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The Study of Morphology, Phytochemical, and Distribution of *Pittosporum moluccanum* in Mount Merbabu National Park (TNGM)For the Development of Educationl Teaching Materials

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Abstract

This study aims to 1) observe the morphology of *P. moluccanum*, 2) analyze the content of chemical compounds in *P. moluccanum*, and 3) determine Normalized Difference Vegetation Index (NDVI) and Enhanced Vegetation Index (EVI) of *P. moluccanum*. The research was conducted in the form of morphology studies by conducting field studies, phytochemical compounds sample test by conducting sample preparation, chemical screening, and detection of aromatic compounds and quercetin, and remote sensing of Landsat 8.0 OLI satellite imagery. The results of morphological studies show that *P. moluccanum* have the same characteristics as plants in the literature, whereas the phytochemical test shows that *P. moluccanum*'s leaves and fruit contain flavonoids, tannins, saponins, steroids, and alkaloids. These compounds are known to have many functions for medicines. NIR analysis shows that leaf and fruit samples are detected to have aromatic compounds (essential oils) which are widely used as raw materials for fragrance oils or cosmetics. The results of remote sensing using NDVI in the Selo region, Boyolali produce a maximum vegetation index value of 0.464, meaning that the area has a high level of greenness, while the EVI used to see the level of health and fertility of plants in August 2018 shows the same figure, which is 0.464, meaning that the plant is in healthy condition. Remote sensing with satellite imagery helps analyze the distribution of sample plants in a large area. The results of this study can be used as a development of teaching materials both at junior and senior high school levels.

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INTRODUCTION

One of the plants that have potential as medicinal plants in Mount Merbabu National Park (TNGM) is Sengiran (*Pittosporum mollucanum*) (Zaelani 2018). This plant is one of 160 species which belong to family Pittosporaceae. In TNGM, *P. moluccanum* are found on the slopes of Selo climbing track, Boyolali.

This study aims to 1) observe the morphology of *P. moluccanum*, 2) analyze the types of phytochemical compounds contained in *P. moluccanum*, and 3) determine value of Normalized Different Vegetation Index (NDVI) and Enhanced Vegetation Index (EVI) of *P. moluccanum* in Mount Merbabu National Park.

To achieve the goal of the study, field study was done by taking samples in the form of leaves and fruit. Furthermore, phytochemical screening was carried out at the Laboratory to obtain data on the content of compounds in the leaves and fruit. NIR (Near Infrared) analysis was performed to see the closeness of certain compounds to standard compounds. To find out the distribution of *P. moluccanum* in Mount Merbabu National Park, this study used remote sensing of Landsat 8.0 OLI satellite imagery which measures NDVI (Normalized Different Vegetation Index), and EVI (Enhanced Vegetation Index).

METHOD

This study was done in the form of field study. Field study was conducted to collect *P. moluccanum* samples in the slopes of Selo climbing track in Mount Merbabu National Park, Boyolali. The research was conducted in the form of morphology studies by conducting field studies, phytochemical compounds sample test by conducting sample preparation, chemical screening, and detection of aromatic compounds and quercetin, and remote sensing of Landsat 8.0 OLI satellite imagery.

RESULTS AND DISCUSSION

Phytochemical screening

Compound testing on the sample was done by phytochemical screening on flavonoids, tannin, quinone, saponin, steroid, and alkaloid.

Flavonoids Identification (John et al. 2014)

- Add 100 ml of hot aqua dest to 5 grams of sample and boil for 15 minutes. Filter while it is hot.
- Put 5 ml of filtrate to a test tube, add 0,1 gram of Magnesium (Mg), 1 ml of concentrated hydrochloric acid, and 5 ml of amyl alcohol
- Shake the solution and let it separate
- The positive result will show orange color

Tannin Identification (Sangi et al. 2012)

- Add 100 ml of hot aqua dest to 5 grams of sample and boil for 15 minutes. Filter while it is hot.
- Put the filtrate to four test tubes (5 ml each)
  - Tube I : add 1 ml of Iron(III) chloride 5%
  - Tube II : add 1 ml of gelatin solution 1%
  - Tube III : add 1 ml of Stiasny Reagent
  - Tube IV : add 1 ml of Sodium Acetate 1M and then add 1 ml of FeCl3 5%
- The positive result shows specific condition:
  - Tube I : shows black blue color
  - Tube II : forms white sediment
  - Tube III : forms rosy-colored sediment ➔ Katekotannin
  - Tube IV : shows black blue color ➔ Galotannin

Quinone Identification (Saito, 2005)

- Add 100 ml of hot aqua dest to 5 grams of sample and boil for 15 minutes. Filter while it is hot.
- Put 5 ml of filtrate to a test tube and add a drop of NaOH 1N
- The positive result shows red color

Saponin Identification (Depkes RI, 1979)

- Add 100 ml of hot aqua dest to 5 grams of sample and boil for 15 minutes. Filter while it is hot.
- Put 5 ml of filtrate to a test tube and shake it vertically in 10 minutes until it forms 1-10 cm of foam

- Add 3 drops of chloride acid 2N
- The positive result is shown by the stabilized foam which doesn't disappear

Steroid Identification (Farnsworth, 1966)

- Add 20 ml of ether to 1 gram of sample. Crush them in a mortar and filter it.
- Put the filtrate to the evaporating dish porcelain and let it dry
- Add 3 drops of Liebermann-Burchard reagent
- The positive result shows greenish blue or violet-red

Alkaloid Identification (Depkes RI, 1979)

- Add 10 ml of HCl 2N to 2 grams of sample. Crush them in a mortar and filter it.
- Put the filtrate to a test tube and add 5 ml of ammonia 25%
- Extract it with 20 ml of chloroform
- Take the chloroform layer
  - Test 1 : put chloroform on a filter paper
  - Test 2 : put chloroform in a test tube and add Dragendorff reagent
- Take the water layer
  - Test 3 : put water in a test tube and add Dragendorff reagent
  - Test 4 : Put in a test tube and add Mayer reagent
- The positive result shows specific conditions:
  - Test 1: shows orange color
  - Test 2: forms sedimentation
  - Test 3: forms brick red sediment which lasts for 15 minutes
  - Test 4: forms white sediment

Aromatic Compound and Quercetin Detection using NIR

To determine aromatic and quercetin compounds in a qualitative way, NIRFlex N-500 was used. Leaves and fruits of *Pittosporum moluccanum* were dried without sun exposure and then put on the oven in 40°C. The dried samples were then crushed in a porcelain mortar and filtered by using tea filter. The filtered samples were then analyzed by using NIRFlex N-500.

Samples of leaves and fruit of *P. moluccanum* were placed in the sample cup on NIRFlex N-500. The test of leaves and fruit samples were carried out alternately. The samples were then exposed to NIR radiation. This infrared light generated energy in the samples so that it triggered vibrations in the form of compression and rarefaction in the bonds of O-H, N-H, and C-H atoms. These atoms are the main components that make up organic compounds. The energy produced is partly absorbed whereas the other part will be reflected back. In comparison, in other organic materials that are exposed to NIR light, about 4% of the light will be reflected back by the outer surface, while 96% will enter the sample and will undergo the process of absorption, reflection, spread, and light forwarding (Mohsenin, 1984).

Test results on leaves and fruit samples produced a spectrum that provides information about the material. To ensure the information about these materials, standard materials were also analyzed using NIR under the same conditions as sample analysis. In this case, pure quercetin was used as a standard.

Distribution of P. moluccanum by Determining Its' NDVI and EVI

A vegetation index, known as the NDVI (Normalized Difference Vegetation Index) is a green vegetation index obtained from processing digital signals from vegetation brightness data of several satellite sensor data channels. To obtain vegetation data, two brightness channels are compared, namely the red-light channel (Red) and the NIR light channel (Near Infrared). In areas with dense vegetation, the ratio of the two canals will be high, because the chlorophyll found in the leaves is able to absorb NIR (Near Infrared) light and also the ability of the leaf mesophyll to reflect near infrared light. This will make the brightness values received by the satellite sensors on the two channels be very different (Sudiana and Diasmara 2008). One signal processing that can observe the state of vegetation is NDVI algorithm generated by NASA's ERTS earth observation satellite and NOAA's AVHRR. This algorithm works by utilizing the phenomenon of physical reflection of light waves coming from the leaves. The greenness value of an area's vegetation is a scale that has a

range between -1 (minimum value) and 1 (maximum value). This value is obtained by comparing the vegetation reflectance received by the sensor in the red band (RED) and near infrared band (NIR) (Sudiana and Diasmara 2008). To obtain NDVI scores, NDVI formulation is used (Hayati, Yulianto, and Prasetyo 2018):

$$\text{NDVI} = \frac{NIR-RED}{NIR*RED}$$

Information:
NIR = reflectance value of near infrared channel
RED = reflectance value of red channel
NDVI values and interpretations are shown in Table 1 below.

Table 1. Value and NDVI Interpretation (Min, Muchtar, Bahar & Udin, 2016)

NDVI Value	Interpretation
0,5 – 0	Water bodies, rivers, seas, or wetlands
< 0,1	Badlands, rocks, sand, or snow
0,2 – 0,3	Grass and shrubs
> 0,5	Tropical rainforest, land cover, and thick vegetation

Table 2. Value and NDVI Interpretation (Liu et al.., 2016)

NDVI Value	Interpretation
-1 - 1	Inland vegetation canopy and biomass ratio
0,2 – 0,3	Grass and shrubs
> 0,5	Thick green vegetation

EVI (Enhanced Vegetation Index). This is a vegetation index used to monitor and study the health and growth of vegetation while at the same time to explain the level of greenness of the vegetation (Hayati, Yulianto, and Prasetyo 2018). EVI has better sensitivity in areas with higher biomass. To determine the EVI value, the formula used is : $2.5x \frac{(NIR-RED)}{(NIR+C1xRED-C2xBlue+L)}$ (Ginting and Jadera 2018)
Note:
NIR: Near Infrared
RED: Red Channel Reflectance
Blue: Blue Channel Reflectance
L: 1
C1: 6
C2: 7.5

EVI values and interpretations can be seen in the following Table 3.

Table 3. EVI Value and Interpretation

EVI Value	Interpretation
<= (-1)	Very unhealthy vegetation
(-0.99) – (-0.5)	Unhealthy vegetation
(-0.51) – (-0.1)	Rather Unhealthy
0.1 - 0.5	vegetation
0.51 - 1.00	Rather Healthy vegetation
> 1.00	Healthy vegetation
	Very healthy vegetation

Morphology Study of Pittosporum moluccanum

Pittosporum moluccanum has a synonym of *P. microcalya* R. & V. and P. *monticolum* Miq. (Bakker and van Steenis, 1957). These plants are generally tree-shaped with heights reaching 5-20 meters (Backer, 1957). The young stems have pubic fur which look like rust red in color. The branching looks tight at the end of the plant. The shape of the leaves varies, but in general they are pointed, or inverted lancet with a size of 6-17 cm x 4 - 5 cm. The leaf edge is slightly wavy, with the edges tapered. The leaf has a shiny surface and reticulate venation with stem between 1 - 2.5 cm long. The position of the leaves is spiral or clustered at the end of the twigs.

The fruit of *P. moluccanum* is oval in shape with a size of 3 x 1.5 cm. The color is green when it is young and becomes orange when it is ripe. It has a strong smell and emits a lot of sticky lymph fluid.

The flowers are white in color and they smell nice. The number of crowns and flower petals are 5, sticking together at the base and splitting at the tip of the flower. The number of pistils and stamens are 5 each.

The results of observations of *P. moluccanum* in this study are not much different from the characterization done by Steenis in the book "The Mountain Flora of Java", translated by Hamzah (2010). The book states that trees which have a height of 4-15 meters were rarely in the form of shrubs. The shape and size vary, with a length of 5-15 cm and a width of 2.5 - 5.5 cm. Leaves are often

seen clustered at the tips of twigs. The flowers smell good, whether they are male or female. The fruit is pointed, red – orange colored. If the fruit is ripe, the fruit will pop up with two valves containing black seeds and is tightly bound by a sticky resin with a strong turpentine smell.

Phytochemicals of P.moluccanum Leaves and Fruits

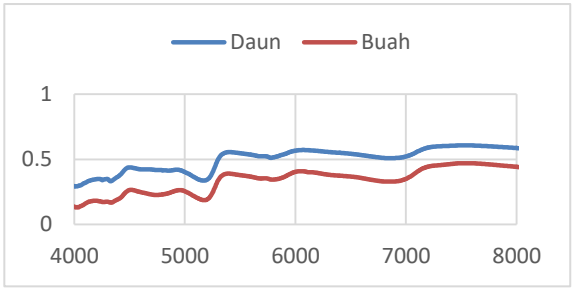
Phytochemical screening is a method to determine the presence of chemical content in materials using certain solvents. The solvent commonly used in phytochemical screening is methanol which has the ability to dissolve almost all organic compounds present in a sample. Methanol is also volatile and easily released from extracts (Malik et al. 2007). Based on the phytochemical tests conducted, there were five types of compound contained in the leaves and fruits of *Pittosporum moluccanum*, namely Flavonoids, Tannins, Saponins, Steroids, and Alkaloids. Look at tabel Phytochemical screening result of *P. moluccanum* leaves and fruit

Chemical Group	Test Type	Result
Flavonoid	Mg powder	Positive
Tannin	$FeCl_3$	Positive
	Gelatin salt	
	Stiasny	
Quinone	NaOH	Negative
Saponin	Soap test	Positive
Steroids	Liberman Burchard	Positive
Alkaloids	Dragendorff (filter paper)	Positive
	Mayer	
	Dragendorf	

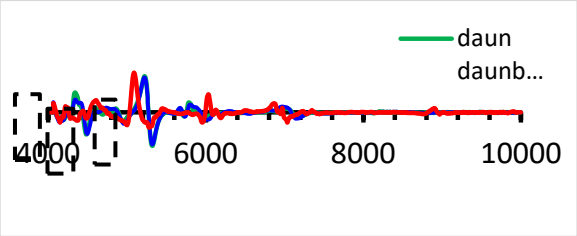
Detection of Aromatic Compounds and Quercetin with NIR

The analysis using NIR was done qualitatively because in general, the contents of metabolite compounds in plants are very small so that they cannot be detected for quantitative analysis. Qualitative testing is more focused on the type of chemical content in a substance contained in the test material (Manual n.d). The NIR spectrum of P. *moluccanum* leaves and fruits is similar. This shows that there are similarities in the compounds present in the leaves and fruit of *P. moluccanum.*

In table 2, it appears that the compounds making up *P. moluccanum* and fruit are very similar. At peak 12 (on leaves) and peaks 12 and 13 (on fruit) it appears that the sample contains essential oil compounds. This supports field data that both ingredients smell good, especially fruit. Essential oil is an ingredient contained in plants that functions as raw material for perfume and cosmetics. The special feature of plants containing essential oils is the emergence of certain peculiar smells and usually the smell is strong. Essential oils contained in the body of plants can be removed by refinement (Berlin, 2014).



The NIR data shows that there are aromatic compounds in the leaves and more in the fruit. Physically, the smell that arises from the leaves and especially in the fruit which is stronger can predict the presence of aromatic compounds. Other compounds determined using NIR are quercetin compounds. Quercetin is a compound that is widely contained in plants, *including P. moluccanum*. Quercetin is a derivative of Flavonoids, found in almost all plants, both in fruits and vegetables. Quercetin also has strong antioxidants because of its ability to cope with free radicals (Baghel et al. 2016). Based on the literature referred to, the Quercetin contained in the leaves and fruit of *Pittosporum moluccanum* also has the same function as an antioxidant. The NIR spectrum of leaves and fruits is combined with the spectra of standard quercetin compounds. It appears that both samples contain quercetin.



Distribution of P. moluccanum with NDVI and EVI

The data in this study were raster data in the form of Landsat 8 OLI 3 satellite images for a period of one year. Data extraction where satellite imagery data is extracted used NDVI and EVI analysis using QGIS 2.1.8 (Quantum Geographic Information System). Extraction results obtained will then be cut according to Mount Merapi-Merbabu National Park vector maps to produce values from NDVI and EVI for each district. After that, they were processed with NDVI and EVI algorithms to get the value of vegetation density in Mount Merbabu National Park area. The coordinates were taken in July 2018 on Selo and Tuk Babon climbing tracks.

NDVI values are divided into 3 data intervals namely Minimum, Average and Maximum. The Minimum Interval represents areas with similar land uses that include low vegetation index characteristics, dominated by barren, rocky and sandy areas of low NDVI. The Average Interval represents areas with similar land uses that include the characteristics of low vegetation index dominated by grasses and shrubs. The Maximum interval represents an area of similar land use that includes characteristics of forests with land cover and thick vegetation.

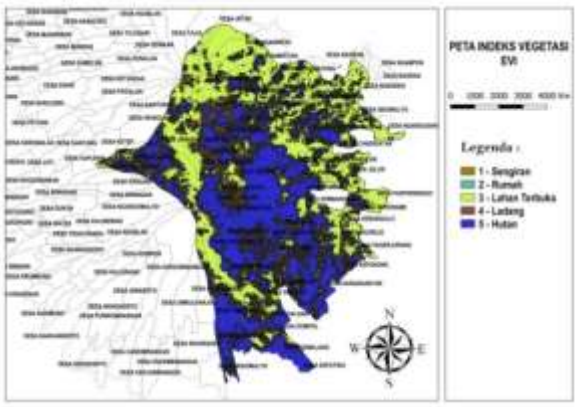
NDVI results in Selo area from February to August with the help of remote sensing satellite imagery showed a maximum vegetation index value of 0.464. This vegetation index looks higher than the surrounding area. The high NDVI vegetation index shows that Selo area has a high level of greenness because the area is dominated by forests, land cover, and thick vegetation. The results of this study are in line with previous studies, that areas which have NDVI vegetation index values more than 0.5 are tropical rain forests, land cover, and thick vegetation (Prasetyo el al. 2017). The comparison of research results with the literature shows evidence that Selo Boyolali region, precisely Selo climbing track, has many plants that have thick canopies, one of which is *P.* moluccanum or often referred to as *Sengiran*.

The EVI (Enhanced Vegetation Index) value of *P. moluccanum* showed a value of 0.464 which was also higher than other regions. Based on the theory, if the EVI value is in the range between 0.1 - 0.5, the vegetation that lives in the area is said to be healthy vegetation (Ravi, et.al 2016).

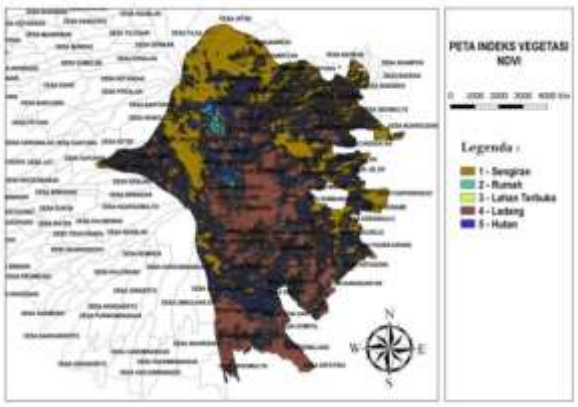
The use of satellite imagery in monitoring process is very helpful in determining the location points as well as the distribution of *P. moluccanum*. Mount Merbabu National Park is very large and therefore it is not possible for the researchers to walk around the area to see the distribution of these plants.

After the vegetation results are obtained, land cover classification was carried out using the supervised classification method, index value image with study area in Mount Merbabu National Park. The results of the classification of images extracted by the NDVI and EVI algorithms have differences that can be seen from the distribution of land cover and the area of each land cover produced. After getting the map of satellite image analysis results, the results were validated by assessing / determining the accuracy of the results of visual image interpretation by conducting a field check and applying confusion matrix.

The coordinates of the results of the field validation were then overlaid with a map of zoning satellite imagery with the aim to further clarify the initial data and validation data. The data obtained were depicted in NDVI and EVI in February, taking into account the best satellite imagery results in February 2018. *P. moluccanum* were seen scattered along Selo and Tuk Babon climbing tracks with NDVI index values showing high levels of greenness. In February, the rainfall was still quite high, so the NDVI index value was quite high, i.e. 0.464. The NDVI map of the results of field validation is shown in Figure.



Besides NDVI, the results of field validation were also overlaid with EVI which produced EVI index validation which can be seen in Figure.



CONCLUSIONS

Based on the observations, field studies, literature studies, phytochemical screening, NIR, and also remote monitoring of satellite images of *P. moluccanum*, it can be concluded:

1. *P. moluccanum* are generally trees with a height of up to 20 meters. They have branched stems and various leaf shapes. The flowers have 5 parts. *P. moluccanum* fruit is oval in shape with a size of 3 x 1.5 cm. The young fruit is green while the ripe one is orange. It has a strong smell and emits a lot of sticky lymph fluid.
2. Compounds contained in P. moluccanum which are tested phytochemically include flavonoids, steroids, tannins, saponins, alkaloids, whereas the NIR test shows the presence of aromatic compounds containing essential oils.
3. The Maximum NDVI index value and the EVI index value are 0.464, meaning that *P. moluccanum* have good greenness and health.

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