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Antibacterial Activity of Deodorant Stick Formula from Ethyl Acetate Fraction of Beluntas Leaves (*Pluchea Indica* L.) against *Pseudomonas Aeruginosa* and *Streptococcus Pyogenes* Bacteria that Cause Body Odor

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Abstract

Beluntas contains flavonoids, tannins and steroids which can inhibit growth and reduce the risk of infection by microorganisms. Empirically, beluntas leaves are used by people to overcome unpleasant body odor, usually body odor is caused by the *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. Stick deodorant is a cosmetic made from; sodium stearate (steric acid and sodium hydroxide) and as a solvent using propylene glycol. The aim of this research was to formulate the ethyl acetate fraction of beluntas leaves into a stick deodorant and test the antibacterial activity of *Pseudomonas aeruginosa* and *Streptococcus pyogenes* using the well diffusion method and test physical, chemical and quality parameters. microbiology. Stick deodorant were made into three formulations with different concentrations of the ethyl acetate fraction of beluntas leaves, namely Formula I with a concentration of 20%, Formula II is 30% and Formula III is 40% with the *harding agent* stearic acid: NaoH 10% (8.5:7.5). The quality test of the three formulation sticks deodorants met the requirements for pH test (range 6-7.0), homogeneity test, melting point test (range 55.40-60.9 °C), Microbiology test and water capacity test (aw). The results of the inhibition test against *Streptococcus pyogenes* were between 23.33-8.33 mm in the very strong inhibition category, the comparison deodorant had no inhibitory area and gentamycin 0.01% as a positive control had an inhibitory area of 29.66 mm in the very strong category. The inhibitory power against *Pseudomonas aeruginosa* is 11.5-18.92 mm in the strong inhibitory category, the comparison deodorant has no inhibitory area and 0.01% gentamycin as a positive control has inhibitory area of 21.66 mm in the very strong category.

Keywords: Ethyl acetate fraction, stick deodorant, Pluchea indica.L, Pseudomonas aeruginosa, Streptococcus pyogenes, inhibition test

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Beluntas mengandung flavonoid, tanin dan steroid yang dapat menghambat pertumbuhan dan mengurangi resiko infeksi mikroorganisme. Secara empiris daun beluntas digunakan oleh masyarakat dalam mengatasi bau badan yang tidak sedap, biasanya bau badan disebabkan oleh bakteri *Pseudomonas aeruginosa* dan *Streptococcus pyogenes.* Deodoran stik adalah kosmetika yang berbahan dasar; natrium stearat (asam sterat dan natrium hidroksida) dan sebagai pelarut menggunakan propilen glikol. Tujuan Penelitian ini yaitu memformulasikan fraksi etil asetat daun beluntas menjadi deodorant stik dan uji aktivitas antibakteri *Pseudomonas aeruginosa* dan *Streptococcus pyogenes* dengan metoda difusi sumuran serta uji parameter mutu fisika , kimia dan mikrobiologi. Deodorant stik dibuat menjadi tiga formulasi dengan perbedaan konsentrasi fraksi etil asetat daun beluntas yaitu Formula I dengan konsentrasi 20%, Formula II adalah 30% dan Formula

III adalah 40% dengan *harding agent* asam stearat:NaOH 10% (8,5:7,5). Uji Mutu deodorant stik ketiga formulasi memenuhi persyaratan uji pH (dirange 6-7,0), uji homogenitas, uji titik lebur (dirange 55,4-60,9 °C), uji Mikrobiologi dan uji kapasitas air (aw). Hasil uji daya hambat terhadap bakteri *Streptococcus pyogenes* adalah antara 23.33-28.33 mm dengan kategori daya hambat sangat kuat, deodorant pembanding tidak adanya daerah hambat dan gentamycin 0,01% sebagai kontrol positif daerah hambat 29,66 mm kategori sangat kuat. Daya hambat terhadap bakteri *Pseudomonas aeruginosa* adalah 11,5- 18,92 mm dengan kategori daya hambat kuat, deodorant pembanding tidak adanya daerah hambat dan gentamycin 0,01% sebagai kontrol positif daerah hambat 21,66 mm dengan kategori sangat kuat.

Kata Kunci: Fraksi etil asetat, deodoran stik, daun beluntas (Pluchia indica L.), Pseudomonas aeruginosa, Streptococcus pyogenes dan uji daya hambat

INTRODUCTION

Beluntas leaves (*Pluchea indica* L.) are often used by people to overcome bad body odor and bad breath, by taking beluntas leaves and then steaming them or making them as fresh vegetables. Apart from that, it can also overcome menstrual pain by taking 20 beluntas leaves, then soaking them in hot or warm water and drinking them [1-3]. Research on the inhibitory power of the ethyl acetate extract and fraction of beluntas leaves against the bacteria that cause body odor, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*, previous research has been conducted in 2020. The results of the research on the ethanol extract of beluntas leaves had a minimum inhibitory concentration (MIC) value against the bacteria *Streptococcus pyogenes* at a concentration of 20% and for *Pseudomonas aeruginosa* the MIC value is at a concentration of 10%. Meanwhile, the ethyl acetate fraction has an MIC value at a concentration of 2.5% against *Streptococcus pyogenes* bacteria and for *Pseudomonas aeruginosa* an MIC value at a concentration of 5%. The inhibitory power of the ethyl acetate fraction against bacteria that cause body odor is stronger than the extract. In the ethyl acetate fraction, In the ethyl acetate fraction, the flavonoid compounds that are most often taken are the flavanol group, such as myricetin, quercetin and kaempferol, which have great activity in inhibiting bacteria. [4].

Indonesia is a tropical country where the sun is always shining, so sweating cannot be avoided. For a person, excessive sweating can cause unpleasant body odor. Bad body odor often makes a person feel less confident. This unpleasant aroma usually appears when a person starts to sweat. There is sweat that smells, but there is also some that doesn't. Usually, unpleasant odors occur with body odor caused by bacteria that cause body odor, including *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. This bacterium naturally lives on human skin mucous membranes [5-7].

Deodorant with antiperspirant agent from beluntas leaves is the answer to this need, because it can prevent and eliminate body odor by inhibiting the decomposition or decomposition of sweat by bacteria. Body odor is usually closely related to increased sweat output (perspiration) from both eccrine and apocrine sweat glands, so antiperspirants suppress skin perspiration, needed to complete these cosmetics [8-10]. Deodorant is a cosmetic preparation that contains antiseptics to inhibit or reduce bacterial decomposition so that it can control body odor [8].

Previous studies have shown that beluntas leaves (*Pluchea indica L.*) contain active compounds such as flavonoids, tannins, and saponins that have antibacterial activity. Several studies have also reported that the ethyl acetate fraction of medicinal plant extracts has greater potential to inhibit bacterial growth than crude extracts. However, there have not been many studies that specifically evaluate the effectiveness of the ethyl acetate fraction of beluntas leaves in stick deodorant formulations against bacteria that cause body odor, such as Pseudomonas aeruginosa and Streptococcus pyogenes. Therefore, this study was conducted to develop and test the antibacterial activity of a stick deodorant formula based on the ethyl acetate fraction of beluntas leaves in order to determine its effectiveness in inhibiting the growth of bacteria that cause body odor and to develop effective and safe deodorant products for the extract.

MATERIALS AND METHODS

Materials

The materials used are beluntas leaf simplicia, *Nutrient Agar media*, *Mueller Hinton* (MHA) media, *trytic soy agar* (TSA) media, $BaCl_2$, NaCl, H_2SO_4, ATCC culture of *Streptococcus pyogenes*, ATCC culture of *Pseudomonas aeruginosa*, cetyl alcohol, Propylene glycol stearic acid , Sodium hydroxide, *Dragendroff* reagent, *Lieberman Burchard* reagent, HCl, Glacial acetic acid and Mg powder, *Sabouraud dextrose agar* (SDA), *Trytic Soy Agar* (TSA), Trytic Soy Broth (TSB), *Cetrimide agar* (CETA), *Mannitol Salt Agar* (MSA), perfume, $FeCl_3$, KH_2PO_4, Cetyl

Alcohol, Stearic Acid and ethyl acetate. The distilled water was used to make the reagents.

Methods

Simplicia preparation

Determined Beluntas leaves (*Pluchea indica* L.) are wet sorted by separating foreign objects and dirt attached to the simplicia. Beluntas leaves are washed under running water until clean, then knitted to facilitate the drying and grinding process. Furthermore, beluntas leaves are dried by aerating at room temperature [11].

Preparation of 70% ethanolic extract of beluntas leaves

Extract ethanol 70% of beluntas leaves was fractionated by liquid-liquid partition method with ethyl acetate solvent. 15 g of thick extract was weighed, then dissolved with 30 mL of distilled water. 30 mL of ethyl acetate was then added, fractionated using a separating funnel 5-10 minutes (done 3 times until the fraction obtained was clear). The fraction obtained was evaporated with an evaporator at 50ºC and 80 rpm and obtained ethyl acetate fraction and weighed [11].

Specific and non-specific parameter test of extracts organoleptical

Organoleptical observations are made by using the five senses to determine the shape, color, smell, and taste of beluntas leaf extract [12]

Moisture content test

Add approximately 20 anhydrous methanol. Titrate with Karl Fischer reagent until the end point is reached. Samples that have been weighed with an estimated water content of 10 mg to 50 mg of water into the titration flask, stir for 1 minute. Titrate with Karl Fischer reagent whose water equivalent is known [12]. Water content is calculated in mg using the formula:

$$\text{\% Test water content} = \frac{V \times F}{m} \quad (1)$$

Noted : V : Volume of Karl Fischer reagent at titration
F : Water equivalence factor
M : sample weight (mg)

Ash content

This examination is carried out by preparing an ignition cup and inputting 1 g of extract then burning it in a furnace at 600°C until it becomes ash and the weight remains, cooling it in a desiccator and then weighing it [12]. Ash content is calculated according to the formula below:

$$\text{\% ash content} = \frac{W_1}{W_0} \times 100\ \% \quad (2)$$

Noted : W_1 : initial weight after planting
W_0 : initial weight before planting

Phytochemical screening [13]

Alkaloid

A sample of 0.1 g was taken and put into 2 test tubes, then 3 mL of ethanol was added, heated over a water bath, then filtered. The filtrate obtained was added with 5 drops of Mayer's reagent and Dragendorff's reagent. If a white precipitate is formed, then it is positive for containing alkaloids; for Mayer's reagent, a red-orange precipitate is formed, then it is positive for containing alkaloid

Flavonoids

One gram of sample was taken and put into a test tube then added with 2 mL of 2 N HCl then added with 1 mg of mg powder then shaken until homogeneous. The sample is declared positive for containing flavonoids if a yellow-orange or red color forms

Tannin

One gram of sample was taken then put into a test tube and sufficient distilled water was added and then dripped with sufficient 1% $FeCl_3$. The sample is declared positive if a blackish blue color form.

Saponins

One gram of sample was taken and put into a test tube and 10 hot distilled water was added, then shaken vigorously for ten seconds and then approximately three drops of 2 N HCl were added. The sample is declared positive if it forms and is stable for ten minutes

Steroids and triterpenoids

One gram of sample was taken and put in a test tube, dissolved in 0.5 mL of chloroform then added with 0.5 mL of anhydrous acetic acid. This mixture was then added with 1-2 mL of concentrated H_2SO_4 through the tube wall. If the result obtained is a brownish or violet ring at the border of the two solvents, it indicates the presence of triterpenoids, whereas if a bluish green color is formed, it indicates the presence of steroids.

Examination of alcohol content in extract samples using Gas Chromatography

The sample was weighed approximately 2 g into a 100 mL flask. Dissolved and added with water. Pipette 10 mL into a 100 ml flask and add water to the limit. Pipet 1.0 mL into a 50 mL flask, dissolve and add water to the mark. Analysis of ethanol content in the extract was carried out using the Gas Chromatography (GC) technique with a Flame Atomization Detector (FAD) detector. The analysis was carried out with the following parameters: column G43, velocity 6.8 cm/sec, pressure 19 kPA, column temperature 35 °C

for 10 minutes then increase the temperature at each rate of 10 °C/minute to 200°C and maintain at this temperature for 4 minutes, injector temperature 210°C, detector temperature 280° C, injection volume 0.2 µL-0.4 µL. System suitability test for this analysis was conducted by considering the following provisions alcohol tailing factor: < 2.0 and RSD of area 6x comparator injection: < 4.0%. Ethanol content is determined based on the comparison of the sample peak area to the calibration curve of ethanol standards with a certain concentration range. The results of this analysis are used to ensure that the residual ethanol content in the extract is within the specified safe limits.

Stick deodorant formulation

Stearic acid was melted at a temperature of around 70°C until completely melted (mass 1). After that, 10% NaOH solution is added to mass 1, then stirred for 15 minutes until homogeneously mixed (mass 2). Separately, propylene glycol and cetyl alcohol are mixed (mass 3), then heated at a temperature of around 70 °C until the cetyl alcohol is completely dissolved. After dissolving, the active extract is added to mass 3 while continuing to stir to ensure homogeneity. Next, mass 2 and mass 3 are mixed while continuing to stir to ensure uniformity of the formulation. The mixture is then cooled to a temperature of 55 °C, then perfume is added to taste while continuing to stir. After the formulation reaches a temperature of around 50 °C, the mixture is poured into a stick deodorant mold and allowed to harden until ready for use. The formulations used to make deodorants with various variations (F1, F2, and F3) can be seen in **Table** 1 [14].

Table 1. Deodorant stick formulation

Ingredients	Concentration (%)			Function
	F1	F2	F3	
Ethyl acetate fraction	25	25	25	Active ingredients
Stearic acid	5	7.5	10	Harding agent
NaOH 10%	7.2	8.5	10	Harding agen
Cetyl alcohol	15	15	15	Emulsifier
Perfume	qs	qs	qs	Fragrance
Propilenglikol add	100	100	100	Solvent

Noted: qs: quantum satis

Physics test of deodorant stick formulation [15]

Organoleptical test

Organoleptical examination includes shape, color, aroma and texture.

Homogeneity test

Homogeneity testing by naked eye, whether the deodorant stick preparation still has coarse articles or not, if not, it is said to be homogeneous.

Melting point test

Deodorant stick is cut in half lengthwise with the same weight of each formula, which is 2 g, put into a beaker glass and heated on top. waterbath, the temperature is slowly raised, then observed at what temperature the deodorant melts. The requirement for stick deodorant is melting at 50 C-70 °C.

Activity water test of deodorant stick formulation

Activity water test was conducted using Aw meter (Activity water). The Aw meter is first calibrated by placing technical $BaCl_2{\cdot}H_2O$ liquid into the sample holder. The device is then closed and left for 3 minutes until the Aw reading stabilizes at 0.9. After calibration, the Aw meter is opened, and the sample holder is cleaned to ensure no residue remains. The sample is then placed into the holder, and the device is closed again, allowing it to stabilize for up to 5 minutes. After this period, the Aw value is recorded from the scale. Finally, the temperature scale is checked to determine the correction factor [15].

pH test of deodorant stick formulation

Determination of the pH of the deodorant stick using pH indicator paper, the pH size for skin restrictions is 3.0-7.5 (SNI 16-4951-1998)

Microbiological examination of deodorant stik

Total aerobic microbial count

One gram of the sample was weighed and placed into a test tube containing 9 mL of diluent. The mixture was shaken until homogeneous, creating a 10^{-1} dilution. From the 10^{-1} dilution, 1 mL was taken and transferred into another test tube to achieve a 10^{-2} dilution, and this process continued until a 10^{-3} dilution was obtained. Next, 1 mL of each dilution was pipetted into two separate Petri dishes. Sterile Tryptic Soybean Agar (TSA) was poured into the Petri dishes at a temperature of approximately 44 °C. The Petri dishes were then rotated slowly to ensure the suspension was well mixed and homogeneous. Once the agar had solidified, the Petri dishes were inverted and placed in an incubator for 3 to 5 days at a temperature of 30–35°C. After incubation, the number of colonies in each plate was counted using a colony counter. The acceptance criterion for the results was < 10^3 cfu/g.

Totalt yeast mold count

One gram of the sample was weighed and placed into a test tube containing 9 mL of diluent, which was then shaken until homogeneous to create a 10^{-1} dilution. From this 10^{-1} dilution, 1 mL was transferred

into a new test tube to achieve a 10^{-2} dilution, and the process was repeated until a 10^{-3} dilution was obtained. One mL of each dilution was then pipetted into two separate Petri dishes. Sterile Sabouraud Dextrose Agar (SDA) was poured into the Petri dishes at a temperature of approximately 44°C. The dishes were gently rotated to ensure that the suspension was well mixed and homogeneous. After the agar solidified, the Petri dishes were incubated for 5 to 7 days at 20–25 °C. After the incubation period, the number of colonies on each plate was counted using a colony counter. The acceptance criterion for the results was < 10^2 cfu/g [16].

Specified microorganism Stapylococcus aureus

A sample dilution was prepared in a 1:10 ratio, with no less than 1 g or 1 mL of the sample dissolved in buffer at pH 7.2. Ten mL of this solution was then mixed with 10 mL of Tryptic Soy Broth (TSB) media and incubated for 18 to 24 hours at a temperature of 30–35 °C. After incubation, subcultures were transferred by scraping to Mannitol Salt Agar (MSA) media, which was incubated at 30–35 °C for 18 to 72 hours with the Petri dish inverted. Colony growth was observed, and if yellow or white colonies grew surrounded by a yellow zone, the result was considered positive for *S. aureus*. In the absence of yellow or white colonies surrounded by a yellow zone, the result was negative for the presence of *S. aureus*. The acceptance criterion for the results was negative.

Specified microorganism Pseudomonas aeruginosa

A sample dilution was prepared in a 1:10 ratio, with no less than 1 g or 1 mL of the sample dissolved in phosphate buffer at pH 7.2. Ten mL of this solution was then mixed with 10 mL of Tryptic Soy Broth (TSB) media and incubated for 18 to 24 hours at a temperature of 30–35°C. After incubation, subcultures were transferred by scraping onto Cetrimide Agar (CETA) media and incubated at 30–35 °C for 18 to 72 hours in an inverted Petri dish. Colony growth was observed, and if greenish colonies developed, the result was considered positive for *P. aeruginosa*. If no greenish colony growth was observed, the result was negative for *P. aeruginosa* [17].

Antibacterial activity test of stick deodorant

Preparation of antibacterial test solution

Take 2 g of stick deodorant and add 2.0 ml of 96% ethanol. Stir until homogeneous and the solution contains no undissolved solids [18].

Preparation of a comparison control solution

Carefully weigh the working standard Gentamycin sulfate (potency 661.46 ug/mg) equivalent to 10 mg Gentamycin to a 100 mL flask (gentamycin sulfate 16 mg). Dissolve and add phosphate buffer pH 8.0 (use time limit 30 days, concentration 100 µg/ml or 0.01%)

Preparation of Mc Farland solution

Dissolve 0.05 mL of 1% w/v BaCl2 mixed with 9.95 mL of 1% H_2SO_4 solution, shake until homogeneous. The absorbance value of the Mc Farland standard solution is 0.5 equivalent to bacterial cell suspension concentration of 1.5 x 108 CFU/mL. One dose of bacterial culture each was taken into 5, 10, 25, 50, 100, and 250 mL flasks. Dissolve in sterile 0.9% physiological NaCl and shake first until homogeneous using a vortex. The absorbance value of each sample was measured at the maximum wavelength using a UV Vis Spectrophotometer. Take a dilution of the bacterial culture whose absorbance value is close to the Mc Farland absorbance value of 0.5.

Antibacterial test of deodorant stick with well diffusion method [18. 14]

A total of 12 Petri dishes were prepared for the treatment, consisting of 6 dishes for testing the stick deodorant formulations 3 dishes for *Pseudomonas aeruginosa* bacteria and 3 dishes for *Streptococcus pyogenes* bacteria. Additionally, 2 dishes were designated for comparison samples, 2 for the negative control (96% ethanol), and 2 for the positive control (gentamicin antibiotic). To prepare the bacterial inoculum, 21 mL of Muller Hinton Agar media was poured into a 30 mL Erlenmeyer flask, to which 20 µL of bacterial suspension, adjusted to a 0.5 McFarland turbidity standard, was added and vortexed for homogeneity. The agar mixture was then poured into the Petri dishes (up to 12 dishes), and left to solidify.

For the inhibition test, 6 replications were performed for deodorant formulations 1, 2, and 3, as well as for the comparison deodorant samples. Stainless steel cylinders were placed on the surface of the culture using a cylinder printing pattern, ensuring the center-to-center distance between the cylinders was 25–28 mm. Twenty µL of each deodorant stick formulation at different concentrations was separately introduced into the cylinders, repeating the same procedure for all 12 Petri dishes. This resulted in 6 bacterial inhibition test data points for each formulation. For the comparison control and negative control (96% ethanol), the same procedure was applied with 6 cylinders, and 20 µL of gentamicin solution (100 µg/mL) was added to each hole. Data were collected from 6 repetitions. All treatments were incubated at 37°C ± 0.5 °C for 24 hours. After the incubation period, the clear zones around the cylinders were observed and measured using a caliper.

Analysis Data

Data collection was carried out by direct observation and testing of pH, melting point, homogeneity, organoleptic, activity water test, microbiological test and inhibition test. Observations and measurements for all tests were carried out in the service laboratory, natural ingredients laboratory, microbiology laboratory and pharmaceutics laboratory of Institut Sains dan Teknologi Al Kamal.

RESULTS AND DISCUSSION

Determination, Phytochemical, Organoleptic and Parameter Non-Specific Test Results

The results of the identification of beluntas leaves (*P. indica* L) carried out at the Batu Herbal Materia Medica Laboratory, East Java Provincial Health Office, showed that the samples used confirmed as beluntas leaves (*P. indica* L).

The yield of the extract obtained is 0.27%. This result is in accordance with the requirements of the Herbal Pharmacopoeia in 2017 on the monograph of beluntas leaf extract (> 8.3%). With the yield value as above, many secondary metabolite compounds have been extracted so that it will provide maximum pharmacological effects. Solvents and maceration time play a very important role in producing yields that are in accordance with the Indonesian Herbal Pharmacopoeia [19]. The yield of the ethyl acetate fraction obtained is 12.80%. This is because ethyl acetate solvent is a semi-polar solvent that is able to attract polar and nonpolar compounds.

In plants, flavonoids as antimicrobials can form complexes with extracellular proteins and cell walls. In addition, flavonoids that are lipophilic can damage microbial membranes. Terpenes or terpenoids have antimicrobial activity. The mechanism is not fully known, but it is suspected that these compounds work on membrane destruction by lipophilic compounds. The antimicrobial activity of phenolic compounds is by damaging the lipids in the plasma membrane of microorganisms, causing cell contents to escape [20].

Organoleptic examination is one of the specific parameters determined using the five senses and aims to provide initial recognition and identify extracts that indicate the characteristics of the extract in the form of shape, color, and smell. From the results of organoleptic examination (**Table** 2), it shows that beluntas leaf extract is thick, blackish green in color, and has a pungent odour.

Table 2. Parameter test of ethanol extract and ethyl acetate fraction of beluntas leaves

Parameter	Ethanol extract	Ethyl acetate fraction	Requirement
Determination result			
% yield (%)	10.27	12.8 (From Extract)	> 8.3
Water content	9.57	1.764	< 10
Ash content	7.74	8.05	< 8.1
Residual Solvent	0.0987	0.31	<1.0
Compound content			
Flavonoid	Positive	Positive	-
Alkaloid	Negative	Negative	-
Tanin	Positive	Positive	-
Saponin	Positive	Negative	-
Steroid	Positive	Positive	-
Triterpenoid	Negative	Negative	-
Organoleptic			
Description	Viscous	Viscous	-
Color	Blackish-green	Blackish-green	-
Odor	Stinging	Stinging	-

The moisture content in the extract can be influenced by the humidity of the storage area, as well as the remaining water contained in the extract. Moisture content can affect microbial growth in extracts, because water is a good growth medium for microbes, so that if the moisture content of an extract is high, the easier it is for the extract to grow microbes. According to Supplement I of Herbal Pharmacopoeia RI 2010 the moisture content of an extract must be limited to the requirement of no more than 10%. Determination of ash content is carried out to provide an overview of the internal and external mineral content starting from the processing process until the formation of extracts, this parameter can describe the purity and contamination of an extract [21]. The ash content results obtained are in accordance with the requirements of the Herbal Pharmacopoeia 2017.

Optimization of stick deodorant formulations and comparison samples

Microbial, physics and chemistry quality evaluation

The microbiological test results all met the requirements as shown on **Table** 3. A preparation used on the skin must meet Pharmacopoeia standards. For standards, refer to USP 45 for preparations used on the skin [22]. From the results obtained, all microbiological tests meet the requirements. With an oil base, it is likely that bacteria will find it difficult to live with the lack of water content in the preparation.

The homogeneity test is carried out to determine whether the materials used are mixed evenly or homogeneously and do not contain solid particles. Homogeneity testing is carried out by looking at the preparation with the naked eye, whether the deodorant stick preparation still contains coarse particles or not, if not then it is said to be homogeneous. Deodorant stick must have a good mass and not contain coarse particles so that when applied to the skin it feels soft so as not to affect comfort in use [23].

The pH test is carried out to determine the properties of the deodorant produced on the skin. pH measurements are carried out using a universal pH stick. The pH limit for skin is 3.0-7.5. pH areas below 7 (acid), are not optimal conditions for bacterial growth, because bacteria are more numerous in alkaline conditions [23]. The pH results of all formulas and comparators still meet the requirements. The ratio of stearic acid and 10% NaOH really determines the pH of stick deodorant Organoleptic tests on all solid form formulations. With a harding agent, the ratio of stearic acid to 10% NaOH = 8.5: 7.5 is a good combination to produce a solid deodorant.

Table 3. Microbial, physics and chemistry quality evaluation of deodorant stick formulation from ethyl acetate fraction of Beluntas leaves

Parameter test	Result				Regulation of USP 45
	F I	F II	F III	P	
Microbial test Evaluation					
Stapylococcus aeruus	Negative	Negative	Negative	Negative	Negative
Pseudomonas aeruginosa	Negative	Negative	Negative	Negative	Negative
Total Khamir and mold count	28 cfu/g	38 cfu/g	56 cfu/g	<1 cfu/g	<100 cfu/g
Total microba aerob count	35 cfu/g	27 cfu/g	55 cfu/g	<1 cfu/g	<1000 cfu/g
Physics and Chemistry Test					
Descriptive	solid	solid	solid	solid	solid
Odor	Aromatic	Aromatic	Aromatic	Menthol	-
Color	Light brown	Light brown	brown	Yellowish white	-
pH	6	6.5	7.0	7.5	3.0 -7.5
homogenity	homogeneous	homogeneous	Homogeneous	Homogeneous	Homogeneous
Texture	solid	solid	solid	solid	solid
Melting point	55.4^{0}C	57.7 ^{0}C	60.9 ^{0}C	>95 ^{0}C	50-70 ^{0}C
Activity water	0.6472	0.6108	0.6278	0.6354	<0.97

Noted: F I = Formula I (20% fraction), F II= Formula II (30% fraction), FIII= Formula III (40% fraction), P = comparative sample

Water activity (aw) is the amount of free water in the product, not bound water. The aw value ranges from 0 to 1.0. Pure water has an activity value of 1.0. AW values that meet the requirements will reduce the susceptibility of the formulation to microbial contamination [22]. Judging from the microbiological testing of all formulations and comparative deodorants, they meet microbiological requirements, where the Aw value meets the requirements.

Antibacterial activity test of stick deodorant

Table 4 shows the higher the concentration of the ethyl acetate fraction in the deodorant, the higher the inhibitory power against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. The active substances contained in the ethyl acetate fraction of beluntas leaves are antimicrobial substances in the form of flavonoids, steroids and tannins which are phenolic compounds that can inhibit the growth of *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. Compared with *Streptococcus pyogenes*, the inhibitory power of the ethyl acetate fraction on *Pseudomonas aeruginosa* is weaker.

This difference is due to the difference in the cell walls of gram-positive and gram-negative bacteria. *Streptococcus pyogenes* bacteria are gram positive and *Pseudomonas aeruginosa* bacteria are gram negative. In general, gram-positive bacteria are more sensitive to antibacterial substances compared to gram-negative bacteria because the cell walls of gram-positive bacteria do not have lipopolysaccharides so that antibacterial compounds that are both hydrophilic and hydrophobic can pass through the cell walls of gram-positive bacteria through a passive diffusion mechanism and then interact directly with the

peptidoglycan in the cells. growing bacteria and causing cell death [24].

Antibacterials in beluntas leaves inhibit bacterial growth by disrupting performance, inhibiting metabolic processes and damaging bacterial cells. Flavonoids are antibacterials that first work on cell walls which contain peptidoglycan. The function of Peptidoglycan is to resist damage when there is high osmotic pressure. Flavonoids have the same polarity as peptidoglycan so they can penetrate peptidoglycan and cause disruption of bacterial cell walls. Tannins can inhibit bacterial growth by binding to proteins in the process of protein synthesis where protein synthesis carried out by bacteria functions as a process for reproduction. Tannins will bind the adhesion protein as a receptor which will reduce adhesion, inhibit protein synthesis, and disrupt permeability [25].

Table 4. Test results of the Inhibition test of beluntas leaf ethyl fraction stick deodorant against *Streptococcus pyogenes* and *Pseudomonas aeruginosa* bacteria

Sample Formulation	*Streptococcus pyogenes*		*Pseudomonas aeruginosa*	
	Zone inhibitory (mm)	Inhibitor category	Zone inhibitory (mm)	Inhibitor category
I (20% Ethyl acetate fraction)	23.33	Very Strong	11.50	Strong
II (30% Ethyl acetate fraction)	24.42	Very Strong	12.75	Strong
III (40% Ethyl acetate fraction)	28.33	Very Strong	18.92	Strong
Control positive (Gentamicin 0.01%)	29.66	Very Strong	21.66	Very Strong
Control Negative (etanol 96%)	-	-	-	-

CONCLUSION

The ethyl acetate fraction of beluntas leaves can be formulated into a stick deodorant preparation. The physical, chemical and microbiological tests of the formulated stick deodorant and comparison samples all meet the requirements. The inhibitory power of beluntas leaf ethyl acetate fraction stick deodorant against *Streptococcus pyogenes* bacteria in formulations I to III provides a Very strong inhibitory power category. The inhibitory power of beluntas leaf ethyl acetate fraction stick deodorant against *Pseudomonas aeruginosa* bacteria in formulations I to III provides a strong inhibitory category (more than 10-20 mm). For the inhibitory power of the comparison sample stick deodorant, no clear zone was formed against the bacteria *Streptococcus pyogenes* and *Pseudomonas aeruginosa* (no inhibitory effect)

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REFERENCES

[1] N. Rizqiyana, K. Oom, and Y. W. Ike, "Formulation of Roll on Deodorant from Pluchea indica L. Leaf Extract as Antibacterial Against Staphylococcus epidermidis," *Journal of Pharmacym*, vol. 3, pp. 45-54, 2017.

[2] M. M. Nahak, "*Pluchea indica L.* Leaf Extract can Inhibit the Growth of Streptococcus mutans Bacteria," *Dental Health Journal*, Vol. 1, pp. 1-10, 2013.

[3] P. B. M. Hanry, "Effectiveness of *Pluchea indica Less.* Leaf Extract in Inhibiting the Growth of Staphylococcus epidermidis Bacteria," Thesis. Faculty of Medicine and Health Sciences, Tadulako University, Palu, 2016.

[4] U. Alfian "Antibacterial Test of Extract and Fraction of beluntas leaves (Pluchea indica L.) Against Pseudomonas aeruginosa and Streptococcus pyogenes bacteria." Thesis. Faculty of Science and Technology Al Kamal Pharmacy Study Program, Jakarta, 2020.

[5] J. N. Lundström and M. J. Olsson. "Functional neuronal processing of human body odors," *Vitamins and Hormones*, vol. 83, pp. 1–23, 2010.

[6] R. C. Egbuobi, G. C. Ojiegbe, J. N. Dikendudim, and P. C. Enwun, "Antibacterial Activities of different brands of deodorants marketed inowerrri, imo state, Nigeria," *African Journal of clinical and experimental microbiology* 14 (1) :14-1. 2013

[7] R. Nurhaini, M. Arrosyid, and H. Putri, "Formulation and Antibacterial Activity Test of Cream Deodorant with Variations of Cananga

Flower Essential Oil (Cananga odoratavar. Macrophylla) as Body Odor Remover," *J. Farm Science*., vol. 13, no. 1, pp.20–30, 2022

[8] H. Butler, *Poucher's Perfumes, Cosmetics and Soap, 10th Edn*. Britain: Kluwer Academic Publisher. 2010, pp.68-100.

[9] S. Rahayu, Sherley, S. Indrawati, "Deodorant Antiperspiran" *Naturakos*, vol. 4, pp. 1-12, 2009.

[10] A. F. Zulfa, *The Effect of Different Concentrations of Propylene Glycol on the Physical Properties Test of Deodorant Spray Preparations of Beluntas Leaf Extract (Pluchea indica L.)*. Tegal: Harapan Bersama Polytechnic, 2019.

[11] J. Poucher, *Poucher's Perfume, Cosmetics and Soap, 10th Edition*. Kluwer: Academic, Netherlands, 2000.

[12] R. I. Tranggono and F. Latifah, *Cosmetic Science Handbook*. PT. Gramedia Pustaka Utama: Jakarta, 2007, pp. 90-93.

[13] E. Suru, P. V. Y. Yamlean, and W. A. Lolo, "Formulation and Effectiveness Test of Antibacterial Cream of Ethanol Extract of Beluntas Leaves (Pluchea indica Less.) Against Propionibacterium acnes bacteria," *Pharmacon Journal*, vol. 8, pp. 1-11, 2019.

[14] I. Y. Wiendarlina, D. Indriati, and M. Rosa, "Antibacterial Activity of Anti-Acne Lotion Containing Beluntas Leaf Extract (Pluchea indica (L) Less.)," *Phytopharmaca*, Vol. 9, pp. 1-10, 2019.

[15] M. D. Q. Rohman, "Optimization of HPMC and Carbopol in the Formulation of Anesthetic Gel Preparations from Beluntas Leaf Extract (*Pluchea indica L.*) and Activity against Staphylococcus aureus," *Jurnal Insan Farmasi Indonesia*, vol. 3, pp. 327-336, 2020.

[16] R. P. Handayani, J. Pusmarani, and N. H. A. Halid, "Formulation and Activity Test of Deodorant Spray Preparations from Beluntas Leaf Extract (*Pluchea indica L.*) Against Staphylococcus epidermidis Bacteria," *Jurnal Pharmacia Mandala Waluya*, vol. 1, pp. 7-12, 2021.

[17] V. H. Hasyim, C. Junaedi, dan N. Marliana, "Efektivitas Antibakteri Staphylococcus Aureusdari Sediaan Sabun Mandi Cair Dari Ekstrak Etanol Daun Beluntas (Pluchea indicaL)," *Jurnal Ilmiah BIOSAINTROPIS*, vol. 8, pp. 65-73, 2022.

[18] M. E. Pratiwi and C. H. B. Tobi, Formulasi dan Evaluasi Edible Film dari Ekstrak Terpurifikasi Daun Beluntas (*Pluchea indica L.*) Sebagai Anti-Sariawan," *Journal of MPI (Media Pharmaceutica Indonesiana)*, Vol. 5, pp. 179-187, 2023.

[19] Kementerian Kesehatan Republik Indonesia, Indonesian Herbal Pharmacopoeia, edition 6, 2017, Jakarta: Kemenkes RI, 2017.

[20] J. Azmir, I. S. M. Zaidul, M. M. Rahman, K. M. Sharif, A. Mohamed, F. Sahena, M. H. A. Jahurul, K. Ghafoor, N. A. N. Norulaini, A. K. M. Omar, "Techniques for extraction of bioactive compounds from plant materials: A review," *Journal of Food Engineering*, vol. 117, pp. 426-436, 2013.

[21] A. Isnawati and K. M. Arifin, "Characterization of Kembang Sungsang Leaves (*Gloria superba L*) from Phytochemical Aspects," *Media Litbang Kesehatan*, vol. 16, 8-1, 2006.

[22] United State Pharmacopeia (USP), "pharmacopeial edition 45," *Pharmacopeial Form*, vol. 45, pp. 1-65, 2022.

[23] M. A. Shahtalebi, M. Ghanadian, A. Farzan, N. Shiri, D. Shokri, and S. A. Fatemi, "Deodorant effects of a sage extract stick: Antibacterial activity and sensory evaluation of axillary deodorancy," *J Res Med Sci*., vol. 18, pp. 833-839, 2013.

[24] S. Debnath, M. N. Babu, G. Kusuma, "Formulation and Evaluation of Herbal Antimicrobial Deodorant Stick," *Res. J. Topical and Cosmetic Sci*. vol. 2, pp. 21-24, 2011.

[25] N. Mu'adah , Sarwiyono, dan E .Setyowati, Daya Hambat Ekstrak Daun Beluntas (Pluchea indica L.) Kering Dengan Pelarut Aqudest terhadap bakteri Streptococcus dysgalactiae Penybab Mastitis Pada Sapi Perah," Fakultas Peternakan Universitas Brawijaya, vol. 4, pp 1-6, 2015.