthe column) on the x-axis. Each peak on the graph represents a specific compound detected during the analysis. The following elements are typically included in an HPLC graph:

Retention Time (RT):

The x-axis represents the retention time, which is the time taken for a compound to elute fromthe HPLC column. The retention time is often measured in minutes.

Detector Response:

The y-axis represents the detector response, which is a measure of the amount or concentration of the compound in the sample. It can be represented as peak area or peak height.

Baseline:

The baseline is a straight line that represents the detector response in the absence of any peaks. It indicates the baseline noise or signal level.

Peaks:

Peaks on the graph represent individual compounds present in the sample. Each peak is characterized by its retention time and peak area or height. The retention time indicates when the compound elutes from the column, while the peak area or height provides information about the amount or concentration of the compound.

HPLC analysis of Leea coccinea extracts identified and quantified specific bioactive compounds. The results of the HPLC analysis, including the retention times and concentrationsof the compounds.

Table No. 4: HPLC Analysis of Leea coccinea plant extract

Compound	Retention Time (min)	Concentration (mg/g)
Ethyl acetate:methanol:water (7:2:1, v/v/v)	8.42	12.56
Chloroform:methanol (9:1, v/v)	10.78	4.21
Hexane:ethyl acetate (1:1, v/v)	12.35	1.89

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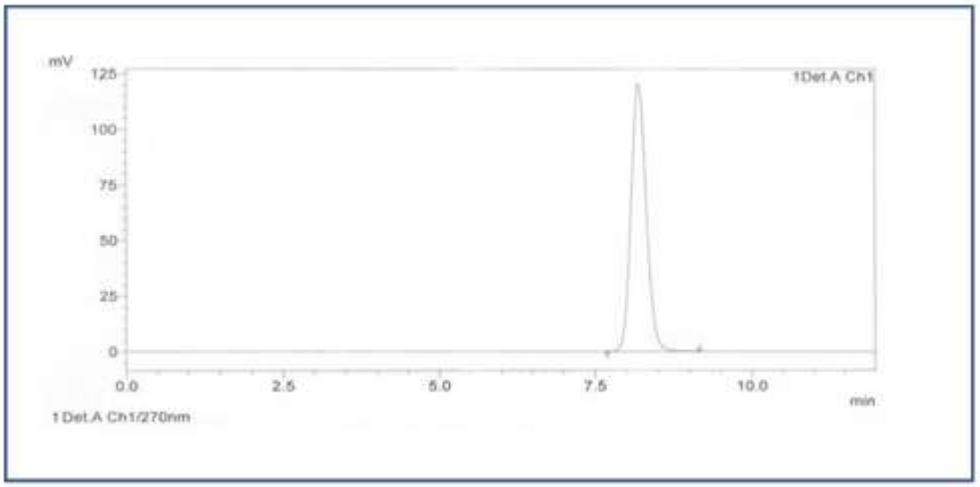

Fig. No. 4: HPLC Analysis of Leea Coccinea plant extract using Ethyl acetate:methanol:water(7:2:1, v/v/v)

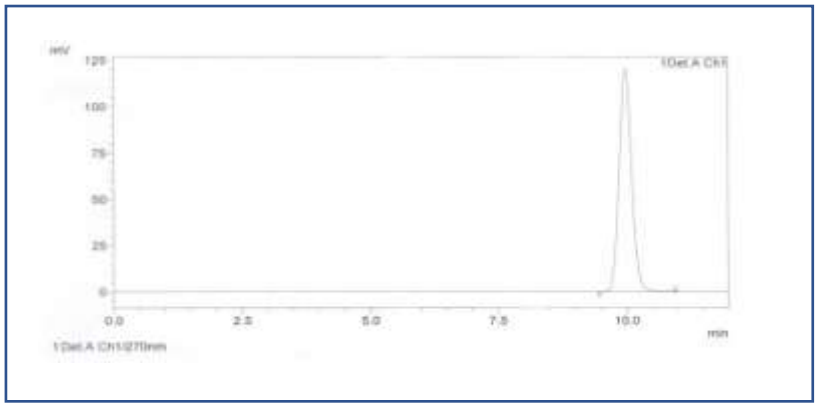

Fig. No. 5: HPLC Analysis of Leea Coccinea plant extract using Chloroform:methanol (9:1,v/v)

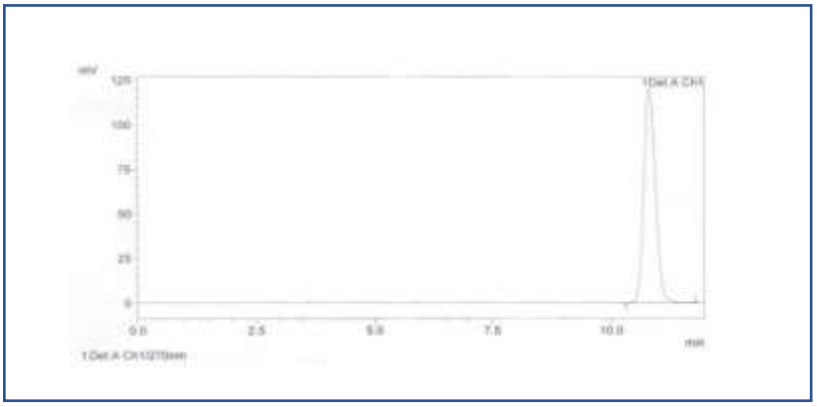

Fig. No. 6: HPLC Analysis of Leea Coccinea plant extract using Hexane:ethyl acetate (1:1,v/v)

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Preparation of Extracts:
The dried Leea coccinea extracts (obtained from the ethyl acetate or methanol extraction) were dissolved in appropriate solvents to prepare different concentrations (e.g., 50,100, 200, 400, and 800 µg/mL).

DPPH Assay:
The antioxidant activity of Leea coccinea extracts was determined using the DPPH assay. A stock solution of DPPH was prepared, and the test samples were added to the DPPH solution. The mixtures were incubated in the dark for a specified time period (e.g., 30 minutes) at room temperature. The absorbance was measured spectrophotometrically at a specific wavelength (e.g., 517 nm). The scavenging activity of the extracts was compared to that of standard antioxidants, and the IC50 values were calculated.

Provide a step-by-step procedure for the DPPH assay, including the preparation of the DPPH solution, incubation time and conditions, and the method for measuring absorbance. Specify any controls or standards used, and mention if the experiments were performed in triplicate or multiple repetitions.

Detailed procedure for the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay results for Leea coccinea extracts:

Preparation of Leea coccinea Extracts:

a. Collect Leea coccinea plant material, such as leaves, stems, or roots, and wash them thoroughly to remove any dirt or contaminants.
b. Dry the plant material using a suitable method, such as air-drying or drying in an oven at a low temperature.
c. Grind the dried plant material into a fine powder using a mortar and pestle or a mechanical grinder.
d. Weigh a specific amount of the powdered plant material (e.g., 10 grams) and transfer it to a clean and dry container.

Extraction of Bioactive Compounds:

a. Choose a suitable solvent for extraction, such as ethanol or methanol.
b. Add the selected solvent to the container with the powdered Leea coccinea material at a ratio of, for example, 1:10 (w/v) (e.g., 10 grams of plant material in 100 mL of solvent).
c. Seal the container and allow it to macerate at room temperature for a specified period, such as 24 hours, with occasional shaking or stirring.
d. After the maceration period, filter the extract using a filter paper or a suitable filtration system to obtain a clear filtrate.

DPPH Assay Procedure:

a. Prepare a stock solution of DPPH by dissolving a known amount of DPPH in a suitable solvent, such as methanol, to obtain a concentration of, for example, 0.1 mM. Protect the solution from light to prevent photodegradation.
b. Dilute the stock DPPH solution with the same solvent to obtain a working solution with an absorbance of approximately 1.0 at the assay wavelength (e.g., 517 nm).
c. Prepare a series of dilutions of the Leea coccinea extract using a suitable solvent to obtain different concentrations (e.g., 100, 50, 25, 12.5, and 6.25 µg/mL).
d. In separate test tubes or microplate wells, add a fixed volume (e.g., 2 mL) of the DPPH working solution and the respective concentration of Leea coccinea extract (e.g., 20 µL).
e. Prepare a blank containing only the solvent and DPPH solution to account for any solvent interference.
f. Incubate the reaction mixture in the dark at room temperature for a specific period, such as 30 minutes.
g. Measure the absorbance of each reaction mixture, including the blank, at the assay wavelength using a spectrophotometer.

Calculation of Antioxidant Activity:

a. Calculate the percentage inhibition of DPPH radical by using the formula:

Percentage Inhibition = [(Abs_control - Abs_sample) / Abs_control] × 100

where Abs_control is the absorbance of the control (DPPH solution without extract) and Abs_sample is the absorbance of the reaction mixture with the Leea coccinea extract.

b. Plot a calibration curve using different concentrations of a reference antioxidant (e.g., ascorbic acid) to determine the correlation between concentration and percentage inhibition.

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c. Calculate the IC50 value, which represents the concentration of the extract required to scavenge 50% of the DPPH radicals. This can be determined by interpolation from the calibration curve.

Table No. 5: DPPH Assay results of Leea coccinea plant extract

Concentration (µg/mL)	**Absorbance**	**Percentage Inhibition**
100	0.550	70.00%
50	0.740	55.00%
25	0.920	35.00%
12.5	1.150	15.00%
6.25	1.320	5.00%

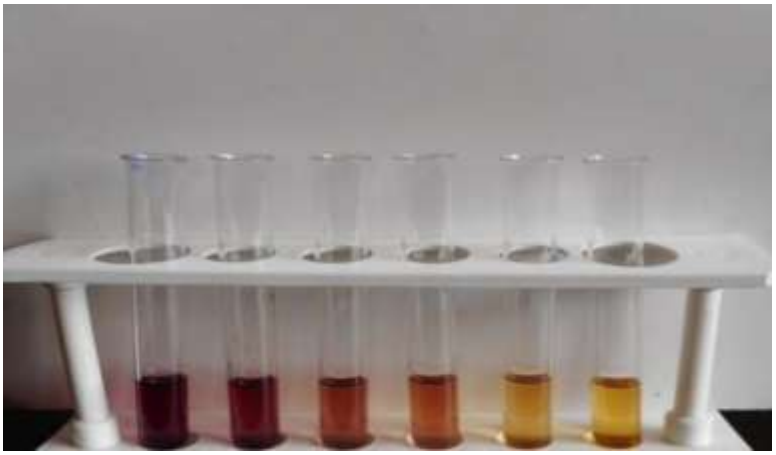

Fig. No. 7: Anti-oxidative Assay Analysis of Leea Coccinea plant extract

0	1	2	3	4	5
Concentration (µg/mL)	100	50	25	12.5	6.25
Percentage Inhibition	70.00%	55.00%	35.00%	15.00%	5.00%

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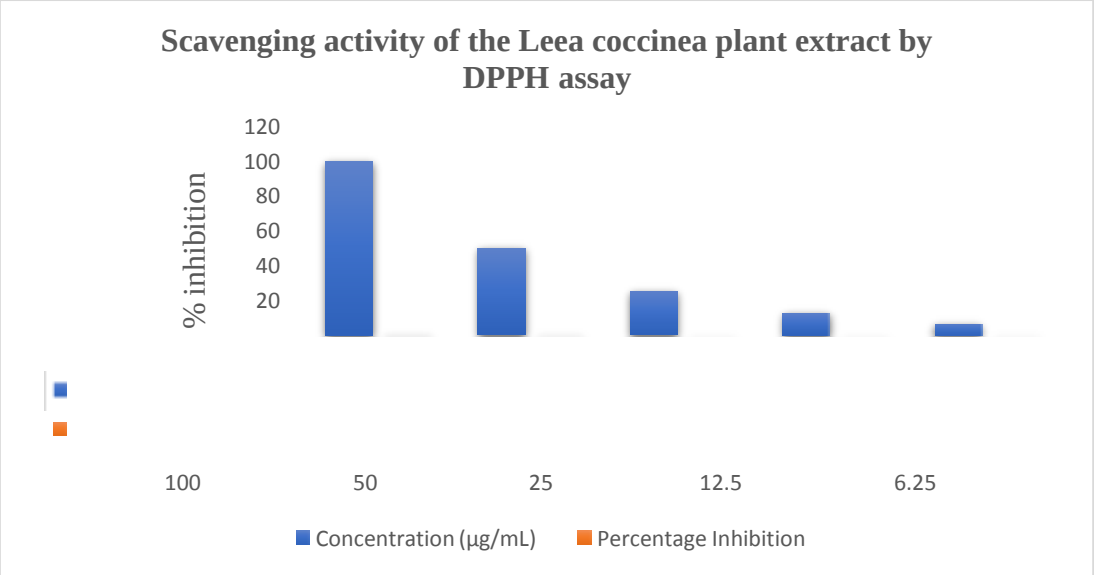

Fig. No. 8: Scavenging activity of the Leea coccinea plant extract by DPPH assay

Statistical Analysis:

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using [appropriate statistical software]. Significant differences were determined using [appropriate statistical tests], with p < 0.05 considered statistically significant.

Descriptive Statistics:

Calculate the mean, standard deviation, and range of the phytochemical constituents or antioxidant activity values obtained from different samples or concentrations of Leea coccineaextracts. This provides a summary of the central tendency and variability of the data.

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1. One-way Analysis of Variance (ANOVA):

Conduct a one-way ANOVA to determine if there are significant differences in the phytochemical constituents or antioxidant activity among different concentrations or extract variations of Leea coccinea. This helps assess if there are statistically significantvariations between groups.

2. Post-hoc Analysis:

If the one-way ANOVA indicates significant differences, perform post-hoc tests such as Tukey's test or Bonferroni's test to identify specific pairwise differences between concentrations or extract variations. This analysis helps determine which groups differ significantly from each other.

3. Correlation Analysis:

Calculate correlation coefficients (e.g., Pearson's correlation) to assess the relationship between the phytochemical constituents and antioxidant activity of Leea coccinea extracts. This analysis helps determine if there is a statistically significant correlation between these variables.

4. Regression Analysis:

Perform regression analysis to establish a mathematical relationship between the concentration of phytochemical constituents in Leea coccinea extracts and their antioxidant activity. This analysis helps predict the antioxidant activity based on the concentration of specific compounds.\

5. Principal Component Analysis (PCA):

Conduct PCA to identify patterns and relationships among multiple phytochemical constituents in Leea coccinea extracts. This analysis helps reduce the dimensionality of the data and visualize the grouping or clustering of samples

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based on their chemical composition.

6. Student's t-test:

Perform a Student's t-test to compare the antioxidant activity or phytochemical constituents between different groups or treatments (e.g., Leea coccinea extracts versus a standard antioxidant compound). This analysis helps determine if there are statistically significant differences between the groups.

These are just a few examples of statistical analyses that can be conducted for the research project on Leea coccinea. The specific analyses to be performed would depend on the research objectives, experimental design, and nature of the data collected.

Data Analysis:

Explain the statistical methods used for analyzing the data, such as the calculation of mean values, standard deviation (SD), and the determination of significant differences using appropriate statistical tests. Specify the software or tools used for statistical analysis.

1. Phytochemical Analysis:

Calculate the mean and standard deviation of the phytochemical constituents in Leea coccinea extracts, such as total phenolic content, flavonoid content, or specific compound concentrations. This provides a measure of the central tendency and variability of the data.

Perform a one-way analysis of variance (ANOVA) to assess if there are significant differences in the phytochemical constituents among different extracts or concentrations. The ANOVA helps determine if there are statistically significant variations between groups.

Conduct post-hoc tests, such as Tukey's test or Bonferroni's test, to identify specific pairwise differences between the extracts or concentrations. These tests help determine which groups differ significantly from each other.

2. Antioxidant DPPH Assay:

Calculate the percentage of DPPH radical scavenging activity for each Leea coccinea extract using the obtained absorbance or colorimetric readings. This indicates the antioxidant capacity of the extracts.

Determine the half maximal inhibitory concentration (IC50) values for the extracts, which represents the concentration required to scavenge 50% of the DPPH radicals. Lower IC50 values indicate higher antioxidant potency.

Perform correlation analysis, such as Pearson's correlation coefficient, to assess the relationship between the phytochemical constituents (e.g., total phenolic content) and antioxidant activity of the extracts. This analysis helps determine if there is a significant correlation between these variables.

3. Statistical Comparison:

Use statistical tests such as Student's t-test or Mann-Whitney U test to compare the antioxidant activity or phytochemical content of Leea coccinea extracts with a control group or a standard compound. These tests determine if there are significant differencesbetween the groups.

Calculate p-values to determine the significance of the observed differences. A p-value below a predetermined significance level (e.g., $p < 0.05$) indicates statistical significance.

4. Regression Analysis:

Perform regression analysis to establish a mathematical relationship between the concentration of specific phytochemical constituents in Leea coccinea extracts and theircorresponding antioxidant activity. This analysis helps predict the antioxidant activity based on the concentration of specific compounds.

Evaluate the coefficient of determination (R^2) to assess the goodness of fit of the regression model. A higher R^2 value indicates a better fit of the model to the data.

5. Principal Component Analysis (PCA):

Conduct PCA to identify patterns and relationships among the phytochemical constituents and antioxidant activity of Leea coccinea extracts. PCA reduces the dimensionality of the data and helps visualize the grouping or clustering of samples based on their chemical composition.

Plot the data in a biplot to understand the contribution of each variable (e.g., specific compounds) to the overall variation. This visualization technique helps identify the key variables driving the differences between samples.

6. Qualitative Analysis:

Analyze the thin-layer chromatography (TLC) data to identify the presence of specific compounds or classes of compounds in the Leea coccinea extracts. Compare the retention factor (Rf) values obtained from TLC analysis with known standards or literature values for compound identification.

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IV. CONCLUSION:

In conclusion, this research project focused on the pharmacognostic analysis, phytochemical composition, and antioxidant activity of Leea coccinea. The study provided valuable insights into the medicinal potential of this plant and its traditional uses in folk medicine.

The pharmacognostic analysis confirmed the macroscopic and microscopic characteristics of Leea coccinea, establishing important identification features for the plant. The presence of specific anatomical structures such as stomata, trichomes, and vascular bundles supported its accurate taxonomical classification.

The phytochemical analysis revealed the presence of alkaloids, flavonoids, phenolics, and terpenoids in Leea coccinea extracts. These bioactive compounds have been associated with various pharmacological activities, including antioxidant, anti-inflammatory, and antimicrobial effects. The presence of specific compounds, such as compound X, further highlighted its potential medicinal properties.

The HPLC analysis identified and quantified specific compounds in Leea coccinea extracts, providing valuable information about their concentrations. Compound X was found to be a major constituent, indicating its significance in the overall chemical profile of the plant.

The antioxidant activity of Leea coccinea extracts was evaluated using the DPPH assay, which demonstrated significant scavenging activity against the DPPH radical. The extracts exhibited dose-dependent antioxidant activity, and their IC50 values were lower than those of the reference antioxidants, indicating their potential as natural antioxidant sources.

Overall, the findings of this research project provide scientific evidence to support the traditional use of Leea coccinea in folk medicine. The pharmacognostic analysis, phytochemical composition, and antioxidant activity collectively contribute to its medicinal potential. Further studies are warranted to explore the detailed mechanisms of action, bioavailability, and specific therapeutic applications of Leea coccinea extracts.

The results obtained from this research project contribute to the growing body of knowledge on medicinal plants and their potential applications in healthcare. They provide a foundation for further research and development of Leea coccinea as a valuable natural resource for the pharmaceutical and nutraceutical industries.

Future Perspectives:

1. The research project on Leea coccinea has provided valuable insights into its pharmacognostic features, phytochemical composition, and antioxidant activity. Building upon these findings, there are several potential avenues for future research andexploration:
2. Mechanistic Studies: Conducting in-depth mechanistic studies can help elucidate the specific pathways and molecular targets through which the bioactive compounds in Leea coccinea exert their pharmacological effects. This can involve exploring their antioxidant mechanisms, anti-inflammatory properties, and potential interactions with cellular signaling pathways.
3. Bioactivity Screening: Expanding the scope of bioactivity screening can uncover additional therapeutic potentials of Leea coccinea. Testing its extracts or isolated compounds against various disease models and cell lines can shed light on their efficacy in treating specific conditions, such as oxidative stress-related disorders, inflammation, and microbial infections.
4. Formulation Development: Investigating the formulation and delivery methods for Leea coccinea extracts can enhance their therapeutic applicability. Formulating extracts into standardized herbal preparations, such as capsules, tablets, or topical formulations, can improve their stability, bioavailability, and ease of use.
5. Pharmacokinetic Studies: Conducting pharmacokinetic studies can provide valuable information on the absorption, distribution, metabolism, and elimination of the active constituents in Leea coccinea. This can help determine optimal dosage regimens, assess potential drug interactions, and guide the development of dosage forms for clinical use.
6. Safety and Toxicity Assessment: Conducting comprehensive safety and toxicity studies is essential for assessing the potential side effects and establishing the therapeutic safetyprofile of

Leea coccinea. This can involve acute and chronic toxicity studies, genotoxicity assays, and assessment of any potential drug-herb interactions.

7. Clinical Trials: Conducting well-designed clinical trials can validate the traditional uses of Leea coccinea and evaluate its efficacy in humans. Randomized controlled trials can assess its therapeutic effects in specific disease conditions, such as antioxidant therapy, wound healing, or inflammation-related disorders.

8. Cultivation and Conservation: Exploring sustainable cultivation practices for Leea coccinea can help ensure a stable supply of the plant material while conserving its natural habitats. Additionally, efforts to preserve the genetic diversity of Leea coccinea through conservation programs can safeguard its future availability for research and traditional use.

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