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Utilization of *Trichoderma viride* to Increase Patchouli Alcohol from Crude Extract of Acehnese Patchouli Leaves

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Abstract. Acehnese Patchouli var. Sidikalang can produce patchouli oil, one of the country's sources of foreign exchange, and it is advantageous in many industries. However, patchouli alcohol (PA) content in patchouli oil is still low. The higher PA content determines the better quality of patchouli oil. So, delignification with *Trichoderma viride* was carried out to increase PA and yield of patchouli oil. The duration of delignification patchouli leaves is one factor affecting the increase in PA and yield. This study aimed to determine the effect of delignification duration on PA content and yield. The research method was completely randomized with 8 treatments (a combination of 2 treatments: with and without delignification, and 4 delignification durations: 0, 3, 6, and 9 days). The inoculum used for delignification was an inoculum ball, and then patchouli leaf samples were extracted using microwave-assisted extraction (MAE). Gas chromatography was carried out for PA analysis. Delignification of patchouli leaves, with a delignification time of 9 days, showed high PA and yield, which were (0.3129 ± 0.1557)% and (1.4543 ± 0.7717)%, respectively. The success of delignification at 9 days was also shown by increased phenolic content. *Trichoderma viride* is often utilized in delignification process, but the optimal duration of its delignification of Aceh patchouli leaves that can help improve patchouli oil quality is not yet known. This research is hoped to help related industries improve the secondary metabolites of plants, which has many benefits for the community. Implication/benefit for science development/society

Keywords: Delignification; Duration of delignification; Phenolic; Yield

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INTRODUCTION

Patchouli belongs to the Lamiaceae family and is a highly commercial aromatic plant widely utilized because it produces patchouli oil, which is highly valued in the global market. In this study, Acehnese Patchouli (*Pogostemon cablin* Benth.) var. Sidikalang was used because of its better quality and higher patchouli oil content. Based on Nisak et al. (2024), geographical and varietal influences on oil composition.

Patchouli oil is widely used in several industries because it has biological properties, such as anti-inflammatory, aromatherapy, bactericide, fungicide, and many more (Swamy & Sinniah, 2015). Patchouli oil is rich in sesquiterpenes, with the main content being patchouli alcohol (Patchoulol), which belongs to tricyclic sesquiterpenes and is used in perfumes, soaps, and other cosmetic products (Swamy & Sinniah, 2015). Patchouli alcohol is a major constituent that regulates and controls the quality of patchouli oil, where the higher the patchouli alcohol content, the better the quality of patchouli oil.

In 2020, the Ministry of Agriculture Directorate General of Plantations reported that Indonesia is the world's leading producer of patchouli oil. Patchouli oil exports dominate essential oil exports in Indonesia by around 80-90% (Sukardi et al., 2017). Patchouli oil production in Indonesia is one of the country's sources of foreign exchange, so developing patchouli plant cultivation to produce quality patchouli oil can help meet market demand (Mukhtar et al., 2020). However, patchouli oil produced in Indonesia has a low PA content of < 30% and fluctuates due to the influence of the

origin of raw materials, improper cultivation techniques, poor post-harvest handling of leaves, and the extraction process is not optimal. This results in low prices and does not meet the quality standards of market demand. Therefore, the PA content of patchouli oil needs to be increased (> 40%) to meet global market demand standards.

Increasing patchouli alcohol levels can be optimized through the patchouli oil extraction process so that the pretreatment process (proper post-harvest handling) is carried out to maximize the patchouli oil extraction process. Pretreatment is done biologically, namely delignification with *Trichoderma viride* mold. The pretreatment process is needed to damage the cell walls of plant cells, which inhibits the extraction of patchouli oil. Plant cell walls are composed of lignocellulosic, which consists of cellulose (30-50%), hemicellulose (20-30%), and lignin (15-25%) (Vasic et al., 2021). *Trichoderma* sp. molds are often utilized to degrade agro-waste biomass through the degradation of polysaccharide complexes. Several studies have been conducted regarding applying *Trichoderma* sp. in biomass degradation benefits, such as Bisphenol A degradation (Zhang et al., 2023; Zhao et al., 2018). *Trichoderma viride* is often used to degrade agro-waste from floral, tea leaves, fruits, vegetables, sugarcane, etc., using solid-state fermentation (Ahuja & Bhatt, 2018).

Delignification with *Trichoderma viride* mold is also proven to increase patchouli alcohol levels. Based on research by Muharam et al. (2017), the delignification of Aceh patchouli with *Trichoderma viride* caused an increase in patchoulol levels by 35.6%/400 grams. The delignification process can also use several other molds, especially those with cellulase enzymes, such as *Trichoderma viride* (Muharam et al., 2017), *Phanerochaete chrysosporium* (Rulianah et al., 2015), *Aspergillus niger* (Pawestri & Fitri, 2019), *Rhizopus* sp. (Fitrihidajati et al., 2015), and others. Fitrihidajati et al. (2015) showed that *Rhizopus* sp. (tempe yeast) has lignocellulolytic enzymes that help reduce crude fiber levels in ruminant feed. This also indicates that other molds with lignocellulolytic enzymes can reduce crude fiber levels from plants, such as the components that make up the plant cell wall (lignin, hemicellulose, and cellulose). Thus, it is hoped that using *Trichoderma viride*, which has lignocellulolytic enzymes and is often used in delignification, can help optimize the patchouli oil extraction process.

Delignification in plants that aim to improve the quality of yield or yield content is also familiar. The lignin layer in the leaves needs to be broken down to facilitate the extraction of patchouli oil. Some species of molds can degrade lignin. By applying suitable mold, research can be carried out to determine delignification procedures and mold species that can add value to the patchouli oil production process (Slamet & Rahmi, 2019).

The success of delignification is influenced by various factors, such as delignification temperature, delignification room humidity, delignification initiation procedure, delignification medium used, delignification period chosen, pH, and substrate concentration (Irshad et al., 2013). These factors contribute to ensuring the running of delignification, especially the delignification period. With an optimal period, lignin degradation can be completed, and no further biosynthesis process of *Trichoderma viride* will occur.

Furthermore, extraction of patchouli oil by microwave-assisted extraction (MAE) method using ethanol solvent showed better extraction results than conventional methods (such as ultrasonic extraction and soxhlet extraction) because it uses microwaves as a source of extraction energy with high temperature in a closed vessel.

Based on various obstacles and previous research that has been carried out, in this study, efforts were made to increase patchouli alcohol levels by optimizing the length of patchouli leaf delignification by *Trichoderma viride* molds to facilitate the release of patchouli oil from patchouli leaves during the extraction process, so that the level of patchouli oil obtained is high. This research describes the optimal duration of patchouli leaf delignification as a biological pretreatment process of patchouli leaves before extracting patchouli oil to increase the level of patchouli oil extracted. So, the extraction process can be optimized in the following research, such as using the distillation method to obtain pure patchouli oil to support the optimized pretreatment process. Also, this research is hoped to help related industries increase PA content or other secondary metabolites from plants. Not only that, this research is expected to provide an overview of the utilization of *Trichoderma viride* with optimal delignification duration for solid fermentation, which can be used to reduce agro-waste, delignified bioethanol production, facilitate the delignification process in the paper industry, and others.

METHODS

The plant materials used in this research were Acehnese Patchouli var. Sidikalang is from the greenhouse, Faculty of Biotechnology, University of Surabaya. Acehnese patchouli leaves of var. Sidikalang (diameter ±7-8 cm) will be dried in a cabinet dryer at 60°C until the moisture content remains at 15%.

The leaves to be harvested have a diameter of approximately 7-8 cm, are dark green, and do not experience pest attacks or contract diseases. Harvesting is done in the morning, the leaves are weighed by wet weight and wind-dried on a tampah for 1 day. The wind-dried patchouli leaves will be further dried in a cabinet dryer at 60 °C for 1 day. After the drying process from the cabinet, the dry weight of the leaves will be weighed, and then the leaves will be stored at room temperature.

Trichoderma viride Preparation and Delignification Process

Propagation of molds was initiated from the *Trichoderma viride* mold mother culture. Potato dextrose agar (PDA) media were overgrown with *Trichoderma viride* molds cut with a size of 1x1 cm and transferred sterile on media. The media that had been planted was then incubated at 28 °C for 5 d. After the mold grows evenly on the PDA media, cut the PDA media that has been overgrown with *Trichoderma viride* by 1x1 cm. Reculture on 20 mL potato dextrose broth (PDB) media and incubate in a shaker incubator at 125 rpm (temperature 28 °C for 5 d). The molds will be shaped like cotton balls and washed with distilled water. Next, centrifugation at 2,000 rpm for 5 minutes. Washing and centrifugation are repeated until the obtained mold has been cleared from the PDB media.

Delignification was done by taking 1 gram of leaves (15% moisture content) and adding 0.2 grams of *Trichoderma viride* mold. Dry leaves were moisturized by watering 0.6 ml of sterile PDB media and incubated at 28 °C for a predetermined delignification duration (0 days, 3 days, 6 days, and 9 days). The same treatment was also carried out without adding *Trichoderma viride* as a control treatment.

Total Phenolic Content Test

Analysis of phenolic content for all samples was carried out to determine the success of delignification. Small pieces of patchouli leaves, as much as 0.1 grams, are put in a test tube, and 5 mL of distilled water is added. Then, it is homogenized and heated in boiling water for 15 minutes. Next, the supernatant will be taken for further testing.

The test supernatant obtained will be taken as much as 0.2 mL. Then, 1 mL of folin-ciocalteau reagent is added, homogenized, and allowed to stand for 5 minutes. Then, 0.8 mL of $CaCO_3$ is added, homogenized, and allowed to stand for 30 minutes. When the incubation is complete, the absorbance is measured at a wavelength of 760 nm.

Microwave-Assisted Extraction (MAE) of Patchouli Oil

The dried leaves (see Plant Materials method) will be ground into powder and sieved with a 40-mesh sieve. The sieving results are powdered Acehnese patchouli leaves var. Sidikalang, which will be stored in a vacuum desiccator.

The dried Acehnese patchouli leaf powder was taken in as much as 1 gram, and 96% ethanol, as much as 50 mL, was added to an extraction jar. Then, put into the microwave with 1000 watts power for 60 seconds so that the crude extract is obtained and filtered with filter paper so that the supernatant of the crude extract is obtained. Then, the supernatant is transferred into a vial, and the solvent will be evaporated using an 80 °C water-bath for 90 minutes. The crude extract from MAE will be weighed as crude extract yield, and then the solventless extraction results can be continued in gas chromatography (GC) analysis.

Gas Chromatography for PA Analysis

After that, the gas chromatography (GC) method was carried out to determine the patchouli alcohol content. The crude extract sample in the vial bottle will be pretreated before being injected into the gas chromatography device. Sample preparation is done by dissolving the sample using ethanol 96% pro analysis, as much as 2 mL, then homogenized and centrifuged so that impurities settle. Take the supernatant/liquid part and inject as much as 100 µL into the gas chromatography column. Then, adjust the flow rate and wait until the chromatogram results are obtained.

Conditioning of the column for the GC instrument (HP6890) is shown below:

Table 1. Conditioning of column for GC instrument

Capillary column model number	J&W 19095N-123 (HP-INNOWAX)
Maximum temperature	240°C
Nominal length	30 m
Nominal diameter	530 um
Nominal film thickness	100 um
Initial flow	15 mL/min
Initial pressure	10.38 psi
Average velocity	95 cm/sec

Experimental Design and Statistical Analysis

The experiment was designed in a completely randomized design with two treatments (without delignification and through delignification) with four duration of delignification (0 days, 3 days, 6 days, and 9 days). Data was analyzed using two-way ANOVA (Analysis of Variance). Duncan's Multiple Range Test (DMRT) at 5% error level (α=0.05) was used in case a significant difference was observed. Phenolic content, yield crude extract, and patchouli content were observed in this research.

RESULTS AND DISCUSSION

Delignification of Acehnese Patchouli leaf var. Sidikalang used *Trichoderma viride* mold to increase patchouli alcohol (PA) content and yield produced from the crude extract of Acehnese patchouli var. Sidikalang. Several factors, including the duration of the delignification, can influence the delignification process. In this study, delignification was carried out for 0, 3, 6, and 9 days. To determine the success of delignification, total phenolic content was tested to determine which phenolic content would be higher if the delignification was optimal. Based on Table 2, it is known that 9 days of delignification is the optimal treatment to increase patchouli alcohol content and yield.

Trichoderma viride is a mesophilic mold with an optimal growth temperature of around 25 °C – 30 °C. In this study, a delignification temperature of 28 °C was used. In addition, this mold is facultative aerobic so it can live in microaerobic conditions through the delignification pathway. The delignification process with *Trichoderma viride* is carried out in Petri containers so that only a little air is in the container, with the aim of the remaining air and the addition of PDB media in the container can help the growth of mold hyphae first, because the hyphae of the *Trichoderma viride* mold mediate the production of lignocellulose enzymes.

Table 2 shows that the total phenolic content in the delignification treatment is higher than in the control treatment when compared on the same day. This indicates that delignification affects the total phenolic content because the delignification of leaves makes it easier for phenolic compounds to be extracted during sample preparation so that more free phenolics can be measured. When viewed based on the duration of delignification, it can be seen that there is an increase in total phenolic content along with the duration of delignification, which is getting longer, namely in 9 days, delignification shows total phenolic content of (58.46 ± 2.55) $mg.L^{-1}$ which was initially only (19.51 ± 0.46) $mg.L^{-1}$.

Table 2. Delignification of Acehnese Patchouli var. Sidikalang with *Trichoderma viride* to Increase Total Phenol Content

Treatment	Phenolic Content ($mg.L^{-1}$)			
	Duration of Delignification (Days)			
	0	3	6	9
Without delignification (Control)	21.89 ± 5.75[a]	26.23 ± 0.49 [a]	27.99 ± 1.55 [a]	42.61 ± 0.76[b]
Pretreatment: delignification process	19.51 ± 0.46[a]	29.89 ± 0.15 [a]	48.38 ± 2.76[b]	58.46 ± 2.55[b]

Note: Values followed by the different letter were significantly different at $p < 0.05$ (α=5%) by the DMRT test

The total phenolic content is used as a parameter for the success of delignification because this delignification aims to facilitate the extraction process of patchouli alcohol (PA) contained in patchouli oil. PA is classified as sesquiterpenes, which have a structure similar to terpenes and phenolics. This is also shown by the increasing PA content along with the length of the delignification process (Figure 2), which on days 6 and 9 showed a more significant increase in PA content. Thus, the increase in total phenolic content correlates with the increase in PA content.

In addition, the patchouli plant also includes many other phenolic compounds that are released as free phenols after the delignification process. When the delignification process is successful, the higher phenolic content will be released, making the blue color more intense. This is because the high levels of phenolics are equivalent to the higher phenol ions that can reduce hetero-poly acids (phosphotungstate-phosphomolybdenum) to molybdenum-tungstate (blue). Thus, if delignification is successful, the measured free phenolic content will increase (Michiu et al., 2022).

Based on Sholahuddin et al. (2024), delignification with a physicochemical method (steam and using chemical reagent) for palm-oil mill agro-waste can break down the lignin-carbohydrate complex (LCC), which this method changes the physical and chemical properties into lignin-derived products, including phenolic compounds. So, we know that the delignification process and duration can also increase the total phenol compound (TPC).

Table 2 shows that on day 9, the control treatment also increased phenolic content to (42.61 ± 0.76) mg.L^{-1} even though it was not inoculated with *Trichoderma viride* mold. However, the increase was insignificant compared to the treatment with the delignification process by *Trichoderma viride*. This is because the patchouli plant has several endophytic microorganisms already present in the plant. The endophytic fungi will grow when incubated at the optimal temperature for these microorganisms, namely 28 °C (optimal temperature for fungal growth). These endophytic fungi can survive in the plant for a specific time and form colonies but do not harm the host. There are several types of endophytic fungi found in Aceh patchouli plants almost all of them were genus of *Daldinia* sp. (Ascomycetes), but not all of them can be explicitly determined by the name of the endophytic fungi (Arif, 2023). Based on Chanyal (2018), *Daldinia* sp. can produce the laccase enzyme, which can play a role in decolorizing textile dyes and help in the delignification process to break down plant cell walls into simpler structures. Thus, this can support that endophytic fungi from patchouli leaves can also play a role in the delignification process of patchouli leaves themselves, which is characterized by an increase in total phenolic content on day 9 even though they were not given *Trichoderma viride* inoculum. In addition, the redox-potential of the laccase enzyme is lower than non-phenolic compounds of lignin composition, due to this restriction, laccase can only oxidize phenolic units of lignin into the free phenolic-derivated product, which is measured on folin-ciocalteau reaction with spectrophotometry (Ali et al., 2018). Almost all fungi, such as Ascomycetes, Basidiomycetes, and Deuteromycetes, can produce laccase enzymes to help degrade lignin compounds.

After determining the success of delignification based on the increased total phenolic content, patchouli oil extraction was carried out to obtain the crude extract using the microwave-assisted extraction method. The following is the appearance of the extraction of patchouli oil by microwave-assisted extraction (MAE) method:

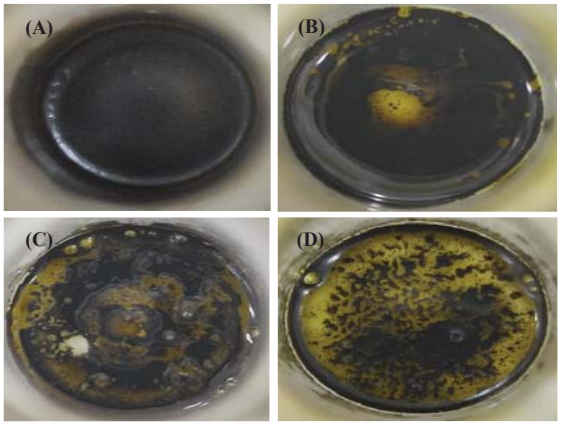

Figure 1. Extraction Results of Patchouli Oil by Microwave Assisted Extraction (MAE) Method
Note: (A) 0 days after delignification; (B) 3 days after delignification; (C) 6 days after delignification; (D) 9 days after delignification

The extraction process used is microwave-assisted extraction (MAE) because this method only requires a small amount of sample, is more efficient than conventional extraction methods, saves time, does not require a lot of solvents, and is more effective in extracting plant secondary metabolites. In addition, the energy from

microwaves can help damage the glandular trichomes of patchouli plants, which are the storage sites for patchouli oil. Microwaves can transfer ions and electrons so that an electric field is generated and ionic conduction occurs. This will be converted into heat and evaporation of water vapor from the plant cells (Marić et al., 2018). The accumulated water vapor in plant cells will cause cell swelling, which eventually causes cell lysis (Zhi et al., 2017). In the extraction process, ethanol solvent is used because it utilizes the principle of electromagnetic radiation, which will be sent to the polar solvent and absorbed by the simplistic/material, which, over time, the energy increases due to the mobility of the intermolecular and intramolecular resulting in the release of heat by the simplistic/material so that it breaks. The concentration and volume of ethanol determined the effectiveness of extraction (Le et al., 2018). In addition, ethanol has several advantages as a solvent: it is cheap, has a low boiling point so that it is quickly evaporated and separated from the extraction results, is less dangerous than other solvents, and so on. The use of some other solvents is allowed with the following considerations: solvents that are polar, easy to absorb heat and distribute, volatile, harmless, and others.

The extraction results from this method are crude extracts, so the extracts are not pure and only contain patchouli oil, especially patchouli alcohol. The content of the crude extract can be compounds from patchouli leaves that can be extracted by 96% ethanol, such as fat, other patchouli oil content, plant cell wall constituent components, especially hemicellulose components, and other impurities that can be extracted. Based on Figure 1, it can be seen that as the duration of delignification increases, the appearance of patchouli oil extraction results gets yellowish. This can be because when not delignification, the components that make up the cell wall, especially the hemicellulose part, are still many and are extracted, so the color of the extract tends to be brown to black (Geng et al., 2019). However, after the delignification process for several days, the hemicellulose content can be reduced, and the color of other crude extract compounds that have a yellow color begins to appear. The color of patchouli oil, which is increasingly yellow and clear, indicates the quality of the oil is getting better too, so if extracted by distillation method, there is a possibility that the extraction results from leaves through the delignification process will be more transparent and purer without other impurities from the leaf simplistic.

Furthermore, the patchouli alcohol (PA) content in the crude extract was determined, where the higher the PA value, the better the quality of patchouli oil. Based on Figure 2, it is known that the highest PA content is in the delignification treatment with *Trichoderma viride* with a delignification duration of 6 days:

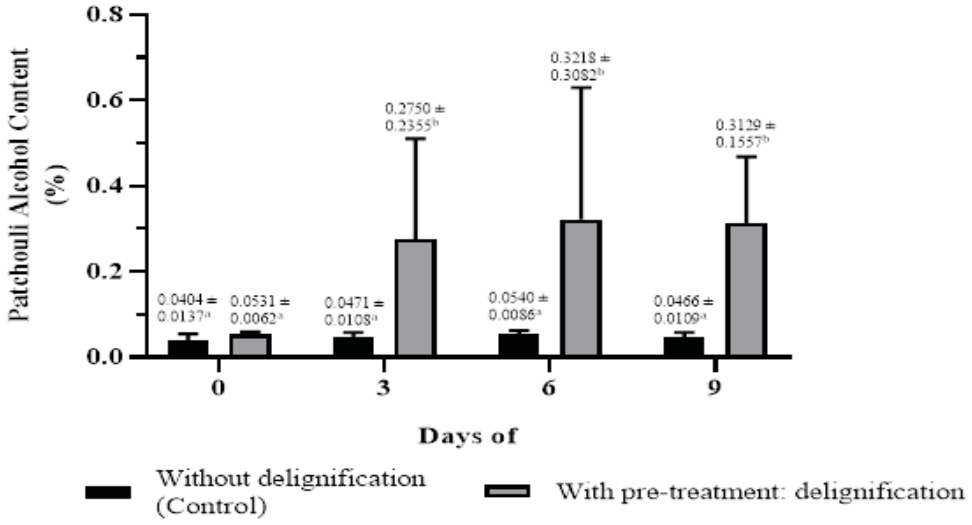

Figure 2. Patchouli Alcohol Content of Crude Extract of Acehnese Patchouli var. Sidikalang
Note: Values followed by the different letter in the same column or line were significantly different at $p < 0.05$ (α=5%) by the DMRT test

Based on Figure 2, the PA content of Acehnese patchouli after the delignification process was known. Sidikalang leaves are higher than in the control treatment. This shows that more PA can be extracted due to the pretreatment process, namely delignification with *Trichoderma viride*. The increase can be due to delignification, which causes the components that make up the cell wall to become loose so that the components of patchouli oil stored in the glandular tissue/trichomes are more accessible to come out during extraction and the higher the chance of PA obtained.

Compared to the control, it can be seen that the control treatment does not increase PA levels, which indicates that the delignification process of *Trichoderma viride* influences the increase in PA. Based on Figure 2, it can be seen that increasing the duration of delignification can also cause an increase in PA because the longer the delignification process, the more plant cell walls can be degraded or stretched, causing the extraction process to be optimal. However, on day 9, there was a decrease in PA, although it was not significant compared to day 6. This shows that if the delignification is extended to 10 days or more, PA levels may be decreased. That is because each microorganism also has its optimal growth time, which correlates with the role of the enzymes it produces. If mold growth begins to be inhibited, the enzyme activity produced will also decrease, and the delignification process will no longer be optimal. Based on the research of Ming et al. (2019) showed that the growth of *Trichoderma viride* mold reached the exponential phase on 0-84 hours (0-4 days), so day 4 should be the right time for harvesting the mold and used in the delignification process, while 84-144 hours (4-6 days) there was a slight decrease (stable period) in *Trichoderma viride* growth, but not significant. That is reflected in the increased PA content on day 6 of this study, which indicates that on day 6, the lignocellulolytic enzyme activity of *Trichoderma viride* was still optimal.

The delignification duration that produced the most PA content was day 6, with PA content of (0.3218 ± 0.3082)%, but it was not significantly different from the PA content with a delignification duration of 9 days, which was (0.3129 ± 0.1557)%. That shows that the length of delignification can affect the PA content that can be extracted. In this study, the PA content on day 6 in the treatment without delignification was only (0.0540 ± 0.0086)%. The increase in PA content in the delignification treatment can be caused by the work of *Trichoderma viride* mold enzymes, namely lignocellulolytic enzymes that can break β(1,4)-glycosidic bonds between lignin and cellulose that make up the plant cell wall. Thus, the plant cell wall bonds become loose, so patchouli oil (with its main constituent PA) can be more easily extracted from plant cells/tissues, even its glandular trichomes (Sugiarto & Hamidi, 2019).

Other parameters are also considered to determine the optimal delignification duration, such as the yield of crude extract obtained, so it is not only concerned with total phenolic and PA content. Based on Table 3, it can be seen that the yield of crude extract from patchouli oil obtained is the highest at a delignification duration of 9 days.

The crude extract is the result of extracting Acehnese Patchouli leaves using the MAE method, which is still in the form of impurities/constituents of 96% ethanol-soluble patchouli leaves, such as fat. In addition, it can be compounds contained in patchouli oil, such as patchouli alcohol (patchoulol), patchoulene, caryophyllene, pogostol, seychellene, guaiene, bulnesene (Jain et al., 2022), and other essential components of patchouli oil.

Table 3. The yield of Crude Extract of Acehnese Patchouli var. Sidikalang

Treatment	Yield content (%)			
	Duration of Delignification (Days)			
	0	3	6	9
Without delignificatin (Control)	0.6798 ± 0.01301[a]	0.8864 ± 0.1607[ab]	0.9150 ± 0.1203[ab]	1.1096 ± 0.2693[ab]
Pretreatment: delignification process	0.6716 ± 0.0473[a]	0.8990 ± 0.1428[ab]	0.9133 ± 0.1227[ab]	1.4543 ± 0.7717[b]

Note: Values followed by the different letter in the same column or line were significantly different at $p < 0.05$ (α=5%) by the DMRT test

Based on the literature, after the delignification process, the yield of crude extract of Acehnese patchouli var. Sidikalang will increase. That is because solid delignification causes the process of delignification of patchouli leaves by *Trichoderma viride*, so that the lignocellulose that makes up the leaf cell wall becomes dismembered, releasing lignin, cellulose, and hemicellulose. The process occurs when environmental conditions lack nutrients. *Trichoderma viride* will begin to secrete the enzyme laccase to consume fermented substrates (Lakshmanan & Sadasivan, 2016) and cause secondary metabolites previously trapped in the cell wall constituents to come out more quickly for extraction. Based on Ghorbani et al. (2015), delignification process of rice straw, *Trichoderma viride* also secrete other lignin-degrading enzymes, such as lignin peroxidase and manganese peroxidase. Also, Ndapamuri et al. (2021) show that the hydrolysis process with *Trichoderma viride* for ten days can help increase the production of sugar content in sweet sorghum stem bagasse. Like Geng et al. (2018), the delignification process can increase hemicellulose extract yield to 50% compared to without delignification on pine (3.4)%. Besides that, Sugiarto & Hamidi (2019) also research the pretreatment of *Pogostemon cablin* Benth. using *Rhizopus oligosporus* to increase crude extract yield, fermentation during 6 and 8 days can meet SNI standards of patchouli oil. Fermentation with *Rhizopus oligosporus* can also increase yield because phytase enzymes can degrade plant cell walls and open the crystalline cellulose structure, so when distillation can extract more easily the oil in vacuole, oil glands, vessels, oil sacs, or glandular hair. This is similar to the role of the laccase enzyme, which is produced from *Trichoderma viride*.

In this study, it can be seen that delignification can increase the yield of crude extract when compared to control. Namely, the highest yield obtained in the 9-day delignification treatment was (1.4543 ± 0.7717)%, while the control on day 9 was (1.1096 ± 0.2693)%. However, compared to the day 0 delignification treatment, the yield obtained after delignification showed an increase of (0.6716 ± 0.0473)% (Table 3). This indicates that the delignification process can help optimize the extraction process to obtain a higher yield even though it is still the yield of the crude extract, so the yield does not only contain patchouli alcohol.

This study illustrates that biological delignification can also be done directly without extraction, so the delignification process will be more practical and cost-effective without the enzyme extraction method. Biological delignification also has many advantages, such as a low energy supply and environmental impact compared to physical and chemical pretreatments (Zabed et al., 2019). Many biological delignification methods have been carried out. Still, only a few utilize the *Trichoderma viride* mold for delignification on patchouli leaves, especially to increase plant secondary metabolites. Even though the *Trichoderma viride* mold is one of the molds that can produce high levels of lignin-degrading enzymes.

This research can help related communities/industries that want to carry out the biological delignification process at a lower cost and in an easier way. This is because the delignification process is quite much needed in society, such as for secondary metabolite production, bioethanol production, reducing agro-waste, papermaking processes, and much more utilization of delignification that can help increase the selling value of a product or the production process of a new product.

CONCLUSION

Delignification with *Trichoderma viride* can increase PA content and crude extract yield, which is the optimal delignification duration of Acehnese Patchouli leaf var. Sidikalang (9 days) resulted in high patchouli alcohol content and crude extract yield: (0.3129 ± 0.1557)% and (1.4543 ± 0.7717)%, respectively, and the appearance of patchouli oil extraction results is yellowish. So, in the next research, the extraction process can be optimized using another extraction method to obtain pure patchouli oil to support the optimized pretreatment process.

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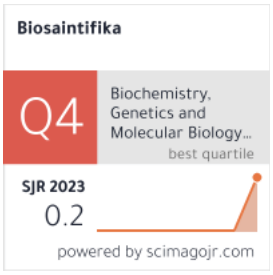

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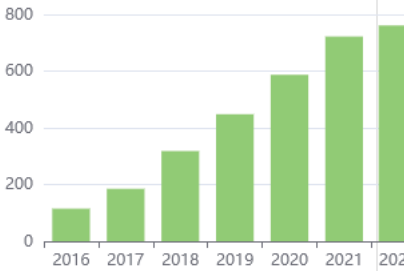

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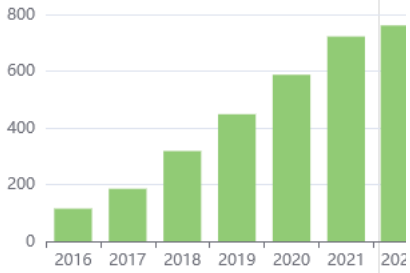

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