Leaf epidermis and phytochemical studies of sambiloto 
(*Andrographis paniculata* (Burm. F.) Nees)

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Abstract. This study was conducted to observe leaf epidermis and phytochemicals of Sambiloto (*Andrographis paniculata* (Burm. F.) Nees) that lived in different areas, i.e. Kp. Lapai, district Nanggalo, municipality Padang (altitude 7 m above sea level) (AP 00A1); and Sawah Dangka jorong Gaduik, district Tilatang Kamang, Agam (altitude 885 m asl) (AP 00B1). *A. paniculata* is a herbaceous plant, that is known as one of traditional medicinal ingredients. For observation of leaf epidermis, abaxial region from the 4th leaves of 8 weeks old seedlings were Analyzed by using SEM (Scanning Electron Micrograph). Phytochemicals observation include qualitative analysis of phenolics, flavonoids, saponins, terpenoids and alkaloids compounds. The results showed that *A. paniculata* (AP 00A1) have average width and length 57.3-68.8 µm of stomatal size, 28.7 - 40.8 μm guard cell area, average of Stomatal Index ± SE (25.4% ± 0.09), average of Stomatal Density ± SE (172 stomata / mm² ± 0.13), with multicellular glandular trichome presence (++) and multicellular trichome (+++). Whereas *A. paniculata* (AP 00B1) have 43.3 - 57.7 µm average width and length of stomatal size with of 28.8 - 33.7 μm guard cell are, Stomatal Index ± SE (34.3% ± 0.11), and Stomata Density ± SE (352 stomata / mm ± 0.08 ), with. multicellular glandular trichome (++) and multicellular trichome (++++). The stomata Index, Stomata Density and trichome presence on abaxial epidermis of Sambiloto (*A. paniculata*) leaf at Sawah Dangka jorong Gaduik, high altitude (AP 00B1) were greater than those growth at Kp. Lapai municipality Padang, a low altitude (AP 00A1). We believe that appear as plant adaptation for lower partial pressure of CO₂ and to more protection from external interference at high altitude region. Phytochemicals results showed that both of *A. paniculata* (AP 00A1) and (AP 00B1) accumulated of phenolics (+), flavonoids (+), saponins (-), terpenoids (+) and alkaloids compounds (-).

1. Introduction

*Andrographis paniculata* (Burm.F.) Nees is one of 28 species, from genus Andrographis in the world. This plant belongs to the Acanthaceae family. This plant is a traditional medicinal plants used in the treatment of colds, fever, laryngitis, as well as gastrointestinal infections such as dysentery and diarrhea, in some countries such as China, India to Southeast Asia. In Chinese Traditional Medicine (TCM) this plant determined as an herb that has a significant value in "cold property"i.e. for the treatment of fever and eliminate toxins from the body [7], and is also used in paste form for the
treatment of periodontitis supplement cattle feed in large scale [6]. This plant is known worldwide as ‘the
creat “or” King of Bitters “ [7], while in Indonesia known by several names such as Papaitan, bile
land, bidara, sadilata and the most popular is sambiloto. Active compounds contained in A. paniculata,
is actually of secondary metabolites, including economic stakes are diterpene classes, such as:
andrographolid, neoandrographolid and dehydro-andrographolid [7].

Several research reports indicate that A. paniculata have morphological variations, levels of
chemical compounds contained, as well as proving the existence of diversity because of differences in
growth and the environment. [8], conducting research in the area of Forest Management Surakarta
Central Java, getting that content andrographolid of A. paniculata, which grows in the middle plains of
2.27%, in the lowlands and height respectively of 1.37 and 0. 89%. Meanwhile, in another study
conducted by [2] proves that the stomata density of Greenleaf Manzanita (Arctostaphylos patula) were
higher in the leaves that were collected from the plateau (30.03 stomata / mm²) compared to lower
plants (19.3 stomata / mm²).

In theory known that the layers of the epidermis is the most sensitive to changes in the external
environment, wherein the epidermal layer, the structure of stomata and trichome, a row is a structure
that is directly related to the diffusion of water evaporation of water and release of other
compounds to the atmosphere [9]. Reports of [5], on plant leaves Nothofagus solandri that grow on the
slopes of Mt. Ruapehu, the stomata index and stomatal density increases with increasing altitude grow.

Based on the above information, this study was performed to evaluate the leaf epidermis which
includes the size of the stomata, stomatal index, stomatal density, guard cell area, trichome type and
presence, as well as the phytochemical content of plant A. paniculata that grows at Kp Lapai, district.

Nanggalo, municipality Padang (height of 7 m above sea level) and Sawah Dangka jorong Gaduik,
district. Tilatang Kamang, Agam (altitude of 885 m above sea level).

2. Materials and Methods

A total of 79 and 56 plants of A. paniculata were in living in the village Kp. Lapai, Kotamadya
Padang (7 m asl) and Sawah Dangka jorong Gaduik, Kab. Agam (885m asl), was taken and observed
in Plant Physiology and Plant Structure & Development laboratory of the Department of Biology
UNAND. For leaf epidermis studies, the material used is the fourth of leaf from tip apical region of A.
paniculata plant, which originated from a seedling of A. paniculata. The epidermis of leaves observed
stomatal size, stomatal index, guard cell area, shape and hair trichome glands onabaxial the leaves of
A. paniculata by using SEM (Scanning Electron Microscope), in the Laboratory of the Biology
Department of the State University of Padang.

Stomata index be determined in accordance Metcalfe and Chalk (1979) using the following formula
[4].

\[
\text{Stomatal index} = \frac{S}{E + S} \times 100
\]

where \(S\) = number of stomata per unit area; \(E\) = the number of epidermal cells in the same area.

Stomata number per area of 1 mm² = Stomata Density

Phytochemical Studies conducted in UPT Biological Resources Laboratory Biota Sumatra
Andalas University. The material in the form of aerial parts of the plants A. paniculata that are
flowering. Analysis of phenolic, flavonoids, saponins, terpenoids and steroids according to the method
[10], while the alkaloid analysis according to the method [1].

2.1 Analysis of Phenolic

Simplicia (1 g) was macerated with methanol heat for 5 minutes. Maserat separated and evaporated the
solvent until thick. Then add 5 ml of distilled water and 5 ml of chloroform, fractionation. Taken layer
of distilled water and place it on a plate drops, add 1-2 drops of FeCl₃ 1% and visible color change. (+)
Phenolic = green form the color changes to dark green or blue to blue-black; (-) Phenolic = no change of color) [10].

2.2 Analysis of Flavonoid

simplicia (1 g) was macerated with methanol heat for 5 minutes. Maserat separated and the solvent evaporated to half of the initial volume. Then drip drip a few drops on a plate and add a pinch of Mg powder, then add 1-2 drops of concentrated HCl, and then see the color change. (++) Flavonoids = the color changes to orange to red; (-) flavonoids = no change of color.) [10].

2.3 Analysis of saponin

Simplicia (1 g) heat macerated with methanol for 5 minutes. Maserat separated and evaporated the solvent until thick. Then add 5 ml of distilled water and 5 ml of chloroform, fractionation, and then taken a few layers of water, put in a test tube, then shaken strongly during 1 minute and allowed to stand for 1 minute. Judging changes. Added 1 drop of concentrated HCl, and then see the changes. ((+) Saponin = the formation of foam that lasts for 1 minute or more and remain there after the addition of concentrated HCl; (-) saponin = not happen foaming or the formation of foam but missing after the addition of concentrated HCl) [10].

2.4 Steroid Analysis Terpenoids

Simplicia (1 g) was macerated with methanol heat for 5 minutes. Maserat separated and evaporated the solvent until thick, add 5 ml of distilled water and 5 ml of chloroform, fractionation. Then retrieved the chloroform layer and miss out on a small column containing activated charcoal and dropped on the plate and let it drip dry up, so add 1-2 drops of acetic anhydride and add 1-2 drops H$_2$SO$_4$ of concentrated. Then see the color change. (++) Steroids = change color becoming blue; (+) terpenoids = change color becoming red; (+) steroids and terpenoids = discoloration becoming purple; (-) steroids and terpenoids = no color change) [10].

2.5 Alkaloids Analysis

Simplicia (2 g) finely ground and then added with chloroform (5 ml) was then added chloroform ammonia (0.05N) (5 ml). Taken chloroform extract is added 5-10 drops of H$_2$SO$_4$ 2N, shaken gently for a few times and let stand for 1 minute. Taken acid layer, put in a small test tube, add 1-2 drops of reagent in mayer through the tube wall and see the changes. ((+) Alkaloid = the formation of white when the reagent solution mayer contact with the test substance; (-) alkaloid = no change of any kind.) [1].

3. Result and Discussion

3.1 Leaf epidermis of Andrographis paniculata

The observation by SEM showed that the region abaxial leaves epidermis layer of A. paniculata consists of cells of irregular elongated shape, the most of stomata type is diacytic (Figure 1 and Figure 2), and multicellular trichome and glandular multicellular trichome (Figure 2). The other type of stomata in A. paniculata was anisocyct type (Figure 1 and Figure 2) and, so cryptophore type or sunken stomata (not shown). In the other research on Ixora plant have been done by [4], their found several stomata types i.e.anisocytic, anomocytic, staurocyclic, paracytic, laterocytic, brachyparacytic.

Based on Table 1, it is seen that average wide and length stomatal size and guard cells area of A. paniculata at Kp Lapai, Padang (AP 00A1) tends to be greater than plant A. paniculata at Sawah Dangka jorong Gaduik, Agam (AP 00B1). Although in Figure 2B shows that the cell size (AP 00B1)
is larger, it is because of the difference of magnification in Figure 2B using a magnification of up to 500x, while Figure 1A, 1B and 2A respectively using a magnification of 310x, and 350x 310X.

Table 1. Leaf epidermis include the average Stomatal Density, average width and length guard cell area and average width and length stomatal size. Average Stomatal Index, and Trichome Presence on the abaxial leaf Sambiloto (A. paniculata) who live in Kp. Lapai, Kec. Nanggalo, Padang and Sawah Dangka Jorong Gaduik, Kec. Tilatang Kamang, Agam.

| Source of Plant | Average Stomatal Density ± SE | Average width and length of stomatal size (µm) | Average width and length of stomatal size (µm) | Average Stomatal Index (%) ± SE | Trichome Quality |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| (AP 00A1) A. paniculata from lowland 7 m asl (Kp. Lapai Padang) | 172 ±0.13 | 28.7-40.8 | 57.3-68.8 | 25.4± 0.09 | +++ ++ |
| (AP 00B1) A. paniculata from altitude 885m asl Sawah Dangka Jorong Gaduik, Agam) | 352 ±0.08 | 28.8-33.7 | 43.3-57.7 | 34.3±0.11 | ++++ +++ |

Description: Category amount of Trichome (ΣT) is: (+) if (ΣT) ≤ 2; (++) if 2 < (ΣT) < 30 ; (+++) if (100≥ ΣT) > 30) ; (++++) if 100 <ΣT), SE = Standard Error . N (9)

In Table 1. A. paniculata (AP 00B1) that living in Sawah Dangka, jorong Gaduik, district Tilatang Kamang, Agam, showing the average Stomatal Index of 34.3% ± 0.11, it is higher than A. paniculata (AP 00A1) at Kp. Lapai, Padang (25.4% ± 0.09). Likewise with the Stomatal Density on A. paniculata (AP 00B1) that growth at Sawah Dangka, jorong Gaduik, district Tilatang Kamang, Agam, showing Stomatal Density of 352 ± 0.08 per 1 mm² area,
**Figure 1.** Leaf epidermis *Andrographis paniculata* Nees (A. AP00A1; B. AP00B1) stomata (s); multicellular glandular trichome (gt)

**Figure 2.** Leaf epidermis *Andrographis paniculata* Nees (A. AP00A1; B. AP00B1) stomata (s); multicellular glandular trichome (gt); multicellular trichome (mt).

It is higher than the Stomatal Density *A. paniculata* (AP 00A1) that growth at Kp. Lapai, Padang (172 ± 0.13 per 1 mm² area). This condition was the same that [5], found on plant leaves *Nothofagus solandri* that grow on the slopes of Mt. Ruapehu, and he suggests that stomata index and stomatal density will be increase by high elevation are related to carbon dioxide uptake [5].

At **Figure 2A** and **B** showed that in *A. paniculata* layer epidermis abaxial region there were multicellular trichome and multicellular glandular trichome. These fact is the same with [3] report, that their found multicellular and glandular trichome in anatomical *A. paniculata*. 
3.2 Phytochemical Andrographis paniculata

As shown in Table 2, A. Paniculata that growth at low land Kp. Lapai, municipality Padang (AP 00A1) have phytochemical compound just the same with higher altitude at Sawah Dangka jorong Gaduik, district Tilatang Kamang, Agam (AP 00B1).

Table 2. Phytochemical analysis A. paniculata

| Phytochemicals | A. paniculata from lowland – 7 m asl (Kp.Lapai - Padang (AP00A1)) | A. paniculata from altitude 885 m asl Rice Dangka, jorong Gaduik, Agam (AP00B1) |
|----------------|-------------------------------------------------|-------------------------------------------------|
| Phenolic       | +                                               | +                                               |
| Flavonoids     | +                                               | +                                               |
| Saponins       | -                                               | -                                               |
| Steroids       | -                                               | -                                               |
| Terpenoids     | +                                               | +                                               |
| Alkaloids      | -                                               | -                                               |

4. Conclusion

1. Sambiloto (Andrographis paniculata (Burm F.) Nees plant that growth at Kp. Lapai, district Nanggalo, municipality Padang (at lowland until 7 m above sea level) (AP 00A1) have 57.3-68.8 μm average width and length of stomatal size with 28.7-40.8 μm average width and length of stomatal guard cell area, 25.4% ± 0.09 of Stomatal Index, 172 ± 0.13 of Stomatal Density, with multicellular glandular trichome (++) and multicellular trichome (+++) presence. Whereas A. Paniculata that growth at Sawah Dangka, jorong Gaduik, Agam (altitude 885 m asl) (AP 00B1) have average width and length of stomatal size 43.3 - 57.7 μm, with average width and length of guard cell area is 28.8 - 33.7 μm, 34.3% ± 0.11 of Stomatal Index, 352 ± 0.08 of Stomatal Density, with multicellular glandular trichome (+++) and multicellular trichome (++++) presence.

2. Phytochemical analysis results showed that both of A. paniculata (AP 00A1) and A. paniculata (AP 00B1) accumulated phenolic ie secondary metabolite (+), flavonoids (+), saponins (-), terpenoids (+) and alkaloids (-).

3. The average of Somatal Index, and Stomatal Density and the presence of trichome on abaxial leaf A. paniculata (AP 00B1) describes the adaptation to changes caused by the decrease in the partial pressure CO₂ at high altitudes, as well as greater protection against outside interference.

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