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Antibacterial activity roll on deodorant with *Pluchea indica* (L.) leaf extract against *Staphylococcus epidermidis* (Evans 1916) *in vitro*

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Abstract. *Pluchea indica* L. is a medicinal plant that is often used to eliminate body odor. This research aims to test effectiveness roll on deodorant as an antibacterial against *Staphylococcus epidermidis* (Evans, 1916) and it's stability test. Antibacterial activity test used dilution method. Phytochemical analysis were carried out qualitatively. Roll on deodorant test for four types stored at 28 °C to 30 °C for 8 wk includes observation of the color, odour and homogeneity, irritation test, pH test, viscosity and specific gravity. The results showed *P. indica* leaf extract's roll on deodorant have antibacterial effect against *S. epidermidis*. *P. indica* leaf extract have compounds consisting of flavonoids, saponins, tannin and alkaloids. Roll on deodorant stored on 28 °C to 30 °C for 8 wk showed stable for color, odour and homogeneity. Deodorant roll on have a pH of 4.52 to 5.53, viscosity 331.8 cP to 340.8 cP, specific gravity 1.0107 g mL⁻¹ to 1.0397 g mL⁻¹, and do not cause irritation.

Keywords: Body odor, microorganism on the skin, phytochemical compound, underarm skin.

1. Introduction

Leather continuous contact with the surrounding environment so easily exposed to microorganisms, some of the microorganisms commonly found on the skin is *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Sarcina* sp., *Mycobacterium* and *Acinetobacter*. The bacteria that can cause body odor as *Staphylococcus epidermidis*, *S. aureus*, *S. pyogenes*, *C. acne* (difteroid), and *Pseudomonas aeruginosa*. *S. epidermidis* is a normal flora of bacteria are dominant on the skin, especially underarm skin that cause body odor [1]. Deodorant used to eliminate body odor. Cosmetics deodorant ingredient is the substance or mixture of substances that can be used to eliminate or reduce the unpleasant body odor. Generally, the deodorant is often used the liquid form (liquid), for example the form of roll on. Deodorants generally contain antibacterial active substances can be derived from natural or synthetic materials. *Pluchea indica* L. [2] is a source of phytochemical compounds, and antioxidants that can protect and prevent damage to cells against free radicals. The main antioxidants in *P. indica* [3] are tannin, terpenes, lignin glycosides, triterpenoids, including some flavonoids polyphenols, quersetin, quinic acid and derivatives eudesmane. *P. indica* widely grown in Indonesia. This study aimed to test effectiveness roll on deodorant as an antibacterial against *S. epidermidis* and it's stability test.



2. Material and methods

The macerated extract with 96 % ethanol solvent concentrated with a vacuum evaporator (Buchi 461) until thick extract is obtained. Thick extract was analyzed qualitatively phytochemical and used for the manufacture of roll on deodorant. Phytochemical analysis conducted to know saponin, flavonoids and alkaloids [4], tannins [5]. Roll on deodorant is formulated using a base roll on which is added a variety of *P. indica* leaf extract has a minimum inhibitory concentration. Roll on deodorant was made with four formulas (table 1) using extracts with different concentrations, as shown in figure 1. *S. epidermidis* obtain from the Laboratory of Microbiology, Faculty of Medicine, University of Indonesia. The Antibacterial activity determined by dilution method (figure 2). Bacteria from culture media dissolved in 5 mL 0.9 % physiological NaCl, turbidity is measured using nefrometer diagnostic in accordance with the standard 0.5 McFarland [6]. Incubated for 24 h at 35 °C to 37 °C. The growth of bacteria observed.

Table 1. Formulation roll on deodorant.

Material	Formula (%)			
	F1 (blank)	F2	F3	F4
<i>P. indica</i> leaf extract	0	3*	4*	5*
HPC-m (hydroxy propyl cellulose-medium)	3	3	3	3
Propylene Glycol	15	15	15	15
BHT (Butylated hydroxy toluene)	0.1	0.1	0.1	0.1
Sodium metabi-sulfite	0.1	0.1	0.1	0.1
Ethanol 96 %	40	40	40	40
Perfume (Apples smell)	0.5	0.5	0.5	0.5
Distilled water added till	100	100	100	100

* Note: The extract is added to the formula is a value and upper of the Minimum Inhibitory Concentration (MIC) of the extract.

Evaluation of roll on deodorant based on antibacterial activity test. Test of irritation were carried out directly on ten male and female volunteers with patch test method. Approximately 0.1 g of the sample is applied to the inner arm with a diameter 2 cm, then covered with gauze [7]. After 24 h observed symptoms. The organoleptic tests include color, odor, and homogeneity, observed for 8 wk. Test of the pH used a pH meter. Viscosity used a Brookfield viscometer. Specific gravity of roll on deodorant is determined by pycnometer.

3.Result and discussion

The average *P. indica* L. powder water content of 4.725 %, and the average of ash content 7.5 %. Water content and ash simplicia adjusted to regulation of Ministry of Health Republic of Indonesia [8]. Result of phytochemicals leaf extract *P. indica* is in table 2.

Table 2. Phytochemicals results of *P. indica* leaf extract.

Identification of Compounds		Parameter	Results of analysis	Conclusion
Saponin		Stable foam	Stable foam	+
Tanin		White precipitate	White precipitate	+
Flavonoids		Red-purple/yellow orange	Yellow orange	+
Bou-chardat		Precipitate dark brown	Precipitate dark brown	+
Alkaloids	Mayer	White precipitate/yellow late	White precipitate	+
	Drag-endorf	Precipitate Orange brown	Precipitate Orange brown	+

Note: (+) there is compound, (-) No compound

Based on minimum inhibitory concentration the results of *P. indica* leaf extract conducted at a concentration of 0.5 %, 1 %, 2 %, 3 %, 4 %, 5 %, 10 %, 15 %, 20 % and 25 %, above concentrations 3 % of *P. indica* leaf extract showed inhibition that is characterized by the absence of bacterial growth. *P. indica* leaf extract has antibacterial activity against *Enterococcus faecalis* at the concentrations of 100 % and 50 % with strong effect and *Fusobacterium nucleatum* only at the concentration of 100 % with moderate effect [9]. So that the minimum inhibitory concentration (MIC) of ethanol extract of *P. indica* leaf to *S. epidermidis* is concentration 3 %. There are four formulas of roll on deodorant of *P. indica* leaf extract consisting of 0 % (F1), 3 % (F2), 4 % (F3), 5 % (F4), as shown in figure 1. 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 increasingly green. As shown in figure 1.



Figure 1. Roll on deodorant of *P. indica* leaf extract. Description: F1 = 0 % Extract, F2 = 3 % Extract, F3 = 4 % Extract, F4 = 5 % Extract.

The observation shows that roll on deodorant containing extracts of *P. indica* leaves 3 % (F2), 4 % (F3) and 5 % (F4) no bacterial growth, based on this results deodorants have effect antibacterial against *S. epidermidis*. While formula 1 which does not contain extracts of *P. indica* leaves overgrown with bacteria *S. epidermidis* (figure 2). The results showed that the roll on deodorant does not cause skin irritation in both men and women (table 4).

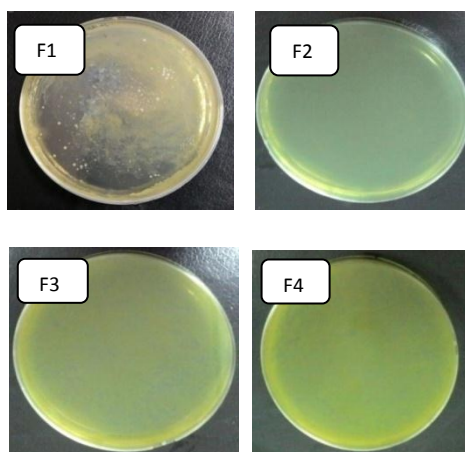


Figure 2. Test results of minimum inhibitory concentration (MIC) of roll on deodorant *P. indica* leaf extract. Note: F1 = There is bacterial growth, F2 = 3 % extract, F3 = 4 % extract and F4 = 5 % extract no bacterial growth.

Table 3. Test results appearance of roll on deodorant

week	Formula											
	F1			F2			F3			F4		
	0 % Extract			3 % Extract			4 % Extract			5 % Extract		
	C	A	H	C	A	H	C	A	H	C	A	H
0	●	++	◆◆	●●	++	◆◆	●●●	++	◆◆	●●●●	++	◆◆
2	●	++	◆◆	●●	++	◆◆	●●●	++	◆◆	●●●●	++	◆◆
4	●	++	◆◆	●●	++	◆◆	●●●	++	◆◆	●●●●	++	◆◆
6	●	++	◆◆	●●	++	◆◆	●●●	++	◆◆	●●●●	++	◆◆
8	●	++	◆◆	●●	++	◆◆	●●●	++	◆◆	●●●●	++	◆◆

Note:
 C = color
 ● = White
 ●● = Light Green
 ●●● = Dark Green
 ●●●● = Brownish Green
 H = homogeneity
 ◆ = Not Homogeneous
 ◆◆ = Homogeneous
 A = Apple smell
 + = Weak
 ++ = Medium
 +++ = Strong

The colors of each roll on deodorant which is studied from 0 wk until 8 wk was relatively stable does not change color. Smell of deodorant which are stored for up to 8 wk of relatively stable is green apples smell. Homogeneity of roll on deodorant Formula 1, Formula 2, Formula 3 and Formula 4 from start until to 8 wk which stored at room temperature (28 °C to 30 °C) remained stable, there was no separation among components. During the storage period 8 wk of deodorant pH which was observed at room temperature (28 °C to 30 °C) ranged from 4.52 till 5.53, which means that the pH of roll on deodorant *P. indica* leaf extract still in underarm skin pH range is 4.0 till 6.8 so it can be safely used.

Viscosity of roll on deodorant ranges from 331.8 cP to 340.8 cP. Results the evaluation of the viscosity Formula 1, 2, 3 and 4 decreased every week but this is not too significant, this showed that roll on deodorant has a good and stable condition. There is increase and decrease in specific gravity but decrease or increase values is not much different from the range 1 g mL^{-1} , so that it can be concluded the measurement result specific gravity roll on deodorant during storage 8 wk relatively stable.

Table 4. Irritation test results for roll-on deodorant.

Formula	Reaction									
	Women					Men				
	1	2	3	4	5	1	2	3	4	5
1	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-

Note: - does not cause reaction

+ reddened skin

++ red and itchy skin

+++ swells skin

Based on these research that *P. indica* leaf extract can be made deodorant have antibacterial activity against *S. epidermidis*. The ethanol extract 96 % more potent in inhibiting bacteria than water extract. If ethanol, hexane and methanol is used as a solvent to extract the plant, mostly from the extraction may indicate an inhibitory effect on gram-positive and gram-negative [10]. Many studies *P. indica* leaves have activity against *Entamoeba* [11] against *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 10031 in urinary tract infections [12]. Saponin, tannins, flavonoids and alkaloids compound are the source anti-oxidants and antibacterial [6]. Flavonoids in *P. indica* leaves have antibacterial activity against *Staphylococcus* sp., *Corynebacterium* sp. and *Propionibacterium*. Flavonoid contains a phenol compound is an acidic alcohol also known as carboic acid. Most the structure of the bacterial cell wall is protein and fat, presence flavonoids in plant can damage protein and damage of cell membrane so antibacterial of *P. indica* leaves can penetrate into bacterial cells *S. epidermidis*. Leaf extract of *P. indica* can be made roll on deodorant. Roll on deodorant containing 3 % (F2), 4 % (F3) and 5 % (F4) of *P. indica* leaf extract result in no bacterial growth. Based on this result, the deodorant have function effective as antibacterial against *S. epidermidis*. Flavonoid compounds in ethanol extract of *P. indica* leaves have function antibacterial because flavonoids contains a phenol compound which is an acidic alcohol that is also called carboic acid. Bacterial cell growth may be interrupted because a phenol compound contained in the ethanol extract of the leaves *P. indica*. The presence of phenol in acidic conditions can affect the growth of *S. epidermidis*. Phenol has the ability to damage protein and cell membrane, phenol binds to the protein through hydrogen bonding resulting protein structure becomes damaged. Instability of the cell wall and the cytoplasmic membrane of bacteria cause the function selective permeability, active transport function, controlling the proteins composition from bacterial cells to be disrupted. Cytoplasmic integrity disruption resulted macromolecules and ions from the cell, the bacteria become lost its shape and lysis. It leads to the imbalance of cell environment which results in the cells death [13]. Bacterial cell death means the loss of the ability of bacteria to reproduce (grow and divide). On media there is not colony *S. epidermidis* growth showed that the ethanol extract of the *P. indica* leaves roll on deodorant can to be bactericide. It is not cause irritation to the men and women who were tested table 4. The color of each formula is stable from 0 wk to 8 wk does not change. Roll on deodorant with apple smell are stored for up to 8 wk at room temperature (28 °C to 30 °C) relatively stable, homogeneous remained stable there was no separation between the components. During the storage period of 8 wk the pH the deodorant at room temperature (28 °C to 30 °C) ranged from 4.52 to 5.53, that the pH roll on deodorant *P. indica* leaf extract still in underarm skin pH range is 4.0 to 6.8 so it can be used safely [14]. Viscosity of deodorant roll on ranges from 331.8 cP to 340.8 cP. The evaluation results of the

viscosity Formula 1, 2, 3 and 4 decreased which is not too significant every week, this happens because of thickening agent used in the formula HPC-m incompatible with derivatives of phenols contained in the extract of *P. indica* leaves so that the viscosity gradually decreased during storage. This decreases the viscosity in accordance with the Arrhenius law says that if a material were stored for a certain period of time it will decrease the viscosity so the longer the shelf life of the material will the more dilute. Decrease of viscosity was not significant indicates that deodorant has a good and stable condition. Specific gravity of deodorant during storage of 8 wk relatively stable. Formula 2 roll on deodorant with the addition of 3 % *P. indica* leaf extract is a formula most excellent, F3 and F4 are darker is less preferred by panelists.

4. Conclusion

Roll on deodorant with *P. indica* leaf extract have antibacterial activity against *S. epidermidis* *in vitro*. Formula 2 deodorant roll on with the addition of 3 % *P. indica* leaf extract is a formula most excellent and effective against *S. epidermidis* than the other formula to overcome the problem of body odor.

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