Antibacterial activities test of Cajuput Leaf Waste extract (*Melaleuca cajuputi* Powell) on Pathogenic Bacteria

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**Abstract.** Cajuput plant (*Melaleuca cajuputi* Powell) and its waste is one of the plants that can produce atsiri oils. Cajuput plant essential oil has the largest compound component, 1.8 cineol which has the ability as an antibacterial to kill pathogenic bacteria. The purpose of this study was to determine the antibacterial activity of cajuput leaf waste extract against pathogenic bacteria and find out the category of inhibition zones formed from extracts of cajuput leaf waste. This study used a completely randomized design method. Cajuput leaf waste used has a shelf life of 0, 2 and 4 months old. The pathogenic bacteria used consisted of 17 isolates, each treatment was repeated twice. The results showed that cajuput leaf extract had antibacterial activity known by the formation of inhibitory zones. The inhibitory zone in the extract of the 0-month leaf waste was greater than in the leaf extract of leaf of 2 months and 4 months. The diameter of the inhibition zone is at most 13 mm and at least 1 mm. With these results, it can be concluded that the antibacterial activity of cajuput leaf waste belongs to the category of low to strong.

1. **Introduction**

Cajuput plant is one of the plants that can produce atsiri oils. Other studies show 26 compounds have been identified in cajuput plant such as cineol, limonene, 4-terpineol, α-terpineol, caryophyllene and caryophyllene oxide etc. which have the ability as antibacterial, antioxidant, immune, analgesic [1,2]. In Indonesia, there are several industries that produce essential oils from cajuput leaf such as the cajuput leaf processing plant managed by Perum Perhutani (State Forest Company) in West, Central and East Java Provinces. However does not produce only oil, but also waste. The utilization of cajuput leaf waste is still very limited, only for making briquettes and compost [3]. Cajuput leaf waste is produced every day and causing environmental pollution. Not only fresh leaf, but cajuput leaf waste also has a stinking odor.

Cajuput waste which still contains atsiri oils has the ability as an antibacterial like fresh cajuput leaf. Some studies show that plant sources such as coffee, tea, grapes, sorghum, and some herbs such as oregano and spices have been shown to have antibacterial activity due to their content of phenolic compounds [4]. But, Research on the antibacterial activity of cajuput leaf waste (*Melaleuca cajuputi* Powell) has not been done much.
2. Methods
Cajuput leaf waste samples were taken from the Kesatuan Pengelolaan Hutan (KPH) Jatimunggul, Indramayu Regency. The sample age is 0, 2 and 4 months from the distillation of cajuput leaf. The leaf of cajuput leaf are dried in the open air, then the waste is mashed to powder and then weighed 60 grams of each age of the waste.

Maceration and extraction. Cajuput leaf waste with age 0, 2 and 4 months were put into jar bottles and then soaked using methanol solvent. Soaking is carried out for 24 hours, after which it is filtered using filter paper, and carried out until three times (3x24 hours). Extract of cajuput leaf waste concentrated using a rotary evaporator at 40°C until in the pasta form. Solid extracts of cajuput leaf waste are weighed and put in vial bottles [5]. Solid extract of cajuput leaf waste weighed as much as 0.1 grams was dissolved by adding 100% DMSO as much as 1 ml and shaken until homogeneous. Extract solution of cajuput leaf was taken 0.1 ml then added 0.1 ml of distilled water and then shaken until homogeneous [6].

The rejuvenation of the test bacteria began with the manufacture of media, so that the age of the bacteria was obtained 24-48 hours with a temperature of 30°C. The solution extract from cajuput leaf waste (10µl) was dropped on disc paper. Disc paper that has been dripped by a solution of cajuput extract is placed on the media that has been drilled with test bacteria, then incubated for 24-48 hours, observed and measured inhibition zones were formed [7].

3. Results and discussion
Cajuput leaf waste was obtained from KPH Jatimunggul, Indramayu. The condition of cajuput leaf waste (LDKP) can be seen in Table 1.

| LDKP age | Temperature | Moisture | pH  |
|----------|-------------|----------|-----|
| 0 month  | 36.0        | 60.3     | 6.9 |
| 2 month  | 38.5        | 50.0     | 7.0 |
| 4 month  | 37.1        | 53.3     | 7.0 |

The condition of cajuput leaf waste in storage and the shelf life of cajuput leaf waste can affect secondary metabolites in cajuput leaf waste. Long-stored will reduce compounds volatile or disappear due to the oxidation process. The oxidation of chemical compounds can be influenced by time, air moisture, material water content, temperature, and surface area of material [8]. pH is related to the shelf life of waste. The longer storage of waste will result in increased activity of microorganisms in the waste which will eventually decomposition. The decomposition process is accompanied by an increase in pH, the greater the pH value, the lower the metabolite content in waste [9].

The temperature in cajuput leaf waste is caused by microorganisms fermentation. The hot temperature in cajuput leaf waste increases during fermentation due to exothermic reactions [10]. Cajuput leaf waste that is 0-months old has a smaller temperature and moisture compared to temperatures in 2 and 4 months. This is because 0-month is waste that has just come out from the process of refining cajuput oil so that it has not undergone a fermentation process. The moisture in cajuput leaf waste is influenced by temperature. The higher temperature cause less water content in the leaf because it is influenced by the water evaporation process. The evaporation process of water is affected by air, the lower moisture of the air cause the higher evaporation of water [11].

The extracts result initially for medicine agents in the solutions form and liquid extracts but require further processing [12]. Maceration is an extraction method that is carried out by immersing plant material with a solvent at certain time [13]. Methanol solvents were chosen because the sample used was cajuput leaf waste where the non-polar compounds had been taken and the possibility of the polar compounds in them had not been taken. According to Romadanu et al., methanol is a solvent that can
dissolve polar and non-polar compounds [14]. The results of extracting cajuput leaf can be seen in Table 2.

Table 2. The extract results of cajuput leaf waste (Melaleuca cajuputi Powell).

| No | Cajuput leaf waste sample   | Weight (gr) | LDKP extract (gr) |
|----|-----------------------------|-------------|-------------------|
| 1  | Cajuput leaf waste 0-month  | 60          | 3.30              |
| 2  | Cajuput leaf waste 2-month  | 60          | 2.50              |
| 3  | Cajuput leaf waste 4-month  | 60          | 1.00              |

The cajuput leaf waste at 4 months produces fewer extracts compared to 0 and 2 months (Table 1 and Figure 2). The factors that can influence the production of atsiri oils are the type of cajuput, leaf storage, and age of leaf [15]. Moreover, the compounds contained in cajuput leaf waste are volatile compounds which can evaporate caused by the oxidation process with the length of waste storage [8]. The extract weight is related to the heavy fraction of compounds present in cajuput leaf waste. The greater the concentration and proportion of its constituent components, the more extract heavy produced will be [16]. High immersion requires the same polarity between solvents and their bioactive compounds. Methanol solvents have a higher polarity compared to n-hexane and ethyl acetate solvents [13].

Antibacterial testing uses a disc diffusion method to determine the sensitivity of bacteria to an antibacterial compound which is indicated by the large inhibition zone of bacterial growth [17]. The inhibitory zone proves that cajuput leaf extract has antibacterial properties against test bacteria. Antibacterial testing using age levels of cajuput leaf waste. The results of measuring the diameter of the inhibition zone can be seen in Table 3.

Table 3. Diameters average of inhibition zone from cajuput leaf waste to several bacteria.

| No | Test of Bacteria   | Diameters average of inhibition zone (mm) |
|----|--------------------|------------------------------------------|
|    |                    | 0 month         | 2 month         | 4 month         |
| ---|--------------------|-----------------|-----------------|-----------------|
|    | Gram-Positive Bacteria |                |                 |                 |
| 1  | Bacillus sp.       | 13.87 ± 0.53a   | 8.00 ± 0.00b    | 3.00 ± 0.00b    |
| 2  | B. licheniformis   | -               | -               | -               |
| 3  | Bacillus sp.       | 5.25 ± 0.00a    | 5.00 ± 1.41a    | 3.00 ± 0.00a    |
| 4  | B. subtilis 1      | 8.25 ± 0.35b    | 5.12 ± 0.88a    | 4.25 ± 0.35a    |
| 5  | B. subtilis 2      | 4.50 ± 0.70b    | 1.75 ± 0.35a    | 1.50 ± 0.70a    |
| 6  | B. subtilis 3      | 5.12 ± 0.17b    | 2.62 ± 0.17a    | 2.62 ± 0.17a    |
| 7  | B. subtilis 4      | 5.00 ± 0.00a    | 3.75 ± 0.00b    | 3.12 ± 1.23a    |
| 8  | B. subtilis 5      | 6.00 ± 0.35b    | 1.12 ± 0.17a    | 2.37 ± 0.53a    |
| 9  | B. subtilis 6      | 4.87 ± 0.17c    | 2.00 ± 0.00b    | 1.00 ± 0.00a    |
| 10 | B. licheniformis   | 4.75 ± 0.00a    | 2.00 ± 0.00a    | 3.00 ± 1.41a    |
The largest inhibition zones are found in Bacillus sp. (13.87 ± 0.53 mm) (Table 3). Based on Repi et al., the size of the inhibition zone below 5 mm is categorized as low, the size of the inhibition zone 6-10 mm is categorized as medium, the size of the inhibition zone 11-20 is strong, and the size of the inhibition zone more than 21 mm is categorized as very strong [18]. Then it can be said that the inhibitory zone results shown in Table 3 included in the category of low to strong against the test bacteria.

The biggest inhibition zone was found in cajuput extract leaf waste which is 0 months old. This is because the extract of 0-month-old cajuput leaf waste comes from leaf waste that has just come out from the process of distillation cajuput oil so that it still has a considerable amount of compound content. The smallest inhibition zone is produced by cajuput extracts of 4-month-old because most of the compounds contained in them have been lost, so they are less able to kill bacterial cells. According to Khabibi the length of storage time, it will result in the loss of components or atsiri oils caused by evaporation, oxidation processes, resignification, and other chemical reactions [19]. The secondary metabolites that are predominantly found in cajuput leaf (Melaleuca cajuputi Powell) are cineol, limonene, 4-terpineol, α-terpineol, carophyllene, and carophyllene oxide, all of these compounds are terpenoid compounds [1]. Terpenoids are plant-derived hydrocarbons with the general formula (C5H8) and oxygenation, hydrogenated and dehydrogenated derivatives. Sineol has antibacterial and anti-inflammatory properties [20]. Terpenoids are used for the manufacture of perfumes, cosmetics, home cleaning products and medicines [21]. Carophyllene and carophyllene oxide has antibacterial, anti-inflammatory, antifungal and insect killer abilities [22]. Linalool can be used as an antibacterial, increase permeability, anti-inflammatory, antioxidant and spasmolytic [23]. Extract of cajuput leaf can kill Bacillus sp., Bacillus subtilis, Bacillus licheniformis, Enterococcus faecalis, and Staphylococcus aureus which are Gram positive bacteria, while Vibrio cholerae and Shigella dysentriae bacteria are Gram negative bacteria. Terpenoids result in disruption of cell membrane permeability. This situation causes lysis of the cell membrane and eventually die [24]. The mechanism of terpenoids action is not yet fully understood but is thought to involve membrane disruption by lipophilic compounds [25].

The result of the inhibition zone diameter obtained from Gram positive bacteria is greater than Gram negative because Gram positive bacteria have simple cell walls. Gram positive bacteria have many peptidoglycan on their cell walls, have little lipids, and their cell walls contain teicoic acid. This causes antibacterial compounds to enter the cells more easily so that the antibacterial activity in Gram positive bacteria is relatively high [26]. Cajuput leaf waste extract could not inhibit the growth of Bacillus licheniformis and Gram negative bacteria such as Pseudomonas aeruginosa, Proteus mirabilis, and Escherichia coli because the compounds in cajuput leaf waste extract cannot penetrate the cell walls of Gram negative bacteria. Gram negative bacteria also have thicker cell walls because they have fewer lipids and peptidoglycan. This bacterium also has an outer layer consisting of phospholipid and lipopolysaccharide and has high permeability [19].

Cajuput leaf waste has antibacterial activity against Gram positive bacteria (Bacillus sp., Bacillus subtilis, Bacillus licheniformis, Enterococcus faecalis, Staphylococcus aureus) and Gram negative bacteria (Vibrio cholerae and Shigella dysentriae) and the resulting inhibition zone diameter is
categorized as low to strong. Cajuput leaf waste with 0 months old has an antibacterial activity that is better than the antibacterial activity with age 2 months and 4 months. The use of natural products against pathogenic bacteria becomes very popular because side effects that not significant and often the intended results can be achieved [27].

4. Conclusion
Based on the results of this research, leaf waste was greater at 0 months than leaf extract at 2 months and 4 months. The diameter of the inhibition zone is at most 13 mm and at least 1 mm.

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