Original Research Article

Analysis of Antiacne Effectiveness of Red Dragon Fruit Peel Methanolic Extract Gel

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ABSTRACT

Aim: To determine the effectiveness of Anti-Acne from the methanol extract of dragon fruit peel (*Hylocereus polyrhizus*) by in Vitro and In Vivo assay.
Methods: This study was an experimental study using disc diffusion and microdilution methods as in vitro methods. Meanwhile, the topical application of dragon fruit peel extracts to P. acne-injected rats by in vivo method.
Results: dragon fruit peels methanolic extract had MIC and MKC values of 50 mg/ml by in vitro assay. In addition, the dragon fruit peels methanolic extract as a gel showed significant improvement in acne vulgaris lesions after 7-14 days of extract administration (P value < 0.05). The lesion improvement was demonstrated by decreasing the size of the lesion. It was supported by the regeneration of the epidermal structure of all groups that received dragon fruit peels extract gel compared to the control group that only received gel base.
Conclusion: The dragon fruit peels methanol extract has an antiacne effect in a physically stable gel form.

Keywords: Dragon fruit, peel, methanol, gel, antiacne

1. INTRODUCTION

Acne vulgaris is a chronic inflammation of the pilosebaceous unit with or without the involvement of adjacent tissue due to blockage of the pilosebaceous unit (Heng et al., 2021; Tan et al., 2021). Acne vulgaris can be suffered by any age group. However, it is more common in the adolescent group. Around 85% of these cases were found in the adolescent group with various severity and persisted into older age [1]**.**

Global Burden of Disease reported that the global prevalence rate of acne vulgaris in 2016 was 28.41% from 39,319 cases aged between 10-24 years old. Meanwhile, a higher prevalence rate was found in Southeast Asia, which was 27.96%. At the same time, Indonesia reported a higher prevalence rate, 31.79% from 43,322 cases. This prevalence rate was higher than the previous report in 2010, which was 26.88%. A local institution, Indonesia Cosmetic Dermatology, also reported that the prevalence rate in 2006, 2007, and 2009 were 60%, 80%, and 90%, respectively. Although acne vulgaris is not life-threatening, scar formation as a complication of severe acne vulgaris may cause psychological problems, such as a lack of self-confidence**.** Furthermore, some studies reported that acne vulgaris caused functional and emotional problems. Thus, treating acne vulgaris is challenging to improve patients' mental health. Due to this, the newer modality of acne vulgaris treatment with minimal cost and side effects is concerned [2] [3].

The development of acne vulgaris treatment is based on four key factors: increased sebum production, follicular hyperkeratinisation, colonisation of Propionibacterium acnes, and induction of inflammation. Agarwal et al. (2016) reported that the most prescribed class of

drugs in treating acne vulgaris was Oral Vitamin A-Derivates, 37%, followed by topical clindamycin, 28%. Irrational use of these agents may cause consequences such as resistance to therapy due to down-regulation of the drug receptor's expression, teratogenicity effects, and even a tendency to develop antibiotic resistance. Therefore, herbs are quite popular nowadays and can be an alternative to acne vulgaris treatment.

Recently, various studies on natural materials have been performed. Subositi and Wahyono (2019) reported that Indonesia had various biotics like algae, spermatophytes, tracheophytes, and others. Moreover, Subositi and Wahyono reported that around 15.5% of this biotics were found in Indonesia. According to this information, Indonesia riches in various plants with potential herbs value. The utility of herbs was high in some developing countries. Eko (2014) reported that around 4 million people in developing countries used the herb as a primary treatment [4].

Dragon fruit (*Hylocereus polyrhizus*) is one of the plants from Indonesian biodiversity. The flesh is widely consumed as a fiber-sourced fruit. However, dragon fruit peels always become a wasted product. Several studies have investigated the pharmacological effects of these fruit peels. Astridwiyanti et al. (2019) reported that the red dragon fruit peels ethanol extract had an antibacterial effect against *Staphylococcus aureus* ATCC 25923. The in vitro assay showed this extract's minimum inhibitory concentration (MIC) was 25%. Not only against *Staphylococcus aureus*, but Hendrea et al. (2019) also reported that n-hexane, ethyl acetate, and pigment extracts from dragon fruit peels also had antibacterial effects against some bacteria, including *Bacillus subtilis* ATCC 11774, *Escherichia coli* ATCC 35218, and *Vibrio algynolyticus*. Another study also reported an inline result, Ridwan et al. (2020) reported that the dragon fruit peels ethanol extract had an antibacterial effect against *Streptococcus mutans* ATCC 29212 and *Enterococcus faecalis* ATCC 29212. It was shown that the MIC and MBC values were 6.25% and 12.5%, respectively [5].

Moreover, Ridwan et al. also reported the fungicidal effect against *Candida albicans* ATCC 10231. The anti-fungal effect was reported as the MIC and MFC (Minimum Fungicidal Concentration), which were 12.5% and 25%, respectively. These previous studies were limited to in vitro studies. Afandi et al. (2017) also developed a dragon fruit peel aqueous extract as a pigment for lipstick formulations; hence, this formulation has an antibacterial effect against several gram-negative and gram-positive bacteria [6].

Although many studies have been performed to investigate the antibacterial effect of dragon fruit peel, there are still few studies exploring the antibacterial effect of dragon fruit peel against bacteria that cause acne vulgaris. The little study still develops the dragon fruit peel as a cosmetic. A study that developed dragon fruit peel extract as a cosmetic product was performed by Afandi et al. (2017). Therefore, this study was designed to develop the dragon fruit peel extract (*Hylocereus polyrhizus*) in treating acne vulgaris through its antiacne effect in both in vitro and in vivo assay in a topical gel form [6].

2. MATERIAL AND METHODS

2.1 Materials

Dragon fruit peels, 98% methanol, Dimethyl sulfoxide (DMSO), phytochemical screening reagent, ciprofloxacin, NA and NB Media Powder, Propionibacterium acne isolate in slant agar, McFarland Standard, disc diffusion, Carbopol 940, propylene glycol, propylparaben, TEA, glycerine, distilled water, 10% Buffer Formalin Solution, and PBS.

2.2 Study Design

This study was an experimental study with pre and post-test control group design and was performed in May 2022- June 2022 at the Microbiology and Pharmacology Laboratory of

Universitas Prima Indonesia. All procedure of this study has been approved by Health Research Ethics Committee from Universitas Prima Indonesia with No. Letter: 038/ KEPK/ UNPRI/ III/ 2022.

2.3 Extract Process

The extraction process was performed by maceration methods using 98% methanol as solvent. Five-Hundred grams of dragon fruit peel simplicial powder was soaked into 1.5 litres of 98% methanol solution. This mixture was kept from any lights at room temperature for three days and regularly stirred. After three days, this mixture was filtered, and the residue was re-macerated as before two times. The filtrate from the first maceration and re-maceration were collected to evaporate by rotary evaporator at a temperature of 40-50^{o}C until it formed a concentrated extract. After that, the extract yield was determined by dividing the mass of the extract by the mass of the simplicial powder and multiple by 100% [7] [8], [9].

2.4 Phytochemical Screening

This study underwent a phytochemical screening based on the standard manual for phytochemical screening from the Pharmacology Laboratory of Universitas Prima Indonesia. This procedure investigated the presence of phenolic, flavonoid, alkaloid, terpenoid/ steroid, tannin, and saponin [10], [11].

2.5 Determination of Total Polyphenol Compound Level

After the phytochemical screening, this study continued to determine the total polyphenol level, including phenol, flavonoid, and tannin. Initially, one millilitre of the sample was added in 1 ml of 50% ethanol, and then 0.1 ml of 10% AlCl3 solution was added after being incubated for 30 minutes. Absorbance readings were carried out at the maximum wavelength. The equation determined determination of total flavonoid content:

TFC = (Equivalent Quercetin Mass)/Concentration

After that, analysis was continued to determine the total tannin content. A millilitre sample solution was put into a 10 ml volumetric flask. Add 0.5 ml of Folin Denis reagent and 1 ml of saturated sodium carbonate solution (35%) (Na_2CO_3), then add distilled water up to 10 ml. The blank and standard solutions were distilled water and tannic acid, respectively. Total tannin content is expressed in units of mg equivalent of tannic acid/ grams extract (mg TAE/ gram extract). The equation determines determination of total tannin content:

TTC = (Equivalent Tannic Acid Mass)/Concentration

At last, the analysis was continued to determine the total phenolic content. One hundred microliter extract was added with 0.5 mL of Folin-Ciocalteu reagent. Stir the solution and let it stand for 6 minutes. Add 2.5 mL of 5% sodium carbonate solution. Then the mixture was incubated for 30 minutes at room temperature. Absorbance readings were carried out at the maximum wavelength. As a blank, distilled water was used instead of the sample. Gallic acid is used as a standard at various concentrations. Phenolic content is expressed in units of mg equivalent of gallic acid/g sample (mg GAE/g). The equation determined determination of total phenolic content:

TPC = (Equivalent Gallic acid Mass)/Concentration

2.6 In Vitro Antiacne Assay

This study used two in vitro methods to evaluate the antiacne effect of dragon fruit peels methanolic extract. These methods were disc diffusion methods and microdilution assay.

2.7 Disc Diffusion Assay

Initially, the concentrated form of dragon fruit peels extract was diluted into five different concentrations, including 250 mg/ml, 200 mg/ml, 150 mg/ml, 100 mg/ml, and 50 mg/ml, which was used DMSO as the solvent by the volumetric flask. Meanwhile, the standard and control used ciprofloxacin and DMSO, respectively. After that, Nutrient Agar and Nutrient Broth, the media for bacteria growth, were made by diluting 3.8 gram NA and 1.3 gram NB into 100 ml distilled water, respectively. Then, these media and other instruments were sterilised by autoclave at 121^{o}C for 15 minutes.

On the other hand, *Propionibacterium acnes* isolate from slant agar was inoculated in NA media by four quadrants streak plate methods. This media was incubated at 37^{o}C for 24 hours. Afterwards, a colony was suspended in a millilitre of normal saline in a reaction tube and incubated for 24 hours. Then, it was compared to McFarland Standard 0.5 [12], [13].

An amount of 20 ml NA Media was poured into some petri dishes filled with 1 ml of Propionibacterium acne suspension. Then, it was homogenised. After that, these media was placed in the disc diffusion that had been diffused by extract, ciprofloxacin, or DMSO. Each dish was placed in five-disc diffusions, except the standard and control, which only placed two disc diffusions. All Petri dishes were incubated at 35-37^{o}C for 18-24 hours. At last, the width of the inhibition zone was measured by a calliper [14].

2.8 Microdilution Method

This method was performed to look for the Minimal Inhibitory Concentration (MIC) and Minimal Killing Concentration (MKC). One hundred microlitres of NB media was filled into 12 columns on 96-well plates. After that, 100 µL NB media and 100 µL Propionibacterium acnes suspension was filled into the 12th (Strile control) and 11th (Growth control) columns, respectively. Then, 100 µL dragon fruit peels extract that showed the lowest antibacterial effect in the disc diffusion methods was filled into the first column and homogenised it. Then, a millilitre of the mixture in the first column was filled and homogenised in the second column, and it was repeated for the remaining tube (until the tenth column). In the tenth column, 100 µL of the mixture in this column was discarded. Finally, 100 µL of Propionibacterium acnes suspension was filled and homogenised into each column from the first to the tenth column. This 96-well plate was then incubated at 35-37^{o}C for 18-24 hours in an incubator. MIC was determined according to the turbidity, and the lowest concentration showed a clear appearance without any bacterial growth known as the MIC.

Furthermore, some columns which showed a clear appearance were subcultured into the NB agar by pour and spread plate methods. This agar was then incubated at 35-37^{o}C for 18-24 hours. The lowest concentration which did not show any bacterial growth in NA media was known as the MKC [13] [14] [14].

2.9 Gel Formulation

It was begun by formulating the gel base, which was made by dissolving 0.3-gram Carbopol 940 into 15 ml distilled water until homogeneous. On the other hand, 0.06 grams of methyl paraben and 0.03 grams of propyl paraben were dissolved into 5 ml of distilled water until homogeneous, and 1.5 ml of propylene glycol was added. After that, the weight of 0.75 grams, 1.50 grams, and 3.00 grams of dragon fruit peels extract was added into this mixture to obtain 2.5%, 5%, and 10% dragon fruit peel extract gel, respectively. Then, these mixtures were stirred gradually into the initial Carbopol 940 mixture. Then, the remaining distilled

water was dissolved into the mixture. In addition, TEA and glycerin can be added before adding up to 30 ml of distilled water [15].

2.10 Physical Stability

Physical stability was evaluated by the Freeze-Thaw Cycle method. Each cycle consists of a temperature of 4^{o}C for 24 hours (freeze), after which it is stored at 40^{o}C for 24 hours (thaw). This study used six freeze-thaw cycles, and each cycle ended with an evaluation of physical stability, including organoleptic, homogeneity, pH, and spreading ability [15], [16].

2.11 In Vivo Antiacne Assay

This assay was begun by preparation of *Propionibacterium acnes* suspension. *Propionibacterium acnes* was obtained from the Microbiology Laboratory, Universitas Prima Indonesia and cultured into 10 ml NB at 37°C until OD600 = 1.0. In this phase, 10 ml of this suspension is estimated to be 1.34 x 10^{9} CFU. Then, this broth was centrifuged at 4000 rpm for 15 minutes at 4^{o}C to remove the supernatant. Meanwhile, the remaining bacterial pellets were washed three times with 10 ml of PBS and then suspended in one millilitre of PBS solution. This suspension was then incubated at 80^{o}C for 30 minutes for the heat-killing reaction. At last, this suspension can be reserved at a temperature of 4^{o}C until it is used [17], [18].

In vivo antiacne assay used 25 male Wistar rats. These rats were grouped into five groups, including control (gel base), standard (clindamycin), 2.5%, 5%, and 10% dragon fruit peels extract gel. One hour before the injection of *Propionibacterium acnes* suspension, all rats received an intervention base on their groups. Then, the backs of all rats were injected intradermally with 10 µL of Propionibacterium acnes suspension. Moreover, the diameter of the lesion was measured by a calliper immediately, 7th day, and 14th day after the injection. At the end of the observation period, the neck dislocation method sacrificed the rats, and the lesion was excised for histological examination [19], [20].

2.12 Data Analysis

Data analysis in this study was performed by IBM SPSS 25. Initially, all data were analysed by descriptive statistics, including central tendency and dispersion. Then, the data analysis was continued with the bivariate analysis of the extract concentration variable in the in vitro assay and the diameter of the inhibition zone formed by using the One-Way Anova test if the data were normally distributed and using the Kruskal-Wallis test as an alternative test for data that were not normally distributed. Similar bivariate analyses were also performed on the variable gel concentration and diameter of the lesion in rats' backs caused by *Propionibacterium acne* suspension injection.

3. RESULTS AND DISCUSSION

This study extracted the dragon fruit peels by maceration method and the obtained extract has some characteristics, as described in Table 1.

Table 1. **Characteristics of Dragon Fruit Peels Methanolic Extract**

Characteristics	Value
Fresh Simplicial Mass(gr)	2000.0
Simplicial powder (gr)	1896.9
Solvent Volume (ml)	7500
Extract Weight (gr)	79.97
Yield (%)	4.22

Based on Table 1, it can be seen that this study used 2,000 grams of fresh dragon fruit peels. These dragon fruit peels are dried and mesh into 1,896.9 grams of simplicial powder. After that, the simplicial powder was macerated by 7,500 ml solvent and obtained 79.97 grams of a concentrated extract. According to these data, the yield extract was 4.22%. The obtained dragon fruit peels methanolic extract underwent a phytochemical screening, and the result of the phytochemical screening is described in Table 2.

Table 2. **Phytochemical Screening of Dragon Fruit Peels Methanolic Extract**

Phytochemical	Methods	Result	Interpretation
Phenol	FeCl3	++++	Positive
Flavonoid	Pb (CH3COO)2	++++	Positive
	Alkaline (NaOH)	++++	
	Sinoda Test (Mg+HCl)	++++	
Alkaloid	Mayer	-	Positive
	Dragendorf	+++	
Terpenoid/ Steroid	Lieberman-Burchard	-	Negative
	Salkovski	-	
Tannin	FeCl3	++++	Positive
Saponin	Foam Test	-	Negative

Table 2 shows that the dragon fruit peels methanolic extract had some phytochemicals, including phenol, flavonoid, alkaloid, and tannin. After that, the analysis continued to measure the total polyphenol compound content described in Table 3.

Table 3. Quantitative Analysis of Polyphenolic Group Compound in Dragon Fruit Peels Methanolic Extract

Phytochemicals	Value
Phenol (GAE mg/ gram extract)	10.52 ± 0.89
Tannin (TAE mg/ gram extract)	3.41 ± 0.27
Flavonoid (QE mg/ gram extract)	1.37 ± 0.07

GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent; TAE: Tannic Acid *Equivalent*.

Table 3 shows that the total phenol, tannin, and flavonoid of dragon fruit peel methanolic extract were 10.52 ± 0.89 GAE mg/ gram extract, 3.41 ± 0.27 TAE mg/ gram extract, and 1.37 ± 0.07 QE mg/ gram extract, respectively. Then, the dragon fruit peels methanolic extract underwent in vivo analysis for antibacterial assay against Propionibacterium acnes by disc diffusion methods. The result of the disc diffusion assay is described in Table 4.

Table 4. **Antibacterial Activity of Dragon Fruit Peels Methanolic Extract by Disc Diffusion Method in *Propionibacterium acnes***

Concentration	Width of Inhibition Zone (mm)		P-Value
	Mean	SD	
250 mg/ ml[a]	13.80	0.73	< 0.05
200 mg/ ml[b]	11.08	0.72	
150 mg/ ml[bc]	9.18	0.56	
100 mg/ ml[cd]	7.81	0.53	
50 mg/ ml[d]	6.63	0.45	
Standard[e]	27.63	1.38	
Control[d]	6.00	0.00	

P-value was obtained from the One Way ANOVA; Different superscripts in the same column show significant differences based on Post Hoc Test Tukey HSD

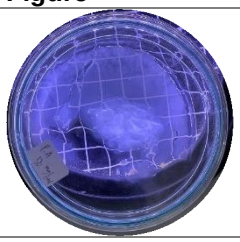
Based on Table 4, it can be seen that there was a significant difference in the width of the inhibition zone among all concentrations (P value < 0.05). The highest concentration of dragon fruit peels methanolic extract did not show any significant difference width of the inhibition zone to the standard group. It indicated that the highest concentration extract did not have an antibacterial activity as well as the standard group. Meanwhile, the two lowest concentrations of dragon fruit peels methanolic extract (100 mg/ml and 50 mg/ml) showed no significant differences compared to the control group. Therefore, the lowest concentration of dragon fruit extract still showed an antibacterial effect against *Propionibacterium acnes* was 150 mg/ ml. However, the highest concentration of dragon fruit peel methanol extract (250 mg/ml) still did not show an antibacterial effect against *Propionibacterium acnes* that was as well as or better than the standard group (Ciprofloxacin). Then the antibacterial activity analysis was continued with the microdilution method to obtain the MIC and MKC values. The analysis of MIC is described in Table 5.

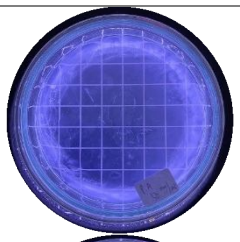
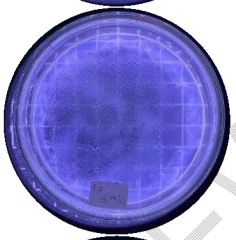
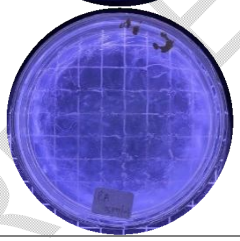
Table 5. **Determination of MIC Value in Dragon Fruit Peels Methanolic Extract by Microdilution Method in *Propionibacterium acnes***

Concentration	Turbidity	
	I	II
Media Control	-	-
Bacterial Growth Control	+	+
Extract Control	-	-
50 mg/ ml	-	-
25 mg/ ml	+	+
12.50 mg/ ml	+	+
6.25 mg/ ml	+	+
3.13 mg/ ml	+	+
1.56 mg/ ml	+	+
0.78 mg/ ml	+	+
0.39 mg/ ml	+	+
0.20 mg/ ml	+	+

Table 5 shows that at a diluted level of 50 mg/ml, the dragon fruit peels methanolic extract showed a clear-appearance media. It indicates that 50 mg/ml of dragon fruit peel methanolic extract inhibited the growth of *Propionibacterium acnes* bacteria. Based on the observation of the broth media from the microdilution method above, the MIC or Minimal Inhibitory Concentration of dragon fruit peels methanol extract against *Propionibacterium acnes* was 50 mg/ml. After that, the column with the MIC concentration and one level lower serial concentration (25 mg/ ml) was subcultured into the NA media to determine the MKC or Minimal Killing Concentration. Thus, the analysis of MKC is described in Table 6.

Table 6. **Determination of MKC Value in Dragon Fruit Peels Methanolic Extract by Microdilution Method in *Propionibacterium acnes***

Concentration	Repetition	Bacterial Growth	Figure
50 mg/ ml	I	-	

	II	-	
25 mg/ ml	I	+	
	II	+	

Based on Table 6, it can be seen that the MKC value of dragon fruit peels extract was 50 mg/ml. At this concentration, there was not find any *Propionibacterium acnes* bacteria growth. Meanwhile, the higher concentration of dragon fruit peels methanol extract still showed some *Propionibacterium acnes* bacteria growth. Thus, the MKC value of dragon fruit peels methanol extract against *Propionibacterium acnes* was the same as the MIC value of 50 mg/ml. Furthermore, the dragon fruit peels methanolic fruit extract was formulated into some concentration of gels.

The obtained dragon fruit peels extract gels underwent an evaluation of physical stability by the Freeze-Thaw method for six cycles. The physical characteristic includes organoleptic, homogeneity, pH, and spreading ability with or without load. All physical stability parameters for all concentrations of dragon fruit peels extract gels were stable for six cycles. All gels' concentrations were semisolid without any odour for six cycles. All concentrations of gels also did not show any colour changes within six freeze-thaw cycles. The original colour of 5%, 7.5%, and 10% dragon fruit peels extract gels were yellow, darkish-yellow, and brown, respectively. All concentration of dragon fruit peels extract gels also has stabile homogeneity, which was homogenous for six freeze-thaw cycles.

Moreover, the level of acidity or pH from dragon fruit peels extract gels was also stable for six freeze-thaw cycles, which had neutral pH (6.5-6.9). The pH of 5%, 7.5%, and 10% dragon fruit peels extract gels ranged from 6.6-6.9, 6.5-6.8, and 6.5-6.8, respectively. Finally, the spreading ability of all gels also showed a stabile spreading ability with or without load. The highest spreading ability without load was found in the 10% dragon fruit peels extract gel (3.7 cm), followed by 7.5% dragon fruit peels extract gel (3.5 cm), and the lowest was 5% dragon fruit peels extract gel (3.2 cm). In addition, the spreading ability with load showed an inverse value. The spreading ability after adding 100 grams of load only wides the diameter of gel spreading. The spreading ability with 100 grams load in 5%, 7.5%, and 10% dragon fruit peels extract gels were 5.5cm, 5.2 cm, and 5.0cm, respectively.

The optimal dragon fruit peels extract gels were then analysed for antiacne activity by in vivo assay, including evaluation of lesion size and histology study. The comparison of acne lesion size in all groups is described in Table 7.

Table 7. Analysis of Acne Lesion Size in All Groups

Groups	Lesion Size, cm	
	7th Day	**14th day**
Control	1.32 ± 0.40a	1.23 ± 0.41a
Standard	0.27 ± 0.10b	0.12 ± 0.06b
Dragon Fruit Peel Extract Gel-I	1.02 ± 0.05ab	0.95 ± 0.05ac
Dragon Fruit Peel Extract Gel-II	0.76 ± 0.16b	0.70 ± 0.12c
Dragon Fruit Peel Extract Gel-III	0.46 ± 0.09c	0.40 ± 0.12b
P-Value*	**< 0.05**	**< 0.05**

**P-value was obtained from the One Way ANOVA analysis of the transformation value of the lesion size; Different superscripts in the same column show significant differences based on Post Hoc Test Tukey HSD*

Based on Table 7, it can be seen that after seven days and 14 days after the intervention, there was a significant change in the acne lesions size (P-value < 0.05). After 14 days of intervention, the highest concentration of dragon fruit peels extract gel showed no significant differences in the acne lesions size compared to the standard group that received clindamycin. However, at a lower concentration, the extract was reported to differ from the standard group significantly. Based on the information above, the highest concentration of dragon fruit peels extract gel at a concentration reported to have good antiacne effects was as well as the standard group. The improvement of acne lesion size was supported by the result of the skin histology study described in Figure 1.

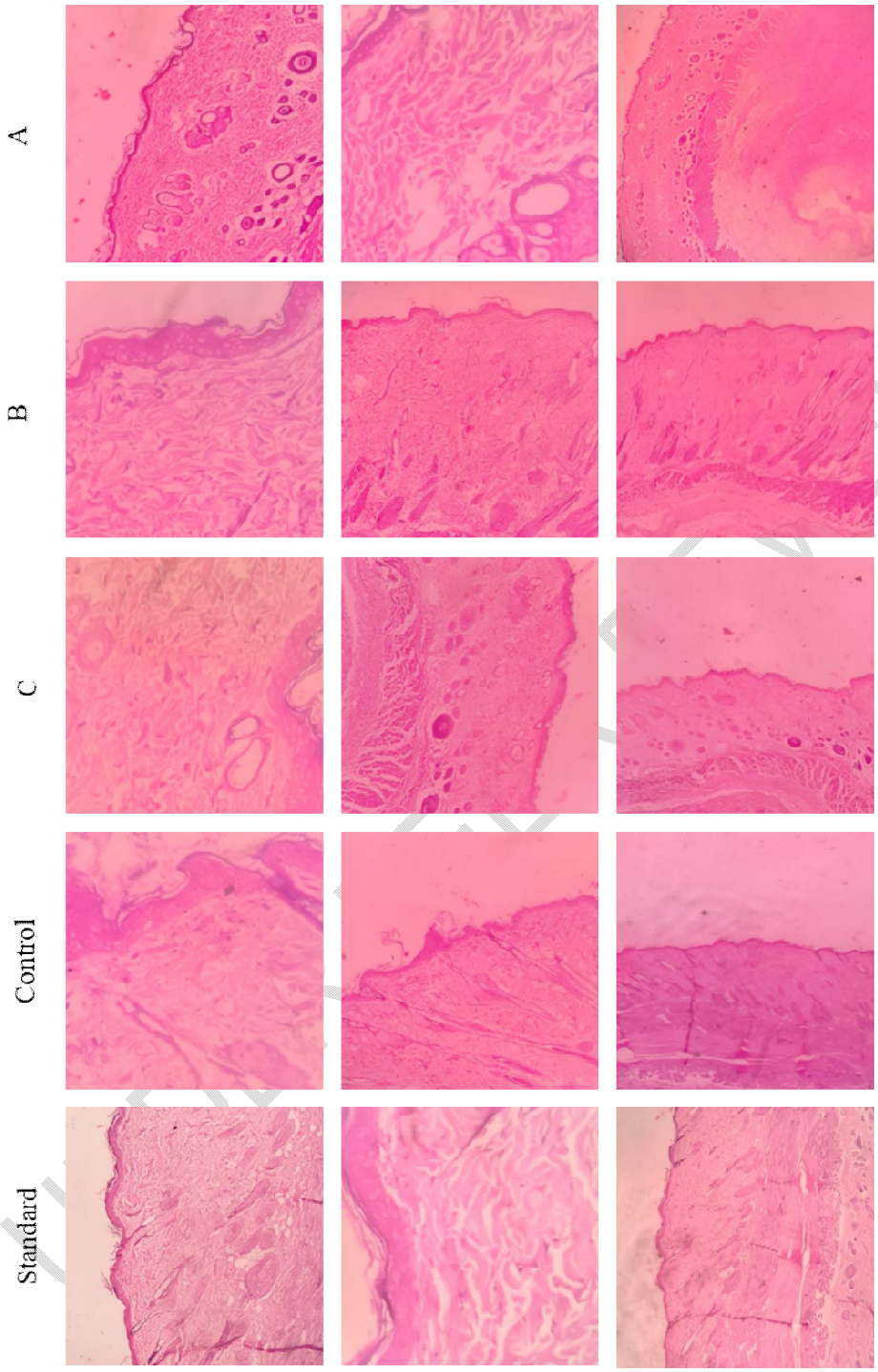


Fig. 1. Histological View of the Skin Tissue in All Groups; (A) 5% Dragon Fruit Peel Methanol Extract Gel; (B) 7.5% Dragon Fruit Peel Methanol Extract Gel; (C) 10% Dragon Fruit Skin Methanol Extract Gel. Staining: Haematoxylin and Eosin. Magnification: 400x

Based on Figure 1, it can be seen that all groups had an intact epidermis structure. This improvement was also found in the standard group due to the repairing epidermis effect from the dragon fruit peels gel and clindamycin as the standard. Meanwhile, the control group showed an erosion of the epidermis structure. Overall, it can be seen that the application of dragon fruit peels gel could improve the skin barrier from Propionibacterium acnes infection, which was injected intracutaneously. The improvement of the skin barrier by dragon fruit peels gel was better than the control group, which only received a gel base.

It can be obviously seen that the dragon fruit peels methanol extract with a yield of 4.22% contains several phytochemicals, including phenols, flavonoids, alkaloids, and tannins. These phytochemicals are responsible for the antibacterial activity of the dragon fruit peel methanol extract against *Propionibacterium acnes* bacteria with MIC and MKC values of 50 mg/ml. Then, dragon fruit peel methanol extract was formulated into dragon fruit peel methanol extract gel with concentrations of 2.5%, 5%, and 10%. All dragon fruit peel extract gel had well physical stability during six freeze-thaw cycles. Furthermore, all of the gels were analysed for their antiacne activity by an in vivo assay, and it showed that the dragon fruit peel extract gel significantly improved the lesion acne after seven days of application, but this antiacne effect was not as good as the standard group (Clindamycin gel). Interestingly, after 14 days of treatment, dragon fruit peel extract gel at the highest concentrations showed an antiacne effect that was as good as the standard group.

The yield of the dragon fruit peel methanol extract in this study was lower than the results of other previous studies. Dewi et al. (2020) reported that dragon fruit peel extract extracted with 10% citric acid had yield values ranging from 18.20 to 60.91 %. Furthermore, Dewi et al. (2020) reported that dragon fruit peel extract extracted with 10% citric acid was influenced by the duration of maceration; the longer the maceration may increase the yield value of the extract. This result showed the opposite value to the current study. It can be caused by the difference in the solvent solution and maceration duration between the current study and previous studies. Moreover, Pandey and Tripahu (2014) reported that several factors might affect the quality of the extracts, including the part of the plant, the solvent used for extraction, and the extraction procedure [21], [22].

Various phytochemicals as secondary metabolites may be extracted from simplicial powder. Pandey and Tripathi (2014) also reported that several factors might affect the level of phytochemicals, including extraction methods, extraction time, temperature, solvent solubility, solvent concentration, and solvent polarity. According to the current study result, the dragon fruit methanol extract contained some phytochemicals, including phenols, flavonoids, alkaloids, and tannins. Cruz et al. (2022) reported a similar result. Cruz et al. reported that the dragon fruit methanol extract had some phytochemicals, including alkaloids, cardenolides and bufadienolides, flavonoids, coumarins, and terpenoids. Furthermore, Fidrianny et al. (2017) also reported various polyphenol compounds in dragon fruit peel ethyl acetate extract. These compounds that were total Phenolic Contents and Flavonoid Contents were 4.56 g GAE/ 100g and 12.63 QE/ 100g, respectively. [21], [23], [24]

The current study demonstrated that the dragon fruit methanol extract had an antiacne property by inhibiting the growth of *Propionibacterium acnes*. Wahdaningsih et al. (2014) reported that the n-hexane fraction of dragon fruit peel chloroform extract had well antibacterial activity against *Propionibacterium acnes.* Wahdaningsih et al. reported the antibacterial effect as inhibition zone diameter. The inhibition zone diameter from the concentration of 20 mg/ml, 40 mg/ml and 80 mg/mL were 9 mm, 10.25mm, and 10.5mm, respectively. Furthermore, Wahdaningsih and Untari (2021) also reported that the dragon fruit peel ethanol extract had good antibacterial activity against *Propionibacterium acnes*.

The inhibition zone diameter from concentrations of 100 mg/ml, 50 mg/ml and 25 mg/mL were 10.5 mm, 10.00mm, and 8.5mm, respectively. [25], [26]

These antibacterial effects were due to phytochemicals like alkaloids and some polyphenol compounds. Alkaloid was found as berberine and harmane that inhibits bacterial DNA synthesis. In addition, alkaloids can also interfere with the formation of peptidoglycan in the bacterial cell wall. Thus, the bacterial cell wall does not form and leading to cell death. Moreover, alkaloid compounds contain primary nitrogen groups, which will react with amino acid compounds that synthesise bacterial cell walls and DNA. These changes lead to a genetic imbalance in the DNA chain and lead to bacterial cell lysis, which will cause cell death in bacteria. [25], [26]

Meanwhile, the polyphenolic compounds may denature cell proteins and disturb the synthesis of bacterial cell walls until the bacteria die. Other mechanisms that can occur are active protein precipitation and the breakdown of lipids in cell membranes through a voltage drop mechanism on the cell membrane surface. The antibacterial activity of flavonoid compounds has the same mechanism as phenolic compounds, and then flavonoids act on bacteria by damaging the cytoplasmic membrane. If the cytoplasmic membrane is damaged, essential metabolites in bacteria are released, and food materials to produce energy cannot enter; hence the bacterial cell cannot grow and reproduce—finally, the bacterial cell dead. [25]

An in vivo study reported that the dragon fruit peel methanol extract gel has the potential to be an antiacne gel. The results of this study are supported by other studies performed by Azhari et al. (2021), who reported that acne spot gel preparations with an active ingredient in the form of red dragon fruit peel had an antiacne effect by inhibiting the growth of *Propionibacterium acne* and *Staphylococcus aureus* bacteria. **A**nother study by Azhari et al. (2021) reported that the dragon fruit peel extract acne gel spot was physically stability and had inhibition zones against *Propionibacterium acne* bacteria of 23.855 mm (F1) and 23,671 mm (F2) and *Staphylococcus aureus* bacteria of 22.127 mm (F1) and 23,410 mm (F2). However, none of the previous studies evaluated the antiacne effect of dragon fruit peel extract by in vivo methods. Therefore, the current study evaluated the antiacne effects not only by in vitro method but also in vivo method. Dragon fruit peel extract has been studied in some previous studies, not only as a gel but also as a peel-off facial mask. It was supported by Jani et al. (2020), who also reported that dragon fruit peel could be used as a peel-off facial mask that is rich in various antioxidants. [27], [28]

4. CONCLUSION

Overall, it can be concluded that the dragon fruit peels extract has an antibacterial effect against *Propionibacterium acne* with MIC and MKC of 50 mg/ ml. Moreover, dragon fruit peels extract as a gel also showed significant improvement in acne vulgaris lesions after 7-14 days of gel administration (P value < 0.05). This improvement was shown as decreasing the acne lesion size and repairing the epidermal in all dragon fruit peels extract gel groups, which was better than the control group (gel base).

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (Seven WHO Standard 2011) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee (Health Research

Ethics Committee from Universitas Prima Indonesia) with No. Letter: 038/ KEPK/ UNPRI/ III/ 2022

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