

Secretory Structure, Histochemistry and Phytochemistry Analyses of Stimulant Plant

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Abstract. Plants that are used as stimulant supposed to contains various metabolit compounds that are produced or secreted by secretory structures. This study aimed to identify the secretory structure of plant used as stimulant and chemical compounds accumulated in it. The secretory structure and its histochemistry were observed on plant material that are used as herbal ingredient. Phytochemical content was analyzed by using a qualitative test. The result showed that the idioblast cells and secretory cavities were found in the leaves of *Decaspermum fruticosum*, and *Polyalthia rumphii*. Most idioblast cells contained lipophilic substances and terpenoids or alkaloids, while secretory cavity contained alkaloid. Phytochemical analysis for *D. fruticosum*, and *P. rumphii* contain terpenoids, phenols, steroids, and flavonoids

Keywords: phytochemistry, histochemistry, secretory structure, stimulant.

1. Introduction

Tonic is a medicine that can strengthen or give additional energy to the body. It can play an important role tightening or strengthening body physiological system. Tonic plants are kinds of plants that can raise and keeping body stamina againts disease attack and protect body vitality to keep in perfect health [1]. Plants that are potential as tonic are often used to keep health by traditional society, for example Anak Dalam Tribe who live in in the area of Bukit Duabelas National Park.

Bukit National Park is one of tropical rain forest Jambi in lowland in that area were found 110 kind of plants used as medicinal. From 110 plants spend as medicine, there are 22 spesies have been examined their chemical contents including their availability of alkaloid, saponin, flavonoid, tannin and polyphenol [2-3].

Anak Dalam Tribe utilize a traditional medicines to maintain health based on knowledge to maintain health based on knowledge, belief experience and direct practices which have been conducted from generation to generation from their ancestor. Knowlegde about traditional medicines was the foundation of new medicine invention on the modern therapy system [4]. Plants that are used as medicinal material contain metabolite compounds which are potential as active ingredient among others are alkaloid, terpenoid, polyphenol, saponin, flavonoid, tannin, triterpen, and steroid. Compounds contents found on the plants utilized as tonic are among others alkaloid compounds terpenoid, quinon, phenol, polysaccharide, and glycoprotein [5].



Scientific study on secretory structure producing metabolite compounds and histochemical test on tonic plants used by Anak Dalam Tribe have never been carried out. The scientific study enabled to develop information about metabolite compounds content on plants used as tonic material as well as developing through tissue culture or cell culture.

The objective of this research was to indentify plant secretory structure used as tonic and accumulated compounds in the structure.

2. Materials and Methods

2.1. Plant Collection and Identification

Plant collection was conducted in Bukit Duabelas National Park area, Jambi. Plant material used were leaf of saledemo (*Decaspermum fruticosum* and ganja sayur (*Polyalthia rumpii*) (Table 1). For light microscope observation, leaves were fixed using 70% ethanol. Fresh leaves were collected for the histochemistry test, while for qualitative phytochemical content analysis the samples were dried under the sun light for two days, and then dried using oven at 50°C for five days. Identification of plant local name originated from Bukit Duabelas National Park was helped by the head of Anak Dalam Tribe (Tumenggung Tarip). Further plant identification was carried in Herbarium Bogoriense, LIPI, Bogor.

Table 1. Kinds of tonic plant, usage and used methods.

Local name	Scientific name (Family)	Part of plant used	Usage*	Usage methods*
Saledemo	<i>D. fruticosum</i>	Leaf	cold medicinal	Boiled leaves, drunk and used for taking a bath
Ganja sayur	<i>P. rumphii</i> (Annonaceae)	Leaf	Appetizer	Raw eaten

* Tumenggung Tarip, Anak Dalam Tribe pers.comm, 2014

2.2. Histological Analysis

Leaves paradermal section were prepared as a semi permanent preparation by using wholemount method [6]. Fixative leaves in ethanol 70% were washed with distilled water and soaked in 50% HNO₃ solution, rinsed in the water and then the adaxial and abaxial of the leaves were peeled by using razor blade. The section result was soaked in the clorox solution for 3-5 minutes, then was coloured by safranin 1%. The samples were observed by using a light microscope (Olympus CX21).

2.3. Histochemistry Test

Leaf samples of *D fruticosum* and *P.rumphii* were sliced transversally as thick as 15-20 µm using freezing microtome (Yamato RV-240). The sliced result were tested by using several reagent. Alkaloid compounds test on cell or tissue. Lamina leaf section was soaked in Wagner reagent for 24 hours. A positive result was indicated by presence of reddis-brown deposit. As negative control alkaloid, the leaf section pre-treated with 5% tartaric acid for 48 hours to dissolve alkaloid [7]. Lipophilic compounds content test by using sliced samples washed in 70% ethanol for one minute, then coloured with 0.03% sudan IV and heated in a water bath at 40 °C for 30 minute. The sliced sample were then washed with ethanol 70%. The presence of lipophilic compounds was indicated by colour orange [8]. Terpenoid compounds test in the leaf tissue was identified by soaking samples slices in 5% cupric acetate reagent for 24 hours. Terpenoid compound content will be shown if there is yellow or brownish-yellow color [9].

2.4. Qualitative Phytochemical Analyses

Leaf samples that had been air dried will be made into powder by using blender. The powder is qualitatively tested to detect the existence of terpenoid, steroid, flavonoid, alkaloid, and phenol compounds according to the methods of Harborne [9].

2.4.1. *Triterpen and Steroid Compounds Test.* Approximately 3 gram of powder sample will be added 5 ml concentrated ethanol while being heated then it will be filtered. The obtained filtrate will be dropped on the drop plate and will be heated until dry, then it will be added with 1 ml diethyl ether, and Lieberman-Burchard reagent (3 drops of unhydrous acetic acid + 1 drop of sulphuric acid). If the colour of red or purple is obtained it shows triterpen compounds is positive, but if the colour of green or blue appears it shows steroid compounds is positive.

2.4.2. *Alkaloid Compounds Test.* Approximately 3 gram of sample powder will be extracted by a little chloroform, then it was added with 3-5 drops of ammonia and was filtered. Filtrate was added with 1-2 ml H₂SO₄ 2M, then was shaken until two layers will be formed. Acid layer (no colour) will be dropped on three test plates, the first plate will be dropped with Dragendorff reagent, the second plate will be dropped with Mayer reagent and the third plate will be dropped with Wagner reagent. If there is orange, white and brown precipitation consequently against the three reagents mentioned above it means the result is positive against alkaloid compounds.

2.4.3. *Flavonoid Compounds Test.* Approximately 3 gram of sample powder will be given a little distilled water and was heated for 5 minutes, then it will be given filtered. The filtrate was added a little Mg powder, several drops of concentrated chloric acid and 2 ml amyl ethanol, if the colour of orange appears it means flavonoid compounds is positive.

2.4.4. *Phenol Compounds Test.* Approximately 3 gram of sample powder was added with hot 70% methanol, then it will be filtered. The filtrate was dropped with 10% NaOH. If there is yellow until red colour it means phenol compounds is positive.

3. Results and Discussion

3.1. Secretory Structure

Secretory structure was plant cell or tissue plant function as secretory place of metabolite compounds such as essential oils, gum, resin, latex, alkaloid, glycoside, and mineral salts. Secretory structure were differentiated into two based on location, namely external and internal secretory structure. External secretory structure comprises trichome, nectary or honey gland, hydathode and stigma, while internal secretory structure can consist of idioblast, secretory cavity, secretory duct and laticifer [10].

Secretory structure found in the two studied tonic plants, consist of idioblast cell and secretory cavity (Figure 1). *D. fruticosum* plant as included in the Myrtaceae family [11]. In *D. fruticosum* leaves were found secretory structure as idioblast cells and secretory cavities which are spread in palisade and sponge tissues (Figure 1).

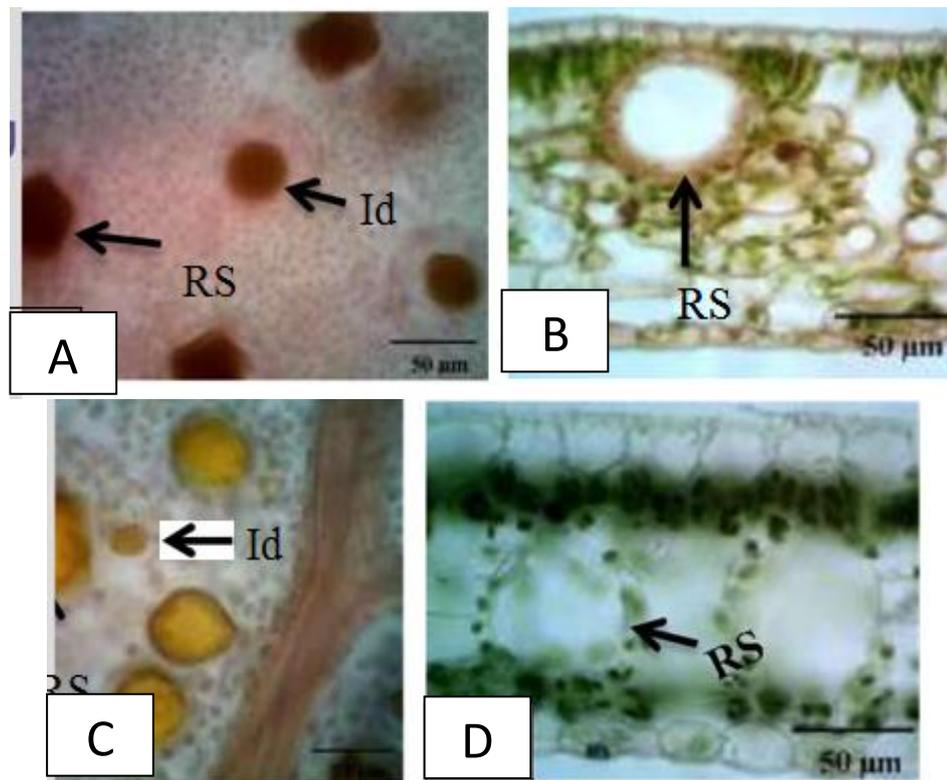


Figure 1. Secretory structure on tonic plant *D. fruticosum* leaf (A, B) *P. rumphii* leaf (C, D), idioblast cell (Id), RS) secretory cavity.

D. fruticosum leaf idioblast cell was round with various measurements. The measurements of idioblast cell in palisade and sponge tissues are not different but the higher density was in sponge tissues (Table 2). Secretory cavity on this plant are round with epithel cell surround it. Secretory cavity measurement was bigger than idioblast cell do not the same as in the palisade and sponge tissue, while density was higher in the palisade tissue (Table 2). On the leaves of *Ugni molinae* Turcz as included in the Myrtaceae family there is also a secretory cavities. Metcalfe and Chalk [12] reported the availability of secretory cavity is a general characteristic of Myrtaceae family, even though the structure is also found in other families.

Table 2. Measurement and density of various tonic plant structure secretory.

Plant name	Organ	Secretory structure	Diameter measurement (µm)		Density (mm-2)	
			Palisade	Sponge	Palisade	Sponge
<i>D. Fruticosum</i> (Myrtaceae)	Leaf	Idioblas cell	26.3-36.1	31.6-39.6	16.8-18.6	20.4-33.4
		Secretory cavity	46.1-58.3	52.7-56.1	26.3-35.5	16.6-20.2
<i>P. Rumphii</i> (Annonaceae)	Leaf	Idioblas cell	21.4-25.4	17.7-26.1	20.4-33.4	25.5-48.0
		Secretory cavity	30.4-63.0	43.4-69.6	41.8-43.6	30.2-48.8

P. Rumphii plant which is the member of the Annonaceae family [13] has the secretory structure as idioblast cells spread in the whole mesophilic tissues beside there are also secretory cavity spread

among palisade and sponge tissue (Figure 1). Secretory cavity size is bigger than idioblast cell. Idioblast cells round with the size and density that are not different between palisade and sponge tissue. Secretory cavity is round with the size and density which are really varies (Table 2).

3.2. Histochemistry Analysis

Idioblast cell in *D. fruticosum* contain lipophilic compounds and terpenoids, whereas the lipophilic compounds containing in the *P. rumphii*. Secretory cavity in *D. fruticosum* and *P. rumphii* leaves contain alkaloid compounds. On *Peganum harmala* leaves idioblast cell is specific cell which accumulate alkaloid compounds [14]. On *Mandevilla guanabara* leaf (Apocynaceae) idioblast cell produces lipophilic compounds [15].

The result of *D. fruticosum* leaf histochemistry test showed the availability of alkaloids, terpenoids and lipophilic compounds (Table 3). Alkaloid compounds was detected in the secretory cavity, lipophilic and terpenoid compounds were found in the different idioblast cells (Figure 2). From several study results, besides producing alkaloid compounds, secretory cavity also produce other secretes, such as on *Ugni molinae* Turcz (Myrtaceae) produces lipophilic compounds [16], while in leaf of *Myrrhinium atropurpureum* Schott var. *atropurpureum* (Myrtaceae) produces lipophilic and terpenoid compounds [17]. *P. rumphii* leaf contains alkaloid compounds which was detected on the secretory cavity and lipophilic compounds was detected in the idioblast cell (Figure. 3)

Table 3. The results of histochemistry tests of tonic plants.

Secretory structure	Plant name	Test result		
		Alkaloids	Terpenoids	Lipophilic compounds
Idioblast cell	<i>D. fruticosum</i>	-	+	+
	<i>P. rumphii</i>	-	-	+
Secretory cavity	<i>D. fruticosum</i>	+	-	-
	<i>P. rumphii</i>	+	-	-

+ = detected secondary metabolite compounds

- = undetected secondary metabolite compounds

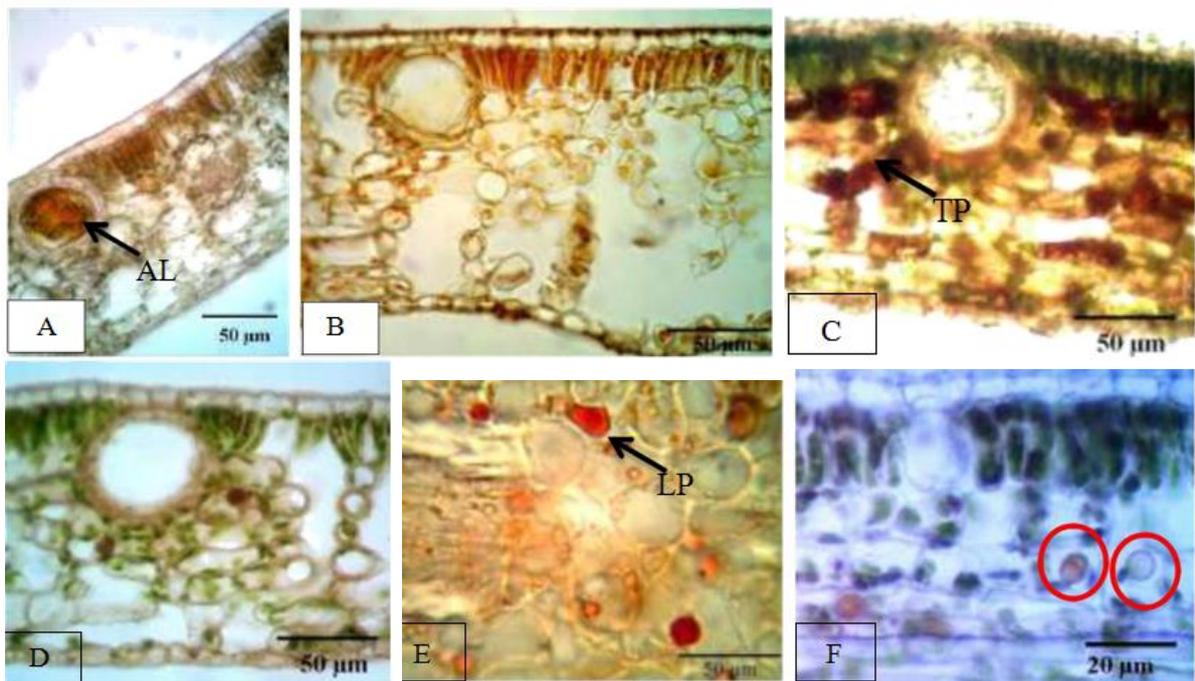


Figure 2. Histochemistry test result on *D. fruticosum* leaf: compound (A) alkaloids control (B) terpenoids (C), terpenoids control (D), lipophilic compounds (E), lipophilic compounds control (F): Al) alkaloid compounds, TP) terpenoid compounds, LP) lipophilic compounds.

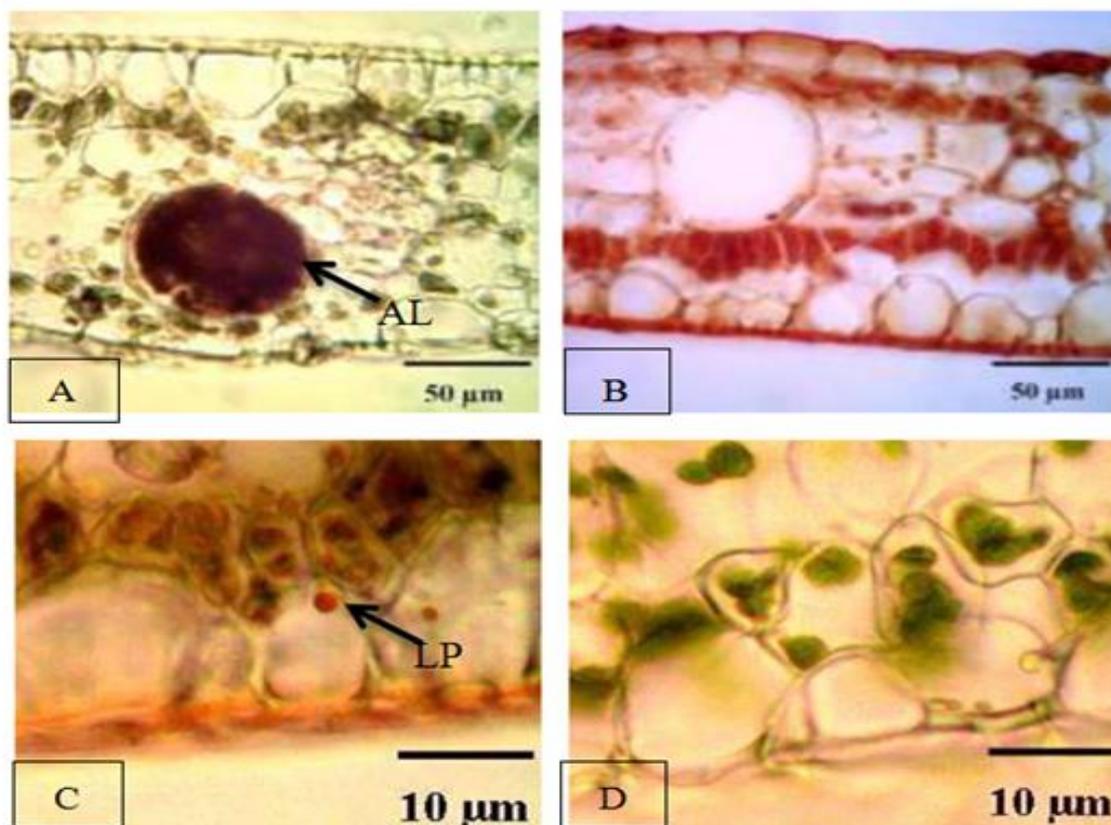


Figure 3. Histochemistry test result *P. rumphii* leaf: Alkaloid compounds (A), alkaloids control (B), lipophilic compounds (C), lipophilic compounds control (D):AL) alkaloid compounds, (LP) lipophilic compounds.

3.3. Phytochemical Analysis

Qualitative phytochemical analysis result of *D. fruticosum* and *P. rumphii* leaf showed there phenolic, flavonoid, steroid and triterpene compounds, but alkaloid compounds was undetected (Table 4). Alkaloid compounds was undetected on phytochemistry test was shown by no precipitation occur when it was given Mayer reagent, Wagner reagent and Dragendrof reagent. Different from the phytochemical result, histochemistry result on *D. fruticosum* and *P. rumphii* showed there was alkaloid compounds. Alkaloid compounds was undetected on phytochemical test because the test was carried out against powder originated from the whole plant organ tissue, so that metabolic compounds concentration was low. Histochemistry test showed positive result because it was conducted against tissues obtained from organ slices so that the test was directly observed on the secretory structure.

The highest triterpenes content was found on *D. fruticosum* leaf. The highest steroids content was on the *P. rumphii* leaf. On the *D. fruticosum* leaf, triterpenes was the main component on metabolite compounds, while other compounds were available in low number (Table 4). According to Wagner [5] alkaloid, terpenoid, quinonic, phenol, polysaccharide, glycoprotein compounds are potential as tonic, as well as flavonoids group such as flavones and flavonols [18]. Phytochemical test result of *P. rumphii* showed the existence of phenol, flavonoid, and steroid compounds (Table 4). Paarakh and Khosa [19] reported that several species from genus *Polyalthia* also contain alkaloid compounds.

Table 4. Qualitative analysis result of phytochemistry compounds on tonic plants.

Plant	Test result				
	Alkaloids	Phenols	Flavonoids	Steroids	Triterpenes
<i>D. fruticosum</i>	-	+	+	++	++++
<i>P. rumphii</i>	-	++	++	+++	+++

Explanation:

- : no compounds
- + : a little bit
- ++ : moderate
- +++ : a lot
- ++++ : very much

4. Conclusion

D. fruticosum and *P. rumphii* used has tonic have secretory structure in the form idioblast cell and secretory cavity. Idioblast cell on *D. fruticosum* leaves contain lipophilic and terpenoid compounds, while on *P. rumphii* only contain lipophilic compounds. In the secretorial cavity structure on *D. fruticosum* and *P. rumphii* contain alkaloid compounds. Phytochemical compounds content produced by *D. fruticosum* and *P. rumphii* are phenol, flavonoid, steroid and triterpene compounds.

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