

Histochemical Testing and Microscopic Structure of *Leea indica* (Burm.f.) Merr. (Memaye) as a Medicinal Plant of the Besemah Tribe for Anti-infective and Degenerative Purposes in Lahat, South Sumatra

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ABSTRACT

Leea indica (Burm.f.) Merr. (Memaye) is traditionally used by the Besemah Tribe in Lahat, South Sumatra, as a medicinal plant to treat infectious and degenerative diseases, including hepatitis, liver disorders, and warts. The utilization of this plant as an herbal remedy requires comprehensive information on its microscopic characteristics and secondary metabolite content. This research aimed to determine the classes of secondary metabolite compounds with anti-infective and anti-degenerative potential and their distribution in the vegetative organs of *L. indica* through histochemical testing, as well as to identify specific diagnostic fragments from microscopic observation of *simplicia* powder and stem maceration preparations. Histochemical tests were performed on fresh cross-sections of leaves, stems, and roots using specific reagents: Wagner's reagent for alkaloids (reddish-brown positive), 10% NaOH for flavonoids (yellow), 5% copper acetate for terpenoids (yellow to brownish), 10% FeCl₃ for phenols (greenish-black), and Lugol's iodine for tannins (black or purple). Microscopic observations were conducted on chloral hydrate-mounted *simplicia* powder and safranin-stained maceration preparations. Results showed that alkaloids, terpenoids, tannins, and phenols were distributed in all vegetative organs, while flavonoids were detected exclusively in leaves. Diagnostic fragments, including various crystal types (raphide, druse, rosette), trichomes (glandular, non-glandular, scale, and septate), and vascular elements (tracheae, tracheids, fibers, and xylem parenchyma), were identified across leaves, stems, and roots. Notably, scale trichomes and septate trichomes with basal sacs serve as specific diagnostic fragments for *L. indica*, providing reliable authentication markers for quality control in medicinal applications and future research.

KEYWORDS

Leea indica, Identifying Fragment, Histochemistry, Antiinfectious, Antidegenerative



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INTRODUCTION

Traditional medicine derived from plants is widely used by Indonesian people as an initial treatment or as a complement to medical treatment for various types of diseases, including infectious and degenerative diseases. The World Health Organization estimates that approximately 80% of the population in developing countries use traditional medicine as a primary healthcare necessity, and most herbal extracts are used in health therapy (Rivai, 2020). The Besemah Tribe in Lahat Regency, South Sumatra, utilizes *L. indica*, a plant typical of the region they inhabit, as a remedy for degenerative diseases and infections (Tanzerina et al., 2023). The potential of *L. indica* as a medicinal plant is further evidenced by the existence of secretory structures in its organs. According to (Tanzerina et al., 2024), *L. indica* has been proven to possess secretory structures whose distribution spans the leaves, stems, and roots. The use of *L. indica* by the Besemah Tribe as an anti-infective and anti-degenerative agent demonstrates the potential of this plant as a raw material for herbal medicine. This potential can be further supported by information on the presence and distribution of secondary metabolite compounds that function as anti-infective and anti-degenerative agents in *L. indica*. Various Histochemical Testing and Microscopic Structure of *Leea indica* (Burm.f.) Merr. (Memaye) as a Medicinal Plant of the Besemah Tribe for Anti-infective and Degenerative Purposes in Lahat, South Sumatra

testing methods have been developed to detect the presence of secondary metabolite compounds, among which histochemical testing is particularly relevant. (Wick, 2012) in (Badria & Aboelmaaty, 2019) stated that histochemistry is specifically used to study the identification and distribution of chemical compounds within cells using color indicators observed under a microscope.

The characterization of a medicinal plant requires not only information on secondary metabolite content and its distribution, but also microscopic identification to determine the microscopic structures that can serve as distinctive diagnostic fragments for verifying plant authenticity. Microscopic analysis is necessary to confirm the correct species and to ensure that the correct plant part is present in the sample; for example, the presence of certain microscopic structures such as leaf stomata can be used to identify the plant part utilized (Alamgir, 2017). Several previous studies have examined the anatomical structure of *L. indica*. (Tanzerina et al., 2024) identified the presence of secretory structures in the vegetative organs of *L. indica*, demonstrating the distribution of secretory tissues in leaves, stems, and roots. Additionally, (Najmaddin et al., 2013) conducted a comparative leaf anatomy study of selected species in Vitaceae and Leeaceae, revealing the presence of druse crystals and secretory cells in *L. indica* leaves. (Greeshma and Kumar, 2023) performed a pharmacognostical analysis of *L. indica* roots, identifying acicular crystals, calcium oxalate crystals, and rosette crystals in elongated parenchyma cells. However, these previous anatomical studies were limited to describing structural features without providing information on the specific localization of secondary metabolite compounds within tissues. Furthermore, these studies did not comprehensively identify the diagnostic fragments from all vegetative organ's leaves, stems, and roots that can serve as authentication markers for pharmacognostic standardization.

This study differs from previous research in several fundamental aspects. First, this study employs histochemical testing to localize the distribution of specific secondary metabolite classes alkaloids, flavonoids, terpenoids, tannins, and phenols within the tissues of leaves, stems, and roots of *L. indica*, thereby providing insight into the sites of biosynthesis and accumulation of bioactive compounds with anti-infective and anti-degenerative properties. Second, this study comprehensively identifies diagnostic fragments from all three vegetative organs through both *simplicia* powder observation and maceration preparations, enabling the establishment of specific authentication markers for *L. indica*. Third, this study integrates histochemical localization with microscopic fragment identification to provide a holistic understanding of the relationship between tissue structure and secondary metabolite accumulation.

The research gap addressed by this study is the lack of integrated information on both the histochemical localization of secondary metabolites and the comprehensive identification of diagnostic fragments from all vegetative organs of *L. indica*. While previous studies have examined either anatomical structure or phytochemical content separately, no study has simultaneously investigated the tissue-specific distribution of bioactive compounds and established comprehensive microscopic authentication markers for *L. indica*. The novelty of this research lies in: (1) the first comprehensive histochemical mapping of five major secondary metabolite classes across leaves, stems, and roots of *L. indica*; (2) the identification of specific diagnostic fragments from each vegetative organ, including the discovery of scale trichomes

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and septate trichomes with basal sacs as unique authentication markers; and (3) the integration of histochemical and microscopic approaches to support the ethnopharmacological use of *L. indica* by the Besemah Tribe.

This research contributes significantly to the pharmacognostic standardization of *L. indica* as a raw material for herbal medicine. The identified diagnostic fragments particularly scale trichomes and septate trichomes with basal sacs provide reliable authentication markers that can be used for verification of *L. indica* identity in herbal drug manufacturing, quality control, and scientific research. The histochemical localization of bioactive compounds provides crucial information for determining which plant organs should be harvested for specific therapeutic applications, ensuring optimal utilization of the plant's medicinal potential. These findings support the development of a comprehensive pharmacognostic monograph for *L. indica*, facilitating its integration into formal herbal medicine systems and contributing to the conservation and sustainable use of this valuable medicinal plant resource of the Besemah Tribe.

METHOD

This study was exploratory descriptive research with a qualitative approach that aims to describe the distribution of secondary metabolite compounds through histochemical assays as well as to identify distinctive diagnostic fragments of the vegetative organs of *Leea indica* (Burm.f.) Merr. through microscopic structure observation. This approach was chosen to provide a comprehensive overview of the *pharmacognostic* potential of *L. indica* as a raw material for traditional medicine of the Besemah Tribe.

Sampling and Research Locations

This research was carried out in January 2025. Sampling of *Leea indica* (Burm.f.) Merr. using convenience sampling took place in Sukamerindu District, Lahat Regency, South Sumatra. Histochemical tests and microscopic observations of vegetative organs were conducted in the Laboratory of Physiology and Development and the Microengineering Room, Department of Biology, Faculty of Mathematics and Natural Sciences, Sriwijaya University.

Histochemical Test

Cross-sections were made of the leaf, stem, and root organs of *L. indica*. Each section was placed on a separate glass slide, then the fresh sections were treated with drops of the following specific reagents: 10% NaOH for the flavonoid test, with a positive indicator of yellow discoloration; Wagner's reagent for the alkaloid test, with a positive indicator of reddish-brown discoloration; 5% copper acetate for the terpenoid test, with a positive indicator of yellow to yellowish-brown discoloration; 10% FeCl₃ for the phenol test, with a positive indicator of greenish-black discoloration; and Lugol's iodine for the tannin test, with a positive indicator of black or purple discoloration. The preparations were then allowed to stand for 10–30 minutes and covered with a cover glass. Color changes in the preparations were subsequently observed under a microscope.

Microscopic Structure Observation

Observation of microscopic structures was carried out using two methods: *simplicia* powder observation and maceration preparations. For *simplicia* powder observation, *simplicia* powder was prepared from the leaves, stems, and roots of *L. indica*. Each *simplicia* powder was placed on separate glass slides, dripped with chloral hydrate solution, covered with a cover glass, and then observed under a microscope.

Maceration preparations used the stem organ of *L. indica* as the material. The material was cut to a thickness of 3 mm and a length of 1 cm. The cut pieces were placed into a test tube containing 20% KOH and brought to a boil. The material was then washed under running water for 15 minutes, followed by immersion in a mixture of 20% chromic acid and 20% nitric acid in a 1:1 ratio for 2–3 hours until the material became soft. The softened material was washed with water for 20 minutes, then dehydrated through a graded alcohol series of 30%, 50%, 70%, 96%, and 100%, followed by clearing in pure xylol, replaced twice. Each step of the dehydration process lasted 30 minutes. Staining was then performed using 1% safranin. The stained material was subsequently spread on a glass slide, dripped with Canada balsam, and covered with a cover glass. The preparations were observed under a microscope.

RESULT AND DISCUSSION

1. Distribution of Alkaloid Compounds in *Leea indica* (Burm.f.) Merr

The results of alkaloid tests on the root, stem, and leaf organs of *L. indica* showed positive alkaloid compounds that accumulated in the root and stem vascular tissues, stem and leaf sclerenchyma, trichomes, and stem and leaf idioblast cells. Based on the results of research by (Kuntorini et al., 2023), the accumulation of alkaloid compounds in leaves is mostly contained in leaf epidermal tissue, mesophyllia, xylem and phloem vascular tissue, leaf bone mother parenchyma, idioblast cells, and trichomes. The content of alkaloid compounds is likely to be more abundant in the bark compared to the content of other metabolite compounds due to the accumulation of abundant stored alkaloid compounds in the secondary cortex and phloem (Pratiwi et al., 2020).

Two types of trichomes were found to contain alkaloid compounds, of which glandular trichomes with three head cells were found on *L. indica* leaves, while headless and branched-haired trichomes were found on stems with red trichomes partitions. Trichomes as a form of secretory structure in plant organs that play a role in the storage and secretion of secondary metabolites, are one of the places for the accumulation of alkaloid compounds. Glandular trichomes and non-glandular trichomes include secretory structures, as well as secretory cavities, idioblast cells, latispheres, resin channels, and oil glands (Mulyah et al., 2019).

Alkaloid compounds as one of the secondary metabolites found in various plant organs make these plant organs can be used as herbal medicines. According to Widi and Indriati (2007) in (Rahmani et al., 2022), the role of alkaloid compounds in the health sector, for example, is used as an anti-infectious and degenerative drug. The Besemah tribe uses the root part of the *L. indica* plant as a medicine for hepatitis (Tanzerina et al., 2023).

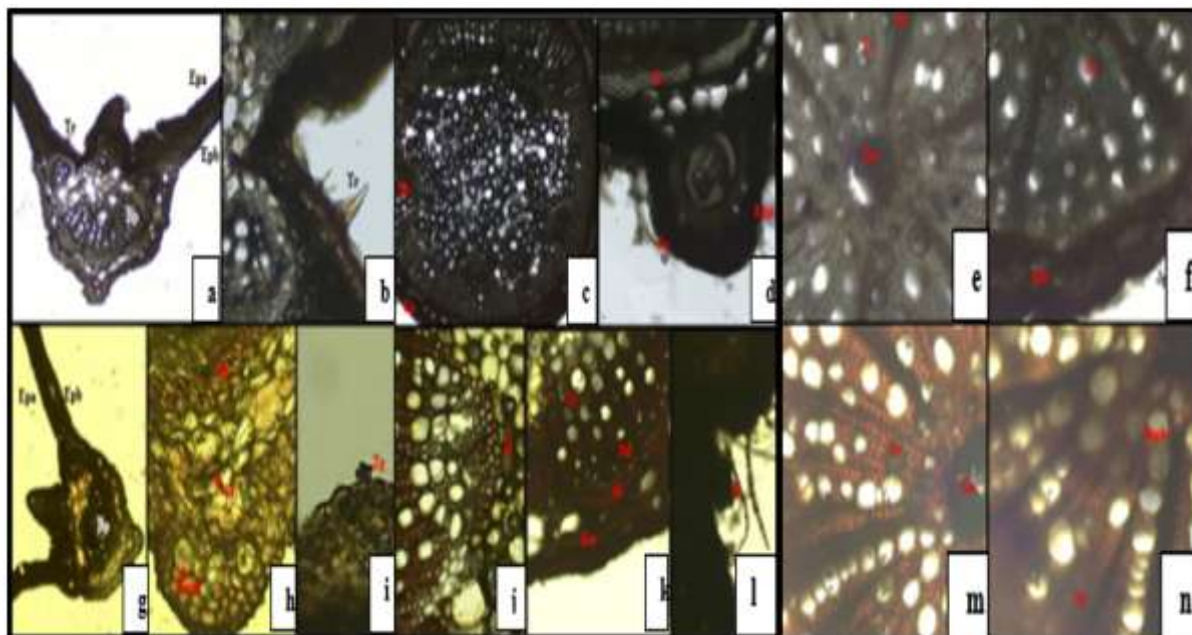


Figure 1. *L. indica* alkaloid test. a-b: leaf cross-incision as negative control; C-D: Cross cut of the rod as negative control; E-F: Cross incision of the root as negative control; g-i: positive result of leaf alkaloids; j-l: positive results of stem alkaloids; M-N: Positive results of root alkaloids. EPA: Upper epidermis; Epb: Lower epidermis; Em: Pular; Jg: Cork tissue; Jm: Fingers of pith; Phl: Phloem; Xy: Xylem; Tb: Branched partitioned trichomes; Tg: Glandular trichoma; Sk: Sklerenkim; Color: Cortex; Id: Idioblas; Raph: Rafida crystal. (a, c, and e: Magnification 4×10; b, d, and f-n: Magnification 10×10).

Source: Research documentation, 2025

Table 1. Distribution of Secondary Metabolite Compounds *L. indica*

Secondary Metabolites	Reaction Results			Location		
	Leaves	Rod	Roots	Leaves	Rod	Roots
Alkaloids	+	+	+	Idioblast cells, glandular trichomes, sclerenchymal tissue	Sclerenchymal tissues, vessels, cortex, idioblast cells, blocked trichomes	Vascular network
Flavonoids	+	-	-	Lower epidermis	-	-
Terpenoids	+	+	+	Upper epidermis, idioblast cells near vessel bundles, glandular trichomes, non-glandular trichomes	Idioblast cells located in parenchymal tissue	Vascular network
Tannins	+	+	+	Vascular tissues, idioblast cells	Cortic, vascular tissue, glandular trichomes, non-glandular trichomes	Vascular tissue, pith parenchyma

Phenol	+	+	+	Epidermal tissues, vessels, glandular trichomes, non-glandular trichomes, idioblast cells in the parenchyma	Glandular trichomes, parenchymal tissues, idioblast cells	Vascular tissue, pith parenchyma
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Remark: (+): Positive reaction result; (-): Negative Reaction Result

Source: Primary data from histochemical testing, 2025

2. Distribution of Flavonoid Compounds in *Leea indica* (Burm.f.) Merr

The test of flavonoid compounds of *L. indica* leaves which showed positive results was supported by the results of (Emran et al., 2012) research, which stated that *L. indica* leaves contain flavonoid compounds based on qualitative tests of secondary metabolite compounds. Meanwhile, the roots and stems of *L. indica* show negative flavonoid test results. According to (Khuniad et al., 2022), based on several studies on the content of secondary metabolites in *L. indica* that have been carried out previously, it can be known that flavonoid compounds in *L. indica* can be found in leaf organs. Based on research on *Larrea* species conducted by (Mercado et al., 2018), the accumulation of flavonoid compounds can be detected in the cuticle area, palisade cells, and also *mesophyllia*.

Previous research has described flavonoid compounds that are widely used as medicinal ingredients, both in traditional and modern medicine. Its ability to ward off free radicals that cause oxidative stress in the body makes flavonoid compounds a compound with strong antioxidant properties. Oxidative stress contributes to various degenerative diseases such as cancer, diabetes, and cardiovascular disease. Flavonoids can help delay the aging of the nervous system, immune organs, reproductive system, liver, skin, and also contribute to the prevention of osteoporosis, cardiovascular disease, Alzheimer's, and breast cancer (Fernandes et al., 2017; Imran et al., 2019; Liu et al., 2021; Selvakumar et al., 2020).

The accumulation of flavonoid compounds contained in the leaf organs of *L. indica* then indicates that the leaf organ has the potential to be an ingredient in herbal medicine to treat degenerative diseases and as an *antiinfectant*. According to (Mohammad et al., 2012), it is stated that the leaf organs of *L. indica* are used by boiling the leaves and then drinking directly as an effort to treat diabetes.

3. Distribution of Terpenoid Compounds in *Leea indica* (Burm.f.) Merr

Terpenoid tests of the roots, stems, and leaves of *L. indica* showed positive results with the appearance of yellow to brownish patches on the parts containing terpenoid compounds. *L. indica* leaves contain terpenoids that accumulate in upper epidermal tissues, idioblast cells, non-glandular trichomes and glandular trichomes. Meanwhile, the stem part of *L. indica* contains terpenoids that accumulate in idioblast cells in the stem parenchyma tissue. According to (Lusa et al., 2016), the results of histochemical tests show that terpenoid compounds are found in glandular trichomes and stem and leaf parenchymal tissues in plants from the subtribe

Lychnophorinae. Glandular trichomes contain specialized cells as a function of biosynthesis and secretion of certain secretory products (Lange and Turner, 2013).

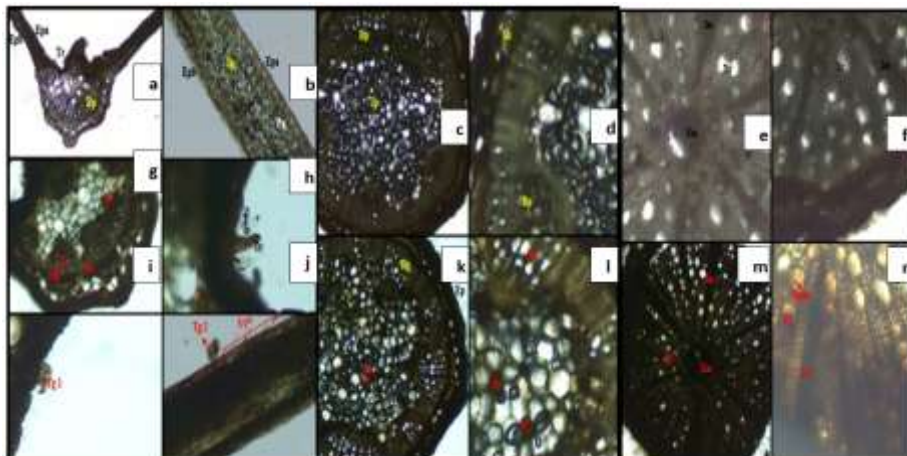


Figure 2. *L. indica* terpenoid test. a-b: leaf cross-incision as negative control; C-D: Cross cut of the rod as negative control; E-F: Cross incision of the root as negative control; g-j: positive results of leaf terpenoids; k-l: positive result of the terpenoid of the stem; L-N: Positive results of root terpenoids. EPA: Upper epidermis; Epb: Lower epidermis; Em: Pular; Jg: Cork tissue; Jm: Fingers of pith; Phl: Phloem; Xy: Xylem ; Tr: Nonglandular trichoma; Tg: Glandular trichoma; Sk: Sklerenkim; Color: Cortex; Id: Idioblas; Raph: Rafida crystal. (b-c, k, and m: Magnification 4×10; a, d-j, l, and n: Magnification 10×10).

Source: Research documentation, 2025

Previous research has shown that terpenoid compounds accumulate in glandular trichomes. Glandular trichomes are generally derived from basal cells in the epidermal cell layer, one or more stem cells, and secretory cells at the apex with the latter responsible for the biosynthesis of terpenoids and other metabolites (Lange, 2015). In addition, brownish spots are also shown on the root of *L. indica*, which means that there are terpenoid compounds in the root. This is supported by the research of Harun et al. (2016), which showed the results that terpenoid compounds in *L. indica* are contained in root organs, stems, and leaves.

The content of secondary metabolite compounds in the form of terpenoids accumulated in the roots, stems, and leaves of *L. indica* supports the therapeutic potential of the plant with the local name *Memaye* as an *antiinfectant* and degenerative. According to (Dominic & Jat, 2025), researchers established that terpenoids fall into the category of active antibacterial agents along with flavonoid compounds, tannins, alkaloids, steroids, and phenolic compounds or other free hydroxyl groups. Terpenoids as antibacterial agents can be a solution to health problems caused by bacterial infections. In addition, terpenoids along with phenolic compounds and alkaloids are responsible for antioxidant activity (Harun et al., 2016).

The use of plants with the content of terpenoid compounds that have antioxidant activity and act as an antibacterial such as *L. indica* has been widely carried out by the community. For example, the Besemah Tribe people use *L. indica* as an alternative to treating liver disease using its roots (Irmastika, 2023). In addition, they also use the root organs of *L. indica* to treat hepatitis disease (Tanzerina et al., 2023). *L. indica* leaf organs are used to boil and drink directly with the aim of treating diabetes (Mohammad et al., 2012).

4. Distribution of Phenol Compounds in *Leea indica* (Burm.f.) Merr

The phenol compound content that can be observed based on the results of phenol tests on the root organs, stems, and leaves of *L. indica* is accumulated in the tissues of the epidermis, parenchyma, vascular tissue, and glandular trichomes, respectively. According to (Lusa et al., 2016), the content of phenolic compounds is found mainly accumulated in epidermal cells, chlorophyll parenchyma on leaves and basic parenchyma on stems in plants of the subtribe *Lychnophorinae*. Phenol compounds are found in the tissues of the epidermis and parenchyma, where these compounds play a role in protection against pathogens. In addition, the accumulation of phenols in the parenchyma contributes to the chemical defenses of plants by inhibiting the enzymatic activity of microorganisms that have the potential to damage tissues.

Phenol compounds also accumulate in glandular trichomes found in *L. indica* stems. Based on research on the localization of histochemical secretions in *Plectranthus grandidentatus* conducted by (Mota et al., 2013), phenol compounds are abundant in various types of glandular trichomes found in *Plectranthus grandidentatus*. Glandular trichomes in *Vitis romanetii* (Vitaceae) can secrete mucus and emit volatile substances so that many insects often attach to the glandular trichomes (Ma et al., 2016).

The use of *L. indica* as an *antiinfectant* and a drug for degenerative diseases is supported by the presence of phenol compounds in the root organs, stems, and leaves. Compound content with antioxidant and anti-microbial activity is needed to ensure a plant can be used for anti-infection and degenerative purposes. According to (Marjoni et al., 2015), phenol compounds that have hydroxyl groups are generally beneficial because they have antioxidant activity. The hydroxyl and carbonyl groups in phenols can interact with bacterial cells through hydrogen bonding so that they coagulate proteins and bacterial cell membranes and cause bacteria to lysis, making phenols effective as antibacterial (Januarti et al., 2019).

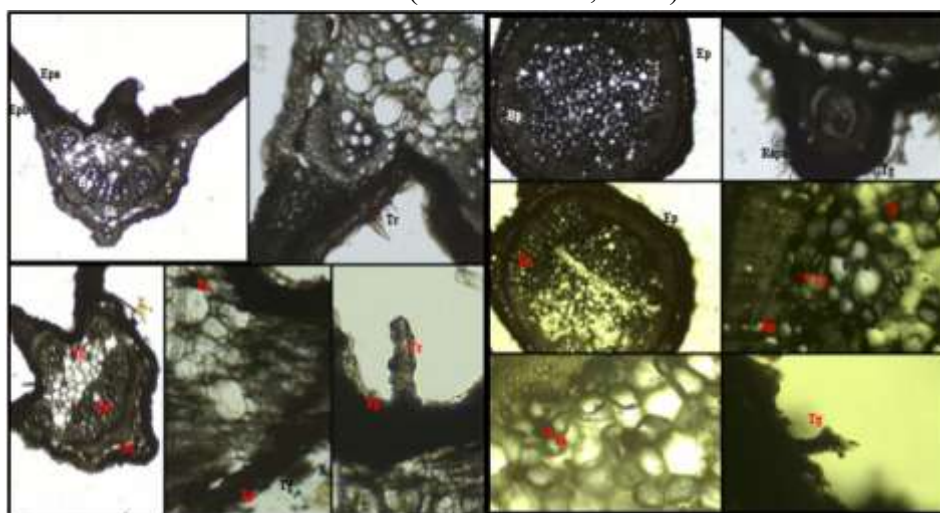


Figure 3. *L. indica* phenol test. a-b: leaf cross-incision as negative control; C-D: Cross cut of the rod as negative control; E-F: Cross incision of the root as negative control; g-i: positive result of leaf phenols; J-M: positive results of stem phenols; N-O: Positive result of root phenol. Ep: Epidermis; EPA: Upper epidermis; Epb: Lower epidermis; Em: Pular; Jg: Cork tissue; Jm: Fingers of pith; Phl: Phloem; Xy: Xylem ; Tr: Nonglandular trichoma; Tg: Glandular trichomes type 3; Sk: Sklerenkim; Color: Cortex; Id: Idioblas; (a, c, f-g, and j: Magnification 4×10; b, d-e, h-i, and k-o: Magnification 10×10).

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Source: Research documentation, 2025

The presence of phenol compounds in the root organs, stems, and leaves of *L. indica* indicates that *L. indica* can be used as a disease treatment ingredient. According to (Temkitthawon et al., 2008), *L. indica* is utilized as an ingredient in preparations to treat vaginal discharge, bowel cancer, and uterine cancer. The root organ of *L. indica* is used by the Besemah Tribe as a medicine for liver disease and hepatitis (Irmastika, 2023; Tanzerina et al., 2023).

5. Distribution of Tannin Compounds in *Leea indica* (Burm.f.) Merr

Tannin compounds in *L. indica* as a whole accumulate in the root organs, stems, and leaves which include vascular tissue, cortex, root pith, trichomes, and idioblasts. The positive results of tannin tests on these parts are supported by the statement of (Oliveira et al., 2012), who explain that the secretory of compounds with the chemical composition of tannin compounds in *Cissus verticillata* (L.) is found in the cortex, stem parenchyma, and parenchyma near leaf veins. According to (Irwanto et al., 2023), based on histochemical tests on *Rhodomirtus tomentosa* leaves, an accumulation of tannin compounds was found in epidermal tissue, sponge parenchyma, vascular bundle or vascular tissue, midrib parenchyma, and secretory chamber.

Tannin tests on roots that showed positive results of tannin accumulation in pith and root vessel tissue were supported by research by (Hermawan et al., 2023), which showed that the presence of tannin compounds was found in the transporter tissue. The existence of glandular trichomes containing black patches adds to the accumulation of tannin compounds on *L. indica* stems. According to (Ma et al., 2016), glandular trichomes found in the species *Vitis* (Vitaceae) can secrete substances and the space between cells is likely to be a temporary storage place for such secretions.

The potential of *L. indica* to be used as a medicinal plant for degenerative diseases and as an anti-infection increases with the discovery of the content of tannin compounds accumulated in the root organs, stems, and leaves. According to Satapathy and Kumar (2020), tannin compounds contribute to the field of health and healing through the mechanism of antioxidant, anti-inflammatory, antibacterial, and especially neuroprotective activity resulting in tannin compounds having high potential as a remedy for the treatment of Parkinson's disease which is an example of a degenerative disease. According to (Tanzerina et al., 2023), *L. indica* root has been used by the Besemah Tribe to treat hepatitis. The Besemah Tribe also uses the *L. indica* root as a medicine for liver disease (Irmastika, 2023).

6. Microscopic Structure

a. Identifier Fragments of *Leea indica* Leaf (Burm.f.) Merr

Based on microscopic observations on *L. indica* leaf *simplisia*, identifier fragments were obtained in the form of druse crystals, rafida crystals, stomata types, glandular trichomes and non-glandular trichomes, trachea, tracheid, and fiber types.

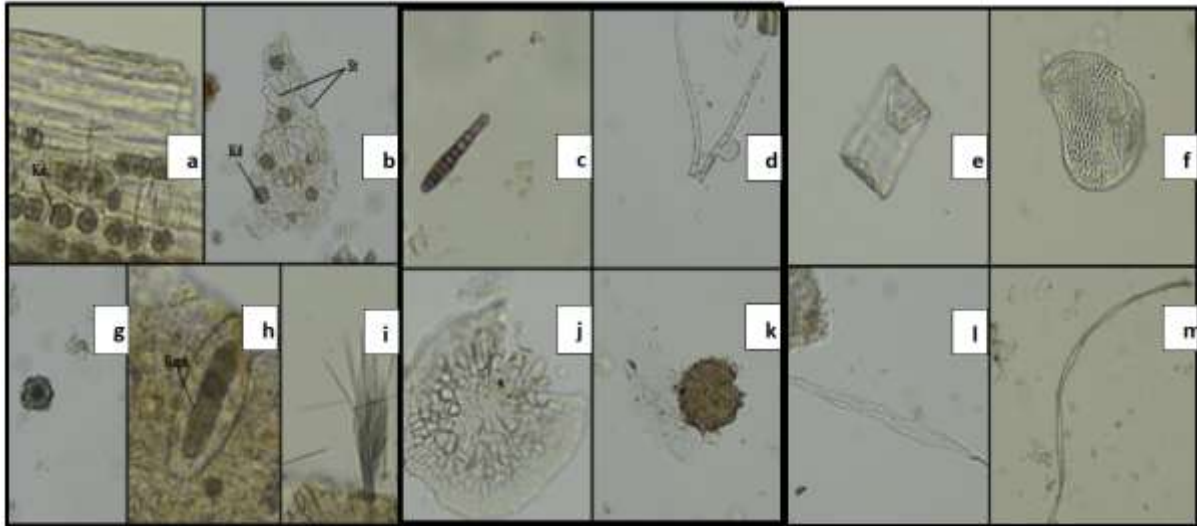


Figure 4. Leaf identifier fragment of *L. indica*. a-b and g: Druse crystals; c: Glandular trichomes; d: Trichomes are partitioned with sacs; e-f: Tracheal fragments; h-i: Crystal rafida; j: Trichoma of scales type 1; k: Trichoma scale type 2; l: Tracheid; m: Fiber. Kd: Druse crystals; Raph: Crystal rafida; St: Stomata. (Magnification 10×10)

Source: Research documentation, 2025

Microscopic observations showed the presence of druse crystals found on the epidermis of *L. indica* leaves (Figure 4. a). The visible stomata are *anisocytic* (Figure 4. b). *L. indica* leaves also store groups of needle crystals or rafida crystals arranged into a single unit in a sac-like structure (Figure 4.h). Crystals of rafida outside the pouch can also be seen on observation (Figure 4. i).

The existence of druse crystals found as one of the identifying fragments of *L. indica* leaf simplicia is supported by research by (Greeshma and Kumar, 2023), who showed that calcium oxalate crystals were found on the cross-section of *L. indica* leaves. Druse crystals as a form of calcium oxalate crystals found in the leaves of *L. indica*, are also found in the leaf organs of other *Leea* species. According to (Najmaddin et al., 2013), the cross-section of *L. indica* and *L. rubra* each indicates the existence of druse crystals, secretory cells, and additional vascular bundles present in both species.

A special identifying fragment also found in *L. indica* leaves is a rafida crystal arranged in a single oval-shaped sac. Based on research on the microscopic structure of *Cissus latifolia* Lam (Vitaceae) conducted by (Sreebala et al., 2021), it can be found that the presence of rafida crystals contained in the sac is one of the markers of members of the Vitaceae family. This statement is supported by the research of (Oliveira et al., 2012), which showed results that *Cissus verticillata* (Vitaceae) has rafida crystals contained in sacks in the tissue part of the vegetative organs.

b. Identifying Fragments of the Stem of *Leea indica* (Burm.f.) Merr

Based on the results of microscopic observations on simplicia preparations and *L. indica* stem maceration preparations carried out, identifying fragments were obtained in the form of roset crystals, rafida crystals, cell types that make up cortical tissues, non-glandular trichomes, types of thickening of the tracheal walls, cells that make up the xylem parenchyma, tracheids, and also long fibers.

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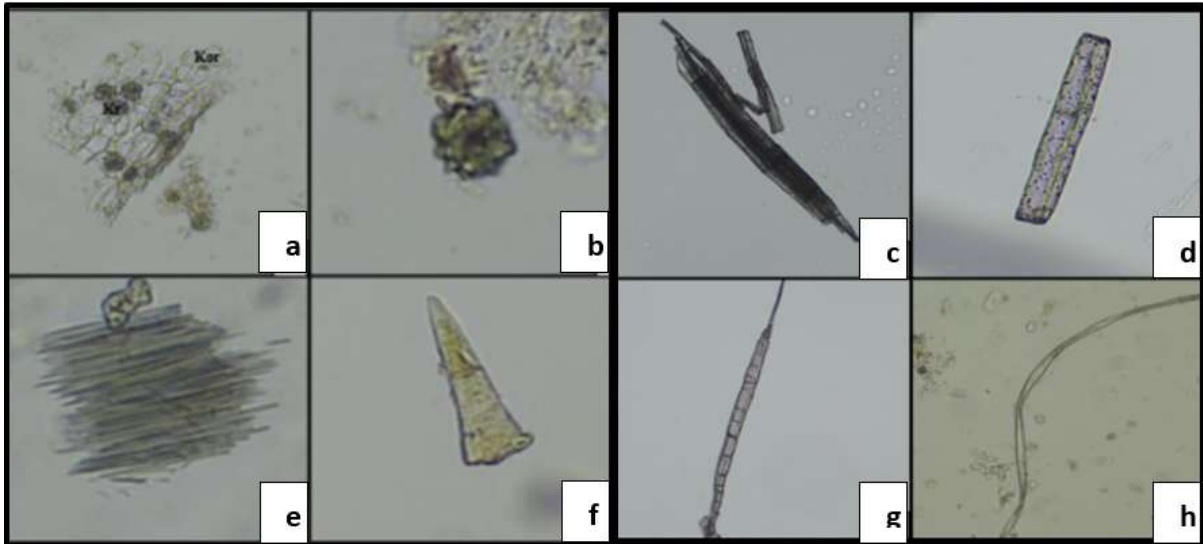


Figure 5. Identifying fragments of the *L. indica* stem. a-b: Rosset crystal; c: Trachea; d: Parenkim xylem ; e: Crystal rafida; f: Nonglandular trichomes; g: Trakeid; h: Fiber. Kr: Crystal rosset; Color: Trunk cortex. (Magnification 10×10).

Source: Research documentation, 2025

A rod simplicia identifier fragment in the form of rosset crystals was found in the trunk cortex tissue of *L. indica*. Based on the research of Tanzerina et al. (2024), the transverse cross-section of the *L. indica* stem shows that rosset-shaped crystals are found in the trunk cortex. Rosset crystals are contained in the stem part of *L. indica* and are mostly scattered on the stem pith (Greeshma and Kumar, 2023).

A rafida crystal without a sac that wraps the crystal on the simplicia of *L. indica* stems was also found. The release of rafida crystals from inside the sac that holds them can be caused by the rupture of the cell wall of the sac. The accumulation of mucus and rafida crystals on the inside of idioblasts increases the pressure on the cell wall, causing the cell to break down and facilitating the crystals to exit the cell (Oliveira et al., 2012). Calcium oxalate crystals contained in plant cells and tissues can play a role in plant protection against herbivores. According to (Konyar et al., 2014), calcium oxalate crystals protect plants from herbivores through toxic chemicals or through the mechanical effects of needle-like crystals that pierce herbivorous animals that are foraging.

Identifying fragments in the form of non-glandular trichomes with a structure composed of one basal cell and one apex cell were also found in the observation of *L. indica* stem simplicia. Simple or multicellular non-glandular trichomes are found in many plants belonging to the Vitaceae family such as in the genus *Vitis* and *Cyphostemma hypoleucum* (Ma et al., 2016; Rambau et al., 2023).

The identifying fragments or distinctive markers found on the stem of *L. indica*, which are part of the constituent part of the xylem parenchyma, have a rectangular shape with dots on their surface. According to (Punjungsari and Ulfa, 2022), the xylem parenchyma is also known to be a living cell that has involvement in the metabolism and protection of xylamine. Wood parenchyma and pith finger parenchyma can be found in secondary xylem (Hindriana and Handayani, 2023).

c. Identifier Fragments of *Leea indica* Root (Burm.f.) Merr

Based on microscopic observations of *L. indica* root simplicia preparations, identifier fragments were obtained that included rosset crystals, rafida crystals, xylem parenchyma cells, and fibers that have a long size.

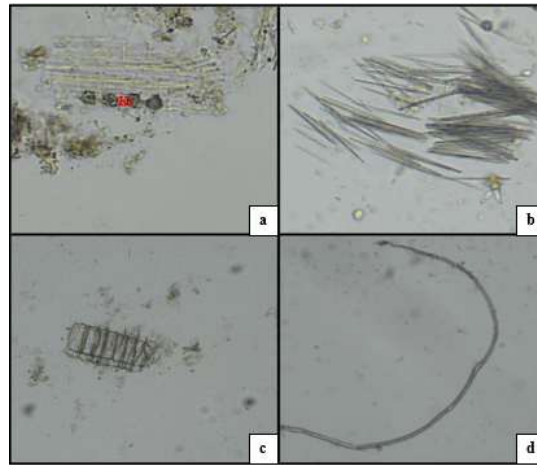


Figure 6. A fragment of the root identifier of *L. indica*. a: Rosset crystal; b: Crystal rafida; c: Parenchym xylem ; d: Fiber. (a-b and d: 10×10 magnification; c: 40×10 magnification).

Source: Research documentation, 2025

The results of the observations showed that two types of crystals were found as identifiable fragments of *L. indica* root simplicia. The crystals found were rosset crystals (Figure 6. a) and rafida crystals (Figure 6. b). Based on research conducted by Greeshma and Kumar (2023), the presence of acicular crystals, calcium oxalate crystals, and also rosset crystals located in elongated parenchymal cells was found as a result of microscopic studies of *L. indica* root simplicia. The study showed that the two types of crystals in question were indeed found in the root of *L. indica*.

The xylem parenchyma found has a rectangular cell shape that is tightly arranged with dots on the surface of the cell. Xylem parenchyma is an important component in plant transport networks that play a role in storing and distributing nutrients and water. The walls of the xylem parenchyma can form secondary thickening and the pair of dots can be shielded, semi-shielded, or simple (Estiti, 1995). The fibers found have a long shape without dots or holes at each end.

Microscopic observations show that each of the vegetative organs of *L. indica* has diverse identifying fragments. The observed microscopic structure is characteristic of *L. indica* so that it can be used for the identification of the plant. The identifying fragments in each plant have their own characteristics, for example in microscopic structures in the form of trichomes. According to Harisha and Jani (2013) in (Dewi, Hindun, & Wahyuni, 2015), both the structure and morphology of trichomes have diversity so that they can be used as the key to the identification of genera, species, subspecies and varieties of various plant families. Fragments of specific identifiers for *L. indica* are found in the leaf organs in the form of multicellular partitioned trichomes with sacs at the base of the trichome, and flattened scaled trichomes with a surface that appears to have a notched partition-like motif that can be used to verify the authenticity of *L. indica* used in medicine and research.

CONCLUSION

Histochemical testing of *L. indica* revealed that alkaloids, terpenoids, tannins, and phenols are distributed across all vegetative organs roots, stems, and leaves while flavonoids were detected exclusively in leaves. Diagnostic fragments identified in the leaves include raphide crystals, druse crystals, stomata, glandular trichomes, scale trichomes, septate trichomes, *tracheae*, tracheids, and fibers; those in the stem comprise rosette crystals, raphide crystals, non-glandular trichomes, *tracheae*, tracheids, xylem parenchyma, and long fibers; while the roots yielded rosette crystals, raphide crystals, xylem parenchyma cells, and long fibers. Notably, scale trichomes and septate trichomes with basal sacs were identified as specific diagnostic fragments of *L. indica*, serving as reliable authentication markers for verifying plant identity in medicinal and research applications. Future research should focus on the isolation and characterization of specific bioactive compounds from each organ, validated through *in vitro* and *in vivo* anti-infective and anti-degenerative assays, alongside pharmacognostic monograph development encompassing diagnostic fragments, marker compound levels, and chromatographic profiles for herbal quality control. Further histochemical and microscopic studies on the reproductive organs of *L. indica* are also warranted, accompanied by cultivation and conservation efforts to ensure a sustainable supply of this valuable medicinal plant, and by the documentation of the Besemah Tribe's traditional knowledge to preserve cultural heritage and support future therapeutic applications.

REFERENCES

- Alamgir, A. N. M. (2017). *Therapeutic Use of Medicinal Plants and Their Extracts: Volume 1. Pharmacognosy*.
- Badria, F. A., & Aboelmaaty, W. S. (2019). Plant Histochemistry: A Versatile and Indispensable Tool in Localization of Gene Expression, Enzymes, Cytokines, Secondary Metabolites and Detection of Plants Infection and Pollution. *Acta Scientifical Pharmaceutical Sciences*, 3(7), 88–100.
- Dewi, V. P., Hindun, I., & Wahyuni, S. (2015). Study of Leaf Trichomes in the Solanaceae Family as a Resource for Biology Learning. *JPBI (Indonesian Journal of Biology Education)*, 1(2).
- Dominic, S., & Jat, R. K. (2025). Evaluation of Antimicrobial Activity of Ethanolic Extract of *Leea indica* Root. *Journal of Pharmacognosy and Phytochemistry*, 14(1), 368–372.
- Emran, T. B., Rahman, M. A., Hosen, S. Z., Rahman, M. M., Islam, A. M. T., Chowdhury, M. A. U., & Uddin, M. E. (2012). Analgesic Activity of *Leea indica* (Burm. f.) Merr. *Phytopharmacology*, 3(1), 150–157.
- Estiti, B. H. (1995). *Anatomy of seed plants*. Bandung: Publisher of the Bandung Institute of Technology (ITB).
- Fernandes, I., Pérez-Gregorio, R., Soares, S., Mateus, N., and De Freitas, V. (2017). Wine Flavonoids in Health and Disease Prevention. *Molecules*. 22(2): 292.
- Giri, I. M. D. S., Wardani, I. G. A. A. K., and Suena, N. M. D. S. (2021). The Role of Plant Secondary Metabolites in the Formation of Collagen in the Skin of Burned Rats. *Usadha*. 1(1).
- Greeshma, K. C., and Kumar, N. M. (2023). Preliminary Pharmacognostical and Phytochemical Analysis of *Leea indica* (Burm. f.) Merr. Root. *Journal of Pharmacognosy and Phytochemistry*. 12(2) : 23-28.

- Harisha, C. R. and Jani, S. (2013). Pharmacognostical Study on Trichomes of Solanaceae and Its Significance. *Universe J Pharm.* 2:100-104.
- Hermawan, L. A., Husna, S., and Akmalia, H. A. (2023). Secondary Metabolite Compounds in the Root and Stem Organs of Water Kale (*Ipomoea aquatica* Forssk.) Based on the histochemistry test. *LenteraBio: Biological Scientific Periodical.* 12(3) : 317-322.
- Hindriana, A. F. and Handayani. (2023). *Plant Anatomy.* Malang: Nusaantara Literacy.
- Konyar, S. T., Öztürk, N., and Dane, F. (2014). Occurrence, Types and Distribution of Calcium Oxalate Crystals in Leaves and Stems of Some Species of Poisonous Plants. *Botanical studies.* 55 : 1-9.
- Kuntorini, E. M., Sari, S. G., and Fariani, R. (2023). The Morphoanatomy, Histochemistry, and Phytochemistry of the Leaves and Fruits of *Rhodomyrtus tomentosa*. *Biodiversity Journal of Biological Diversity.* 24(1).
- Harun, A., Ab Rahim, N. E. A., Abd Jalil, M. A., Rosdi, A. M., Daud, S., Harith, S. S., ... and Hassan, N. M. (2016). Comparative Study of Antioxidant and Antimicrobial Activity of Root, Stem and Leaves of *Leea indica* Species. *Malaysian Journal of Science.* 35(2) : 259-274.
- Hermawan, L. A., Husna, S., and Akmalia, H. A. (2023). Secondary Metabolite Compounds in the Root and Stem Organs of Water Kale (*Ipomoea aquatica* Forssk.) Based on the histochemistry test. *LenteraBio: Biological Scientific Periodical.* 12(3) : 317-322.
- Imran, M., Rauf, A., Abu-Izneid, T., Nadeem, M., Shariati, M. A., Khan, I. A., ... and Mubarak, M. S. (2019). Luteolin, a Flavonoid, as An Anticancer Agent: A review. *Biomedicine & Pharmacotherapy.* 112 : 108612.
- Irmastika, I. (2023). *Ethnobotany of Medicinal Plants for Degenerative Diseases in the Besemah Tribe, Lahat Regency, South Sumatra Province.* Thesis. Sriwijaya University.
- Irwanto, Kuntorini E. M., & Fariani R., 2023, Anatomical structure and histochemical test of tannins in vegetative and reproductive organs (*Rhodomyrtus tomentosa*, Proceedings of the National Seminar of the Indonesian Biodiv Society.
- Januarti, I. B., Wijayanti, R., Wahyuningsih, S. and Nisa, Z. (2019). The Purified Potential of Red Betel Leaf Extract (*Piper crocatum* Ruiz & Pav) as an Antioxidant and Antibacterial. *J Pharm Sci.* 2 : 60-68.
- Khuniad, C., Nahar, L., Ritchie, K. J., and Sarker, S. D. (2022). Therapeutic Potential of *Leea indica* (Vitaceae). *Journal of Natural Products Discovery,* 1(1).
- Lange, B. M. (2015). The Evolution of Plant Secretory Structures and Emergence of Terpenoid Chemical Diversity. *Annual review of plant biology.* 66(1) :139-159.
- Lange, B. M. and Turner, G. W. (2013). Terpenoid Biosynthesis in Trichomes—Current Status and Future Opportunities. *Plant Biotechnology Journal.* 11(1) : 2-22.
- Liu, W., Feng, Y., Yu, S., Fan, Z., Li, X., Li, J., and Yin, H. (2021). The Flavonoid Biosynthesis Network in Plants. *International Journal of Molecular Sciences.* 22(23) : 12824.
- Lusa, M. G., Da Costa, F. B., and Appezzato-da-Glória, B. (2016). Histolocalization of Chemotaxonomic markers in Brazilian *Vernonieae* (Asteraceae). *Botanical Journal of the Linnean Society.* 182(3) : 581-593

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- Ma, Z. Y., Wen, J., Ickert-Bond, S. M., Chen, L. Q., and Liu, X. Q. (2016). Morphology, Structure, and Ontogeny of Trichomes of the Grape Genus (*Vitis*, Vitaceae). *Frontiers in Plant Science*. 7 : 704.
- Marjoni, R, Afrinaldi, & Novita., A, 2015, Total Phenol Content and Antioxidant Activity of Kersen Leaf Water Extract (*Muntingia calabura* L.), *Journal of Yarsi Medicine*, Volume & Page: Vol. 23, No. 3, P. 187-196.
- Najmaddin, C., Hussin, K. and Maideen, H. (2013). Comparative Leaf Anatomy of Selected Species in Vitaceae and Leeaceae. *American Journal of Applied Sciences*. 10(4): 414: 417
- Mercado, M. I., Moreno, M. A., Ruiz, A. I., Rodríguez, I. F., Zampini, I. C., Isla, M. I., and Ponessa, G. I. (2018). Morphoanatomical and Histochemical Characterization of *Larrea* Species from Northwestern of Argentina. *Brazilian Journal of Pharmacology*.
- Mohammad, N. S., Milow, P., and Ong, H. C. (2012). Traditional Medicinal Plants Used by The Kensiu Tribe of Lubuk Ulu Legong, Kedah, Malaysia. *Studies on Ethno-medicine*. 6(3) : 149-153.
- Mota, L, Cristina, A, Figueiredo, Luis G. Pedro, José G. Barroso, Ascensão, L. 2013 Glandular trichomes, histochemical localization of secretion, and essential oil composition in *Plectranthus grandidentatus* growing in Portugal. *Flavaour Fragrance Journal* , Volume 28, Issue 6
- Muliyah, E., Dorly., and Djuita, N. R. (2022). Secretory Structure in *Physalis angulata* as a Medicinal Plant. *Scientific Journal of Biology*. 1(2): 19-24.
- Oliveira, A. B. D., Mendonça, M. S. D., Azevedo, A. A., and Meira, R. M. (2012). Anatomy and Histochemistry of the Vegetative Organs of *Cissus verticillata*: a Native Medicinal Plant of the Brazilian Amazon. *Brazilian Journal of Pharmacology*. 22 : 1201-1211.
- Pratiwi, D. R., Sulistyaningsih, Y. C., and Ratnadewi, D. (2020). Localization of Alkaloids and Other Secondary Metabolites in *Cinchona ledgeriana* Moens: Anatomical and Histochemical Studies on Fresh Tissues and Cultured Cells. *HAYATI Journal of Biosciences*. 27(1) : 1-1.
- Punjungsari, T. N., and Ulfa, F. (2022). Papaya Plant Transport Network (*Carica papaya* L.) Growing on Aluminum Highlands. *VIABEL: Scientific Journal of Agricultural Sciences*. 16(1) : 74-81.
- Rahmani, R., Sutiya, B., and Abidin, Z. (2022). Analysis of Several Secondary Metabolite Compounds in Sungkai (*Peronema canescens* Jack), Mali-Mali (*Leea indica*), and Lerak (*Cyathostemma viridiflorum*) Plants from KHDTK Lambung Mangkurat University. *Sylva Scientiae Journal*. 5(4) : 582-586
- Rivai, A. T. O. (2020). Identification of Compounds Contained in Moringa Leaf Extract (*Moringa oleifera*). *Indonesian Journal of Fundamental Sciences*. 6(2).
- Satapathy, T., and Kumar, D. (2024). A Comprehensive Review and Recent Advancement in the Application of Tannins for Treating Parkinson's Disease. *Pharmacological Research-Modern Chinese Medicine*. 12 : 100499
- Sreebala, G., Eapen, J., and Deepa, M. S. (2021). Pharmacognostic Studies of an Ethnomedicinal Plant: *Cissus latifolia* Lam. *Journal of Pharmacognisys and Phytochemistry*. 10(6): 206-209.

- Selvakumar, P., Badgeley, A., Murphy, P., Anwar, H., Sharma, U., Lawrence, K., and Lakshmikuttyamma, A. (2020). Flavonoids and Other Polyphenols Act as Epigenetic Modifiers in Breast Cancer. *Nutrients*. 12(3) : 761.
- Tanzerina, N., Safitri, D., Harmida, H., Aminasih, N., and Juswardi, J. (2023). Ethnobotany of Medicinal Plants for Infectious Diseases in the Besemah Tribe, Lahat Regency, South Sumatra Province, Indonesia. *Asian Journal of Social and Humanities*. 2(1) : 82-95.
- Tanzerina, N., Yohana, F., Aminasih N, Junaidi, E., and Juswardi (2024) Anatomy And Secretary Structure Of *Leea Indica* (Burm.F.) Merr (Memaye) As A Typical Medicinal Plant Of The Besemah Tribe For Anti- Infective And Degenerative In Lahat Regency South Sumatra Indonesia. *Eduvest – Journal of Universal Studies* Volume 4 Number 10, <https://eduvest.greenvest.co.id/index.php/edv/article/view/17481>
- Temkitthawon, P., Viyoch, J., Limpeanchob, N., Pongamornkul, W., Sirikul, C., Kumpila, A., and Ingkaninan, K. (2008). Screening for Phosphodiesterase Inhibitory Activity of Thai Medicinal Plants. *Journal of Ethnopharmacology*. 119(2) : 214-217.
- Wick, M. R. (2012). Histochemistry as a Tool in Morphological Analysis: A Historical Review. *Annals of Diagnostic Pathology* 16(1): 71-78.