Antibacterial activities of leave extracts as bactericides for soaking of skin or hide

Ono Suparno*, Tania Panandita, Amalia Afifah, Marimin, Rini Purnawati
Department of Agroindustrial Technology, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University (IPB), Darmaga Campus, Bogor 16680, Indonesia
*ono.suparno@apps.ipb.ac.id

Abstract. Antibacteria, a substance inhibiting the growth of bacteria, can be obtained from tropical-almond (*Terminalia catappa*), morinda (*Morinda citrifolia*), and white leadtree (*Leucaena leucocephala*) plants, since the plants have phytochemical content functioning as antibacterial agent. Commonly, part of plant that contains higher antibacterial substances is its leaf. The objectives of this study were to determine antibacterial activity of tropical-almond, morinda, and white leadtree leaves extracts, and to analyse the potency of the three extracts as natural bactericide for soaking of skin or hide. The responses measured in this study were phytochemical contents, total flavonoid, tannin content, the inhibition zone, the minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC). Phytochemical contents containing the three leaves extracts were alkaloid, flavonoid, tannin, saponin, phenolic, and glycoside. Total flavonoid and tannin contents of the three extracts were tropical-almond extract of 1.14 % and 1.51 %, respectively; morinda extract of 0.61 % and 0.36 %, respectively; and white leadtree extract of 0.60 % and 4.82 %, respectively. White leadtree leaf extract gave the highest inhibition zone against *B. subtilis*, *S. aureus* and *E. coli*, i.e. 1.50, 1.3, and 1.65 cm, respectively; and the lowest MIC and MBC against *B. subtilis*, *S. aureus* and *E. coli*, i.e. 1500, 3000, and 1500 μg/ml, respectively. Therefore, the white leadtree leave extract had more potential as bactericide for soaking of skin or hide compared to those of the tropical-almond and morinda leaves extracts.

1. Introduction
Natural antibacterial sources can be obtained from some parts of plants. The part of plant containing more antibacterial substances is leaf [1]. The antibacterial agents can be obtained from plant leaves by extraction. Tropical-almond (*Terminalia catappa*) leaf extract has good antibacterial activity against bacteria *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, and *Salmonella typhi* [2]. White leadtree (*Leucaena leucocephala*) leaf extract can inhibit the growth of bacteria commonly found in skin or hide preservation, such as *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* [3]. Phytochemical contents such as flavonoids, tannins, alkaloids, and saponins contained in leaf extract act as an antibacterial [4][5].

Leather manufacture commences with soaking of cured skin or hide in water to rehydrate and to cleanse it [6]. The soaking is one of the processes in the leather industry that serves to rehydrate the skin [7]. Bactericide is needed in the soaking process to prevent collagen damages by microorganisms [8]. Utilization of plant leaf extract should be developed as a natural bactericide. The use of natural bactericides from plant extracts may be potentially used in eco-friendly soaking.
This research was conducted to determine antibacterial activity of tropical-almond, morinda, and white leadtree leaves extracts, and to analyse the potency of the three leaves extracts as natural bactericide for soaking of skin or hide in leather manufacturing.

2. Materials and Methods

2.1. Materials
The materials used in this study were tropical-almond (*Terminalia catappa*) and morinda (*Morinda citrifolia*) leaves obtained from Darmaga Campus, Bogor Agricultural University (IPB), Bogor, Indonesia. Isolates of *Bacillus subtilis*, *Stapylococcus aureus*, and *Escherichia coli* were obtained from the Department of Biology, IPB, Bogor, Indonesia. Other chemicals used were Muller Hinton agar (MHA), ethanol 70%, chloramphenicol, amoxicillin, ciprofloxacin, nurient broth (NB), and dimethyl sulfoxide (DMSO).

2.2. Chemical Components Tests
The chemical components tests of the leaves were performed by method reported by Harborne [9] and Hasan et al. [10]. The tests consisted of alkaloids, flavonoids, saponins, tannins, phenolics, triterpenoids, steroids and glycosides.

2.3. Preparation of Extracts
The fresh leaves were dried under the sunlight for 2 - 3 days until the constant weight. The dried leaves were grinded using a blender and stored in an airtight container. The extraction technique used in the extraction was maceration. The powder was soaked using 70% ethanol at 1:10 w/v for 24 hours and then stirred with a shaker. Use of a 1:10 w / v ratio between simplicia and solvent based on a study conducted by Margeretha et al. [11]. The 70% mixture of simpilisia and ethanol was filtered through fabric and filter paper. The filtrate was evaporated with a vacuum rotary evaporator, water bath heating at 60 °C for an hour, then the water bath heater temperature was increased to 70 °C, thus leaf condensed extract was obtained [12]. The condensed extract was heated in a water bath to obtain an extract with a solid total of about 50%-60%. The total solid measurement was performed using a moisture analyser.

2.4. Total Flavonoid Test [13]
Test of total flavonoid extract was initiated by making reagent solution. The reagents used were 0.5% w/v HMT (hexamethylenetetramine) solution, 25% HCl solution, 5% glacial acetic acid solution in methanol, and 2% AlCl$_3$ solution in glacial acetic acid solution. The next step was making the mother liquor used as the sample solution. The procedure for making a mother liquor was an extract equivalent to 200 mg of simplicia poured into a round bottom flask and then added 1 ml of HMT solution, 20 ml of acetone, and 2 ml of HCl solution. The mixed solution mixed with the sample is then hydrolysed by reflux for 30 minutes. The mixture was then filtered using cotton, filtrate poured into a 100 ml measuring flask. The next step was the resulting residue refluxed with 20 ml of acetone for 30 minutes, filtered, and mixed in a 100 ml measuring flask. The filtrate mixture in the measuring flask was supplemented with acetone to 100 ml. The filtrate taken 20 ml was poured into the separating funnel, added with 20 ml of water, and extracted three times with 15 ml of ethyl acetate. Ethyl acetate fraction was collected and added with ethyl acetate to 50 ml in a measuring flask. The sample solution to be tested was taken from the mother liquor. The sample solution came from 10 ml of the mother liquor and was added with 1 ml of AlCl$_3$ solution and acetic acid solution up to 25 ml in a measuring flask. In addition to the preparation of the sample solution, the blank solution was made as a comparison. The blank solution was taken 10 ml from the mother liquor, then added with a solution of glacial acetic acid to 25 ml in a measuring flask. Measurements were conducted 30 minutes after the addition of AlCl$_3$ using a spectrophotometer at 425 nm wavelength with a quercetin standard.
Calculation:
\[ \% = \frac{C_p \times (A_s - A_{bs})}{(A_p - A_{bp}) \times 1.25 \times 100}{\text{Sample weight}} \]

where:
- \( C_p \) = standard concentration
- \( A_s \) = absorption of sample
- \( A_{bs} \) = absorption of blank sample
- \( A_p \) = absorption of standard
- \( A_{bp} \) = absorption of blank standard

2.5. Test for Tannin Content [14]

2 grams samples were weighed and then heated with 500 ml boiling water for 30 minutes. Then stuck for a few minutes and poured slowly through a lump of cotton into a 250 ml flask. The filtration was repeated several times until the solution when reacted with iron (III) ammonium sulfate did not show any tannin. Furthermore, the liquid was cooled and added with water up to 250 ml. The 25 ml solution was poured into a 1000 ml flask and added with 750 ml of water and 25 ml of LP indigo sulfonic acid, titrated with 0.1 N potassium permanganate to a golden yellow solution. 1 ml of potassium permanganate 0.1 N was equivalent to 0.004157 grams of tannin. Titration was carried out also for a blank.

The indigo sulfonic acid LP was made with 1 gram of indigo of carmin P dissolved in sulfuric acid P, then added with 25 ml of P sulfuric acid again and diluted with water to 1000 ml.

2.6. Inhibition Zone Test

The inhibition zone test carried out in this study used the well method reported by Mukti [15] and Suparno et al. [3].

2.7. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration Tests

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests of the leaves extracts against E. coli, B. subtilis, and S. aureus were carried out using liquid dilution method repoted by Nuraina [16] and Suparno et al. [3].

3. Results and Discussions

3.1. Phytochemical Contents

Phytochemical test in this study was conducted to show the presence of substances or phytochemicals in tropical-almond, morinda, and white leadtree leaves extracts. Phytochemicals play a role in inhibiting microbial activity. The results of qualitative tests of the three leaves extracts were presented in Table 1. Phytochemical contents of some types of leaves were in accordance with previous studies. According to Kadam et al. [17], the tropical-almond leaves have a blackish-green leaf colour and a distinctive odour. The leaves of this plant contain phytochemicals such as carbohydrates, amino acids, glycosides, flavonoids, essential oils, alkaloids, tannins, and steroids. Krishnaiah et al. [18] reported that the main components of morinda are terpenoids, alkaloids, glucoside flavonoids, and some amino acids. The contents of tannins, saponins, flavonoids, phlobotanins, steroids, and glycosides in white leadtree leaves show good antimicrobial and antioxidant properties [19] [3].

Table 1 shows that morinda leaf contains alcaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, steroids, and glycosides, whereas the tropical-almond and white leadtree leaves did not contain triterpenoids. Widiyati [20] states that triterpenoids are secondary metabolite compounds whose skeletons derived from acyclic C30 hydrocarbon derivatives and have alcohol groups, aldehydes or carboxylic acids. Most of the morinda plant components contain triterpenoids that are used as medicines. Ghalib [21] reported that active compounds such as alkaloids, saponins, and flavonoids in the leaves act as antifungals, thereby preventing mould activity. Quantitatively, the total contents of phytochemicals such as total flavonoids and tannin contents are presented in Table 2.
Table 1. Phytochemical test of the leaves extracts.

| Phytochemicals | Tropical-almond | Morinda | White leadtree* |
|----------------|-----------------|---------|-----------------|
| Alcaloids      | +               | +       | +               |
| Saponins       | +               | +       | +               |
| Tannins        | +               | +       | +               |
| Fenolics       | +               | +       | +               |
| Flavonoids     | +               | +       | +               |
| Triterpenoids  | -               | +       | -               |
| Steroids       | +               | +       | +               |
| Glycosides     | +               | +       | +               |

*Suparno et al. [3]

Table 2. Total flavonoids and tannin content.

| Leaves Extracts | Total Flavonoids (% w/w) | Tannin Content (%) |
|-----------------|---------------------------|--------------------|
| Tropical-almond | 1.14                      | 1.51               |
| Morinda         | 0.61                      | 0.36               |
| White-leadtree  | 0.60                      | 4.82               |

*Suparno et al. [3]

White leadtree leaf extract contained the highest tannin content (4.82%). According to Kumar et al. [22], the morinda fruit extract contains total tannins of about 3.5 mg/g. The tannin content in tropical-almond leaves can be an anti-quorum sensing (QS) in Chromobacterium violaceum and Pseudomonas aeruginosa [23]. High levels of tannin affect the antibacterial inhibition. Tannins can precipitate proteins and inhibit the enzyme reverse transcriptase and DNA topoisomerase, so that no bacterial cell formation [24].

The highest total flavonoid was obtained from tropical-almond leaf extract (1.14%). According to Muryati et al. [25], tropical-almond leaves ethanol extract at concentration of 1% contain total flavonoid of 0.29%. Flavonoids can reduce the thickness of the organism because it has a broad spectrum on antimicrobial activity [26].

3.2. Yields

The yield of the extracted simplicia was measured after the ethanol extract was evaporated using a rotary evaporator. The yield was influenced by the amount of compound which can be extracted by the solvent and had the same polarity level [27]. The yields of the leaves extracts are shown in Table 3.

Table 3. Yields of the leaves extracts.

| Leaves          | Yields (%) |
|-----------------|------------|
| Tropical-almond | 22±0.70    |
| Morinda         | 29±0.70    |
| White leadtree* | 24±0.79    |

*Suparno et al. [3]

Table 3 shows that morinda leaf extract gave the highest yield (29%). It was influenced by the number of polar compounds that can be extracted by 70% ethanol [27]. The yield was a yield of leaf extract with a total solid content of 50%. The morinda leaf contained more polar compounds than the other leaves so that the extracted compounds increased, so that increased the extract yield (Table 3). The compounds
that can be extracted with 70% ethanol were alkaloids, flavonoids, saponins, terpenoids, and tannins [28]. Several extraction yields with 70% ethanol maceration process are 22.6% for purple cabbage powder and 22.53% for the henna tree leaf [29]. The yields obtained from our research were similar with those of some other extracts with the same solvent and type of extraction.

3.3. Inhibition Zones
The antimicrobial activity of the leaf extract was measured by inhibition zone, minimal inhibitory concentrations (MIC), and minimum bactericidal concentrations (MBC) tests. Inhibition zone test is performed to determine the activity of extracts or substances in inhibiting bacterial growth. The inhibitory zone is characterized by a clear zone around the well or sample hole indicating the absence of bacterial activity [30]. Measurement of inhibition zone using concentrated extracts was carried out to show the potential of antimicrobials in the extract. The inhibition zone are presented in Table 4.

Table 4. Inhibition zones (cm) of the leaves extracts against B. subtilis, S. aureus, and E. coli.

| Extracts          | Bacillus subtilis | Staphylococcus aureus | Escherichia coli |
|-------------------|-------------------|-----------------------|------------------|
| Tropical-almond   | 0.91±0.11         | 0.82±0.12             | 1.14±0.25        |
| Morinda           | 0.23±0.14         | 1.04±0.25             | 0.57±0.01        |
| White leadtreea   | 1.50±0.00         | 1.30±0.10             | 1.65±0.06        |
| Chloramfenicolb   | 2.03±0.06         | -                     | -                |
| Ciprofloxacinb    | -                 | 3.26±0.11             | -                |
| Amoxicillinb      | -                 | -                     | 2.63±0.06        |

*aSuparno et al. [3]

Table 4 shows that the inhibition zones of tropical-almond leaf extract against B. subtilis, S. aureus, and E. coli were 0.91 cm, 0.82 cm and 1.14 cm, respectively. The study conducted by Chanda et al. [31] showed that tropical-almond methanol extract is able to inhibit the growth of B. subtilis, S. aureus, and E. coli with the inhibition zones of 0.9 cm, 1.3 cm, and 1.2 cm, respectively. Muhammad and Mudi [2] stated that the difference in results is influenced by the type of solvent used during the extraction of the leaves. The tropical-almond leaf extract contains alkaloids, tannins, and saponins that act as antimicrobials. Riskitavani and Purawani [32] reported that the development of almond-tree plant extracts can also be used to inhibit weeds grass (Cyperus rotundus) because it contains alkaloid, tannin, and saponin metabolite compounds.

The inhibition zones of morinda leaf extracts were 0.23 cm for B. subtilis, 1.04 cm for S. aureus, and 0.57 cm for E. coli. Pandey et al. [33] reported that inhibition zones are resulted in by the presence of flavonoid, alkaloid, and saponin metabolite compounds that inhibit the bacterial activity. The inhibition zone of white leadtree leaf extract was the widest compared to those of moringa, cucumber tree, and cherry leaves extracts [3]. In this research, the white leadtree leaf extract showed also the widest inhibition zone compared to those of tropical-almond and morinda leaves extracts. White leadtree leaf extract can inhibit B. subtilis, S. aureus, and E. coli with inhibition zones of 1.5 cm, 1.3 cm, and 1.7 cm, respectively [3]. Research conducted by Aderibigbe et al. [34], white leadtree leaf extract with n-hexane resulted in inhibition zone of 1.4 cm on 100% extract concentration against B. subtilis, 1.2 cm against S. aureus, and 1.8 cm against E. coli. The inhibition zones formed showed antimicrobial activities of the extracts because of their flavonoids, alkaloids, saponins, tannins, and triterpenoids contents.

The white leadtree leaf contains lupeol, an antibacterial active compound [35]. According to Soedarjo and Borthakur [36], white leadtree plant parts including its leaf contain mimosine that harmful when used as food. Mimosine compound is able to inhibit DNA replication of yeasts [37]. These compounds support the high inhibition zone of white leadtree leaf extract among the other two leaf extracts.
3.4. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration
Tests of minimum inhibitory concentration and minimal bactericidal concentration were performed to measure antibacterial activity of the leaves extracts. According to Nuraina [16], the minimum inhibitory concentration is the lowest concentration of a substance that has the power to inhibit the growth of microorganisms (indicated by the absence of turbidity in the test tube) which has been incubated for 24 hours at 37 °C. The results of minimum inhibitory concentrations are presented in Table 5.

**Table 5.** Minimum inhibitory concentration (μg/ml) of the extracts against *B. subtilis, S. aureus*, dan *E. coli.*

| Extracts    | *Bacillus subtilis* | *Staphylococcus aureus* | *Escherichia coli* |
|-------------|---------------------|-------------------------|-------------------|
| Tropical-almond | 3000                | > 6000                  | 3000              |
| Morinda     | > 6000              | > 6000                  | 6000              |
| White leadtree\(^a\) | 1500                | 3000                    | 1500              |

\(^a\)Suparno et al. [3]

Minimum bactericidal concentration (MBC) is the lowest concentration of a substance that can kill the bacteria characterized by the absence of bacterial growth on the solid medium of 1 ose suspension of the minimum inhibitory concentration tested [16]. The lower the minimum bactericidal concentration, the stronger the antimicrobial activity. Table 6 shows the results of MBC of the leaves extracts. Quantitative results from minimum inhibitory concentrations were performed through the minimum bactericidal concentration test.

**Table 6.** Minimum bactericidal concentration (μg/ml) of the extracts against *B. subtilis, S. aureus*, dan *E. coli.*

| Extracts    | *Bacillus subtilis* | *Staphylococcus aureus* | *Escherichia coli* |
|-------------|---------------------|-------------------------|-------------------|
| Tropical-almond | 3000                | > 6000                  | 3000              |
| Morinda     | > 6000              | > 6000                  | 6000              |
| White leadtree\(^a\) | 1500                | 3000                    | 1500              |

\(^a\)Suparno et al. [3]

Table 5 and Table 6 show that morinda leaf extract was only capable of inhibiting and killing *B. subtilis* at concentration of more than 6000 μg/ml and for *E. coli* at 600 μg/ml. MIC and MBC of tropical-almond leaf extract were at 3000 μg/ml for both bacteria. The antibacterial activity of white leadtree leaf extract against *B. subtilis* and *E. coli* on MIC and MBC of 1500 μg/ml [3]. Low concentrations of MIC and MBC of white leadtree leaf extract compared to the other extracts were supported by its inhibition zone.

MIC and MBC of tropical-almond and morinda leaves extracts against *S. aureus* were more than 6000 μg/ml. The MIC and MBC of white leadtree leaf extract against *S. aureus* were 3000 μg/ml [3]. The presence of tannin in the white leadtree leaf has potential as antioxidant and antimicrobial [38].

Compounds that play a role in the MIC test are metabolite compounds such as alkaloids, flavonoids, steroids, tannins, and quinones [16]. Saponin and flavonoid compounds are capable to alter the permeability of bacterial cell membranes, thus denature proteins in bacterium cell wall [26]. In addition, metabolite compounds that play a role in inhibiting the bacterial activity is tannins.

4. Conclusions
Tropical-almond, morinda, and white leadtree leaves extracts had antibacterial activity. White leadtree (*Leucaena leucocephala*) leaf extract gave the highest antibacterial activity compared to those of tropical-almond and morinda leaves extracts, as indicated by the inhibition zone, minimal inhibitory concentrations (MIC), and minimum bactericidal concentrations (MBC) tests. Therefore, white leadtree leaf extract was more potential as a bactericide for the soaking process in leather manufacturing.
Acknowledgments

The authors acknowledge Direktorat Riset dan Pengabdian Masyarakat (DRPM), Indonesian Ministry of Research Technology and Higher Education for its financial support and Bogor Agricultural University for its facility for conducting the research.

5. References

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