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EPIDERMAL CHARACTERISTICS AND EPIDERMAL DERIVATIVES OF THE LEAVES OF *Ipomoea pes-caprae* (L.) R. Br. IN THE DEPOK BEACH AREA OF YOGYAKARTA

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ABSTRACT

Depok Beach is a sandy beach that has limited vegetation because of strong winds, poor microclimate, and low availability of nutrients. One of the plants that can adapt to these extreme conditions is *Ipomoea pes-caprae* (L.) R. Br. One form of its adaptation to extreme environments is thickening the leaves. Other forms of adaptation can also be studied through the characteristics of the epidermis and the epidermal derivatives of the leaves, as the focus of this study. This type of research is exploratory research, including sampling, plant identification, leaf preparation using the leaf clearing method, and observing microscopic preparations. The data analysis used is descriptive quantitative. The data obtained include the characteristics of the epidermis and its derivatives in the form of stomata and trichomes. The results showed the average number of epidermal cells in the adaxial part of the leaves higher, that is equal to 54.05 ± 2.31 / 0.06 mm^2. Stomata are parasitic and amphistomatic. The size of the stomata and the width of the stomatal pore on the adaxial surface of the leaf is smaller but has a higher average density, which is 5.80 ± 1.15 / 0.06 mm^2. The trichomes found were of the glandular type.

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1. INTRODUCTION

Yogyakarta's environmental conditions vary from the presence of Mount Merapi in the north to the coast in the south. Famous beaches in Yogyakarta include Parangtritis, Parangkusumo, Depok, which stretch from east to west and are located in the Bantul district. This beach is unique compared to beaches in general, has a vast coastline, and the coastline is filled with black sand and rows of dunes in the shape of a crescent moon (barchan type), which typically can only be found in Southeast Asia.

The black sand and sand dunes of Depok Beach come from volcanic ash material that is carried by the river and empties into the south coast, then carried by the waves to form a coastal alluvial plain. Furthermore, lightly dry sand is blown away by the wind and accumulates in vegetated areas, forming sand dunes (Nicolla et al., 2021). The sandy characteristics of Depok Beach are known to have limited vegetation due to stress influenced by strong winds, low availability of nutrients and freshwater, high soil salinity, poor microclimate, and the nature of the sandy soil itself (Prasetyawati & Mangopang, 2013). Plants under stress will be stunted in their development, often experiencing abnormalities or differences in the anatomical structure of roots, stems, and leaves.

One of the plants that can adapt to the extreme conditions on Depok Beach is *Ipomoea pes-caprae* (L.) R. Br. This plant grows along the beach border to the sand dune area. *Ipomoea pes-caprae* (L.) R. Br. is an annual herbaceous plant scattered on tropical and subtropical beaches (Suarez, 2011). Ecologically this plant plays important roles in preventing abrasion because it has the ability to grow to form a dense stretch or "green barrier" to bind and stabilize beach sand. This plant is classified as an extremophile halophyte that is able to adapt to extreme environments by thickening the leaves and increasing the size of the cells inside (Suarez, 2011; Zheng et al., 2018).

Ipomoea pes-caprae (L.) R. Br., also known as tapak kuda or katang-katang, is a member of the Convolvulaceae family. Coastal vegetation can be distinguished from other plants by considering the peculiarities of the plant. For example, the shape of the leaves with split ends and the flowers are typical of Convolvulaceae flowers with attached petals forming a funnel shape and pink-purple and slightly dark at the base of the flower. *Ipomoea pes-caprae* (L.) R. Br. economic potential considering the content of bioactive substances so that it can be used as medicine (Wardhani & Poedjirahajoe, 2020), but its availability is still limited considering that this plant only lives on the coast with stress conditions, especially salinity.

The anatomical structure of plants is influenced by environmental abiotic stresses. Seeing the diversity of Dicotyledonae and the uniqueness of their structure, several studies have been carried out including the study of Ma'ruf (2016), regarding the response of some cultivar of a crop (*A. Mangium* Willd.) against salinity; Puspitasari & Salamah (2021), which raise the characteristics of the epidermis and epidermal derivatives of Gramineae member's leaves living on Depok beach; Prihastanti (2010)**,** on changes in the structure of xylem vessels of cocoa roots (*Theobroma cacao* L.) and *Gliricidia sepium* in drought stress; Djazuli (2010), on morpho-physiology of patchouli plants experiencing drought stress to find superior varieties that are resistant to drought. Identification and structural characterization research of *Ipomoea pes-caprae* (L.) R. Br., which grows in the coastal area of Depok, Yogyakarta, has not been carried out completely. There have not been many studies to reveal how its structure can adapt to changing environmental stresses. Therefore, it is interesting to observe and examine this particular topic further.

2. RESEARCH METHOD

This type of research is exploratory research, including sampling, plant identification, leaf preparation using the leaf clearing method, and observing microscopic preparations. The study was conducted in April-May 2021. Leaf samples were obtained directly from Depok Beach, Yogyakarta (Figure 1). Preparation and observations were carried out at the Biology Laboratory of Ahmad Dahlan University. The data obtained were analysed descriptively quantitatively.

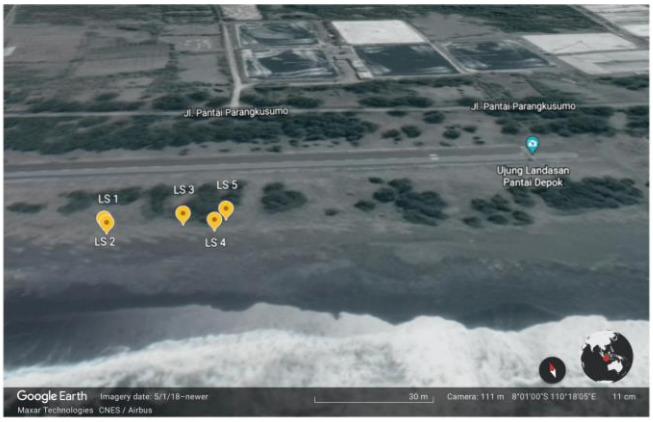

Figure 1.Captured satellite image of the sampling location of Ipomoea pes-caprae (L.) R. Br.

Tools and Materials

The tools used in this research include camera, GPS (Global Positioning System), scissors, flacon bottle, brush, thermo hygrometer, soil tester, lux meter, Optilab IRIS Smart-4 biological microscope, object glass, cover slip, optilab and image raster application version 4.0, pipette drops, wooden tongs, bunsen, and stationery. Meanwhile, the plant identification was done by using PlantNet Plant Identification. PlantNet is an android-based application to identify plants simply by photographing them with a smartphone. The materials used include the leaves of the plant *Ipomoea pes-caprae* (L.) R. Br. from Depok Beach, chloral hydrate solution, aquadest, label paper, spirit, and matches.

Research Procedure

The sampling point was determined by the plotless sampling method. The environmental parameters measured included humidity and air temperature, pH and soil moisture, and light intensity. The sample is the fifth leaf from the shoot, considered optimal expansion and differentiation. Observation of leaf morphology, including length and width was done directly on research site. The leaves were prepared using the leaf clearing method. Epidermal characters and its derivatives were observed under a microscope with an attached optilab. Calculation of density and stomatal index in one field of view is calculated using the following formula:

$$\text{Stomatal density} = \frac{\text{the number of stomata}}{\text{the area of view field } (mm^2)}$$

$$\text{Stomatal index} = \frac{\sum \text{stomata} \times 100\%}{\sum \text{stomata} + \sum \text{epidermis cell}}$$

3. RESULTS AND DISCUSSION

Depok Beach is one of the tourist destinations located in Parangtritis Village, Yogyakarta. The border of Depok Beach is covered by black sand and a row of barchan-type sand dunes. In general, coastal dune areas have high salinity. The influence of wind and high environmental temperatures can accelerate evaporation, leaving little water in the soil (Nicolla et al., 2021). In addition, the influence of sea tides makes the type of sandy beach unable to provide a substrate for organisms to cling to. The poor microclimate and the lack of nutrients make it more difficult for plants to adapt there (Prasetyawati & Mangopang, 2013). The following are the results of measuring environmental parameters at Depok Beach.

Table 1.The results of the measurement of environmental parameters in the Depok Beach area

Environmental Parameters	Measurement result
Light intensity (Lux)	99036.5
Air humidity (%)	60.7
Air temperature (˚C)	35.2
Soil moisture (%)	10
Soil temperature (˚C)	33
Soil pH	6.9

Given the condition of the Depok coast, good adaptability is needed for plants to survive. One of the plants found there, *Ipomoea pes-caprae* (L.) R. Br. This creeper has fast and aggressive growth. Its life in colonies spreads over the surface of the sand, from the area of the highest tide line to the sand dune. Known as halophilous-psammophilous, these plants can live in dry beach sand and high salinity and can survive in direct sunlight and also from the influence of coastal winds (Conrad et al., 2011; Zheng et al., 2018).

The form of plant adaptation that can be observed is from changes in morphology and anatomical structure of its organs. The plant organs that are most likely to be directly exposed to abiotic environmental factors are leaves. Following are the results of observations of leaf morphology of *Ipomoea pes-caprae* (L.) R. Br. found in the Depok Beach area.

Table 2.Observations of leaf morphology of Ipomoea pes-caprae (L.) R. Br. in the Depok Beach area

Aspects observed	Observation Results
Leaf shape	Egg-shaped (Ovate)
Leaf base	Heart-shaped (Cordate)
Leaf apex	Cleft
Leaf margin	Entire
Leaf surface	Hairless (Glabrous)
Leaf veins	Pinnate
Length (cm)	5
Width (cm)	5.5

Based on Table 2 it is known that the leaf size of *Ipomoea pes-caprae* (L.) R. Br. The leaves found in Depok Beach were smaller than the leaves obtained in Porbandar Gujarat, the leaves of Ipomoea pes-caprae (L.) R. Br. found in Porbandar has a length range of 5-7 cm and a width of 7-9 cm (Nilam et al., 2018). The conditions of each sampling location could influence this difference.

Ipomoea pes-caprae (L.) R. Br., which are found in the Depok Beach area, live in open areas, causing plants to be exposed to direct sunlight with high intensity. Beach sand as a growing medium for this plant also has the potential to have higher salinity than the soil in general. According to Setiawati & Syamsi (2019), leaves exposed to high light intensity will be smaller, thicker and more rigid, have a cuticle layer, thick cell walls, and small intercellular spaces. In addition, the results of the research by Purwaningrahayu & Taufiq (2017), on soybeans showed that the higher the salinity of the growing media, the lower the leaf area.

Based on observations, the leaves of *Ipomoea pes-caprae* (L.) R. Br. What was found did look thick and hard. The surface of the leaves was also covered with cuticles. Leaf thickness can be affected by salinity stress. The results of Suarez's research (2011), showed that the leaves of *Ipomoea pes-caprae* (L.) R. Br. exposed to high salinity would have a fixed number of cells, but the size of the cells will increase. The increase in the size of the cells in the mesophyll makes the leaves thicker.

In addition to leaf morphology, various other forms of adjustment can also be observed in the epidermal characters and leaf epidermal derivatives of *Ipomoea pes-caprae* (L.) R. Br. Epidermis is the outermost layer of cells that make up plant organs. This tissue protects plants from biotic threats and stress due to abiotic factors, and also plays a role in the processes of transpiration and respiration.

Table 3.Observation of the epidermal character of the leaves of Ipomoea pes-caprae (L.) R. Br.

Observed components	**Adaxial**	**Abaxial**
Cell shape	Polygonal	
Cell wall	Straight	
Cell length (µm)	46.59 ± 3.09	49.06 ± 2.86
Cell width (µm)	27.37 ± 2.40	28.30 ± 1.96
Total epidermis / 0.06 mm^2	54.05 ± 2.31	46.65 ± 2.39

Based on the observations, the shape of the epidermal cells on both leaf surfaces of *Ipomoea pes-caprae* (L.) R. Br. polygonal or polygonal with the edges of the cell walls slightly curved too straight. Judging from the average measurement of the length and width of the epidermal cells on both leaf surfaces, it is known that the abaxial epidermal cells have a larger size than the adaxial epidermal cells of the leaf. On closer inspection, the adaxial epidermal cells, which are smaller in size, will be seen more in number in one field of view. The abaxial epidermal cells that are larger in size will actually be seen in small numbers when viewed in one field of view.

As stated on Table 3, it is known that the size of epidermal cells is inversely proportional to the number of cells observed in one field of view. One of the factors that can affect the size of epidermal cells is light intensity. The adaxial part of the leaf indeed receives a higher light intensity when compared to the abaxial part. The higher the light intensity received by the leaf surface, the smaller the size of the epidermal cells.

Epidermal cells can be modified in form and function into a pair of guard cells in the middle of a gap. The gap is referred to as a stoma (plural = "stomata"). Stomata are more commonly found in leaf organs, on both surfaces or one surface only. Stomata play a role in the respiration process and a place for water to escape when transpiration occurs (Crang et al., 2018).

Table 4.The results of observations of the stomata character of the leaves of Ipomoea pes-caprae (L.) R. Br.

Observed components	**Adaxial**	**Abaxial**
Stomata distribution	Amphistomatic and distributed throughout the lamina	
Stomata type	Parasitic with 2 subsidiary cells	
Guard cell shape	Kidney-like shape	
Length (µm)	34.66 ± 2.73	35.60 ± 1.37
Width (µm)	25.19 ± 1.98	26.53 ±1.49
Stomatal pore width (µm)	7.61 ± 0.11	9.54 ± 0.28
Number of stomata / 0.06 mm^2	5.80 ± 1.15	4.60 ± 1.31
Stomatal index (%)	9.66 ± 1.64	8.90 ± 2.24

Based on the observations, the leaf stomata of Ipomoea pes-caprae (L.) R. Br. can be found on both leaf surfaces (amphistomatic). Pinnate leaf spines make stomata scattered irregularly over the entire surface of the epidermis. Stomata type on the leaves of Ipomoea pes-caprae (L.) R. Br. was taken from Depok Beach, parasitic with 2 subsidiary cells. The parasitic stomata show that subsidiary cells are arranged parallel to the guard cells, as shown in Figure 1. The larger size of subsidiary cells looks like they are covering the guard cells. The parasitic type of stomata was also found in the leaves of Ipomoea pes-caprae (L.) R. Br. live in Porbandar, Gujarat (Nilam et al., 2018).

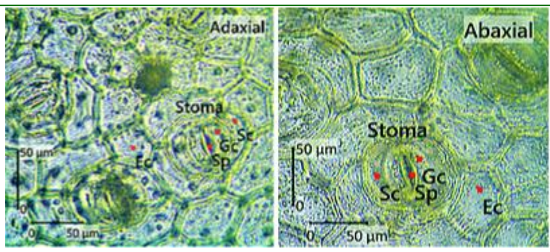

Figure 2.
Parasitic stomata on both leaf surfaces of Ipomoea pes-caprae (L.) R. Br. 400x magnification. Sp = Stomatal pore; Gc = Guard cells; Sc = Subsidiary cells; Ec = Epidermis cell

According to the average measurement of length and width of the stomata observation, the stomata on the abaxial surface is larger than the stomata on the adaxial surface. Stomata are smaller if the leaves are exposed to high light intensity. The adaxial section will undoubtedly be directly exposed to high light intensity compared to the abaxial section. The size of the stomata on the adaxial surface tends to be smaller as well.

The condition of the stomatal pore on both leaf surfaces when sampling was open. The measurement results showed that the stomata on the abaxial surface opened more expansive than the stomata on the adaxial surface of the leaf. *Ipomoea pes-caprae* (L.) R. Br. found in Depok Beach are known to live in open areas, this indicates that these plants are entirely exposed to the sun freely. In the absence of shade, sunlight with high intensity will directly hit the adaxial surface of the leaves. In order to reduce excessive evaporation, *the Ipomoea pes-caprae* (L.) R. Br. tends to narrow the stomatal pore.

Internal and external factors influence the opening of stomata. Internal factors related to the biological timing, stomata usually open in the morning and close at night. External factors, such as light intensity and concentrations of CO_2 and abscisic acid. In addition, the opening of stomata is also supported by structural factors of guard cells. The existence of radial mycelation of the cellulose microfibrils and the two ends of the cells close together/attached only allows the guard cells to grow elongated and curved outwards, when the turgor pressure increased (Nadliroh et al., 2015).

Related to the biological timing, the leaf samples were taken in the morning at 07.00-09.45 WIB time zone, where the stomata still open on both leaf surfaces. In the morning, the guard cells are still fill by the amylum and the presence of sunlight then will raise the chlorophyll to carry out the photosynthesis process. As long as it lasts, some of the CO_2 in the cells will reduce into CH_2O, causes the environment pH increased due to a lack of H^+ ions. So that the concentration inside and outside the cell remains balanced, K^+ ions will enter the cell. This potassium then triggers the phosphorylase enzyme to convert the starch in the guard cells into glucose 1-phosphate. The formation of glucose increases the osmotic potential of the guard cells so that water from subsidiary cells enters and the turgor pressure of the guard cells increases, causing the stomata to open (Nadliroh et al., 2015; Setiawati & Syamsi, 2019).

Based on the data in Table 4, the stomata on the adaxial surface of the leaf have a higher density than the abaxial surface. Light intensity, temperature, water availability, and CO_2 concentration could affect the level of stomatal density. Adaxial surfaces that receive high-intensity light tend to have smaller stomata; this makes the distance between the stomata appear close together. The number of stomata observed in one field of view becomes more.

The level of stomatal density could be known through the value of the stomata index. The stomata index is a percentage, the result of comparing the number of stomata with the total number of epidermises plus stomata. Changes in the number of stomata and epidermis will be seen through the value of the stomata index. Based on the data in Table 4, the stomata index on the adaxial surface of the leaf is higher than on the abaxial surface. It is clearly due to the higher number of stomata on the adaxial part of the leaf (higher stomatal density).

In addition to stomata, epidermal cells can also be modified into trichomes. Trichomes are present in almost all surfaces of plant organs. When touching the surface of a plant with trichomes, it will feel rough, itchy, sticky, and has a pungent smell. The trichomes in various plants vary in shape and size.

Table 5.Observations on the character of leaf trichomes of Ipomoea pes-caprae (L.) R. Br.

Observed Components	**Adaxial**	**Abaxial**
Trichome type	Glandular	
Trichome form	Peltate glands with 10-12 secretory cells	
Trichome head diameter	30.10 ± 3.24	40.52 ± 3.34
Trichome density / 1.00 mm^2	19.10 ± 2.75	9.15 ± 2.21

There are two types of trichomes, namely glandular and non-glandular trichomes. Based on the results of observations on the leaves of *Ipomoea pes-caprae* (L.) R. Br. taken from Depok Beach, glandular trichomes were found on both leaf surfaces. The results of research Noraini et al. (2021) found the presence of peltate glands in the leaf blades and mothers of *Ipomoea pes-caprae* (L.) R. Br. According to Hazzoumi et al. (2020), a peltate gland is a type of glandular trichome composed of a basal cell, a stalk cell, and a trichome head with 4-18 or more flat-shaped secretory cells inside. Based on the observations, it is known that the trichome heads from the leaves *of Ipomoea pes-caprae* (L.) R. Br. found in Depok Beach has about 10-12 secretory cells in it, as shown in Figure 2.

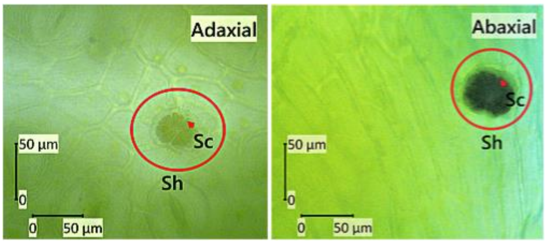

Figure 3.
Glandular trichomes on both leaf surfaces of Ipomoea pes-caprae (L.) R. Br. 400x magnification. Sh = Secretory head; Sc = Secretory cells

Furthermore, the glandular trichome parts of the leaves of Ipomoea pes-caprae (L.) R. Br. can be seen in Figure 3. The head of the trichome consists of thin-walled secretory cells arranged in a circle. The stalk of the trichome is composed of one short, rectangular cell. The basal cells are described as isodiametric, thin-walled, vacuolated, and contain a nucleus at the periphery (Kuster et. al., 2016).

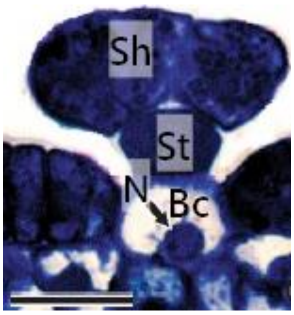

Figure 4.
Sections of glandular trichomes from leaves of Ipomoea pes-caprae (L.) R. Br. Sh = Secretory head; St = Stalk; Bc = Basal cell; N = Nucleus. (Source: Kuster et. all., 2016)

Based on the results of measuring the diameter of the head of the trichomes, it is known that the size of the trichomes on the adaxial surface is smaller than the trichomes found on the abaxial surface. The results of calculating trichomes density showed a higher value for the adaxial part of the leaf. Similar to the previous observations of the epidermis and stomata, the smaller adaxial trichome size will display a higher number of trichomes in one field of view. The higher the number of trichomes, the higher the density.

The density of the trichomes may be affected by the presence of abiotic stress. The presence of drought stress can increase the density of trichomes. Ilahi et al. (2018), in his research, explained that it was increasingly difficult for eggplant (*Solanum melongena* L.) to absorb water from the soil due to the long watering interval, resulting in an increase in the number of trichomes in one field of view. The increase in the number of trichomes is inversely proportional to their smaller size. The high density of trichomes in the face of drought stress also acts like the epidermis, namely as a protective tissue. Tight trichomes can prevent excess water loss and increase the efficiency of water use in leaves (Fu et al., 2013).

Not only that, salinity stress is also known to affect the density of trichomes. The results of Purwaningrahayu & Taufiq's research (2017), showed that soybean genotypes that could withstand salinity stress had higher trichome densities on both leaf surfaces than salinity-sensitive genotypes. The high density of trichomes is one form of plant adaptation in dealing with salinity stress. Tight and long trichomes may play a role in excreting excess salt in plant tissues (Dolatabadian et al., 2011).

Based on the research results of Kuster et al. (2016), it is known that glandular trichomes on the leaves *of Ipomoea pes-caprae* (L.) R. Br are not salt glands. The glandular trichomes found played a role to secrete mucilage that contains polysaccharides (pectin) in it. This mucilage secretion responds to injury, pathogen interactions, regulates water transport, and protects herbivorous animals.

It was further explained that mucilage secretion could help *Ipomoea pes-caprae* (L.) R. Br. withstand light irradiation and high temperatures and from low water availability due to low field capacity and high soil salinity. Mucilage secretion on young leaves causes leaf blades directly exposed to the external environment to curl so that the leaf surface area decreases. This can reduce the rate of transpiration so that the water in the tissue remains sufficient (Kuster et al., 2016).

4. CONCLUSSION

Epidermal characteristics of the leaves of *Ipomoea pes-caprae* (L.) R. Br. grows in Depok Beach, which has polygonal-shaped cells with the edges of the cell walls slightly curved to straight. The size of leaf epidermis cells on the adaxial surface is smaller but greater than on the abaxial surface. The average number of epidermal cells on both leaf surfaces has a difference of 7.40 / 0.06 mm^2.

Leaf stomata of *Ipomoea pes-caprae* (L.) R. Br. irregularly distributed on both leaf surfaces (amphistomatic). Kidney-shaped guard cells. The type of stomata is parasitic with two neighbouring cells. The size of the stomata on the adaxial surface is also more minor but still has a more significant number than the stomata on the adaxial surface. It can also be seen from the higher stomatal index value for adaxial stomata. The difference in the mean stomatal density on the two leaf surfaces was 1.20 / 0.06 mm^2. The types of trichomes found on the leaves of *Ipomoea pes-caprae* (L.) R. Br. The glandular trichomes are peltate glands with 10-12 secretory cells. The mean density of the trichomes also showed a higher value on the adaxial surface, which was 19.10 ± 2.75 / 1.00 mm^2.

5. ACKNOWLEGEMENT

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