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Original Article

ANTI-ACNE CREAM OF LEAVES EXTRACT OF FIG (*FICUS CARICA* L.) FROM CIWIDEY DISTRICT, INDONESIA, AGAINST *PROPIONIBACTERIUM ACNES* AND *STAPHYLOCOCCUS EPIDERMIDIS*

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ABSTRACT

Objective: This study aimed to formulate and evaluate an anti-acne cream of fig leaves extract.

Methods: The methods included formulation and evaluation of anti-acne cream, antibacterial activity assay against *Propionibacterium acnes* and *Staphylococcus epidermidis*, irritancy test, and preference test. The oil-in-water creams were made with various extract concentrations (1, 2, and 3%).

Results: Greenish cream with fig fragrant, viscosity ranging from 332 to 388 cP, pH ranging from 6.69 to 7.23, and oil-in-water type. Antibacterial activity was dose-dependent, without irritancy, erythema, and edema, and the most preferred cream based on the texture and fragrance was the 3% extract formula.

Conclusion: Fig leaves extract can be made into a safe cream with antibacterial activity against *P. acnes* and *S. epidermidis*.

Keywords: Fig leaves extract, *P. acnes, S. epidermidis,* Safe cream, Dose-dependent

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INTRODUCTION

Fig (*Ficus carica* L., family Moraceae) is distributed in Southwest Asia and the Eastern Mediterranean region [1]. In West Java Province, Indonesia, fig is cultivated in Ciwidey district [2, 3]. Traditional medicine uses fruit, leaves, and root of fig to treat the disorder of digestive (colic, diarrhea, and appetite loss), cardiovascular, respiratory (bronchial problem, sore throat, and cough), and as an anti-inflammatory and antispasmodic remedy [4].

The leaves of fig from Ciwidey District have been proven to contain terpenoids, steroids, alkaloids, saponins, phenolic compounds, flavonoids, and tannins. The ethanol leaf extract contains a total flavonoid content of 2.03±0.01 mg RE/g simplicia and a total phenolic content of 2.52±0.24 mg GAE/g simplicia [2]. Increasing the extract concentration from 30% to 60% showed an increase in the inhibition zone, which is proportional to the increasing antibacterial activity against *Propionibacterium acnes* and *Staphylococcus epidermidis* [3].

P. acnes is the main microorganism that is involved in acne pathogenesis and is localized in the follicle [5]. While *S. epidermidis* is a human skin microbiota which contributes to the pathogenesis of common skin diseases like acne [6]. Acne occurs when the skin pores are blocked with dead skin, oil, or bacteria [5]. Alternative remedies are being developed to overcome antibiotic resistance, due to antibiotic long-term use [7].

Preliminary studies showed that fig leaves extract had the potential to be formulated as a topical preparation with antibacterial activity. Although it has been proven that there is a relationship between the concentration of flavonoids and phenolic compounds with antibacterial activity. There is no standardized topical formulation with good efficacy, safety, and stability for fig leaves extract. The use of non-standardized herbal preparations is associated with health risks, such as poisoning, sub-therapeutic effects, and drug-herb interactions [8]. This encourages the importance to scientifically evaluate the efficacy, safety, and quality of herbal preparations [9]. This study aimed to formulate and evaluate anti-acne cream of fig leaves extract, then assess antibacterial activity against *P. acnes* and *S. epidermidis*, irritancy, and preference formula. The oil-in-water creams were made with various extract concentrations (1, 2, and 3%). The novelty of this study was fig leaves extract from domestic cultivation, i.e. Ciwidey district. So, raw materials are easy to obtain and to optimize the use of fig leaves because, generally, fig fruit was being used.

MATERIALS AND METHODS

Materials

The etanol fig leaves extract was obtained from previous studies [3]. Fig leaves were collected from Ciwidey District, West Bandung Regency, West Java, Indonesia. *P. acnes* ATCC 1223 and *S. epidermidis* ATCC 12228 obtained from the Microbiology Laboratory, School of Pharmacy, Bandung Institute of Technology.

Barium chloride, sulfuric acid, gallic acid, and dimethylsulfoxide (DMSO) was analytical grade and purchased from Sigma Aldrich (Germany). Mueller Hinton Agar (MHA) was bacteriology grade and purchased from Oxoid (UK). Liquid paraffin, stearic acid, cera alba, methylparaben, and triethanolamine (TEA) was cosmetic grade and purchased from TTK Science Co. (Thailand).

Cream formulation

The oil phase consists of 25% liquid paraffin, 15% stearic acid, and 8% cera alba) was melted in a water bath at 60 °C. The aqueous phase was prepared by mixing 0.5% methylparaben, 1.5% TEA, and up to 100% distilled water (according to the extract concentration) at 60 °C. The oil and aqueous phases were mixed to form a homogeneous cream base. The extract (1, 2, and 3%) was added and mixed into the cream base to form a homogeneous cream [10].

Cream evaluation

All creams were evaluated for eight weeks of observation. The organoleptic properties of the creams were assessed, such as color and fragrance. Homogeneity was analyzed from visual inspection for the clog existence and cream appearance. The cream type was observed under the microscope after methylene blue addition. pH evaluation of the 10% cream solution was measured using a calibrated pH meter (Beckman, Germany). Viscosity was determined using a CAP-2000 Brookfield viscometer with spindle no 63 at 30 rpm and 25 °C. The results were recorded after a stable value [10].

Antibacterial activity assay

The turbidity of *P. acnes* and *S. epidermidis* suspension was equal to 0.5 Mc Farland solution, prepared from a mixture of 1% sulfuric acid solution and 1.175% barium chloride solution (9.95:0.05). A petri dish containing 20 µl of bacterial suspension and 20 ml of MHA was allowed to solidify. Then, agar was perforated with a perforator and filled with 50 µl of sample or 1% DMSO. Each inhibition zone was measured using a caliper [3]. The sample was prepared by dissolving 1 g of cream with 1 ml of 1% DMSO.

Irritancy and preference test

Irritancy and preference tests were conducted after obtaining ethical approval from the Health Research Ethics Committee of the Government Hospital of Dr. Hasan Sadikin Bandung, West Java, Indonesia. Irritancy test was performed on the dorsal left-hand surface of the volunteers. Irritancy, edema, and erythema were checked and reported every hour for up to 24 h. A preference test was conducted based on color, fragance, and texture. The level of preference was assessed using a numerical scale, i.e. 1 = extremely dislike, 2 = dislike, 3 = like, 4 = extremely like [10].

Statistical analysis

One Way ANOVA for parametric analysis, followed by Kruskal Wallis test and Friedman test for nonparametric analysis ($P<0.05$) [10].

RESULTS AND DISCUSSION

All creams have a smooth and homogeneous texture. The greenish color and fig fragrance increases with the concentration of fig leaves extract. A homogeneity test was conducted to determine whether the oil phase and aqueous phase in the cream were completely dispersed. Homogeneity maintains the cream stability [11], due to the right mixing process, so that the oil and aqueous phase can completely mix. In addition, methylparaben, as a preservative, can prevent the microorganism growth [12]. The homogenous mixture of the creams produces its smooth texture with no change in color and fragrance. An air-tight and light-resistant container is important in maintaining a stable fragrance. All creams contained no clogs during the eight weeks of observation. These results showed that fig leaves extract can disperse in a cream base through good mixing.

Methylene blue is a water-soluble dye, so it will dissolve in the oil-in-water cream base [11]. The results showed that methylene blue was completely dispersed in all creams, which confirms an oil-in-water cream formulation. The formula showed that the volume of the oil phase is smaller than the aqueous phase, so the oil phase will be dispersed into the aqueous phase to form an oil-in-water type emulsion [11]. Stearic acid and TEA as emulsifiers will reduce surface tension and prevent separation of the oil and aqueous phases [13]. The advantage of oil-in-water cream is that it is easily washed off with water [11].

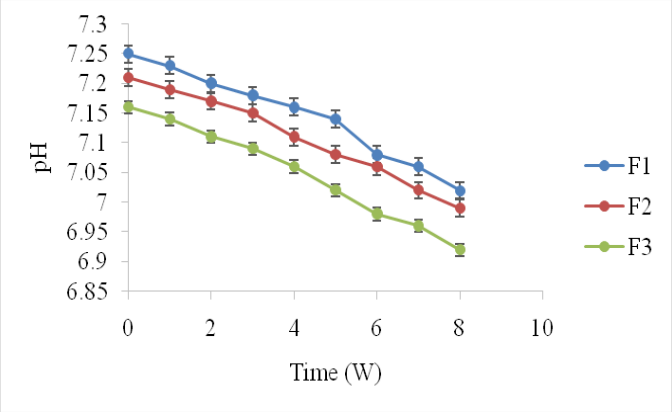

Fig. 1: Cream pH during 8 w of observation (n = 3)

The normal skin pH ranges from 4.0 to 7.0 [14], while the cream pH has a slightly alkaline pH at the beginning, i.e., 7.16-7.25 (fig. 1), due to TEA as an alkalizing and emulsifying agent [13]. There was no difference in pH between the three creams ($p = 0.05$). Storage for 8 w caused a decrease in pH of 0.22 to 0.24, but decreasing pH was not statistically significant ($p = 0.91$). The combination of TEA with stearic acid forms fine-grained and stable oil-in-water emulsions [13]. Increasing the concentration of fig leaves extract showed a decreasing pH of the cream due to fig leaves extract being slightly acidic (pH 6.54±0.14). All creams had a stable pH during 8 w of observation.

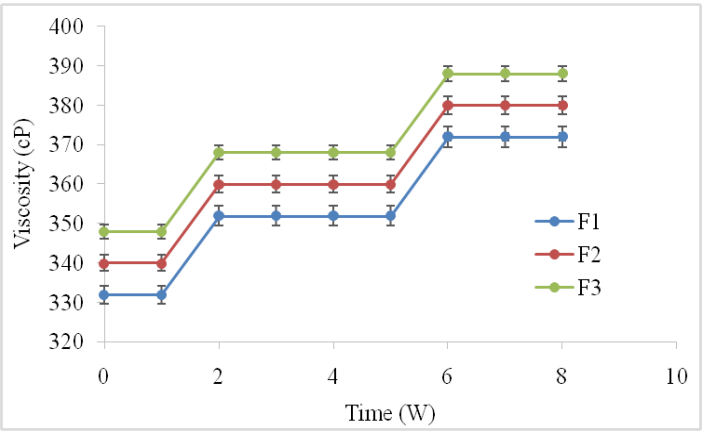

Fig. 2: Cream viscosity during 8 w of observation (n = 3)

The viscosity of the cream is attributed to the excipients being used and the formulation process. The viscosity is being assessed to ensure the cream sensation and behavior on the skin [15]. Cream viscosity was ranging from 332 to 348 cP at the beginning. Increasing the concentration of the fig leaves extract caused an increasing cream viscosity due to the thick extract behavior, but there was no significant difference in viscosity (p = 0.12). The cream viscosity was consistent with the viscosity range of creams made from natural ingredients studied by Tchiennou *et al.*, i.e. 290-480 cP [16]. Storage for 8 w showed an increasing viscosity (fig. 2), which was not significantly different (p = 0.99). This showed all creams meet the requirements of a good cream.

Table 1: Antibacterial activity result of fig leaves extract

Concentration (%)	Inhibition zone (mm)	
	P. acnes	***S. epidermidis***
6.25	18.91±0.31	18.37±0.38
12.5	20.91±0.27	20.31±0.81
25	21.31±0.15	21.67±0.59
50	23.24±0.72	22.74±0.92
Clyndamycin phosphate 250 mg/ml	24.16±0.49	24.46±0.39

Note: Data was shown as mean and deviation standard (n = 3)

According to the study conducted by Saptarini *et al.* [3], increasing the fig leaves extract concentration from 6.25% to 50% showed an increase in the inhibition zone, which was proportional to the increase in antibacterial activity against *P. acnes* and *S. epidermidis*. Based on the results obtained, there was a significant difference between the extract concentration ($p = 1.3 \times 10^{-7}$), but they had the same activity against both bacteria ($p>0.05$).

Table 2: Antibacterial activity result of fig leaves cream

Formula	Inhibition zone (mm)	
	P. acnes	***S. epidermidis***
F1	8.14±1.15	10.97±1.29
F2	9.54±0.70	13.44±1.64
F3	11.14±1.10	15.71±0.95
Cyndamycin phosphate (250 mg/ml)	12.24±0.59	16.53±0.53

Note: Data was shown as mean and deviation standard (n=3)

Increasing the fig leaves extract concentration from 1% to 3% in the cream increased the inhibition zone, which was proportional to the increasing antibacterial activity against *P. acnes* and *S. epidermidis* (table 2). There was a significant difference between the extract concentration in all three cream formulas (p = 0.04). The antibacterial activity of the cream showed significant differences against *P. acnes* and *S. epidermidis*, which showed a p-value of 0.04, 0.02, and 0.005 for formula 1, formula 2, and formula 3, respectively. These results are attributed to the cream base, which supports the extract spreading and cream-bacteria interaction. The cream base is essential to ensure the even distribution of the active metabolites with antibacterial activity to the oil-in-water emulsion. These active metabolites include flavonoids and phenolic compounds, which are more easily distributed through the oil-in-water base as compared to 1% DMSO as solvent. The antibacterial activity of the cream showed that *S. epidermidis* was more sensitive to the cream than to *P. acnes*. The antibacterial activity of creams of fig leaves extract was inversely proportional with the fig leaves extract, i.e. higher activity on *S. epidermidis* compared to *P. acnes* [3].

All creams did not cause irritancy, edema, or erythema showing that the excipients and fig leaves extract are safe for topical preparation. Further study is needed to ensure the safety of using cream of fig leaves extract for 1-4 w, which is the common time used for acne treatment [17].

The preference test was assessed from the sensory test on the skin [10]. Formula 3 was the most preferred formula based on color, texture, and fragrance (fig. 3). The content of 3% fig leaves extract gives a refreshing fragrance and attractive greenish color.

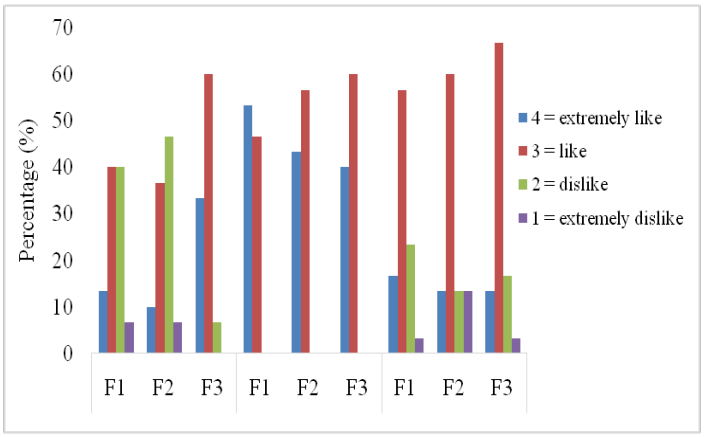

Fig. 3: Fig. leaves cream preference test results

CONCLUSION

Fig. leaves extract can be made into a safe cream without irritancy, erythema, and edema. All formulas were oil-in-water cream and stable during observation. Antibacterial activity against *P. acnes* and *S. epidermidis* was dose-dependent. The most preferred cream based on the texture and fragrance was the 3% extract formula. Further study is needed to ensure the safety of using cream of fig leaves extract.

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AUTHORS CONTRIBUTIONS

Conceptualization: NMS; methodology: IEH; investigation: RH; data curation: NMS; writing of original draft preparation: IEH; review and editing: NMS, MJTC; supervision: RM. All authors have read and agreed to the published version of the article.

CONFLICT OF INTERESTS

Declared none

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