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Antibacterial activity of *Piper betle* L. extract in cream dosage forms against *Staphylococcus aureus* and *Propionibacterium acne*

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Abstract

Acne is an inflammatory skin disease that occurs due to blockages in polysebase and inflammation that are caused by bacteria. Topical and systemic antibiotics are always used for treatment of acne, but the gradual resistance to antibiotics can affect the success rate of acne cure. *Piper betle* L. extract showed that has antibacterial activity against acne-causing bacteria. The aims of this research are to develop cream dosage form from *piper betle* L. extracts, which has antibacterial activity against *Staphylococcus aureus* and *Propionibacterium acnes*. *Piper betle L.* was extracted with the maceration method by using ethanol (90%). Then, the antibacterial activity of the *piper betle* L. extract was determined by the disc-diffusion method to find the minimum inhibitory concentration (MIC). Then, the extract was formulated into the base cream containing variations in concentration of cetyl alcohol (5%, 6%, and 7%) and surfactant (5%, 6% and 7%). The formulations of cream were evaluated for their physical properties, including organoleptic, homogeneity, pH, viscosity and antibacterial activity of cream. The results showed that *piper betle* L. extract has antibacterial activity with MIC value of 4.5 % and 4.0 % , against *S. aureus* and *P. acnes* respectively. Formulations with the best results of physical evaluation were obtained for the formula containing surfactant (containing tween 80 and span 80) 7 %. The antibacterial activity of cream formulation from *piper betle* L. extract occurs and produce inhibition zone 10.21 ± 1.2 mm against *S. aureus* and 15.2 ± 1.6 mm against *P. acnes*.

Key Words: *Piper betle* L. Extract; Cream; Antiacne; *Staphylococcus aureus*; *Propionibacterium acnes*

INTRODUCTION

Acne vulgaris is a skin disease affecting approximately 80-85 % of young people .e., the age range of 12-25 years [1]. Acne is an inflammatory skin disease that occurs due to blockages in polysebase and inflammation that are caused by bacteria. The characteristics of acne on the skin surface is a small lump, it can contain pus, itching and a little pain [2].

The bacterial activity on the surface of the skin becomes one of the main causes of acne [1]. The growth of an acne lesion is due to the presence of *Propionibacterium acnes*, although other organisms, such as *Staphylococci*, have been isolated from acne lesion. These inflammatory organisms are initiated by increased production of sebum, free fatty acids from the sebum itself and abnormal keratinization of the sebaceous canal [3].

Topical and systemic antibiotics are always used for treatment of acne, but the gradual resistance to antibiotics can affect the success rate of acne cure [1]. However, antibiotic resistance has increased in dermatologic prevalence [4]. The previous study shows that *P. acnes* and *S. aereus* have resistance and low sensitivity toward several antibiotics [1]. To solve the problem of antibiotic resistance, medicinal plants have been used as alternative treatments for acne diseases.

One of the natural herbs that act as an antibacterial agent is *piper betle* L. Extract of *piper betle* L. showed that have antibacterial activity against four different pathogenic bacteria: *Streptococcus pyogenes, Staphylococcus aureus, Proteus vulgariss and Escherichia coli* [5]. Other studies showed that the betel leaf oil (Piper betel) from Sri Lanka has anti-bacterial activity against *Staphylococcus aureus bacteria, Staphylococcus epidermidis* and *Pseudomonas aeruginosa, Escherichia coli*

Several reports showed that the leaf of this plant contain many beneficial bioactivities and its extract has a great potential to be used in developing commercial products. Thus, the evaluating study aims to determine the antibacterial activity of *piper betle* L. in cream dosage form with an in vitro study model which may be helpful in developing new novel drugs.

MATERIALS AND METHODS

Materials

The materials used in this study consists of *piper betle* L obtained from Research Institute for Medicinal Plants (BALITRO). Ethanol, Paraffinum Liquidum , propylene glycol, sodium lauryl sulphate, glycerin, hydroxypropyl methylcellulose (HPMC), Mueller-Hinton Agar (MHA), Mueller-Hinton Broth, 0.9% NaCl, ethanol 96%, polyvinyl alcohol (PVA), sodium dodecyl sulfate (SDS), Butylated hydroxytoluene (BHT) and dimetil sulfoksida (DMSO) were purchased from PT. Brataco Chemica, Indonesia. *P. acnes* and *S. aureus* was obtained from Microbiology Laboratory, Faculty of Pharmacy, Universitas Padjadjaran.

Methods

Extraction

10 kg of piper betle was dried by incubation in the oven at 50°C. For extraction, the dried leaves was macerated with 70% ethanol at room temperature. Ethanol was then removed by using a rotary evaporator with a vacuum pressure of 50°C to obtain a crude extract [7].

Piper betle L. Extract Phytochemical Screening

The ethanolic extracts of piper betle were tested for the presence of alkaloids, steroids/triterpenoids, saponins, polyphenols, tannins, flavonoids, quinone, monoterpenes, and sesquiterpenes [8].

Antibacterial Activity Test on *piper betle* L. Extract

Antibacterial activity of this extract was tested with the disc-diffusion method. MHA was used for *S. aureus* and *P. acnes* media. The extract was dissolved in DMSO 0.01% at various concentrations. Paper discs were soaked in 5 mL of the extract solution for 15 min and then dried in a laminar air flow cabinet for 2 h. The paper discs were then placed on the media surface that been inoculated with the bacteria. Petri dishes were incubated at 37°C for 18 h [9].

Table 1: Formulation of Cream from *piper betle* L. extract

Material	Formula (%)					
	I	II	III	IV	V	VI
Piper betle extract	5	5	5	5	5	5
Cetyl alcohol	5	6	7	-	-	-
Sodium Dodecyl Sulfate	0,5	0,5	0,5	-	-	-
Twen 80/Span 80	-	-	-	5	6	7
Paraffinum Liquidum	10	10	10	10	10	10
BHT	0.2	0.2	0.2	0.2	0.2	0.2
Methyl paraben	0.18	0.18	0.18	0.18	0.18	0.18
Propyl paraben	0.2	0.2	0.2	0.2	0.2	0.2
Propylene glycol	10	10	10	10	10	10
Oleum apple	Qs	Qs	Qs	Qs	Qs	Qs
Water	add 100	add 100	add 100	add 100	add 100	add 100

Cream formulation

Creams were prepared by dissolving tween-80, propylene glycol, propyl paraben and methyl paraben in water and span-80, cetyl alcohol, BHT, Paraffinum Liquidum in palm-olein at 70°C. The two phases were mixed together at the same temperature without vortexing to avoid the en- trapment of air. After cooling to 30 – 40°C the cream was homogenized using stirrer at 1500 rpm for 15 min. The most stable cream bases were selected and then were formulated by adding *piper betle* L. extract and homogenized again for 15 min at 1500 rpm. Physical stability of cream formulation from *piper betle* extract was evaluated through organoleptic, homogeneity, pH, and viscosity until 28 days [10].

Antibacterial Activity Test of cream from piper betle extract

Antibacterial activity of cream was tested with the disc diffusion method. Paper discs were soaked in 5 mL of the sample solution for 15 min. The paper discs were then placed on MHA media surface that been inoculated with bacteria *P. acnes* and *S. aureus*. Petri dishes were then incubated at 37°C for 18 h [2].

Statistical analysis

Data are presented as mean ± SD. Data were analyzed by using one-way analysis of variance (ANOVA) with Probability values of 0.05 ($p<0.05$) or less were considered statistically significant [2].

RESULT AND DISCUSSION

Plant Determination

The Plant were determinated at the School of Life Sciences and Technology, ITB, and showed that the plant used was *Piper betle* L.

Extratction

The maceration method is used to protect the compounds contained in the *piper betle* L from degradation, especially potentially antibacterial compounds. The solvent used for the maceration process is ethanol because it is a universal solvent, so it can dissolve polar and nonpolar compounds, and this is safe for topical applications [9]. The use of ethanol is also to optimize the phenol content in the extract. Phenol is a compound that has antibacterial activity. According to Lee et al., ethanol extracts have higher phenol levels than water extracts [11].

Phytochemical Screening

The results of phytochemical screening showed that the ethanol extract of *piper betle* L. has potential antibacterial properties. The screening results are shown in Table 2.

Table 2 Explains the preliminary screening of secondary metabolites obtained in *piper betle* L. extract. It was concluded that ethanol extract had high concentrations of sterols, Monoterpenes and sesquiterpenes, phenols and flavonoids, as well as tannins.

Sterols contained in *piper betle* L. has great potential as an antibacterial. The mechanism of sterols is the interaction with the surface interactions of sterol molecules present in the extracts with cell walls and bacterial membranes that cause changes in the cell wall and membrane primary structures, leading eventually to the formation of pores and degradation of bacterial components. The extract also contains high concentrations of flavonoids and polyphenols. Previous research has shown that flavonoids and polyphenols have potential as antiviral, anti-inflammatory, antitumour, antihaemolytic and antioxidant activity [12].

Tabel 2. Results of phytochemical screening ethanol from extract of *Piper betle* L.

No.	Phytochemical Screening	Result
1	Alkaloids	-
		-
2	Flavonoids	+
3	Polyphenols	+
4	Tannins	+
5	Monoterpenes and sesquiterpenes	+
6	Steroids and triterpenoids	-
7	Quinone	+
8	Saponins	+

The results of the antibacterial activity test from *piper betle* L. can be seen in table 3.

Table 3. Results of Antibacterial Activity Test from Ethanol Extract of *piper betle* L.

Extract Concentration (% b/v)	Inhibition Zone Diameter (mm)	
	S. aureus	***P. acnes***
5	4.5 ± 1.1	4.01 ± 0.4
10	6.40 ± 1.19	5.02 ± 0.3
20	12.3 ± 1.82	5.71 ± 1.64
40	14.33 ± 1.61	12.28 ± 1.49
80	18.66 ± 1.56	16.12 ± 1.03
Solvent control DMSO 0.01%	0	0

The table 3 showed that the extract of *pipe betle* L. has activity against P. acne and S. aureus. This is due to the extract of *pipe betle L.* containing fatty acid compounds (stearic acid and palmitic acid) and hydroxy fatty acid ester (hydroxy ester of stearic, palmitic and myristic acid) and hydroxichavicol. Fatty acids can act as anionic surfactants and have antibacterial and antifungal properties at low pH, in addition to selective against Gram-positive organisms by targeting the structure and function of cell walls and bacterial membranes [13]. Hydroxichavicol compounds also show activity as antibacterial [14].

Organoleptic examination

The result of organoleptic examination from cream of *piper betle* L. extract can be seen in table 4.

Table 4 : Stability test result of cream from piper betle extract

Formula	Evaluation	Days -					
		1	3	7	14	21	28
1	Color	brownish white					
	Odor	Oleum apple					
	Consistency	***	***	***	***	***	***
2	Color	yellowish white					
	Odor	Oleum apple					
	Consistency	**	**	**	**	**	**
3	Color	yellowish white					
	Odor	Oleum apple					
	Consistency	***	***	***	***	***	***
4	Color	brownish white					
	Odor	Oleum apple					
	Consistency	**	**	**	**	**	**
5	Color	yellowish white					
	Odor	Oleum apple					
	Consistency	***	***	***	***	***	***
6	Color	brownish white					
	Odor	Oleum apple					
	Consistency	**	**	**	**	**	**

Notes:
* : thick
** : very thick
*** : semi-fluid

Based on organoleptic observations in table 4, the formula containing tween 80 and span 80 did not change significantly compared to other formulas. In the formulation of this cream preparation, a surfactant was used as an emulsifier. Surfactant affects the rheological properties of the cream. In this study used a combination of surfactants because it is more effective in stabilizing the catalysis of a single surfactant by complementing the properties of each other. The ability of the blend to pack more tightly between formed phases affect to the strength of the surfactant film and the stability of the cream [15].

The result of pH measurement from *piper betle* L. extract cream can be seen in figure 1.

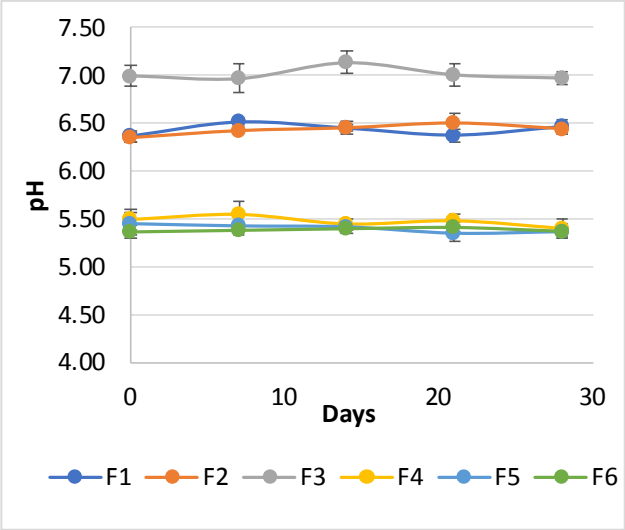

Fig. 1: pH measurement result of *Piper betle* L. extract cream (All the values were calculated as mean±standard deviation; n=3)

For topical preparations, the pH should be in the range of skin pH (4.5-7,0) to avoid any irritation to the skin [2]. During storage, the pH decreases during storage 28 days but is still within the range acceptable level for topical preparation. Based on statistical analysis with ANOVA, the significance value was 0.12 ($P < 0.05$), indicating that there was no influence of the storage time on the pH value of the preparation.

The result of viscosity measurement from *piper betle* L. extract cream can be seen in figure 2.

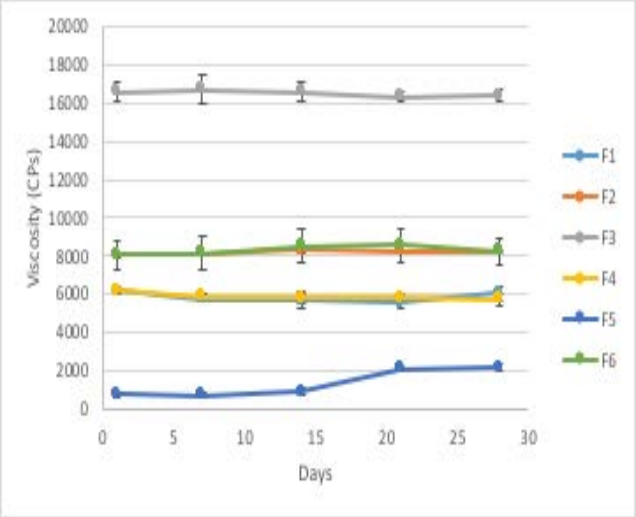

Fig. 2: Viscosity measurement result of *Piper betle* L. extract cream (All the values were calculated as mean±standard deviation; n=3)

Fig 2 showed that the viscosity of the cream preparations did not change significantly during the 28 day storage. Based on the results of ANOVA, the value of significance was 0.23 ($P > 0.05$), which indicated that there was no influence of the storage time on the viscosity of cream.

The results of the antibacterial activity from cream of *piper betle* L. can be seen in table 5.

Table 5: Results of antibacterial activity of cream from *piper betle* L. extract **(**All the values were calculated as mean±standard deviation; n=3)

Formula	Inhibition Zone (mm)	
	S aureus	P acne
Basis	0	0
F 6	10.21 ± 1.2	15.2 ± 1.6

The results showed that cream of *piper betle* L. extract has antibacterial activity by providing inhibit zone. While the base cream has not antibacterial activity so it can not affect the results of extract activity in cream preparation.

CONCLUSION

Extract of *piper betle* L. has antibacterial activity with MIC value of 4.5 % and 4.0 % , against *S. aureus* and *P. acnes* respectively. Formulations with the best results of physical evaluation were obtained for the formula containing surfactant (containing tween 80 and span 80) 7 %. The antibacterial activity of cream formulation from *piper betle* L. extract occurs and produce inhibition zone 10.21 ± 1.2 mm against *S. aureus* and 15.2 ± 1.6 mm against *P. acnes*.

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