

The Antiacne Activity of Sunkist Peels Methanol Extract in Propionibacterium acne-Induced Acne Vulgaris

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Abstrak

Pengobatan acne vulgaris merupakan sebuah tantangan karena patofisiologi acne vulgaris yang kompleks. Salah satu bahan alam yang berpotensi digunakan sebagai obat herbal adalah kulit sunkist. Kulit Sunkist lebih cenderung berakhir sebagai limbah industri dalam negeri. Tujuan dari penelitian ini adalah untuk mengetahui aktivitas antijerawat dari gel ekstrak metanol kulit Sunkist secara in vivo. Penelitian eksperimental menggunakan gel ekstrak kulit Sunkist pada tikus yang disuntik suspensi Propionibacterium acne secara intradermal. Satu jam sebelum penyuntikan suspensi Propionibacterium acnes, seluruh tikus diberi gel topikal berdasarkan kelompoknya. Kontrol, standar, kelompok Sunkist Peel Extract Gel-I, II, dan III masing-masing mendapat basis gel, klindamisin, 5%, 10%, dan 15% gel ekstrak kulit Sunkist. Penelitian ini menunjukkan ekstrak metanol kulit jeruk Sunkist dengan rendemen 8,73% mengungkapkan adanya beberapa fitokimia, antara lain alkaloid, flavonoid, tanin, dan saponin. Gel ekstrak metanol kulit jeruk sunkist secara signifikan mengurangi ukuran lesi akne vulgaris setelah 7-14 hari pengaplikasian ekstrak (p-value <0,05). Hal ini sejalan dengan pemeriksaan histologis bahwa konsentrasi gel meningkat yang diikuti dengan pertumbuhan lapisan epidermis. Penelitian ini menunjukkan dengan kuat bahwa ekstrak metanol kulit jeruk Sunkist mempunyai efek antijerawat dalam bentuk gel yang stabil secara fisik.

Kata Kunci: kelainan kulit; kosmetik; vitamin a; klindamisi; tikus

Abstract

Treatment with acne vulgaris is a challenge because the complex pathophysiology of acne vulgaris. One of the natural products that potentially use as a herbal medicine is Sunkist peel. The peel of Sunkist is more inclined to end up as waste from domestic industries. The aim of this study was to investigate the antiacne activity of the Sunkist peel methanol extract gel in vivo study. The experimental study used by applied Sunkist peel extract gel to rats injected intradermally with Propionibacterium acne suspension. One hour before the injection of Propionibacterium acnes suspension, all rats were applied to a topical gel based on the group. Control, standard, Sunkist Peel Extract Gel-I, II, and III groups received gel base, clindamycin, 5%, 10%, and 15% of Sunkist peel extract gel, respectively. This study indicates the methanol extract of Sunkist orange peel with a yield of 8.73% revealed the presence of some phytochemicals, including alkaloids, flavonoids, tannins, and saponins. Sunkist orange peel methanol extract gel significantly reduced the size of acne vulgaris lesions after 7-14 days of extract application (p-value <0.05). It is in line with the histological study that the gel concentration increased, followed by the growth of the epidermal layer. This study strongly indicates that Sunkist orange peel methanol extract has an antiacne effect in a physically stable gel form.

Keywords: skin disorders; cosmetics; vitamin a; clindamycin; mouse

Introduction

Acne vulgaris is a chronic inflammation of either the pilosebaceous unit or the surrounding tissue due to obstruction of the pilosebaceous unit. Global Burden of

Disease reports that the prevalence of acne vulgaris in 2016 was 28.41% from 39,319 cases aged 10-24 years old over the world. Meanwhile, the prevalence of acne vulgaris in the Southeast Asian Region was 27.96%. Specifically, the rate of acne vulgaris locally (Indonesia) was 26.88% and increased to 31.79% from 43.322 cases of skin disorders in 2016. An older report from Indonesian Cosmetics Dermatology also reported that the prevalence of acne vulgaris in Indonesia increased from 60% in 2006 to 90% in 2009. Acne vulgaris is not a life-threatening condition. However, it can cause acne scars that may affect quality of life and mental health. Hence, treating acne vulgaris becomes more challenging to improve the patient's quality of life and mental health (1,2,3).

Acne vulgaris is a polymorphic disorder with various clinical presentations caused by complex pathophysiology. The four critical pathophysiologies of acne vulgaris include increased sebum secretion, follicular hyperkeratinization, colonization of *Propionibacterium acnes*, and induction of inflammation. Based on this pathophysiology, there are several modalities of acne vulgaris treatment. Agarwal et al. (2016) reported that the most prescribed acne vulgaris drug was Vitamin A derivatives as oral dosage forms (37%) and clindamycin as topical dosage forms (28%). These drugs have some adverse effects if used irrationally, including resistance to therapy due to the down-regulation of each drug's teratogenic drug receptor expression and developing antibiotic resistance. Therefore, herbal medicine, which is quite popular today, promotes an alternative to acne vulgaris treatment (3-5).

Sunkist peels were one of the natural products that could become a herbal medicine for acne vulgaris treatment. Sunkist peel is more likely to be a wasted product from home industries that use citrus as raw material. Several studies have been performed to explore the pharmacological effects of Sunkist peels. Pandey et al. (2017) reported that the *Citrus sinensis* peel ethanol extract (MIC: 0.074; IC₅₀: 0.155) had an antibacterial effect against *Propionibacterium acne* bacteria which was more potent than the *Citrus sinensis* peel petroleum ether extract (MIC: 0.722; IC₅₀: 0.658), essential oils (MIC: 1.357; IC₅₀: 1.301), and acetone extract (MIC: 1.390; IC₅₀: 1.328). Another study performed by Michiko et al. (2020) also demonstrated the antibacterial activity of the sweet orange peel (*Citrus sinensis*) ethanol extract against *Propionibacterium acnes* bacteria at concentrations of 50%, 75%, and 100% by the disc diffusion method (6,7).

Some previous studies also explore the antibacterial effect of the orange peel against other bacteria. Ariani and Wigati (2016) reported that an orange peel peel-off mask gel also had an antibacterial effect against *Staphylococcus aureus*. This antibacterial effect is not limited to Gram-positive bacteria. Another study by Mutia and Manalu (2020) also reported that the sweet orange peel (*Citrus sinensis*) ethanol extract also had antibacterial activity against gram-negative bacteria, *Vibrio cholera*, at a concentration of 50% (12.70mm), 75% (16.57mm), and 100% (25.36mm) (8,9).

Based on the information above, these previous studies have looked for the antibacterial effect of orange peels by in vitro methods. However, none of these studies looked for the antibacterial effects of in vivo study, especially against *Propionibacterium acne*. Hence, this study aimed to investigate the antiacne activity from Sunkist peels methanol extract as a gel pharmaceutical form in *Propionibacterium acne*-induced rats.

Methods

Materials

This study used 25 male Wistar rats grouped into five groups control, standard, Sunkist peel Gel-I, II, and III. The materials used in this study included Sunkist peel, 98% methanol solution, DMSO, phytochemical screening reagent, an inoculum of *Propionibacterium acne*, normal saline, McFarland standard, disc diffusion, Carbopol 940, propylene glycol, propylparaben, TEA, glycerine, distilled water, 10% buffer formalin solution, and PBS. Sunkist peel was cleaned, cut, and dried under a fan for several days. After that, it meshed into simplicial powder. The simplicial powder was then macerated by 98% methanol solution for three days in a ratio of 1:3 and regularly stirred. After three days, it was filtered, and the residue was re-macerated in the same way two times. Obtained filtrate was collected in a container to be evaporated by a rotary evaporator. Thus, it formed a concentrated Sunkist peel methanol extract (10-12).

Phytochemicals Screening and Gel Formulation

The obtained concentrated extract underwent a phytochemical screening to investigate the presence of alkaloids, flavonoids, terpenoids/steroids, tannins, saponins, and glycosides (13,14). Sunkist peel extract gel was formulated using Carbopol 940, propylene glycol, methyl paraben, propyl paraben, TEA (Triethanolamine), glycerine, and distilled water. The exact concentration of all these components is described in the following table.

Table 1. Formulation of Sunkist Peel Methanol Extract Gel

Material	Base Gel	Sunkist Peel Extract Gel		
		5%	10%	15%
Sunkist peel methanol extract (gram)	-	1.50	3.00	4.50
Carbopol 940, 1% (gram)	0.3	0.3	0.3	0.3
Propylene glycol, 5% (ml)	1.5	1.5	1.5	1.5
Methyl paraben, 0.2% (gram)	0.06	0.06	0.06	0.06
Propyl Paraben, 0.1% (gram)	0.03	0.03	0.03	0.03
TEA, 1.2% (ml)	0.36	0.36	0.36	0.36
Glycerin 1% (ml)	0.3	0.3	0.3	0.3
Distilled water	Up to 30 ml	Up to 30 ml	Up to 30 ml	Up to 30 ml

Three hundred milligrams of Carbopol 940 were dissolved into fifteen milliliters of distilled water and left chilled. After that, an amount of 0.06-gram methylparaben and 0.03-gram propyl paraben were dissolved into 5 milliliters of distilled water, then 1.5 ml of propylene glycol and measured Sunkist peel methanol extract were added into this last mixture. Moreover, the dissolved Carbopol 940 was slowly stirred into the last mixture. Finally, distilled water was added up to thirty milliliters, which TEA and glycerine preceded (15).

Physical Stability Assay of Gel

Physical stability assay of the gel used Freeze-Thaw Methods. Freeze-thaw method consists of two different processes: freeze and thawing. The freezing process was performed by storing the gel in a 4°C condition for 24 hours. Meanwhile, the thawing process was performed by storing the gel in a 40°C condition for 24 hours. Physical parameters that were evaluated in this study were organoleptic, homogeneity, pH, spreadability, and adhesion (15,16).

Evaluation of Antiacne

Twenty-five Wistar rats were grouped into five groups: control, standard, and Sunkist Peel Extract Gel-I, II, and III. One hour before the injection of *Propionibacterium acnes* suspension, all rats were applied to a topical gel based on the group. Control, standard, Sunkist Peel Extract Gel-I, II, and III groups received gel base, clindamycin, 5%, 10%, and 15% of Sunkist peel extract gel, respectively. After that, ten microliters of *Propionibacterium acnes* suspension were injected intradermally into the shaved interscapular region. After injection, the lesion size, that was diameter, was measured by a vernier caliper. The lesion size was also measured on the 7th and 14th days. At the end of the evaluation period, all rats were sacrificed by the neck dislocation method, and the lesion was excised for histological evaluation (17-19).

Data Analysis

Initially, all data were analyzed by descriptive statistics describing lesion size's central tendency and dispersion. Meanwhile, the histologic evaluation was narratively described. After that, lesion size data was analyzed for the data distribution. If the data distribution was normal, it was analyzed by one-way ANOVA and expressed as Mean \pm SD. Alternatively, if data distribution was not normal, it was analyzed by Kruskal-Wallis and expressed as Median (Minimum-Maximal).

Research Results

This study used 836 grams of fresh Sunkist peel in 1.500 ml of 98% methanol solution as a solvent. After the maceration, 74 grams of concentrated Sunkist peel methanol extract was obtained. Hence, it obtained a yield of 8.73%. Concentrated Sunkist peel methanol extract underwent a phytochemical screening, and the result of the phytochemical screening was described in the following table.

Table 2 Phytochemical Screening of Sunkist Peel Methanol Extract

Phytochemicals	Methods	Result
Alkaloid	Bouchardart	+
	Maeyer	+
	Dragendorff	+
	Wagner	+
	FeCl ₃ 5%	+
Flavonoid	Sinoda Test (Mg+HCl)	+
	Alkaline (NaOH)	-
	H ₂ SO _{4(p)}	-
Terpenoid/ Steroid	Lieberman-Burchard	-
	Salkovski	-
Tannin	FeCl ₃ 1%	+

Saponin	Distilled Water + 96% Alcohol	+
Glycoside	Mollish	-

Based on table above, concentrated Sunkist peel methanol extract contained phytochemicals, including alkaloid, flavonoid, tannin, and saponin. After that, the concentrated Sunkist peel methanol extract was formulated into some concentration of gels, and these gels were evaluated for physical stability.

The physical stability showed that all physical parameters were stable at six cycles of freeze-thaw. Organoleptic and homogeneity of the Sunkist peel methanol extract gel showed that the color of the gel was yellow to brownish yellow with a characteristic citrus odor and homogeneous for six freeze-thaw cycles. In addition, other parameters of physical stability also show the optimum formulation. The degree of acidity or pH of all gels was within the weak acid range (5.8-6.4). Meanwhile, the spreadability with 100 grams of all gels ranged from 5.0-5.6cm, which is inversely proportional to the concentration of the gel.

Sunkist peel extract gels then underwent antiacne evaluation by *Propionibacterium acne*-induced acne vulgaris. The lesion size of acne vulgaris from all groups was described in the following table.

Table 3 Lesion Size of Acne Vulgaris in All Groups

Groups	Lesion Size, cm		p-Value*
	7 th Day	14 th day	
Control	0.50 ± 0.08	0.51 (0.41-0.57)	0.001
Standard	0.33 ± 0.05	0.17 (0.11-0.24)	
Sunkist Peel Extract Gel-I	0.39 ± 0.07	0.31 (0.25-0.37)	
Sunkist Peel Extract Gel-II	0.35 ± 0.03	0.20 (0.17-0.31)	
Sunkist Peel Extract Gel-III	0.31 ± 0.04	0.18 (0.11-0.19)	

Based on table above, it clearly described that all groups of rats had significant differences in the lesion size, both on the 7th day (P value <0.05) and the 14th day (P value: 0.001). After seven days of treatment, the lesion size significantly decreased in line with the increasing concentration of Sunkist peel extract gel, where the narrowest lesion size was found in the Sunkist Peel Extract Gel-III (0.31 ± 0.04 cm), followed by the Standard group (0.33 ± 0.05 cm), Sunkist Peel Extract Gel-II (0.35 ± 0.03 cm), I

(0.39 ± 0.07 cm), and the widest was the Control group, that was 0.50 ± 0.08 cm. Furthermore, after 14 days of treatment, the lesion size in all groups tended to decrease compared to the seventh day, except for the control group, which experienced an increasing lesion size, whereas the narrowest lesion size was found in the standard group, which was 0.17 (0.11-0.24) cm, followed by the Sunkist Orange Peel Extract Gel group-III, II, I, and the widest was the Control group, that was 0.51 (0.41-0.57) cm. On the 15th day, all rats were sacrificed to excised lesion tissue for histology study, and the microscopic view was described in the following figure.

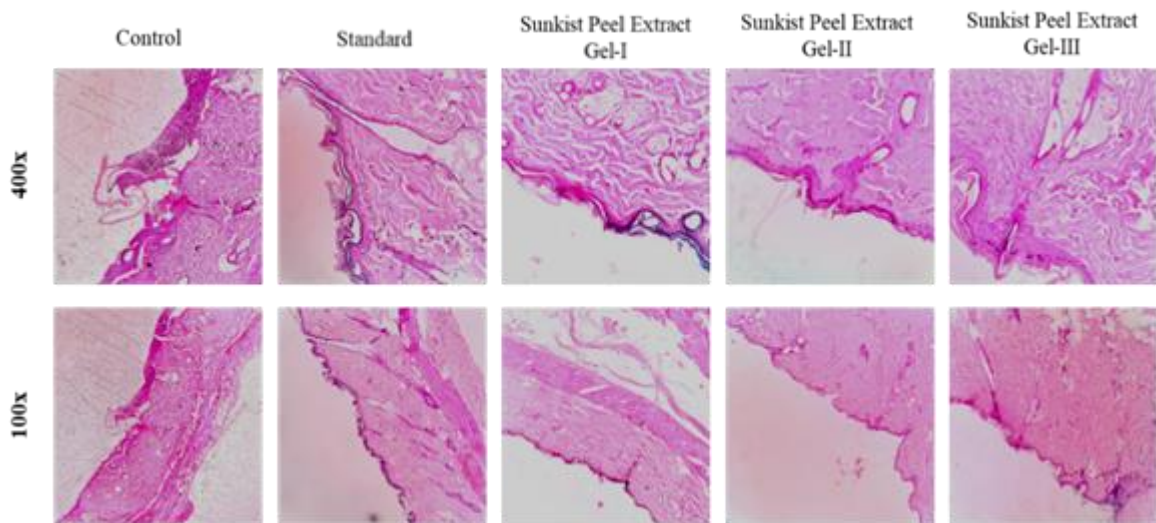


Figure 1 Histology of Acne Vulgaris Lesion Tissue in All Groups. Stain: Hematoxylin and Eosin

Based on the figure above, the improvement of the lesion was revealed in the epidermis layer. The control group still had ongoing structural damage in the epidermis layer, demarked by the epidermis and dermis layer separation. In contrast, the standard group showed an intact skin layer, especially the epidermis layer, that was still complete, and this group also did not show any separation or gap between the epidermis and dermis layer. Meanwhile, the Sunkist peel gel group also showed an intact skin layer like the standard group. However, there was a difference in epidermis layer thickness. The higher concentration of Sunkist peel extract gel, the thicker the epidermal layer was formed. The study results above clearly answered the objectives of this study. Sunkist orange peel methanol extract, yielding 8.73%, contains some phytochemicals, including alkaloids, flavonoids, tannins, and saponins. Sunkist orange peel methanol extract was then formulated into a gel with 5%, 10%, and 15% concentrations. These gels had good

physical stability during six different freeze-thaw cycles. Furthermore, this in vivo assay demonstrated a significant reduction of acne vulgaris lesion size in rats at either the 7th day (P value < 0.05) or the 14th day (P value: 0.001), and it was also supported by a histology study against the skin tissue. The increase in Sunkist peel extract gel concentration was also followed by increasing the rate of epidermal growth, which was marked by the thickness of the epidermis. The yield of Sunkist orange peel methanol extract in this study was lower than in some previous studies. Gulo et al. reported that the yield of orange peels ethanol and ethyl acetate extract was 19.49% and 5.45%, respectively. The yield value inversely correlates to the quality of the extract. The yield of Sunkist peels methanol extract was higher than the ethyl acetate. However, it was not higher than the ethanol extract. Thus, it indicated that the methanol extract had a better quality than the ethanol extract. However, the methanol extract quality was not better than the ethyl acetate. Variation of this yield value may be due to the duration of maceration in this study and previous studies. Pandey and Tripathu (2014) reported that several factors affect the quality of extract, including the plant part used for extraction, the solvent used for extraction, and the extraction methods (11,20).

Discussion

This study showed that the Sunkist peel methanol extract had some phytochemicals, including alkaloids, flavonoids, tannins, and saponins. It showed a similar result to the previous study done by Depari et al. This previous study reported that Sunkist orange peels had phytochemicals, including tannins, saponins, flavonoids, triterpenes, glycosides, and polyphenols. Some factors may affect the extracted phytochemicals compound from a natural product, including the extraction method, duration of extraction, temperature, solvent properties, solvent concentration, and solvent polarity (20,21).

The Sunkist peel methanol extract was then formulated into some concentration of gel form. These gels evaluated the physical stability by the freeze-thaw method. These parameters included organoleptic, homogeneity, pH, spreadability with or without load, and gel adhesion. Organoleptic characteristics and homogeneity of the Sunkist peel methanol extract gel showed that the color of the gel was yellow to brownish yellow with a characteristic citrus odor and homogeneous for six freeze-thaw

cycles. In addition, other parameters of physical stability also show the optimum formulation. The degree of acidity or pH of all gels was within the weak acid range (5.8-6.4), which is still at the normal pH of a preparation (4.5-6.5). Meanwhile, the spreadability with 100 grams of all gels ranged from 5.0-5.6cm, which is inversely proportional to the concentration of the gel. A good semisolid pharmaceutical has a spreadability between 5-7cm, and all Sunkist peel gel has a good spreadability. Wider spreadability indicates the better ability of the gel to spread the active substance on the skin surface. Thereby it increases the active substance concentration that can diffuse into the skin (22).

Sunkist peel extract gel has an antiacne property due to the presence of phytochemicals. This antiacne activity comes from an antibacterial and anti-inflammatory activity that prevents *Propionibacterium acne* colonization and suppresses the inflammatory response. Some previous studies have been performed to explore the antiacne properties of Sunkist peel extract gel. Pandey et al. (2017) reported that the *Citrus sinensis* peel ethanol extract (MIC: 0.074; IC₅₀: 0.155) had an antibacterial effect against *Propionibacterium acne* bacteria that was more potent than petroleum ether extract (MIC: 0.722; IC₅₀: 0.658), essential oils (MIC: 1.357; IC₅₀: 1.301), and acetone extract (MIC: 1.390; IC₅₀: 1.328). Another study by Michiko et al. (2020) also demonstrated the antibacterial effects of *Propionibacterium acne* from *Citrus sinensis* peel extract (50%, 75%, and 100%) by Disc diffusion methods ^{6,7}. Alkaloid is reported to inhibit bacterial DNA synthesis and interfere with the formation of peptidoglycan in the bacterial cell wall so that the bacterial cell wall layer will not form completely and cause cell death. Alkaloid has a base nitrogen group that can react with amino acid in the bacterial cell wall and genomic DNA. This reaction affects the structure and arrangement of amino acids, which leads to DNA damage. Furthermore, this DNA damage lysis of the bacteria cell and induces cell apoptosis. In addition, flavonoids are also reported to have an antibacterial effect by destroying the cytoplasmic membrane and causing the leakage of various chemical compounds in the cytosol responsible for energy production and various metabolism pathways. The failure of energy production and various metabolism pathways causes the failure of bacterial cell growth and leads to cell death (23).

Other than antibacterial activity, Sunkist peel extract also has anti-inflammatory activity. This anti-inflammatory activity is due to phytochemicals, including flavonoids and alkaloids. Flavonoid inhibits the biosynthesis of prostaglandins. Prostaglandin is a product of the cyclooxygenase and lipoxygenase pathways, also involved in various immunological responses in the body. In addition, flavonoids also affect protein kinase, one of the regulatory enzymes that can inhibit the inflammatory process (24). Furthermore, Gaichu et al. (2017) also reported that alkaloid compounds as phytochemical compounds inhibited the biosynthesis of prostaglandins like the flavonoid, especially in the cyclooxygenase pathway²⁵. The cumulative inhibitory effect of the cyclooxygenase pathway from these flavonoids and alkaloids can inhibit the formation of prostaglandins which are inflammatory mediators. Hence the Sunkist peel methanol extract reveals an anti-inflammatory effect.

Conclusions and Suggestions

Sunkist peel methanol extract can be formulated into an antiacne gel that can improve the acne vulgaris lesion after 7 to 14 days of application. The decrease of acne vulgaris lesions required higher Sunkist peel extract gel concentration. This study indicates that Sunkist peel extract gel become a promising extract to treat acne.

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