The Identification of Morphological and Anatomical Structures of *Pluchea indica*

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Abstract. This current research aimed at identifying the morphological and anatomical structures of *Pluchea indica*, specifically in root, stem, leaf, and flower of its. A descriptive approach was applied to this research and was conducted at Biology Laboratory of University of Muhammadiyah Malang from July to August 2019. The identification of morphological structure, furthermore, was focused on the characteristics of root, stem, leaf, and flower organ. In addition, the identification of anatomical structure was through a transverse incision in each organ by means of fresh preparation, safranin coloration, and Scanning Electron Microscope (SEM). The collected data were analyzed descriptively and quantitatively. The identification of morphological structure in the organs was referred to a book entitled *Morfologi Tumbuhan* (Plant Morphology) by Tjitrosoepomo (2007), while the anatomical identification was referring to a book of *Anatomi Tumbuhan* (Plant Anatomy) by Fahn (1995). The result of the identification has indicated that *Pluchea indica* possesses the following morphological characteristics: 1) the leaves are light green, with trichomes in their upper and lower sides; 2) the length of branches signifies 1 cm in average, located at crisscrossing points and in a form of abovatus; 3) the tip of the leaves (apex folli) is sharp-formed (acanthus), with serrated pattern at the edge of the leaves (margo folli); 3) the base of the leaves (basic folli) is blunted (obstitus) and the structure of the leaf bones (nervatio) is pinnate-formed (penninervis), with oval-shaped leaf blades and parchment-like intervenium; and 4) the stem is in a round form with monopodial branches, taproot, and capitulum inflorescent. Further, with the reference to the anatomical structure of the stem and root, there have been found epidermal tissue, cortex, xylem and phloem, sclerenchyma, and pith. The modification on stomatal epidermis is in a form of kidney with two buffer cells of 13.6 µm in length and 9.82 µm in width. Meanwhile, the porous length and width consecutively signify 9.02 µm and 1.32 µm, with glandular trichomes of 551 µm long in average and 39.6 µm wide in average.

1. Introduction

Indonesia has been of fame as a country very rich of plant diversities [1]. Purportedly, among 150-200 species all over the world, there are 60 species that can be found in Indonesia [2]. In nature, the plants are divided into some families with the reference to particular and unique characteristics equipping the plant organs. *Asteraceae* constitutes one of plant families existing in Indonesia. It belongs to a group of plants with the diversity of, more and less, 25,000 species with 1,700 genera spread over the whole parts of the world [3]; [4]. There are three major sub-families of *Asteraceae*, namely *Asteroideae*, *Barnadesioideae*, and *Cichorioideae* [5]. In Asteraceae family, there is *Pluchea* genus; one of the representatives of the genus is *Pluchea indica* [6]. *Pluchea indica* is one of brushwood plants grown as
hedgerows or wilding plants (uncultivated plants) so that this kind of plant is considerably easy to find out at the backyards. *Pluchea indica* can be growing up in dried areas with very rough and rocky contour of land as well as in lowland areas all over Indonesia [7]. *Pluchea indica* is a native plant to Indonesia that has been widespread all over the majority of areas in Indonesia, with significant potency of being cultivated due to its advantages [6].

In nature, every plant must have different morphological and anatomical characteristics [8]. The plants grouped in the same genus will show morphological and anatomical differences along with different ages, or in other words, when they have not been mature yet. Such a phenomenon occurs since the growth and development of morphological and anatomical structures of young plants are not perfectly holistic. Further, the identification of morphological and anatomical characteristics can be an attempt to identify the genus to which the plants should belong [9].

Morphology refers to a surface look of the plants [8]. It is said as the most essential basis for taxonomic description [10]. In addition, morphology is considered an old-fashioned way, but still an essential basis to deal with taxonomic issues [11]. Studying morphology is assumed as an important path to find out the easiest way for the researchers to clarify the genus as the most significant indicator to identify the plants visually lies on the morphological structure. Accordingly, at the end, the identification and classification in respect to plant diversity is supposed to be much easier, especially in giving names to the species, family, or kingdom [8].

The characteristics of plant inner structure have been contributing to plant systematics for, more and less, one-and-half century [12]. Further, anatomical structure in plants has been referred to when identifying and determining phylogenetic relationship. The anatomical characteristics are observed by means of light microscopes, with the modest techniques, and means of electron microscope. One of the most essential and taxonomic aspects in anatomical characteristics refers to anatomy of organs, comprising leaf, stem, root, and flower. The anatomical characteristics, moreover, can contribute to complementing the elaboration on the genetic relationship or phylogenetics [13].

The growth of particular plant species will be based on the condition of surrounding environment and thus there will be found some different morphological occurrences from one to other points [14]. The environments where the plants are growing up will affect the morphological and anatomical structures of the plants [1]. According to [15], the right pace to conduct a morphological identification is by observing such particular organs as root, stem, leaf, and fruit. Meanwhile, in this current research, highlights of the identification of morphological and anatomical structures on *Pluchea indica* are at its stem, leaf, and flower.

2. **Method**

2.1 **Research Design**
A descriptive design was employed.

2.2 **Location and Time of Research**
This research was conducted at Biology Laboratory of University of Muhammadiyah Malang, started from July to August 2019.

2.3 **Materials and Equipment**
There were some materials and equipment used in this research, i.e. alcohol, xylol, safranin, aqua, formalin, binocular microscope, object glass, and Scanning Electron Microscope (SEM).

2.4 **Research Procedures**

2.4.1 **Identification on Morphological Characteristics**
The method of identification was focused on the morphology of leaf, stem, root, and flower referring to a book entitled 'Morfologi Tumbuhan' (Plant Morphology) (Tjitosoepomo, 1990; Dasuki, 1991). Furthermore, the leaf organ was set to choose from those equipped with complex root, stem, leaf, and flower.
2.4.2 Identification on Anatomical Characteristics
The identification on anatomical structure was through observing: 1) fresh preparation; 2) safranin-colored preparation of stem and root with transverse incision method [16], referring to the following procedures: a) organ fixation using FAA solvent; b) organ sample washing; c) alcohol and xylol droppings with gradual levels of concentration; d) sample incision; e) safranin coloration; f) alcohol and xylol droppings with gradual levels of concentration; g) observation using microscope; and h) gluing using enthelan; (3) observation by means of Scanning Electron Microscope (SEM). The observation on the stomata was through [17] method: a) leaves from each of the plants were rubbed with acetone and left for three minutes; b) they were put on the object glass and observed by means of binocular microscope; and c) the samples of the stomata that had been collected were photographed by the microscope.

2.5 Data Analysis
The data were analyzed descriptively by untyling the observation results on the morphological structure of root, stem, leaf, and flower. In addition, the anatomical observation highlighted the anatomy of fresh preparation, safranin coloration, and Scanning Electron Microscope (SEM) observation. The size of stomata and trichome was defined based on the scales displayed on the screen of SEM.

3 Results And Discussion
In the reference to the result of morphological observation in leaf organ (Figure 1.), it has been indicated that the leaves of Pluchea indica have the form of abovatus. The main characteristic of abovatus form is illustrated similarly to common egg’s roundedness, with a wide space nearby the tip of the leaves (apex folli) [8]. The shape of the tip is sharp-formed (acanthus), with both of the edges (margo folli) directing the leaf bones (nervatio) to perennially approach to the peak and form a sharp angle (< 90°) [18]. In addition, the base of Pluchea indica leaves (basic folli) is reportedly sharp-formed, in which this sort of form could be mostly found out in those with abovatus or spathulatus form [8]. The construction of nervatio is in a penninervis form, with a single parent bone heading from the base (basic folli) to the tip (apex folli) and constituting the extension of the base itself. From the parent bone, there have been found branching bones so that the construction bears a resemblance to fins of fish [19]. Further, Pluchea indica intervenium has a shape of parchment – flimsy, but slightly rigid [20]. It has been shown that the color of Pluchea indica is light green, with subtly-white hair decorating its upper and lower sides as a result of modification made by epidermis tissues, called as trichomes. Moreover, referring to Figure 2, the edges of Pluchea indica leaf are in a form of divisus, with the specification of serrated form, in which the sinus and angulus are the same in terms of angle’s degree. According to [21], the leaves are considerably diverse, both in morphological and anatomical senses.

Figure 1 The Morphology of Pluchea indica
Descriptions: apex folli (a), basic folli (b), nervatio construction (c), margo folli (d)
Figure 2 Pluchea indica margo folli
Observation through Scanning Electron Microscope (SEM)

Figure 3 The Morphology of Pluchea indica stem
Description: stem’s color (a), stem’s shape (b), branching direction (c), stem’s branching (d).

From the morphological observation on Pluchea indica stem in Figure 3, it is obvious that the stem is clearly apparent and in a form of lignosus, which is a kind of strong, rigid, and woody construction. Those with woody stem construction are categorized as perennial plants with vascular tissues, equipped with xylem and phloem that undergo secondary growth for woody construction [22]. It has been shown that Pluchea indica stem is not huge and stern because the construction grows up on the mature or the upper side of the plant. The Pluchea indica stem, in addition, is round, with hairy look on the stem’s surface, and grows upright with a number of branches and ground stem greater than its monopodial branches [8].

Figure 4 The Morphology of Root
Description: root’s base (a), taproot (b), root fibers (c), root cap (at the very end) (d).

The result of morphological observation on the root organ shown in Figure 4 has indicated that Pluchea indica is equipped with taproot as its main rooting system. The most basic characteristic of the taproot is that the radicula (prospective root) would keep growing up to become the primary root
which, in forthcoming period, generates root branches with smaller size [8]. Moreover, unbranching taproot (or with a very few amounts of branches) would have unproductive branches with soft-textured root fibers. This sort of taproot is often interrelated to its function as the storage of food reserves and its special shape [60].

\[\text{Figure 5. The Morphology of } \textit{Pluchea indica} \]
\[\text{Description: footstalk (a), calyx (b), corolla (c).}\]

Flower is one of plant organs to function as organ of reproduction for, in general, it is furnished with stamen and stigma. Flower is directly active at the process of attracting pollinating insects so that its morphological and functional characteristics will significantly contribute to the success of plant reproduction [23]; [24]; [25]. Referring to the morphological observation shown in Figure 6, it is evident that \textit{Pluchea indica} is inflorescent, with a branch to accommodate amounts of flowers; each of which has leaves for assimilation process [8]. With respect to inflorescence, \textit{Pluchea indica} could be classified into inflorescent plant because there are stamen and stigma found in one flower. In addition, still regarding Figure 6, \textit{Pluchea indica} comes out of the axilla. Alluding to the origin of its existence, the kinds of flowers are divided into two major groups, namely \textit{flos terminalis} and \textit{flos axillaris/flos lateralis} [26], in a form of capitulum and spikelet. In fact, \textit{Pluchea indica} is decorated with violet corolla. The morphology and color of flowers are closely related to the success and quantity of pollination with the help of insects. The color of corolla is close in relationship to the process of attracting pollinators (insects). In addition, the color of the flower is strongly cohesive to the pollinators [27]. The understanding on the inflorescence morphology is necessary for it constitutes a certain path to approach to basic understanding about reproduction system, especially in setting up the procedures of conservation [28] and plant cultivation [29].

\[\text{Figure 6. Fresh Preparation of } \textit{Pluchea indica} \text{ Anatomy (transverse); 7b SEM} \]
\[\text{Description: lower epidermis (a), vascular tissue (b), trichomes (c), upper epidermis (d), chlorenchyma (e), palisade (f).}\]
The leaf anatomy refers to an inner structure of leaf pinpointing on shape, type, cell structure, and cell content. There are several characteristics referred to for taxonomic classification as proposed by [30]. The result of anatomical observation as illustrated in Figure 7 has shown the characteristic of Pluchea indica anatomy, which is having upper and lower epidermis constructed over the epidermis cells just like brick construction, with perfectly dense cells construction to protect the tissues inside. In addition, epidermis tissue is furnished with the coincident forms of upper and lower epidermis cells, from square to rectangular forms [31].

The vascular tissue is constructed by xylem and phloem which function to transfer the materials and products of photosynthesis. There is a modification formed in epidermis tissue, called as trichome. In nervatio, there have been found chlorenchyma and parenchyma sponges that construct mesophyll (Figure 7c). The mesophyll could be specialized to be palisade and parenchyma sponge tissues [32]. According to [33], mesophyll contains chloroplast in each cell. The palisade cells, moreover, are constructed closely to the upper surface of the leaves, where the sunlight came in through for photosynthesis process.

**Figure 7.** The Anatomy of *Pluchea indica* leaf on Scanning Electron Microscope
Description: chlorenchyma (a), palisade (b).

**Figure 8b.** The Anatomy of *Pluchea indica* Preparation through Safranin Coloration; 8c. Observation through SEM
Descriptions: epidermis (a), xylem (b), phloem (c), pith (d), cortex (e), sclerenchyma (f).
Figure 9. The Anatomy of *Pluchea indica* Root: Safranin Coloration (a), SEM (b).

Description: epidermis (a), xylem (b), phloem (c), pith (d), cortex (e), sclerenchyma (f) hairy layer (g).

Figure 8 and 9 have indicated that epidermis cells have made up epidermis tissue. The construction of epidermis cells is strongly dense, forming a layer (Figure 8). In addition, epidermis tissue functions as mechanical protector, specifically to protect from water loss and to accommodate gas exchange between the outer environment and inner tissue [34]. [21] and [35] also suggest that tissues in dicotyledon stem that have undergone secondary growth consist of epidermis, cortex and pith, vascular system (xylem and phloem) and cambium (located in between xylem and phloem). Moreover, the observational result of anatomical structure on the stem (Figure 8) and on the root (Figure 9) has shown the existence of vascular tissue containing xylem and phloem. According to [33], xylem is constructed by trachea, tracheid, wooden fiber, and parenchyma, whilst phloem is formed by phloem vessels, sink cells, and phloem parenchyma. Furthermore, xylem is responsible for the translocation of water and raw materials as well as for mechanical support for the leaves. Meanwhile, phloem takes charge of the translocation of photosynthesis products from the mesophyll.

Moreover, vascular tissue indicates a bundle of xylem which are separated and alternately located along with a bundle of phloem so that a circular shape with a broad pith in the center is formed [36]. Regarding a research from [37], it has been found that the more mature the wood, the larger the size of sponge’s tangential diameter will be. The size of the sponge’s tangential diameter is also dependent on the activities performed by the cambium that gradually undergoes binary fission during the growth period [38]. Cortex is also found in stem (Figure 8). The tissue of cortex contains parenchyma cells, with bundles of vessels spread over the cortex. The sclerenchyma is considered as fiber due to the physical property, such as having a long size with various fiber sizes in particular plant species. The fiber cell is commonly sharp-tipped, with a thick-layered cell wall and narrow cell lumen. [30] asserts that the cortex layer of dicotyledon root contains parenchyma cells, with separating septum located in between the cells along with the dark cells that exist. Parenchyma constitutes the main part of the ground tissue and is commonly visible in some organs and tissues, such as cortex and pith of stem, root cortex, and ground tissue in leaves petioles and mesophylls. In addition, stomata and trichomes have been frequently found in young stems [39].
Stomata is set up as the target for pollutant absorption [40], which directly interacts with and has access to mesophylls [41]. In addition, stomata are always visible in the plants that are frequently exposed to the air, but more frequently found in leaf organ [42]. According to Sutrian [43], in all green leaves, stomata could be found on both of the surfaces.

As in Figure 10, regarding the modification of stomatal epidermis in *Pluchea indica*, stomata are in a form of kidney in which there are, in nature, 4 (four) different kinds of stomatal types, to name *anomocytic, anisocytic, paracytic, and diacytic* [43]. The stomatal types, furthermore, are distinguished based on the neighbor cells surrounding the guard cells within the stomata [44]. According to [45], stomata is the result of modification made by epidermis tissues which have been specialized to be particular organ responsible for water and air flow exchange on the leaves. [43] postulates that stomata, in common, could be found at the lower surface of the leaves, but there are some species of plants having the stomata at the both surfaces, lower and upper.

Other parts of leaves found out are guard and neighbor cells, also at the both surfaces. Stomata has a form of hole or pore located at the epidermis organ owned by all green plants and are covered by special cells, called as covering cells [46]. The covering cells are surrounded by other epidermis cells, called as neighbor cells [20]. Moreover, the anatomical structure is regarded to stomatal shape, density, epidermis cell construction, and mesophyll structure on the leaves, which constitutes constant identifier for the basis of doing plant identification [47].

![Stomata Image](image1)

**Figure 10a.** Fresh Preparation of *Pluchea indica*; 10b Observation through SEM
Description: Porous stomata (a), guard cell (b), neighbor cell (c), epidermis (d)

![Stomata Size Image](image2)

**Figure 11.** The stomatal size of *Pluchea indica* at the lower surface
The result of measuring the stomata using the scales shown in *Scanning electron microscope* (Figure 11) indicates that the guard cells are 13.6 µm in length and 9.82 µm in width. Meanwhile, the porous is shown 9.02 µm in length and 1.32 µm in width. There are many factors considered to bring about influences upon the stomatal size. Among all, environmental factor is the most significant. The distribution of stomatal amounts and sizes is also influenced by internal and external relationships within the organs [48]. According to [49] and [50], the variations in stomatal sizes exist due to thickening process made by guard cells upon the responses to light, carbon dioxide, and water conservation. Maximum stomatal porous unveiling process occurs in the morning when the plants are in need of carbon dioxide for light-dependent reaction of photosynthesis [42]. In addition, stomata are central to determining the efficiency of water evaporation, as the large and long stomata will be faster when responding to dehydration and transpiration processes [51]. Stomata, furthermore, will be back veiling only if the contents of water vapor in the air and in between the cells are more than the critical point. In addition, the ambient temperature also contributes to stomatal veiling and unveiling processes. If other factors are in constant condition, the stomata will veil wider along with the increase of ambient temperature.

![Figure 12a](image1.png)  

**Figure 12a.** The trichome of *Pluchea indica* leaf shown in fresh preparation; 12b Observation through SEM  
Description: Body of trichome (a), Head of trichome (b)

Stem epidermis could generate a derivate in a form of stomata and trichome [53]. Based on the observational result on epidermis trichome modification (Figure 12), it is obvious that the trichome of *Pluchea indica* is categorized as glandular trichome. Furthermore, trichome is generated from epidermis cells (modification of the cells), containing single or multi cells with significant roles for plants. In general, trichome is divided into two major groups, non-glandular, without secretion products, and glandular, with secretion products [54]. The former functions to resist evaporation, whilst the latter does to protect from drought [21]. According to [55], trichome is generated from epidermis cells functioning to protect the plants from light and evaporation excesses. Besides, the trichome could also be occupied for taxonomic grouping in order to distinguish the plants based on family, genus, and species [30]. The structure and distribution of the trichome are considered effective in distinguishing two different species in one genus [56]. According to [33], the trichome has been shown effective for defining classification by genus and even species. The types of trichome and stomata are very significant to identify the species of plants and to investigate the existing interrelation between the target species.
The trichome and stomata constitute derivatives of epidermis tissue [57]; [58]. The data of stomatal measurement are referred to a scale displayed in the SEM monitor (Figure 13). It has been shown that the trichome sized 551 µm long in average and 39.6 µm wide in average. The various shapes and sizes in trichome occur due to resistance against the herbivores, ecophysiology between water and leaves, and protection from ultraviolet [59]. Further, the shapes and sizes of the trichome depend on what species the plants belong to. Morphologically, the trichome’s wall contains cellulose, lignin, silica, and calcium carbonate [53].

4 Conclusion
As a concluding remark, this current research has proved that Pluchea indica leaves are light green, with the branches’ length of 1 cm in average, located at crisscrossing points, and in the form of abovatus. In addition, the apex folli is sharp-formed (acanthus), with serrated pattern at the margo folli. The basic folli is blunted (obsitus) and the nervatio is penninervis. with oval-shaped leaf blades and parchment-like intervenium. Moreover, the stem is in a form of round with monopodial branches, taproot, and capitulum inflorescent. Further, in reference to the anatomical structure of the stem and root, there are found epidermis tissues, cortex, vascular system, sclerenchyma, and pith. The stomata exists in a form of kidney with the length of both guard cells constituting 13.6µm in length and 9.82µm in width. In terms of porous sizes, it has been shown that the length and width consecutively signify 9.02 µm and 1.32 µm. The trichome is glandular, with the average length of 551 µm and the average width of 39.6 µm.

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