Formulation and Antibacterial Activity Test of Foot Spray with Beluntas Leaf Ethanol Extract (Pluchea Indica L.)

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Abstract. Foot odor is one of the things that is quite disruptive to appearance. the 2014 American Podiatric Medical Association studied a significant increase in the prevalence of foot odor in the community. One of the causes of foot odor is the presence of bacteria, especially the bacteria Bacillus Subtilis. This study used the ethanol extract of beluntas leaf (Pluchea Indica L.) as the active ingredient because the ethanol extract of beluntas leaf has been widely used and proven to have antibacterial activity besides the ethanol extract of beluntas leaf has a high enough compatibility to be used as a cosmetic product, including spray preparations. The spray form was chosen because of its easy and pleasant use. This study aims to make a foot odor control product in the form of a spray with 3 concentrations of beluntas leaf ethanol extract, 0.5%, 1.0% and 1.5% to study the antibacterial activity using the well diffusion method. The results showed that the ethanol extract of beluntas leaf can be formulated into foot spray preparation with the results of the organoleptic examination of the three formulas are clear liquid, and yellow slightly green color. The pH of the preparation is in the range 5.10 - 5.92. In formula 3 (F3) with a concentration of 1.5% beluntas leaf ethanol extract, it has a strong bacterial inhibition zone with an average inhibition zone diameter of 12.5 mm.

1. Introduction
One part of the body that is very important for humans is the feet, both for women and for men, therefore feet need to be kept clean but sometimes they are often ignored, resulting in the appearance of odor problems on the feet. foot odor was found to be derived from isovaleric acid, which is produced when Staphylococcus epidermidis, a resident species of the normal cutaneous microbial flora, degrades leucine present in sweat. In addition, Bacillus Subtilis was detected in the plantar skin of subjects with strong foot odor, and this species was shown to be closely associated with increased foot odor [1]. Bacillus Subtilis is one of the normal flora species which is mostly found on the skin. The Bacillus SP bacteria group is known to have an important role in causing foot odor. Bacillus Subtilis was 11.5% in causing foot odor, while Staphylococcus epidermidis was 86.5% [1].

Many products have been produced for anti-odor, such as anti-bacterial bodyscrub and antibacterial soap. One of the most common cosmetics preparations in the market is the form of spray, which is used on the face, hands and feet. The spray form is chosen based on the nature of the spray which can provide a concentrated content, but at the same time it has a fast drying profile so that it is easy to use and pleasant to the user [2][3]. Pluchea Indica L. [4] is a source of phytochemical compounds, and antioxidants that can protect and prevent damage to cells against free radicals. The main antioxidants in Pluchea Indica L. [5] are triterpenoids, lignin glycosides, tannin, terpenes, including some flavonoids polyphenols, quersetin, quinik acid and derivatives eudesmane. Pluchea. based on the measurement of inhibition, the ethanol extract of beluntas leaf (Pluchea Indica L.) has
anti-bacterial against Staphylococcus aureus, Bacillus Subtilis, and Pseudomonas aeruginosa. [6]. Pluchea Indica widely grown in Indonesia. [7].

This study aimed to determine that the ethanol extract of beluntas leaf can be formulated into foot spray preparation and to test effectiveness foot spray as an antibacterial against Bacillus Subtilis and its stability test.

2. Material and Methods
Beluntas leaf was macerated extract with 96 % ethanol solvent concentrated with a Vacuum rotary evaporator until thick extract is obtained [8]. Thick extract was analyzed qualitatively phytochemical and used for produce of foot spray preparation. Phytochemical analysis conducted to know alkaloids, flavonoids, saponin, tannins, Steroid and triterpenoid. Foot spray is formulated using a base foot spray which is added a variety of Pluchea indica leaf extract. Foot spray was made with four formulas using extracts with different concentrations, as shown in table 1. Basillus subtilis obtain from the Laboratory of Microbiology, Faculty of Pharmacy, University of Buana Perjuangan karawang.

| Table 1. Formulation |
|----------------------|
| Material            | F0 (Blank) | F1 | F2 | F3 |
| Beluntas Leaf Ext    | -          | 0,5 | 1 | 1,5 |
| Glycerin            | 0,2        | 0,2 | 0,2 | 0,2 |
| Alcohol 96%         | 40         | 40 | 40 | 40 |
| Menthol             | 0,5        | 0,5 | 0,5 | 0,5 |
| Perfume             | 0,5        | 0,5 | 0,5 | 0,5 |
| Tween 20            | 4,3        | 4,3 | 4,3 | 4,3 |
| Aqua demineralisata added till | 100 | 100 | 100 | 100 |

Evaluation of foot spray preparations is by organoleptic testing including color, odor, pH test using a pH meter, antibacterial activity test and stability test observed for 12 weeks at room temperature conditions.

The antibacterial activity test of foot spray preparation containing beluntas leaf extract was carried out against Bacillus Subtilis by using the well diffusion method for formulas one (F1), two (F2), three (F3) and negative control or without extract (F0). The test was carried out three times for all formulas. The materials used in the antibacterial power test were MHA (Mueller Hinton Agar), Mac Farland standard solution to make test bacterial suspensions, sterile distilled water as a solvent for testing bacteria and Bacillus Subtilis bacteria.

3. Result and Discussion
Based on the results of the phytochemical screening test, it was found that the ethanol extract of beluntas leaf was positive for alkaloids, flavonoids and tannins. Result of phytochemicals beluntas leaf extract (Pluchea Indica L.) is in table 2.
Table 2. Phytochemicals results of *Pluchea Indica* leaf ethanol extract.

| Secondary Metabolite | Result |
|----------------------|--------|
| Alkaloid             | +      |
| Flavonoid            | +      |
| Saponin              | -      |
| Tannin               | +      |
| Steroid              | -      |
| Triterpenoid         | -      |

Activities of flavonoids, moreover, are related to their ability to form complexes with proteins from the cell wall, which will result in damage to the permeability of the bacterial cell wall. Flavonoids have an antibacterial effect because of its ability to interact with DNA of bacteria [9].

There are four formulas of foot spray of beluntas leaf extract consisting of 0.0 % (F0), 0.5 % (F1), 1.0 % (F2), 1.5 % (F3). The results of the formulas showing there is relationship between concentration of the extract with color, the higher the concentration of extract (active ingredient), the color is getting darker. As shown in figure 1.

![Figure 1. Foot spray of beluntas leaf extract. Description: F0 = 0.0 % Extract, F1 = 0.5 % Extract, F2 = 1.0 % Extract, F3 = 1.5 % Extract](image)

The observation shows that foot spray containing extracts of beluntas leaf 0.5 % (F1), 1.0 % (F2) and 1.5 % (F3) have the same characteristics as F0, but have a yellow slightly green color (Table 3). The colors of F2, F3, and F4 were derived from the ethanol extract of beluntas leaf.

Table 3. Organoleptic Test

| Formula | Appearance | Color                | Smell                  |
|---------|------------|----------------------|------------------------|
| F1      | Clear, Liquid | Colorless            | Floral fruity smell   |
| F2      | Clear, Liquid | Yellow slightly green | Floral fruity smell   |
| F3      | Clear, Liquid | Yellow slightly green | Floral fruity smell   |
| F4      | Clear, Liquid | Yellow slightly green | Floral fruity smell   |
The results of the pH examination, the pH range of the formula F0, F1, F2 and F3 was obtained between 4.58 - 5.92. The results of checking the pH of the four preparations are shown in Table 4.

| Formula | pH  |
|---------|-----|
| F0      | 4.58|
| F1      | 5.10|
| F2      | 5.92|
| F3      | 5.92|

The degree of acidity (pH) of the formula without extract (F0), formula one (F1), formula two (F2) and formula three (F3) is still included in the skin pH range of around 4.5 - 6.5 [10]. This indicates that the pH of 5.10-5.92 in the foot spray with beluntas leaf extract is still in the pH range of the foot skin so it can be used safely for feet.

The results of the antibacterial test using the well diffusion method, it is found that all formulas provide the inhibition zone diameter and the results of the examination can be seen in Figure 2 and the inhibition zone can be seen in Table 5.

Based on the results of the research that has been carried out, formula 0 without active substances has antibacterial activity against Bacillus Subtilis in the weak inhibition category with an average diameter of 4.5 mm, in the inhibition area in Formula 1 with the amount of extract 0.5% has antibacterial ability against Bacillus Subtilis moderate inhibition category with an average diameter of the inhibition area of 6.7 mm, in the inhibition area in Formula 2 with the amount of extract 1.0% has antibacterial ability against Bacillus Subtilis moderate inhibition category with an average diameter of the inhibition area of 9.3 mm, and for formula 3 with a concentration of extract 1.5% had strong antibactery activity with a diameter of the inhibition area of about 12.5 mm [11].

![Figure 2. Antibacterial Activity Test](image)
The observations it can be seen that the increasing concentration of the extract also increases the inhibitory zone of the bacteria Bacillus Subtilis. Formula 0 without beluntas leaf extract provides 4.5 mm inhibition and shows a weak inhibition zone, and with the addition of the extract strengthens the inhibition zone of bacteria to a diameter of 12.5 mm for formula 3 with the addition of beluntas leaf extract was 1.5%.

Formula 0 without beluntas leaf extract provides a weak inhibition zone of 4.5 mm which can be caused by the ethanol content of 40% w/w in the formula. Alcohol is used in this formula as a solvent, provides a cool sensation to the preparation and makes the sprayed preparation dry on the feet quickly after spraying.

From the above results, formula three with a concentration of 1.5% beluntas leaf ethanol extract is effective in blocking the development of the Bacillus Subtilis bacteria which is a group of gram-positive bacteria. The strength of the extract in blocking bacterial development is due to the cell wall properties of the tested bacteria. Gram positive bacteria have a simpler cell wall structure that makes it easier for antibacterial compounds to enter the cells and reach their workplaces, while gram-negative bacteria have better resistance than gram-positive bacteria to antibacterial compounds [12]. Bacteria will die immediately if there is no cell wall, because they are unable to survive the influence of the surrounding environment [13].

Formulas 1, 2 and 3 that are produced are tested by putting them in a spray bottle, from the results of testing formulas 1, 2 and 3 can be sprayed, because formulas 1, 2 and 3 are clear liquids. Foot spray and spray bottle preparations can be seen in Figure 3.

| Formula | Inhibition zone Dia. |
|---------|----------------------|
| F0      | 4.5 mm               |
| F1      | 6.7 mm               |
| F2      | 9.3 mm               |
| F3      | 12.5 mm              |

**Figure 3. Foot Spray Preparation**
Product stability test results for 12 weeks at room temperature (28-30 °C), formula 1, 2 and 3 stable, clear liquid yellow slightly green color, pH 5.11-5.68 and floral fruity smell. Foot spray with beluntas leaf ethanol extract stable and can be used for feet.

4. Conclusion
Beluntas leaf ethanol extract (Pluchea Indica L.) can be formulated into foot spray preparation and have antibacterial activity against Bacillus Subtilis in vitro. Formula 3 (F3) of foot spray on with the addition of 1.5 % Pluchea indica leaf extract is a formula most excellent and effective against Bacillus Subtilis and stable in storage for 12 weeks at room temperature (28-30 °C).

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