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MICROSCOPIC CHARACTERISTICS AND LEAF CONSTANT NUMBERS OF SELECTED *DERRIS* SPECIES IN THAILAND

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ABSTRACT: Medicinal plant identification is necessary for ensuring their efficacy and safety in the quality control of herbal products. The genus *Derris* belongs to the Leguminosae family. This study aimed to investigate the microscopic characteristics of selected *Derris* species in Thailand. Eight *Derris* species (*D. amoena, D. elliptica, D. indica, D. malaccensis, D. recticulata, D. scandens, D. solorioides,* and *D. trifoliata*) existing in Thailand were studied. Anatomical characteristics of the leaves were investigated by the midrib transverse section. The laminae were quantitatively investigated for the stomatal number, stomatal index, epidermal cell area, vein islet number, trichome number, and palisade ratio. Trichomes were found in 4 species (*D. elliptica, D. scanden, D. amoena* and *D. malaccensis)* which presented as unicellular non-glandular trichome on the lower epidermis. Stomatal type found in *Derris* species in this study was paracytic type and presented only on lower epidermis, except *D. trifoliata* which was found on both lower and upper epidermis. The anatomical characteristics of the midrib of eight studied *Derris* species were illustrated, showing the distinguish anatomy. Leaf constant numbers were demonstrated *D. recticulata* showed the highest stomatal index (20.21 ± 1.53) and upper epidermal cell area (1172.99 ± 56.25).

INTRODUCTION: Globally, natural and herbal medications have gained more interest for centuries, promoting the economic values of health services and herbal products. In Thailand, the government has promoted the policy "Thailand 4.0: Herbal Products Roadmap," proposing the development of herbal plants and raising the value of processed herbal products. Medicinal plant identification is necessary for ensuring their efficacy and safety in the quality control of herbal products. Plant authentication plays a crucial role in medicinal plant identification.

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Macroscopic and microscopic examinations are a conservative and easy method for the character-rization of plant species [1]. The *Derris* Lour is a genus member of the Leguminosae family, subfamily Papilionoideae, with small to medium-sized lianas or trees. There are approximately 50 species, and 16 of them are distributed in Thailand [2, 3].

Some species, for instance, *D. malaccensis*, *D. elliptica* and *D. reticulata* are locally used to treat diseases in Thailand and other Southeast Asian countries. Roots, leaves, and stems of *Derris* species and its allies contain isoflavonoid rotenone which can be used as an insecticide and fish poison. Eight taxa were chosen for investigation since they are traditionally used and difficult to separate by their appearance. This research aimed to study the anatomical characteristics of *D. amoena, D. elliptica, D. malaccensis, D. indica, D. scandens,*

D. solorioides, D. trifoliate and *D. reticulatavia* midrib transverse section, and to investigate the microscopic constant values of leaf including stomatal number, stomatal index, epidermal cell area, palisade ratio, vein islet number and trichome number of these eight *Derris* species in Thailand.

MATERIALS AND METHODS:

Plant Collection and Authentication: The aerial parts, including fresh mature leaves of selected *Derris* species were collected from three different locations in Thailand. The specimen numbers of all samples were shown in **Table 1**.

TABLE 1: *DERRIS* SPECIES PLANT SAMPLESUSE IN THE STUDY

Sample	Locality	Collecting Date	Specimen Number
Derris amoena Benth.	Trang	May, 2017	0113010321
	Trang	May, 2017	0113020321
	Nakhon Si Thammarat	May, 2017	0113030321
Derris elliptica (Wall.) Benth.	Bangkok	February, 2016	0512010321
	Nakhon Ratchasima	December, 2017	0512020321
	Nakhon Pathom	December, 2017	0512030321
Derris malaccensis Prain	Trang	May, 2017	1301010321
	Nakhon Ratchasima	December, 2017	1301020321
	Nonthaburi	August, 2017	1301030321
Derrisindica (Lam.) Bennet	Chonburi	December, 2016	0914010321
	Chonburi	December, 2016	0914020321
	SamutSakhon	February, 2016	0914030321
Derris reticulata Craib	Bangkok	June, 2017	1805010321
	Nakhon Pathom	December, 2017	1805020321
	Nonthaburi	August, 2017	1805030321
Derris scandens (Roxb.) Benth.	Bangkok	February, 2016	1903010321
	Nakhon Ratchasima	December, 2017	1903020321
	Nakhon Pathom	December, 2017	1903030321
Derrissolorioides Sirich. & Adema	Bangkok	October, 2016	1915010321
	Bangkok	October, 2016	1915020321
	Nakhon Pathom	December, 2017	1915030321
Derris trifoliata (Lour.) Tabu.	Ang Thong	January, 2018	2018010321
	SamutSakhon	February, 2016	2018020321

All samples were authenticated by Assoc. Prof. Dr. Nijsiri Ruangrungsi. The voucher specimens were deposited at the College of Public Health Sciences, Chulalongkorn University, Thailand.

Midrib Transverse Section: The leaves were transversely cut by razor blade to observe the microscopic structures of the midrib as follows. The leaf section was placed on a glass slide, mounted with a few drops of water, and covered with a coverslip.

Each sample was evaluated under a microscope with an objective lens (magnification of 10X, 20X, or 40X power) and an eyepiece lens (magnification of 10X power). The characteristics of the midrib were illustrated by hand drawing.

Microscopic Leaf Constant Numbers: The mature leaves of all *Derris* species were cleaned and cut at the middle of the leaf between midrib and leaf margin. The laminae were soaked in Haiter® solution (containing 6% w/w sodium hypochlorite) diluted in water (1:1) for a few days and then bleached with chloral hydrate solution (4 g in 1 ml of water) under low heat until they became clear.

The transparent laminae were placed on the microscopic slide and observed under a microscope for leaf constant values. The suitable magnification (10X power eyepiece lens with 10X, 20X or 40X power objective lens) of microscope was chosen with respect to the selected cells.

The interested cells within the defined area of the upper or lower epidermis were traced, photographed, and counted. The complete cells were counted in the area of view, while the incomplete cells were counted in two perimeters of the area of view [4].

The microscopic leaf constant numbers from ninety fields per species (thirty fields of each species from three different places) were averaged and expressed as mean ± standard deviation (SD).

Vein-islet Number: A vein-islet is a small area of tissue surrounded by the veinlets. The vein-islet number per one square millimeter of the upper epidermis was recorded.

Stomatal Number and Stomata Index: The stomatal number and the epidermal cell number per one square millimeter of upper and lower epidermis were recorded. The stomatal index was calculated using the following equation [5]:

$$\text{Stomatal index} = (S / S+E) \times 100$$

S = number of stomata per unit area E = number of epidermal cells in the same unit area, including trichomes or cicatrices

Trichome Number: Trichome number is the average number of trichomes per one square millimeter. It was determined by counting the trichomes or cicatrices in the defined area of the epidermis.

Palisade Ratio: Palisade ratio is the average number of palisade cells beneath one epidermal cell of a leaf. It was defined by counting the palisade cell beneath four continuous epidermal cells. The obtaining palisade cell number was then divided by four.

Upper Epidermal Cell Area: The upper epidermal cell area was calculated regarding to the number of upper epidermal cell per one square millimeter.

RESULTS AND DISCUSSION:

Microscopic Anatomical Characteristics: The midrib transverse section of the leaf of eight *Derris* species were investigated and illustrated in **Fig. 1**. The structures consisted of the upper epidermis, palisade cell, spongy cell, sclerenchyma, xylem tissue, phloem tissue, parenchyma, collenchyma, and lower epidermis, were presented. Trichomes were obviously found only in two species at the lower epidermis of the midrib (*D. elliptica* and *D. scandens*). Based on stomatal type, *Derris* species in this study showed the paracytic type of stomata. Trichomes were found to be unicellular, no glandular type**.**

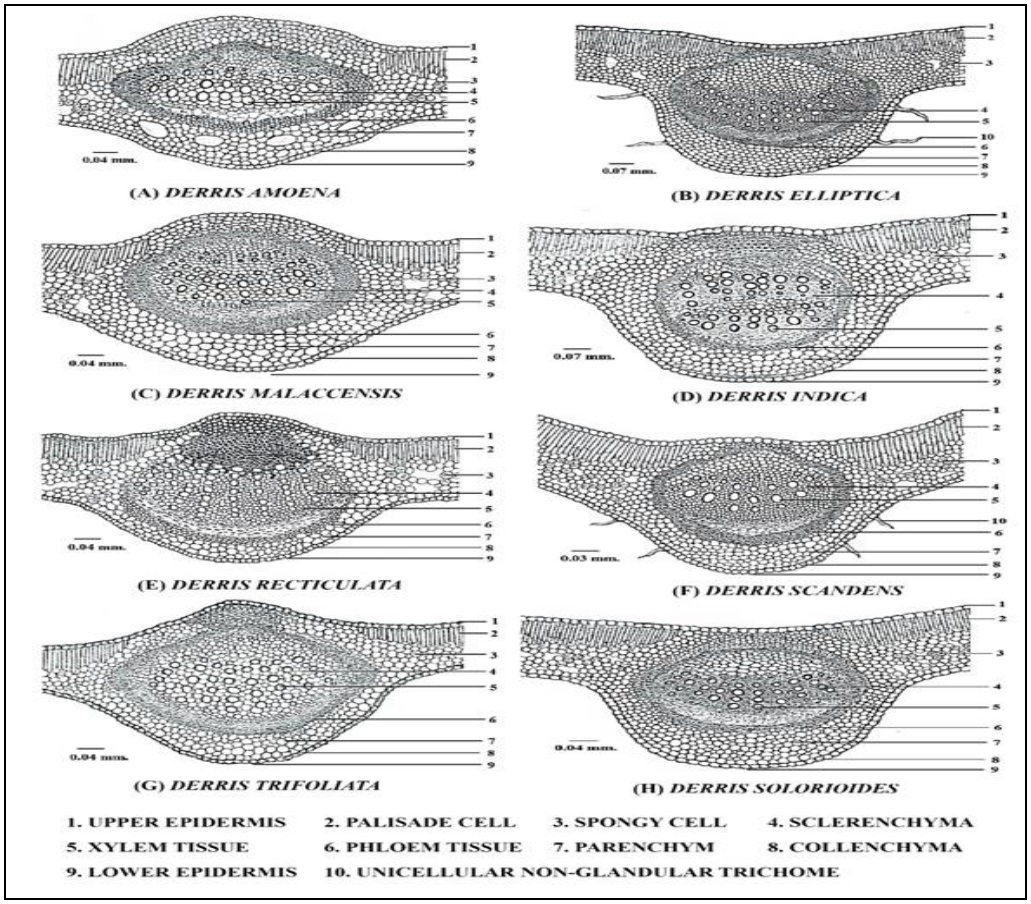

FIG. 1: MIDRIB TRANSVERSE SECTION OF THE LEAF OF EIGHT DERRIS SPECIES

TABLE 2: MICROSCOPIC LEAF CONSTANT NUMBERS OF EIGHT *DERRIS* SPECIES

Derris species	Lower Stomatal Number (Per mm^2)	Lower Stomatal Index	Upper Epidermal Area (µm^2)	Palisade Ratio	Vein Islet Number (Per mm^2)	Trichome Number (Per mm^2)
D. amoena	186.71 ± 20.16 (116 - 324)	13.46 ± 1.33 (10.50-18.66)	886.65 ± 45.34 (586.85 – 1136.36)	7.65 ± 0.87 (4.75 -10.50)	8.88 ± 1.15 (5 – 12.75)	-*
D. elliptica	218.31 ± 21.48 (104 - 334)	11.23 ± 1.17 (6.79 - 16.01)	612.78 ± 27.78 (499 - 817)	10.53 ± 0.84 (6.25 - 17)	18.41 ± 0.87 (17 - 21.75)	16.53 ± 2.75 (6.25 - 26)
D. malaccensis	176 ± 13.43 (128 - 260)	10.27 ± 0.98 (8.09 - 17.08)	656.80 ± 47.55 (537.05 - 825.08)	7.96 ± 0.83 (6 - 10.75)	14.03 ± 1.30 (9 - 16.75)	-*
D. indica	195.11 ± 21.69 (128-264)	8.93 ± 0.91 (5.45 - 11.83)	606.59 ± 30.32 (428.08 - 984.25)	7.70 ± 0.89 (5.5 - 10)	15.36 ± 0.99 (10.50 - 19.37)	-
D. reticulata	316.53 ± 23.03 (264 - 356)	20.21 ± 1.53 (17.07 -22.28)	1172.99 ± 56.25 (1033.06 -1308.90)	9.01 ± 1.96 (6.25 -13.75)	15.05 ± 0.90 (13.50-17.5)	-
D. scandens	223.96 ± 20.38 (132 - 336)	14.35 ± 1.25 (8.66 - 18.77)	756.56 ± 32.93 (683.06 - 836.12)	5.53 ± 0.63 (2.75 - 7.75)	15.37 ± 1.62 (10- 18.50)	14.15 ± 1.92 (8 - 20)
D. solorioides	155.87 ± 11.67 (116 - 192)	9.57 ± 0.70 (7.24 - 12.38)	688.95 ± 32.67 (551.88 - 961.54)	10.66 ± 1.12 (7.50 - 13.75)	12.54 ± 1.07 (9.5 - 14.75)	-
*D. trifoliata***	194.71 ± 17.53 (152 - 272)	9.59 ± 0.86 (8.11 - 11.86)	555.44 ± 25 (422.30 - 737.46)	10.83 ± 1.66 (7.50 - 14.75)	17.97 ± 1.10 (14.50 - 21)	-

-* Could not be quantified but found at the laminae -**upper stomata number 35.46 ± 776/mm2 (34-52); upper stomata index 3.55 ± 0.98 (3.02-5.25)

Microscopic Leaf Constant Numbers: Microscopic leaf constant numbers, such as stomatal index, trichome number, and palisade ratio, are useful indicators for the identification of plants at species level. The results were presented in **Table 2**. Leaf constant numbers are important parameters for the quantitative microscopic evaluation, which can be used to identify and distinguish between some closely related species not easily characterrized by qualitative microscopic evaluation. The characteristics of microscopic leaf constant numbers among eight *Derris* species in Thailand were revealed. The variation within species may be due to age of leaf, environmental condition and geographical source [4, 7]. The results in this study were obtained from the mature leaves collected from 3 different locations. Regarding stomata, only *D. trifoliate* was found as an amphistomatic leaf, whereas the other species were hypostomatic. The paracytic stomata found in the study was in agreement with the previous report [8].

The lower stomatal index of *D. trifoliata* reported by Das & Ghose was found to be 10.74, which was consistent with that of *D. trifoliata* obtained from the study (9.59 ± 0.86). Among eight *Derris* species, *D. reticulata* had the highest value of the stomatal index (20.21 ± 1.53), while *D. indica* showed the lowest value (8.93 ± 0.91). Despite the degrees of overlapping with closely related species, epidermal cell area has been considered to be relatively constant within a narrow range for each species which might be used as a taxonomic tool for the identification of plant materials [9]. Besides The largest value of the stomatal index, *D. reticulata* possessed the greatest amount of epidermal cell area (1,172.99 ± 56.25 µm^2). Type and the density of trichomes were capable of identifying plant samples [10], although the trichome number may be varied caused by seasonal and environmental conditions[11]. In this study, the trichomes that were found on the lower epidermis of laminae of *D. amoena, D. elliptica*, *D. malaccensis* and *D. scandens* were unicellular non-glandular type. *D. scandens* trichome type was in agreement with the previous study that reported as unicellular trichome [12]. The trichome number of *D. elliptica* and *D. scandens* was 16.53 ± 2.75 and 14.15 ± 1.92, respectively, while *D. amoena* and *D. malaccensis* trichomes were too less to be quantified. Based on the presence of trichomes, it could be used to separate eight *Derris* species into 2 groups: trichome-containing group (group 1) and non-trichome-containing group (group 2). Group 1 consisted of four species which were *D. amoena*, *D. elliptica and D. malaccensis*, and *D. scandens*; whereas, group 2 included *D. indica*, *D. reticulata*, *D. solorioides*, and *D. trifoliata.*

The other important leaf constant parameters used as a diagnostic value for differentiating of plant species are palisade ratio and vein islet number. The vein islet is used to point towards the small areas of the photosynthetic tissues encircled by the ultimate division of the vascular strands. The palisade ratio does not alter based on geographical

variations and can be determined on the fine powder of crude drug. The characteristics of selected *Derris* species based on palisade ratio and vein islet number were demonstrated in **Table 2.**

CONCLUSION: The establishment of leaf microscopic characterization, both qualitative and quantitative, of eight *Derris* species can serve as an important identification parameter as well as quality control of these plants.

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CONFLICTS OF INTEREST: The authors declare that there are no conflicts of interest regarding the publication of this paper.

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