Journal of Pharmaceutical Research International

Volume 35, Issue 3, Page 45-57, 2023; Article no.JPRI.97180
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

In vitro and *In vivo* Study of the Red Dragon Fruit Peel (*Hylocereus polyrhizus*) Methanolic Extract Gel Effect on Acne

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CDB, INEL and EF did the conceptualization. Authors CDB and EF did the methodology. Author CDB did the investigation. Author CDB did the discussion of results. Author CDB did the writing – original draft. Authors INEL and EF did the writing – review and editing. Authors INEL and EF did the supervision. Authors CDB, INEL and EF approved the final text.

Article Information

DOI: 10.9734/JPRI/2023/v35i37318

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/97180

Original Research Article

Received: 04/01/2023
Accepted: 08/03/2023
Published: 14/03/2023

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*

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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: 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. 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. [4] 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 [5] 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. Around 4 million people in developing countries used the herb as a primary treatment.

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. [6] 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. [7] 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. [8] 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.

Moreover, Ridwan et al [8] 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. [9] 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.

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. 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.

2. MATERIALS 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 [10–12].

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 [13,14].

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% $AlCl_3$ 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 i*n vitro* methods to evaluate the antiacne effect of dragon fruit peels methanolic extract. These methods were disc diffusion methods and microdilution assay [15,16].

2.7 Disc Diffusion Assay

Initially, the concentrated form of dragon fruit peels extract was diluted into five different concentrations, including 250, 200, 150, 100, 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 g 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 h. Then, it was compared to McFarland Standard 0.5 [15,17].

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 h. At last, the width of the inhibition zone was measured by a calliper [16].

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 h. The lowest concentration which did not show any bacterial growth in NA media was known as the MKC [15,16].

2.9 Gel Formulation

It was begun by formulating the gel base, which was made by dissolving 0.3 g Carbopol 940 into 15 ml distilled water until homogeneous. On the other hand, 0.06 grams of methyl paraben and 0.03 g 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 g, and 3.00 g 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 [18].

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 h (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 [18,19].

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 [20,21].

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, all rats were sacrified by neck dislocation method and the lesion was excised for histological examination [22,23].

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

Table 2. Phytochemical screening of dragon fruit peels methanolic extract

Phytochemical	Methods	Result	Interpretation
Phenol	$FeCl_3$	++++	Positive
Flavonoid	Pb $(CH_3COO)_2$	++++	Positive
	Alkaline (NaOH)	++++	
	Sinoda Test (Mg+HCl)	++++	
Alkaloid	Mayer	-	Positive
	Dragendorf	+++	
Terpenoid/ Steroid	Lieberman-Burchard	-	Negative
	Salkovski	-	
Tannin	$FeCl_3$	++++	Positive
Saponin	Foam Test	-	Negative

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 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 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.

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 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 Fig 1.

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 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	
Controld	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

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	+	+

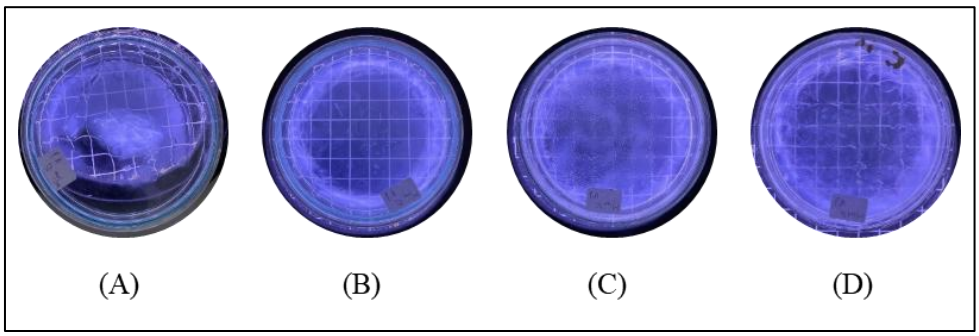

Fig. 1. Determination of MKC in some concentration of dragon fruit peels methanol extract by microdilution method. (A) and (B): Concentration of 50 mg/ ml did not showed any bacterial growth. (C) and (D): Concentration of 100 mg/ ml showed some bacterial growth

Based on Fig. 1, 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 (Fig. 1 (A) and (B)). Meanwhile, the higher concentration (100 mg/ ml) of dragon fruit peels methanol extract still showed some *Propionibacterium acnes* bacteria growth (Fig. 1 (C) and (D)). 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 6.

Based on Table 6, 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 Fig. 2.

Based on Fig. 2, 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 [24] 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 [24,25].

Table 6. 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*

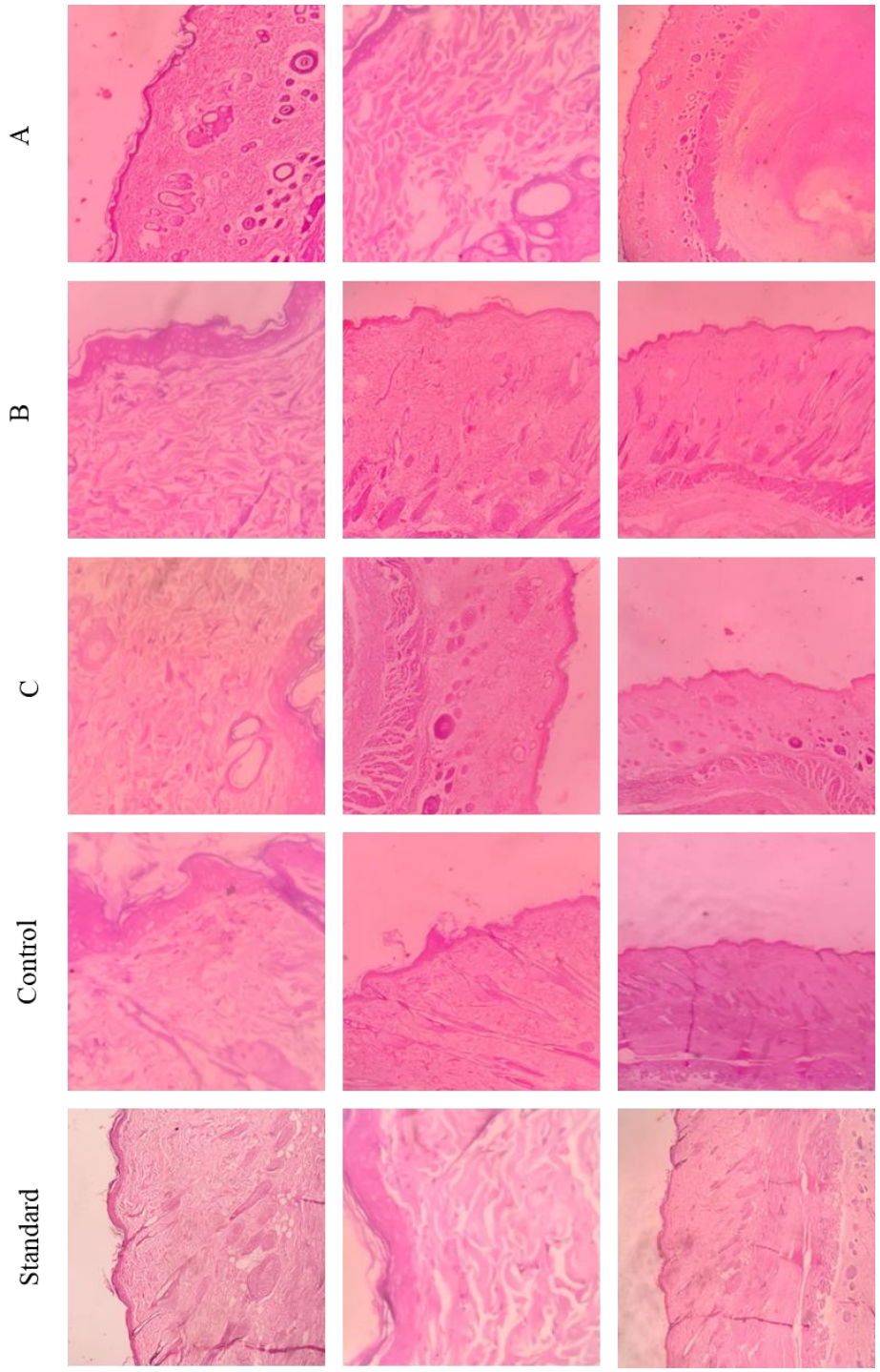

Fig. 2. Histological view of the skin tissue in all groups; (A) 5% dragon fruit peel methanol extract gel; (B) 7.5% dragon fruit peel methanol extract gel; (C) 10% dragon fruit skin methanol extract gel. staining: haematoxylin and eosin. magnification: 400x

Various phytochemicals as secondary metabolites may be extracted from simplicial powder. Pandey and Tripathi [24] 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. [26] 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. [27] 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. [24,26,27].

The current study demonstrated that the dragon fruit methanol extract had an antiacne property by inhibiting the growth of *Propionibacterium acnes*. Wahdaningsih et al. [28] 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 [29] 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. [28,29].

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. [28,29].

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. [28].

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. [30], 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. Another study by Azhari et al. [30] 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. [30,31].

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). Due to these reasons, the Red Dragon Fruit Peel Methanolic Extract gel has an anti-acne effect, that can improve the acne vulgaris after 7-14 days after application of this gel.

CONSENT

It is not applicable.

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.

ACKNOWLEDGEMENTS

A brief acknowledgement section may be given after the conclusion section just before the references. The acknowledgments of people who provided assistance in manuscript preparation, funding for research, etc. should be listed in this section. All sources of funding should be declared as an acknowledgement. Authors should declare the role of funding agency, if any, in the study design, collection, analysis and interpretation of data; in the writing of the manuscript. If the study sponsors had no such involvement, the authors should so state.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ayudianti P, Indramaya DM. Retrospective study: Factors aggravating acne vulgaris (retrospective study: factors aggravating acne vulgaris). Berk Genital Skin Health Sciences 2014;26:41–7. Available:https://doi.org/10.20473/bikk.V26.1.2014.1-7
2. Panonsih RN, Purwaningrum R, Efendi A, Desarta W. The relationship between stress and facial hygiene on the incidence of acne vulgaris in medical students at the University of Malahayati. Malahayati Nurs J 2021;3:11–8. Available:https://doi.org/10.33024/manuju.v3i1.3398
3. Khairunnisa K, Rialita A, Mardhia M. Knowledge and behavior of facial hygiene against the incidence of acne vulgaris in middle school students in Mempawah Hilir. J Doctoral and Health Public Science Faculty of Medicine, Sriwij Univ 2021;8:25–32. Available:https://doi.org/10.32539/v8i1.11886
4. Agarwal S, Pillai A, Singh AP, Pareek R, Bhuptani N V. Drug utilization study of medications used for acne vulgaris in a Tertiary Care Hospital. Eur J Biomed Pharm Sci. 2016;3:311–5.
5. Subositi D, Wahyono S. Study of the Genus curcuma in Indonesia used as Traditional Herbal Medicines. Biodiversity. 2019;20:1356–61. Available:https://doi.org/10.13057/biodiv/d200527
6. Astridwiyanti AAB, Mahendra AN, Dewi NWS. Test the effectiveness of the ethanol extract of red dragon fruit peel (*Hylocereus polyrhizus*) against Staphylococcus aureus ATCC 25923 *In vitro*. Digest of Medical Science 2019;10:482–6. Available:https://doi.org/10.15562/ism.v10i3.425
7. Hendra R, Masdeatresa L, Abdulah R, Haryani Y. Antibacterial activity of red dragon peel (*Hylocereus polyrhizus*) Pigment. J Phys Conf Ser 2019;1351. Available:https://doi.org/10.1088/1742-6596/1351/1/012042
8. Ridwan RD, Diyatri I, Juliastuti WS, Waskita FA, Ananda GC, Juliana NV. The Ability of *Hylocereus polyrhizus* to gram positive bacteria and *Candida albicans*. Biochem Cell Arch. 2020;20:0000–0000.
9. Afandi A, Lazim AM, Azwanida NN, Bakar MA, Airianah OB, Fazry S. Antibacterial properties of crude aqueous *Hylocereus polyrhizus* peel extracts in lipstick formulation against gram-positive and negative bacteria. Malaysian Appl Biol. 2017;46:29–34.
10. Mutia MS. Histology study of liver changes paracetamol-induced wistar rats treated with sunkist (*Citrus sinensis* L. Osbeck) extract. Am Sci Res J Eng Technol Sci. 2019;59:1–7.
11. Chiuman L, Ginting CN, Yulizal O, Suhartomi, Chiuman V. Improvement of liver function from lemon pepper fruit ethanol extract in streptozotocin-induced wistar rats. 2021 IEEE Int. Conf. Heal. Instruments. Meas. Nat. Sci., Medan, Indonesia: IEEE; 2021. Available:https://doi.org/10.1109/InHeNce52833.2021.9537284
12. Suhartomi, Gulo KN, Saragih AD, Martinus AR, Ikhtiari R. Antioxidant properties of sweet orange peels in several fractions of methanolic extract. Proc. int. Conf. Heal. Informatics Med. appl. Technol. - Vol. 1

ICHIMAT, Medan: Scitepress Digital Library. 2020;371–8.
Available:https://doi.org/10.5220/0009515503710378

13. Widowati W, Rani AP, Amir Hamzah R, Arumwardana S, Afifah E, Kusuma HSW, et al. Antioxidant and antiaging assays of *Hibiscus sabdariffa* extract and its compounds. Nat Prod Sci. 2017;23:192–200.
Available:https://doi.org/10.20307/nps.2017.23.3.192
14. Depari SAF, Jihan D, Rambe A, Meilando R, Lisya C, Mutia MS, et al. effectiveness test of sunkist orange peel (*Citrus sinensis* (L.) Osbeck) ethanol extract on blood sugar levels of wistar rats (*Rattus norvegicus*) with hypercholesterolemia. Biospecies. 2021;14:1–9.
15. Milanda T, Zuhrotun A, Nabila U, Gathera VA, Kusuma AS. Antibacterial activity of red yeast rice extract against propionibacterium acnes ATCC 11827 and methicilin-resistant *Staphylococcus aureus* ATCC BAA-1683. Pharmacol Clin Pharm Res. 2021;6:83.
Available:https://doi.org/10.15416/pcpr.v6i2.35062
16. Julianti E, Rajah KK, Fidrianny I. Antibacterial activity of ethanolic extract of Cinnamon bark, honey, and their combination effects against acne-causing bacteria. Sci Pharm. 2017;85.
Available:https://doi.org/10.3390/scipharm85020019
17. Safitri AT, Nur Adiratna N, Drajat S. I. Activity test of durian (*Durio zibethinus* Murr.) peel ethanol extract against propionibacterium acnes and *Staphylococcus aureus* Bacteria. J Farm Udayana 2020;9:66.
Available:https://doi.org/10.24843/jfu.2020.v09.i02.p01
18. Aslani A, Zolfaghari B, Davoodvandi F. Design, formulation and evaluation of an oral gel from Punica granatum flower extract for the treatment of recurrent aphthous stomatitis. Adv Pharm Bull 2016;6:391–8.
Available:https://doi.org/10.15171/apb.2016.051
19. Sing R, Bansal S, Mishra MK. Formulation and evaluation of herbal oral gel containing extracts of powdered *Psidium guajava* linn leaves with *Curcuma longa* linn rhizomes to treat mouth ulcers. Int J Drug Dev Res. 2020;12:1–7.
Available:https://doi.org/10.36648/0975-9344.12.2.150
20. Lim HJ, Jeon YD, Kang SH, Shin MK, Lee KM, Jung SE, et al. Inhibitory effects of *Euphorbia supina* on Propionibacterium acnes-induced skin inflammation *In vitro* and *In vivo*. BMC Complement Altern Med. 2018;18:1–9.
Available:https://doi.org/10.1186/s12906-018-2320-8
21. An HJ, Lee WR, Kim KH, Kim JY, Lee SJ, Han SM, et al. Inhibitory effects of bee venom on propionibacterium acnes-induced inflammatory skin disease in an animal model. Int J Mol Med. 2014;34:1341–8.
Available:https://doi.org/10.3892/ijmm.2014.1933
22. Chen LW, Chung HL, Wang CC, Su JH, Chen YJ, Lee CJ. Anti-acne effects of cembrene diterpenoids from the cultured soft coral sinularia flexibilis. Mar Drugs. 2020;18:1–12.
Available:https://doi.org/10.3390/md18100487
23. Tsai TH, Huang WC, Lien TJ, Huang YH, Chang H, Yu CH, et al. Clove extract and eugenol suppress inflammatory responses elicited by Propionibacterium acnes in vitro and in vivo. Food Agric Immunol. 2017;28:916–31.
Available:https://doi.org/10.1080/09540105.2017.1320357
24. Pandey A, Tripathi S. Concept of standarization, extraction and pre phytochemical screening strategies for herbarl drug. J Pharmacogn Phytochem; 2014.
25. Dewi NPBT, Singapurwa NMAS, Mangku IGP. Extraction and Stability of natural dyes from the skin of red dragon fruit. SEAS (Sustainable Environ Agric Sciience). 2020;4:130–41.
Available:https://doi.org/10.22225/seas.4.2.2622.130-141
26. Vera Cruz DG, Paragas DS, Gutierrez RL, Antonino JP, Morales KS, Dacuycuy EA, et al. Characterization and assessment of phytochemical properties of dragon fruit (*Hylocereus undatus* and *Hylocereus polyrhizus*) Peels. Int J Agric Technol. 2022;18:1307–18.
27. Fidrianny I, Ilham N, Hartati R. Antioxidant profile and phytochemical content of different parts of super red dragon fruit (*Hylocereus costaricensis*) collected from

West Java-Indonesia. Asian J Pharm Clin Res. 2017;10:290–4.
Available:https://doi.org/10.22159/ajpcr.2017.v10i12.21571

28. Wahdaningsih S, Untari EK, Fauziah Y. Antibakteri Fraksi n-Heksana Kulit *Hylocereus polyrhizus* Terhadap *Staphylococcus epidermidis* dan Propionibacterium acnes. Pharm Sci Res 2014;1:180–93.
Available:https://doi.org/10.7454/psr.v1i3.3490

29. Wahdaningsih S, Untari EK. The antibacterial activity of red dragon fruit peel (*Hylocereus polyrhizus* britton & rose) methanolic fraction against *Staphylococcus epidermidis* and *Propionibacterium acnes*. J Pharmascience. 2021;8:51–8.
Available:https://doi.org/10.20527/jps.v8i2.10378

30. Azhari AQ, Mayasari D, Rusli R. Formulasi sediaan gel totol jerawat berbahan aktif ekstrak kulit buah naga merah (*Hylocereus polyrhizus*) terhadap bakteri *Staphylococcus aureus* dan *Propionibacterium acnes*. 14th Proceeding Mulawarman Pharm. Conf. 2021;hal.: 359–65.
Available:https://doi.org/10.25026/mpc.v14i1.603

31. Jani TA, Hakim A, Juliantoni Y. Formulation and Evaluation of antioxidant peel-off face mask containing red dragon fruit rind extract (*Hylocereus polyrhizus* Haw.). J Biol Trop. 2020;20:438–45.
Available:https://doi.org/10.29303/jbt.v20i3.2157

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/97180