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Selected Dicots That can Easily be Sectioned using Hand-Section Method and can Provide Good Anatomical Appearance: For Beginners

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Abstract. This research was conducted to find out the type of dicotyledon which its stem can be sectioned with the method of hand section (HS) and can provide clear anatomy appearances. Research activities start with (1) section using the HS method, (2) observation using a microscope, and the last is (3) limited trial. For the first stage, it was determined that 10 cosmopolitan dicotyledonous found around Universitas Negeri Makassar campus. The stems of all the plants were sectioned by the HS method. The result is 5 of 10 species of dicots were categorized as easy to slash by HS method, namely *Ixora maxima*, *Eclipta prostate*, *Mirabilis jalapa*, *Catharanthus roseus* and *Acalypha siamensis*. In the second stage, the five plant species were sections and observed with a microscope. Based on observations using the monocular XSP-12 light microscope adjusted by Optilab Professional Edition, it can be seen that there are 4 plant species that provide the good anatomical appearance that is *E. prostate*, *M. jalapa*, *C. roseus*, and *A. siamensis*. For the last stage, all four types of plants that provide a good cross-section of the stem anatomy were tested to final semester students and new students. Both groups tried on sectioning and observing and gave their opinion on the level of difficulties. The answer shows that the majority of the groups consider that sectioning and observing of the four dicots are easy or very easy. To sum up, dicots *E. prostate*, *M. jalapa*, *C. roseus*, and *A. siamensis* are easy to section by HS method and to observe by microscope.

Keywords: anatomy plant, dicotyledon, hand section method

1. Introduction

Biological research and teaching often require observation of the anatomy and structure of a plant organ. Primarily in the subject of anatomy and plant structure, the observations are more often, and sectioning becomes very important. Anatomical sectioning can be done by using special tools such as microtomes or by the method of hand section (HS).

The HS give an adequate method for a fast, easy and cheap way to prepare living tissue sections with good quality appearance [1]. However, the problem with the HS method is the difficulty in producing a one-cell-thick incision. Many practices believe, the weakness can be overcome by significant practicing [2]. It means a trained person have more change to get a one-cell-thick incision to compare to untrained one, whereas many beginners often need to do the sections for laboratory



practice and also for research purposes. Hence, for beginners, the problems are not only the person who do the sections, but also what plant materials are proper to section by HS.

Actually, not all plant organs from a plant species can be slashed by the HS method. Some of them are too hard to cut, while the others are too soft. Both of them may give a low quality of the section. Therefore, knowledge of the types of organs and types of plants that can be easily cut by the HS method is important. This research was conducted to select several types of dicotyledonous plants that can be easily slashed by the HS method and can provide a good anatomical appearance. On this study, the incisions will be focused on the stem of shrub and herb dicots. The results of this study will be very useful for people who need HS slicing action, either for research or laboratory practice.

2. Research Method

2.1 Plant materials

Dicotyledonous plants used in this study is a cosmopolitan habitus shrub around the campus of the Universitas Negeri Makassar. The plant species and stem size used in the study can be seen in Table 1.

Table 1. Plant material

Species	Range incision from the apical meristem (cm)	Stem diameter (mm)
<i>Ixora maxima</i>	1 – 2	1.15 – 1.35
<i>Bougenvillae spectabilis</i>	1 – 3	2.00 – 2.07
<i>Olenina syzigum</i>	2 – 3	2.20 – 2.35
<i>Catharanthus roseus</i>	1 – 4	2.25 – 2.33
<i>Mirabilis jalapa</i>	4 – 5	4.11 – 4.15
<i>Zinnia elegans</i>	3 – 5	2.75 – 3.25
<i>Acalypha siamensis</i>	1 – 5	2.24 – 2.27
<i>Eclipta prostate</i>	2 – 5	2.23 – 2.27
<i>Coleus scutellarioles</i>	4 – 5	1.85 – 2.20
<i>Reullia tweediana</i>	2 – 5	2.80 – 3.55

2.2 Stage of sectioning

Plant stems that will be sliced are picked in the morning and soaked in water. The sample is immediately taken to the laboratory for sectioning that is only done no more than 2 hours after picking. Each plant stem was sectioned based on the HS method according to Yeung [3], and the incision should be as thin as possible (one cell thick) and as much as possible in a duration of 10 minutes. This sectioning was repeated 6 times in two days. Furthermore, dicots that provide at least 50 sections are categorized as easy to section and continued to the next stage.

2.3 Stage of microscope observation

Based on the HS method, the stem of the selected plant was cut using a razor blade and transferred to a drop of water on a microscope slide. For instant sections observation, protect the sections with a cover glass and drain the excess water with paper towel. This stage was followed by direct observation by light microscope. For stain added sections observation, the sections should be dropped by safranin for 2-5 second and wash by water until the excess stain are removed. The stained section then covers by cover glass and observe using monocular light microscope XSP-12 adjusted by Optilab Profesional Edition. The rubric of indicator assessment (modify from Wahyuni) [4] was used to assess the appearance quality of all sections (Table 2).

Table 2. The rubric of indicators assessment for clarity slides

Slide clarity	Categories assessment
If the appearance of the cell shape and parts of the tissue can be clearly distinguished on the preparation.	Very clear
If the appearance of the cell shape is indistinguishable, the parts of the tissue can be clearly distinguished on the preparation	Clear
If the appearance of the cell shape and parts of the tissue cannot be distinguished on the preparation	Not clear

2.4 Stage of limited trial

This stage is carried out to find out respondents who are 20 final semester students and 26 new students of Biology Department Universitas Negeri Makassar. They were given instructions by a picture and diagram on how to prepare a one-cell-thick section through HS method and observe it using a microscope. In the implementation of the test, respondents were given time for ten minutes per each plant type, to make at least a one-cell-thick section and observe it using a light microscope. Then they were asked to fill out questionnaires which questioned two things, which is (1) how difficult was the preparation of cross section one-cell thick section? (2) are cells and tissues easy to distinguish? Both questions were given five option answer following the Likert scale modified by Vagias [5], which is very difficult, difficult, neutral, easy and very easy.

3. Results and Discussion

The results of the sectioning stage presented in Table 3. It shows that *A. Siamensis* give the highest number of sections in 10 minutes, while *C. scutellarioles* is the lowest number. In this study, five out of 10 types of plants were considered as easy to section with the HS method, namely *I. maxima*, *E. prostate*, *M. jalapa*, *C. roseus*, and *A. siamensis*. The difficulty of dicots cutting seems to depend on the level of hardness of the stem. The difference in hardness of dicotyledonous stems can be caused by differences in lignification processes in each plant tissue, namely the process of thickening of the lignin structure physically and chemically which can cause changes in the structure to become stiff and hard.

Table 3. Dicots species and its sections characters

Plant Species	Average number sections per 10 minutes	Easily sectioned (Yes/No)	Hardness of stem
<i>Reullia tweediana</i>	38	No	Too soft
<i>Catharantus roseus</i>	64	Yes	Proper
<i>Acalypha siamensis</i>	91	Yes	Proper
<i>Ixora maxima</i>	51	Yes	Proper
<i>Mirabilis japala</i>	52	Yes	Proper
<i>Ecalipta prostate</i>	51	Yes	Proper
<i>Bougainvillea spectabilis</i>	47	No	Too hard
<i>Zinnia elegans</i>	26	No	Too soft
<i>Oleina syzigum</i>	33	No	Too hard
<i>Coleus scutellarioles</i>	23	No	Too soft

Based on the study, it is known that *A. siamensis* and *C. roseus* are plants that are easy to cut. This is because these two types of plants have a rod structure stem that is proper hardness. Apparently, the structure of these stem allows the ease of obtaining a good stem for slicing. Especially for *A. siamensis*, the sectioning can be done from 1 to 5 cm from the shoots, while *C. roseus* can be slashed about 3-5 cm from the tops of plants.

Plants of *I. maxima*, *M. jalapa*, and *E. prostate* are also easily cut. However, based on its morphological structure, the three types of plants have different stem structures. Plant *I. maxima* have a woody, stem structure on the shoots and rounds on the trunk to the base of the stem. The sectioning can only take place up to 1 cm from the shoot. This is because part *I. maxima* have been hardened, even on the young stem, so that the incision can only be made near the apical.

Plants of *B. spectabilis* and *M. jalapa* are plants from the same family (Nyctaginaceae), but both plants have significant differences in their morphological structure. *B. spectabilis* has a structure of stem morphology that is almost the same as *C. roseus* which is rounded and woody. The shoot portion of *B. spectabilis* stem is too soft to cut so that the part of the stem that can be sliced is about 1 to 3 cm from the shoot.

On the other hand, *Z. elegans* and *C. scutellarioles* are grouped to the category difficult to cut. Based on the morphological structure, the plants have soft stems from the shoot to the base of the stem. Meanwhile, *O. syzigum* are too woody and hard to section. Based on this study, this plant has stems that are difficult to cut. The strengthen and spasticity may be strongly influenced by lignin. According to Barros et al. [6], even though lignin is not as strong as cellulose, lignin add a significant reinforcement to any cell wall and providing tensile strength.

In this study, the five types of dicots which were declared easy to slice, then observed their anatomical cross section using a light microscope and the results can be seen in Figure 1. Based on that figure and referring to Table 1, it can be seen that only one dicot (*I. maxima*) that the cells and tissues cannot be distinguished. The portion of the vessel and cortex tissue of this species looks dark and overlapped, both on stained and unstained slides.

In contrast, cell and tissue forms in *A. Siamensis*, *M. jalapa*, *E. prostate*, and *C. roseus* can be clearly distinguished both in stained and unstained sections. Especially for the section of *E. prostate* cells and tissues can be distinguished when sections are colored with safranin. It was different in *C. roseus* where the cells were easier to distinguish on the unstained section. The other dicots, *A. Siamensis*, and *M. jalapa* gave clear anatomy appearance on stained sections and also unstained sections (Figure 1).

Four types of selected dicots, *A. siamensis*, *C. roseus*, *M. jalapa* and *E. prostate*, were tested to final semester students and new students of Biology Department, Universitas Negeri Makassar. The results show that almost all respondents said that the four types of plants were easy to cut, except for *C. roseus* and *E. prostate* which was considered as very easy (Table 4). Based on this results, it can be recommended that *A. Siamensis*, *C. roseus*, *M. jalapa*, and *E. prostate* to be used in practical laboratory and research.

Table 4. Section difficulty of the plant in test limited

Plant materials	Fresh graduate respondents	New students
<i>A. siamensis</i>	Easy	Easy
<i>C. roseus</i>	Very Easy	Easy
<i>M. jalapa</i>	Easy	Easy
<i>E. prostate</i>	Easy	Very Easy

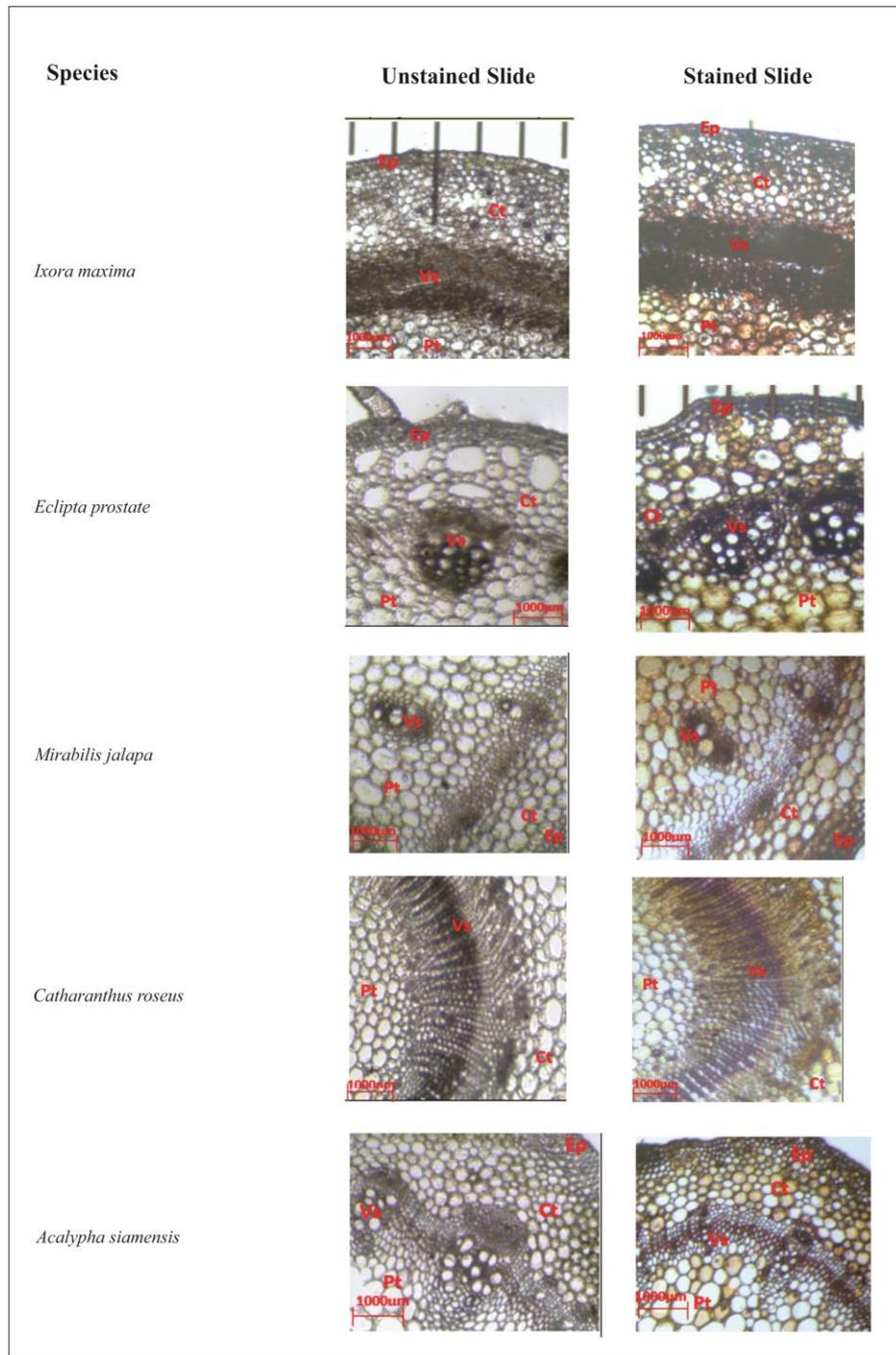


Figure 1. Photomicrograph of selected dicots sectioned by HS method

4. Conclusion

Based on this study, there are four dicots that can be sectioned by a hand-section method and can provide a good anatomical appearance, namely *A. siamensis*, *C. roseus*, *M. jalapa* and *E. prostate*.

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