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Molecular and Morphological Characterization of Vanilla and Pasak Bumi Plants

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Abstract. Vanilla and Pasak Bumi were phytopharmaceutical plants from Indonesia that are important for medicinal purposes and industries. The morphological characterization alone of these plants is often caused misidentification which affected further effort to search their secondary metabolites product. Data about morphological characters accomplished with molecular identification will be objective, more accurate and reliable. This study aims to morphologically and molecularly characterize Vanilla and Pasak Bumi plants using Internal transcribe Spacer region marker. The morphological characterization stage was carried out using a morphological key. Molecular characterization began with DNA isolation with CTAB, then DNA was amplified by polymerase chain reaction (PCR). DNA was then analyzed using the Basic Local Alignment Search Tool (BLAST). The research result showed the variation of morphological character of Vanilla and Pasak Bumi plant in Indonesia supported with the result of molecular identification.

INTRODUCTION

COVID-19 pandemic is the spread of coronavirus that originated from the city of Wuhan, China, to almost all countries in the world since 2019 until now. The form of treatment for this disease is still being pursued [1]. It takes a long time to find effective drugs to treat Covid-19, therefore herbal plants are used for urgency in dealing with covid-19. Although there is no evidence, different studies are being carried out on herbal plants that have the ability to strengthen the immune system and overcome the Covid-19 virus [2,3]. Phytopharmaceuticals are medicinal preparations containing active substances from herbal plants to improve health that have been scientifically standardized. Bioactive compounds in phytopharmaca can be extracted from herbal plants. Phytopharmaceutical products can be applied for diagnosis, treatment, mitigation, and preventing the emergence of disease in an individual [4]. During the Covid 19 pandemic, the investigation of phytopharmaceutical plants is needed to screen the potential use of these plants as the alternative source of medicine in Indonesia.

Previously, the consumption of phytopharmaceutical products is considered to be able increasing the immune system because it has efficacious bioactive ingredients. The active compounds contained in herbal plants have anti-inflammatory, antiviral, and antioxidant properties so that they can indirectly increase the body's immunity [1]. Pasak bumi is one of herbal plant in Indonesia which has plenty benefits related to health. Pasak bumi is an endemic plant from Southeast Asian countries and mostly found in the island of Sumatra (Indonesia) and Kalimantan (Indonesia and Malaysia) [5]. Recent research on *E. longifolia* showed that standardized extract of pasak bumi plant with levels of

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100-200mg in a phytopharmaceutical product has the effect in reducing symptoms of aging and premature fatigue during the consumption time in 2 weeks and 4 weeks [6]. In addition of secondary metabolites analysis, there are several molecular studies of *E. longifolia*. However, the number of studies in this plants in Indonesia, mainly in Borneo island, still has limited number.

Another plant for phytopharmaca sample is vanilla. This plant belongs to the family Archicaceae and contains 2-3% vanillin. Vanillin (4-hydroxy3-methoxy benzaldehyde) is an aromatic aldehyde compound. This plant is a natural source of vanillin. [7]. The secondary metabolites potential of Vanilla such as the content of flavonoids, alkaloids, steroids, saponins, and terpenoids [8]. Therefore, although this plant has not been categorized into medicinal plants, this plant has potential compounds contained in phytopharmaceutical plants.

The purpose of molecular identification and phytopharmaceutical morphology is to determine the molecular character of phytopharmaca plants based on the Internal Transcribed Spacer (ITS) rDNA area. ITS is a sequence of RNA strands from the main transcriptional process that is located between the precursor ribosomal subunits and is removed during the splicing process when the structurally marked molecular marker RNA is processed into a ribosome [9]. ITS is divided into two regions, namely ITS-1 which is between the 18s gene and 5.8S gene, while ITS-2 is between the 5.8S and 38S genes and the three gene regions have a high conversion rate [10]. This research is expected to provide information about the morphological and molecular characters of phytomarca plants in Indonesia which have the potential as immune boosters in the Covid-19 pandemic.

METHODS

Sampling

The study began by obtaining samples of pasak bumi (*Eurycoma longifolia*) leaves taken in the forest area of Balikpapan, East Kalimantan. The second sample, vanilla plants (*Vanilla planifolia*) obtained from retail suppliers at the Kalisari flower market, Jalan dr. Soetomo, Semarang City. Samples were stored in sterile ziplocks for further analysis.

DNA Isolation

DNA isolation of pasak bumi and vanilla was carried out using the CTAB method with slight modifications [11]. The CTAB method is a technique that has been used since ancient times on both dry and fresh samples. The method is carried out by grinding for the lysis of plant cells [12]. The results of DNA isolation were measured using Nanodrop 2000.

PCR dan Electrophoresis

PCR mix consisted of 12.5uL PCR kit (Bioline), 1uL ITS forward primer, 1uL ITS reverse primer, and 8.5uL ddH2O. The PCR cycle consisted of pre-denaturation at 95oC for 1 minute, denaturation at 95oC for 30 seconds, annealing at 52oC (Pasak Bumi) and 55oC (Vanilla) for 30 seconds, extension at 72oC for 15 seconds and post-extension at 7oC for 5 minutes. PCR products were visualized by electrophoresis for 30 minutes (100v) in 1% agarose with 100bp DNA ladder. Visualization of electrophoresis results using UV-TEC doc gel. After the DNA visualization results were obtained, the samples were immediately sent to Genetica Science, Central Jakarta, for sequencing.

Sequencing

DNA sanger sequencing stages was conducted by using BigDye® Terminator v3.1 cycle sequencing kit chemistry in PT Genetika Science.

Phylogenetic Analysis

Phylogenetic tree was constructed through MEGA X Software [13]. Neighbor-Joining tree making method was chosen along with Kimura 2-parameter and 1000X bootstrap replications [14].

RESULT AND DISCUSSION

The concentration of DNA obtained through testing with nanodrop 2000 is presented in the following table:

TABLE 1. Quantitative test results of phytopharmaca plant DNA using nanodrop 2000

Sample	DNA Concentration	260/280	260/230
P	554.8	1.98	1.55
V	145.3	2.05	1.42

Sample description: P = Pasak Bumi (*E. longifolia*)
V = Vanilla (*Vanilla planifolia*)

The results of nanodrop test were DNA purity at 260/Å280 and DNA concentration. Good quality DNA based on the nanodrop test has a purity about 1.8-2.0 and a concentration above 100 ng/µL [15]. Samples V and P have great purity values which range around 1.98 – 2.05 indicating that the samples from DNA isolation carried out for V sample have less contaminants in the form of RNA materials and P sample doesn't have contamination. The value of DNA purity below 1.8 indicates that the extracted DNA process still contains protein [16]. DNA purity values above 2.0 indicate that there are still contaminants in the form of RNA [15]. After obtaining the DNA concentration value from phytopharmaca plants, the process was continued to the DNA amplification stage using a thermocycler and electrophoresis for 30 minutes using an electroporator.

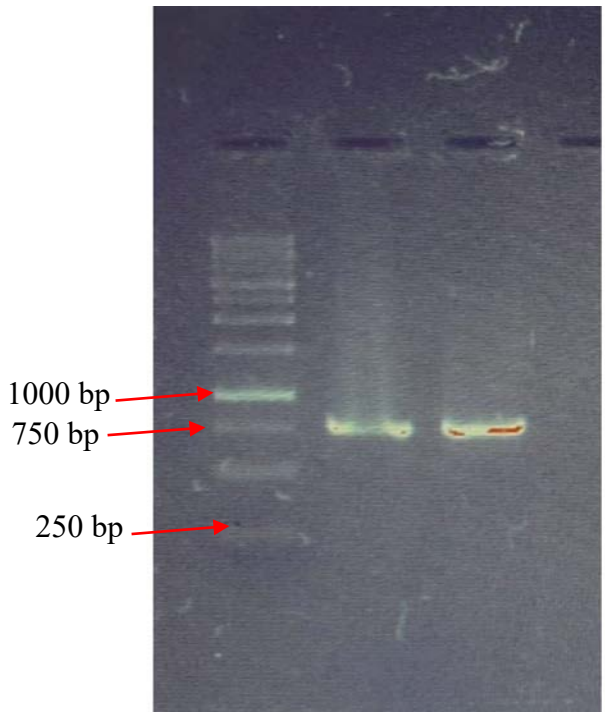


FIGURE 1. Results of DNA visualization using Gel-doc UV-TEC

After the PCR results were out, the samples were then electrophoresed and visualized using a gel-doc with UV-TEC. The gel-doc is the result of visualizing DNA (genetic material) using gel electrophoresis with a 1kb DNA ladder [17]. Based on the results of sample visualization, DNA bands with a base length of 750bp were obtained. There are several samples in the visualization, which are pasak bumi, and vanilla. In the pasak bumi sample, DNA bands were visible, as well as the vanilla sample, although it was quite smeared. The results of the smear contained in the DNA visualization are the residue from the solution that was still carried during the isolation process or in the form of DNA that was degraded in the isolation process [18].

Phylogenetic Analysis

The alignment of Internal Transcribed Spacer (ITS) sequence with National Center for Biotechnological Information (BLAST) showed that the Vanili plant sample in this research is closely related to *Vanilla planifolia* with 97% similarity. Meanwhile, Pasak Bumi Plants showed high similarity with *Eurycoma longifolia*

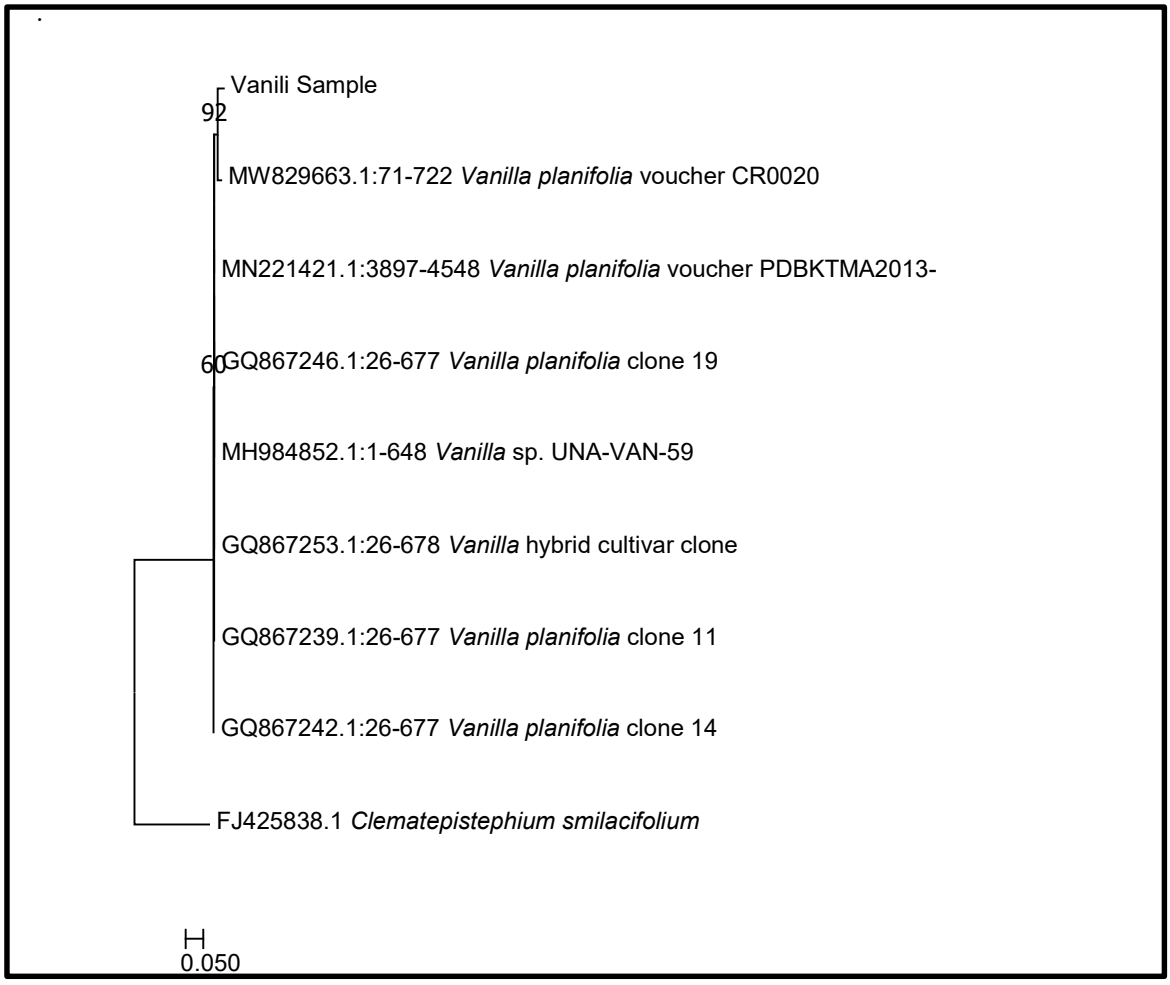
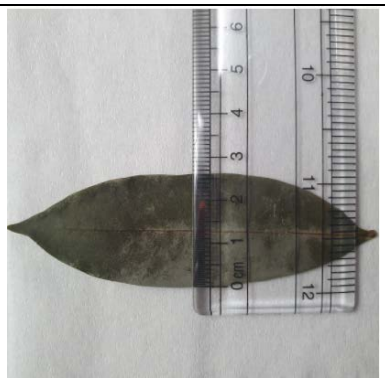


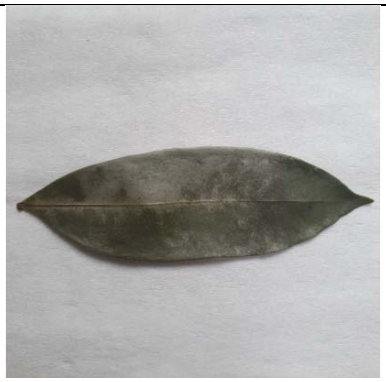
FIGURE 2. Phylogenetic Tree of Vanilli plants by Using Neighbor-joining method with 1000X replications

With regarding to the phylogenetic analysis, the Vanilla sample also showed the closest relation with *Vanilla planifolia* voucher CR 0020 with high bootstrap value of 92%. Other members in the Vanilli's genus also showed the representative bootstrap value (60%). Vanilla plants are classified as one of the main high-value commodities that are widely grown in Asian regions such as Indonesia. There are three types of vanilla that are used, such as *Vanilla planifolia* Andrews, *Vanilla pompona* Schiede, and *Vanilla tahitiensis* JW Moore (Hakim, 2015). Molecular tests were managed by using leaf samples from related plants. Vanilla leaves are similar to most orchid leaves, dark green flattened, rather thick, elongated round and pointed at the tips of the leaves. Leaf length ranges from 9-22 cm with leaf width ranging from 3.5-7 cm [2].

Morphology Identification

TABLE 2. Morphology Identification of Pasak Bumi Leaf

Leaf Characteristic	Description
Length	 **FIGURE 3.** Measurement of Pasak Bumi length 8.45 cm
Width	 **FIGURE 4.** Measurement of Pasak Bumi width 2.6 cm
Leaf shape	Lanceolate
Texture	Smooth and shiny
Tips	Acuminate dan asimetric
Margins	Entire

Leaf Characteristic (continued)	Description (continued)
Surface color	 **FIGURE 5.** Surface of Pasak Bumi leaf Dark green
Base color	 **FIGURE 6.** Base of Pasak Bumi leaf Light green

Measurements on pasak bumi leaves for morphological identification showed that the leaf had a length of 8.45 cm and a width of 2.6 cm. Pasak bumi leaves have a pinnate leaf bone shape with a smooth and shiny leaf texture. In addition, pasak bumi had a clearly visible maternal leaf bone, and the lateral branch bones fused with the other lateral branch bones to form a marginal vein [20]. In addition, the shape of the tip of the leaf is acuminate and asymmetrical with a flat leaf edge. The same thing based on observations in Benuah Hamlet, West Borneo, the tip of the petiole of the pasak bumi leaf is pointed, but it is also common to find a tapered leaf tip, and the leaf base is pointed and asymmetrical and has a flat leaf edge [20]. The color on the surface of the leaf has a darker green color than the base of the leaf.

TABLE 3. Morphology Identification of Vanili Leaf

Leaf Characteristic	Description
Length	8.45 cm
Width	2.6 cm
Leaf shape	elliptic
Texture	Smooth and thick
Tip	Acuminate and simetric
Margins	Entire

Leaf Characteristic (continued)	Description (continued)
Surface color	 **FIGURE 7.** Surface of Vanila leaf Light green
Base color	 **FIGURE 8.** Base of Pasak Bumi leaf Light green

In the morphology carried out on the vanilla leaf sample, the calculation of leaf length was 8.45 cm and leaf width was 2.6 cm. The leaves are oval in shape with a thick and smooth texture. It has a pointed and symmetrical leaf tip, and a fused leaf bone as a whole. Vanila leaf lengths ranged from 8-25 cm [21]. The leaf surface is smooth and shiny green. In addition, *V. planifolia* has a characteristic oval to oval leaf shape. The color of the vanilla leaf for the surface is light green, the same as the underside which shows light green [22]. Therefore, that leaf color is an easy indicator to determine the health of vanilla plants. Leaves that are dull, not shiny, slightly lay, or are an indication of a diseased plant [21].

CONCLUSION

Based on Phylogenetic and Morphological analysis, the Vanilla plant used in this research is closely related to *Vanilla planifolia*.

REFERENCES

1. K. Khanna, S. K. Kohli, R. Kaur, A. Bhardwaj, V. Bhardwaj, P. Ohri, P. Ahmad, Phytomedicine **153361,** 2020.
2. S. Nikhat and M. Fazil. *Overview of Covid-19. Its Prevention and Management in The Light of Unani Medicine* (2020)
3. B. Yang, N. Zhu, D. Zhang, W. Wang, X. Li, J. Song, X. Zhao, B. Huang, W. Shi, R. Lu, P. Niu, F. Zhan, X. Ma, D. Wang, W. Xu, G. Wu, G. F. Gao, W. Tan, N. Engl. J. Med **382**, 727–733 (2019).
4. Z. Nooreen, V. K. Rai, N. P. Yadav, Ann. Phytomed **7**(1), 27-37 (2018)
5. L. S. de Padua, N. Bunyapraphatsara, R. H. M. J. Lemmens, *Plant Resources of South-East Asia Medicinal and Poisonous Plants No 12 (1)* (Leiden, Backhuys Publishers, 1999)
6. S. M. Chinnappan, A. George, P. Pandey, G. Narke and Y. K. Choudhary, Food & Nutr. Research, (2021).
7. Matsjeh, Sabirin, *Utilization of Natural Vegetable Materials that have the Potential as Raw Materials for Medicinal Compounds* (Gadjah Mada University, Yogyakarta, 2009)
8. Purwata, I made Oka Adi, *Obat Tradisional. Bahan Ajar* (Universitas Udayana, Bali, 2017).
9. E.A. Gomes, M. C. Kasaya, E. G. deBarros, A. C. Borgs and E. F. Araujo, Genet Mol Biol **25**(4), 477-488 (2002).
10. H. E. O'Brien, J. L. Parrent, J. A. Jackson, J. M. Moncalvo and R. Vilgalys, Environ. Microbiol **71**, 5544-5550 (2005).
11. H. P. Kusumaningrum, A. Budiharjo, A. Suprihadi, Y. Eshananda, A. Fadillah and D. R. Pangestuti, **10**(3), 26-38 (2018).
12. Z. Khalid, Masoodi Sameena Maqbool, Lone Rovidha Saba Rasool, "Chapter 7 - Genomic DNA extraction from the plant leaves using the CTAB method." in *A Practical Lab Manual,* (2021), pp. 37-44.
13. S. Kumar, G. Stecher, M. Li, C. Knyaz and K. Tamura, Mole. Biol. and evolution **35**(6), 1547 (2018)
14. N. A. Pratama, M. M. Widyarifa, S. L. Farikha, H. P. Kusumaningrum and Y. Eshananda, "Isolation, antibacterial activity, and molecular identification of endophytic fungi from Pogostemon cablin," Journal of Physics: Conference Series (IOP Publishing, Vol. 1943, No. 1, 2021), pp. 012066
15. A. Fatchiyah, L. E. Widyarti, S. Rahayu, *Biologi Molekular Prinsip Dasar Analisis*. (Erlangga, Malang, 2011).
16. A. R. Kartini, "Karakterisasi Molekular Padi Transgenik dengan Beberapa Metode Isolasi DNA," Skripsi, Departemen Biokimia. Fakultas Matematika dan Ilmu Pengetahuan Alam Institut Pertanian Bogor, Bogor, 2012.
17. P. Y. Lee et al., J Vis Exp. **62**, 3923 (2012).