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Original Article

FORMULATION AND EVALUATION OF ANTI ACNE GEL CONTAINING *CITRUS AURANTIFOLIA* FRUIT JUICE USING CARBOPOL AS GELLING AGENT

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ABSTRACT

Objective: The objective of this study was to design a product of anti-acne gel containing *Citrus aurantifolia* fruit juice as an effective antibacterial to treat acne caused by *Propionibacterium acne* and *Staphylococcus epidermidis* using carbopol as a gelling agent.

Methods: The fresh juice of *C. aurantifolia* fruit was obtained by juicer and pasteurized for 30 min at 65-70 °C. The minimum inhibitory concentration (MIC) of the fruit juice was determined using the microdilution method. Then, carbopol in different concentration was incorporated in a gel base formula to obtain a stable gel base. The fresh juice in different formulas (F1, F2 and F3) was evaluated for 28 d. The color, pH and viscosity of each formula were observed. In addition, the antibacterial potency of each formula was analyzed using the agar diffusion method against both tested bacteria.

Results: The citrus MIC values of both test bacteria showed different results, 20-40 % v/v for *P. acne* and 5-10 % v/v for *S. epidermidis*. The MIC values were converted into *in vivo* concentration and the resulted concentrations for each formula were 25, 50 and 75 % v/v. For supporting the formula, the most stable base gel was achieved using carbopol 1 % as the gelling agent. Among three formulas, the anti-acne gel formula containing 75 % fruit juice with carbopol 1% was the best formula based on the physical and microbiological parameter.

Conclusion: Thus, it was concluded that the antiacne gel of fruit juice of *C. aurantifolia* with carbopol as a gelling agent could produce the effective and stable gel of anti-acne product.

Keywords: *Citrus aurantifolia*, Juice, Carbopol, Anti-acne, Gel

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INTRODUCTION

Acne is a skin disease with the highest prevalence among other skin disorders. Almost everyone has experienced acne prone skin, especially in an adolescent. Although it is considered not as a dangerous disease, but in fact, almost all acne sufferers feel disturbed appearance that often leads to lower levels of confidence and interfere with the daily activities. No wonder, if most patients who come to the skin care clinic are those who seek a solution to overcome the acne. According to one of a dermatologist, about 70 percent of patients who come, have acne problems.

The infection of acne vulgaris exhibits wide distribution and its prevalence increase over time [1]. Acne is the most dominant skin disease reported based on large studies in the USA, France, and the UK [2-4]. In Indonesia, about 95-100% of men and 83-85% of women aged 16-17 y suffer acne. The prevalence of acne in adult females is about 12% and in adult males 3%. Another study found that acne is a skin problem of adolescence with a higher prevalence of women than men in the age range of 20 y or older [5].

Acne vulgaris is characterized by various clinical conditions such as scaly red skin (seborrhea), erythematous papules and pustules, comedones, nodules, deep pustules, and sometimes piples [6]. The pathogenicity mechanism of acne was the production of sebums, follicular hyperkeratinization, bacterial colonization, and inflammation [7-10]. *P. acne* plays a role in the development of inflammatory acne by activating complements and can metabolize sebaceous triglycerides into fatty acids, which neutrophils were attracted [11]. In addition, *S. epidermidis* within sebaceous unit responsible in superficial infection [12]. When bacteria colonize into the comedons, then the inflammatory factors are released by those bacteria. This made the comedons transformed into pustules and pimples. The inflamed acne becomes rupture and forms nodulus, also probably forms scars after healing [13].

The type of acne, acne severity grading, number of lesions and anatomic location will determine the treatment. The treatment of acne can be given by topical or systemic therapy. The topical therapies include antibiotics, anti-inflammatory and comedolytic agent [14]. Benzoyl peroxide or its combination with clindamycin or erythromycin can treat acne effectively and recommended as an antibacterial agent for *P. acne* through the release of free oxygen radicals, also reported has a comedolytic agent [15, 16]. But the limitation of benzoyl peroxide therapy is its concentration-dependent irritation, bleaching of bed linen, hair and fabric and causing irritant dermatitis [17, 18]. For systemic therapy, oral antibiotics such as tetracyclines and its derivatives were the first choice [19]. It is indicated mainly for moderate-to-severe inflammatory acne [10]. But long-term therapy of oral antibiotic, not only can induce bacterial resistance but also associated with the incidence of upper respiratory tract infection [20]. The presence of bacterial resistance and unexpected side effects opens the opportunities for traditional medicine to replace the effectiveness of synthetic drugs in overcoming acne vulgaris.

Citrus family is a widely consumed group of fruits which contains several metabolites such as flavonoids, ascorbic acid and carotenoids [21]. *Citrus aurantifolia* is one of the citrus species that widespread and consumed lime species in Indonesia. Traditionally, this fruit juice has long been used as an ant acne herb and it effectively has been proven. But if the fruits must be squeezed first, then the treatment becomes not optimal and not practical. Meanwhile, to overcome the problem of acne against *P. acne* and *S. epidermidis* required anti-acne preparations that have good penetration and long contact time. Therefore, in this study, *C. aurantifolia* juice is formulated in the form of anti-acne gel preparations. Gel preparation can be used as an option for anti-acne preparations. The gel dosage form can last long in the skin and the release of good active substances. Gel formulation makes the preparation more easily removable from the skin then ointment and cream [22, 23]. This study was conducted to develop an anti-acne gel formulation of *C. aurantifolia* fruit juice using carbopol as a gelling agent because another study reported that carbopol showed the superior drug release in gel formulation [24].

MATERIALS AND METHODS

Materials

The mature fresh fruits of *C. aurantifolia* were collected from Tasikmalaya, West Java, Indonesia and authenticated (No.023/ HB/11/2015) by Institute of plant determination in the department of biology, Faculty of mathematics and natural sciences, Padjadjaran University, Jatinangor, Indonesia. The tested bacteria used in this study were *Propionibacterium acnes* and *Staphylococcus epidermidis*, obtained from PT. Biofarma and Microbiology Laboratory, Faculty of Pharmacy Universitas Padjadjaran. The growth medium used was Mueller Hinton Agar (MHA-OXOID) and Mueller Hinton Broth (MHB-OXOID). The chemicals used were amyl alcohol, 10% ammonia, 2N hydrochloric acid, iron (III) chloride, ether, chloroform, anhydrous acetic acid solution in concentrated H2SO4, 1% gelatin, reagent Dragendorff (potassium bismuth iodide solution). Mayer reagents (potassium mercury iodide solution), 10% vanillin solution in concentrated H2SO4, 1N sodium hydroxide, potassium permanganate powder, magnesium powder, and sterile physiological sodium chloride, demineralized water, ethanol, carbopol, propylene glycol, methylparaben/propylparaben and triethanolamine.

Juice fruit preparation

Whole fresh fruits were washed with potassium permanganate 1.5%, then the fruit juice was taken and filtered using a separated funnel with filter paper, then the juice was sterilized by pasteurization at a temperature of 65-70 °C for 30 min.

Phytochemical screening

Phytochemical screening was done by a phytochemical screening of plants method to determine the secondary metabolite group found in the fruit juice of *C. aurantifolia*. Those metabolites include alkaloids, flavonoids, polyphenols, tannins, monoterpenoids, sesquiterpenoids, steroids, triterpenoids, quinones, and saponins [25].

MIC determination

Determination of MIC was performed using microdilution method with microtiter plates 96 wells. Each column is filled with 100 µl MHB. Column 1 was used as a positive control, column 2 as a negative control, column 3 as the control of the juice, and column 4-12 as the test column. Then, as much as 100 µl juices were suspended into column 4 and homogenized by pipetting. From column 4, it was taken as much as 100 µl and put into column 5 to obtain a juice concentration less than half concentration compared to the juice concentration in column 4. Thus, until column 12 and last was removed 100 µl from column 12, so that all columns only contain 100 µl test media suspension. As a positive control filled with 100 µl MHB and 10 µl suspensions of the test bacteria. Then into all columns, except column 2, inoculated with 100 µl suspensions of the test bacteria. The microtiter plate was closed and incubated at 37 ° C for 18-24 h. Petri dishes contain the most active test materials with the smallest concentrations showing the least growth and the last growth of test bacteria was determined as the range of MIC values. The incubation of MIC, observed by its turbidity and concentration resulting in a clear test medium, was taken as much as 10 µl to be reinoculated on a solid MHA surface. The test medium was incubated at 37 °C for 18-24 h. The petri dish contains the most active test material with the smallest concentration that did not show the growth of test bacteria determined as the MBC value range.

Formulation and evaluation of gel base

Gel of fresh juice from C. aurantifolia was made using a varying concentration of carbopol as a gelling agent. In this gel base, distilled water was used as the solvent, methylparaben as a preservative, and propylenglycol as a humectant, the formula can be seen in table 1. The carbopol was dissolved in demineralized water which has been heated to a temperature of 70 °C inside the mortal, then stirred slowly to form a homogeneous dispersion. Each of TEA and propylene glycol was dissolved in demineralized water separately and stirred. The TEA solution was added to carbopol solution and stirred until homogeneous. The pH of that mixed solution was adjusted to 7.4 and the propylene glycol solution was added and stirred until a clear consistent gel base was obtained. The gel was kept for 24 h until the bubbles were disappeared, then the pH and gel viscosity was measured.

Table 1: Preformulation of the base gel

Composition	Formula 1	Formula 2	Formula 3
Juice fruit	-	-	-
methyl paraben	500 mg	500 mg	500 mg
TEA	2 ml	2 ml	2 ml
Carbopol	0.8g	1 g	1.2 g
propylenglycol	10 ml	10 ml	10 ml
demineralized water	Add 100 ml	Add 100 ml	Add 100 ml

Formulation and evaluation of anti-acne gel

To the base, lemon juice which has been added with methyl-paraben and has been diluted with ethanol was added. Stirring was stopped and the gel was stored in a sealed container. The gel was kept for 24 h until the bubbles were disappeared. The detail formula of fruit juice can be seen in table 2. The observation of the physical stability of *C. aurantifolia* juice preparation comprised an examination of organoleptis, pH, and viscosity during storage in climatic chamber, for 28 d. Organoleptic test was performed to see the physical appearance of the preparation by observing the color, odor, and texture of the preparations that have been made [26]. The gel preparation was tested for homogeneity by applying it to a glass of preparation (transparent), thus, the presence or absence of particles/substances that have not been mixed homogeneously can be observed [27]. The preparation was prepared in a 100 ml beaker glass, then the spindle with a certain number and a certain speed (rpm) was set and then dipped into the preparation until the apparatus showed the viscosity value of the preparation. The viscosity value (cPs) shown in the RION Viscometer tool was the viscosity of the dosage. Evaluation of viscosity, done by using spindel R5 with speed of 30 rpm. The pH of gel preparation was measured using a calibrated pH meter [27]. All formulas gel evaluation were observed on 0, 7,14, 21 and 28 d of gel storage at room temperature.

Table 2: Composition of an anti-acne gel formula

Component	Formula I	Formula II	Formula III
Fruit juice	25 ml	50 ml	75 ml
Carbopol	1 g	1 g	1 g
TEA	3.2 ml	4.2 ml	5.3 ml
Propylenglycolm	10 ml	10 ml	10 ml
Ethyl paraben	500 mg	500 mg	500 mg
demineralized water	Ad 100 ml	Ad 100 ml	Ad 100 ml

Preparation of bacterial suspension

McFarland solution consisted of two components, 1% $BaCl_2$ and 1% H_2SO_4. A total of 0.05 ml of 1% $BaCl_2$ solution was mixed with 9.95 ml of 1% H_2SO_4 solution and shaken homogeneously. The turbidity of the solution was measured at a wavelength of 530 nm by using distilled water as a blank. The absorbance value of the standard solution should be in the range of 0.08 to 0.13. The standard McFarland 0.5 solutions are equivalent to a bacterial cell suspension with a concentration of 1.5 x 10^8 CFU/ml. The tested bacteria were scratched on the surface of slant agar, then incubated for 18-24 h at 37 ° C. Each of *P. acnes* and *S. epidermidis* colonies were taken using Ose, then suspended into sterile physiological NaCl. The bacterial turbidity was measured using a spectrophotometer at λ 530 nm, compared with a 0.5 Mc Farland solution [28].

Anti-acne activity

The efficacy of anti-acne gel formulas was performed using the agar diffusion method with perforation technique against *P. acne* and *S. epidermidis*. A total of 20 µl bacterial suspension was fed into sterile petri dishes and suspended in 20 ml of the MHA which was poured into the sterile petri dish. The test medium was homogenized and allowed to solidify. Media that has been solidified, then perforated to make holes for sample reservoir. The tested juice concentrations were 10, 25, 50, and 75 w/w. A total of 50 mg of each concentration was introduced using a sterile syringe into the reservoir on the test medium. The negative and positive control was prepared, where the negative control contains the only medium, meanwhile the positive control consisted of the inoculated bacterial suspension using the streak inoculation method. All test and control media were incubated at 37 °C for 24 h. The inhibitory diameter formed was measured using a caliper.

RESULTS AND DISCUSSION

Fruit juice result

From 10 kg of *C. aurantifolia* fruits, a volume of 3.2 L rendemen juice was obtained. The pH of the juices was 5 and it is in accordance with the pH normal of the face is 4.5-6. The term of pH is used to describe the acid-alkaline ratio of a substance ranging. Skin pH is normally acidic, ranging between 4 and 6, while the body's internal environment maintains a neutral to slightly alkaline pH (~7.4) [29, 30]. Variable skin pH values are being reported in the literature, all in the acidic range, but with a broad range from pH 4.0 to 7.0. In another study, based on the measurement of the biophysical parameters of barrier function, moisturization and scaling, it found that skin with pH values below 5.0 is in a better condition than skin with pH values above 5.0 [31]. The relation between skin pH and acne also had been reported that the majority of acne occurrences in the case group were related to high skin pH [32]. Because one of the natural barriers of skin is the acidic pH of stratum corneum. Thus, a shift in pH of the normal skin causes the barrier dysfunction and finally, acne vulgaris occur [33-35]. As the skin pH rises, normal flora disturbed and diminished the antimicrobial peptide produced by the normal flora. Therefore, the population of acne vulgaris causing bacteria increased and infection resulted [36-38].

Phytochemical screening of fruit juice

The following secondary metabolites were found to present in the fruit juice of *C. aurantifolia*. The metabolites were recorded in table 3. Flavonoid is well known antibacterial agent that had been studied. Flavonoid had been reported showed antimicrobial activities against *P. acnes*, and *S. epidermidis* [39, 40]. In another study, tannins and flavonoids in green tea also proven that possess an anti-acne effect, since they seem to have an antiseptic effect while tannins also have an anti-inflammatory effect [41]. Until now, the popularity of herbal drug increasing because of its advantages such as patient tolerance, long-term use with fewer side-effects and relatively cheap [42].

Table 3: Phytochemical screening

Metabolites	Result
Alkaloids	+
flavonoids	+
polyphenols	-
tannins	+
monoterpenoids	-
sesquiterpenoids	-
steroids and triterpenoids	-
quinones	-
saponins	-

Notes: (+) presence; (-) absence

MIC determination result

The MIC value of the fruit juice is needed to determine the dosage in the formula. This *in vitro* dose was then multiplied 2-4 times to obtain an *in vivo* dose or the test dose used in the formula. MIC results can be seen in table 4. Based on the data in the table, it was known that the MIC value of *C. aurantifolia* juice against *S. epidermidis* was lower than against *P. acnes*. This showed that the juice was more effective work as an anti-acne against *S. epidermidis*. The MIC value of *S. epidermidis* was 5-10 % w/v, whereas against *P. acnes* was 20-40 % w/v.

Table 4: MIC result

Concentration (% w/v)	Bacterial Growth	
	S. epidermidis	*P. acnes*
40	-	-
20	-	+
10	-	+
5	+	+
2.5	+	+
Positive control	+	+
Negative control	-	-

Formulation and evaluation of gel base result

Gels are a semisolid preparation that is used for skin with easier application than lotions and creams. Creams and lotions are rapidly cleared from the skin, thus, provide poor bioavailability of the drug [43]. By applying the gel on the skin, can be convenient because gel can spreadable and washable, hence we are no need to remove it from the skin. In addition, gel application possesses the capability to release

active agents immediately, regardless of the water solubility of the active agent. By applying the juice of *C. aurantifolia* in the form of gel, can produce anti-acne products that are comfortable, effective and easy to use. Considering the use of this juice is generally only squeezed and used directly, so it is not durable and inconvenient. Hence the *in vitro* studies were carried out to formulate the *C. aurantifolia* juice in various concentrations using carbopol as the gelling agent. In this study, carbopol was chosen as a gelling agent because carbopol was reported to have more gelling property than another polymer [44]. The results of the gel base evaluation can be seen in table 5.

Table 5: Evaluation of base gel preformulation

Day	Formula								
	1			2			3		
	Viscosity	pH	Color	Viscosity	pH	Color	Viscosity	pH	Color
1	121±0.50	9±0.00	transparent	145±0.50	9±0.00	transparent	163±0.50	9±0.00	transparent
7	126±0.57	9±0.00	transparent	148±0.00	9±0.00	transparent	152±0.57	9±0.00	transparent
14	134±0.57	9±0.00	transparent	148±0.50	9±0.00	transparent	145±0.00	9±0.00	transparent
21	142±0.50	8±0.00	transparent	152±0.57	9±0.00	transparent	142±0.50	9±0.00	turbid
28	140±0.00	8±0.00	turbid	145±0.50	9±0.00	transparent	150±0.50	9±0.00	turbid

Carbopol also are not absorbed into the body and irritation free. Carbopol polymer proved to be a promising carrier for controlled release of active phytoconstituents in the gel formulation. Another study reported that the gel formulations prepared with Carbopol as a gelling agent were found to be superior to the gel formulations. Hence the effective concentration of formulatingcarbopol was determined for the best base gel formulation. Carbopol is a synthetic polymer made of carbomers that are cross-linked together to form a microgel structure. In nature, carbopol is anionic, therefore, need neutralization for microgel structure by adding triethanolamine [45]. Triethanolamine was added as a neutralizing agent for acidic carbopoles, since triethanolamine contains 56 to 86% carboxylic acid. Triethanolamine also acts as a stabilizer and developer of carbopol and prevents the disruption of disperse from carbopol when exposure to light causes the gel to become cloudy. Methylparaben is added as a preservative. This is because the use of water medium, which is very vulnerable to microbial growth. While the addition of propylenglikol used as humektan. Propylene glycol is reported to be the two best permeation enhancers [46, 47]. Meanwhile, triethanolamine were used in the formulation in order to adjust the pH of the formulation. In this study, three different base gel formulations were prepared using different concentrations of carbopol (0.8;1 and 1.2 %w/v). Carbopol with 1% concentration yields the most stable gel base formula among other formulas. The pH of the base of the gel throughout the formula shows an alkaline pH over the same range, but until the end of storage, the formula with 1% carbopol concentration shows a clear gel base with a stable viscosity. Thus carbopol 1% will be continued as a gelling agent in the next stage of formulation.

The result of anti-acne gel formulation and evaluation

All fruit juice of *C. aurantifolia* gel preparations were evaluated for their physical characterization like pH, viscosity, and color as shown in table 6. Based on the evaluation of the fruit juice extract preparation, the juice could lower the pH of the gel base to a pH corresponding to the normal range pH of the skin. In addition, the viscosity of the all fruit juice gel formula decreased with the increasing of storage time. The viscosity range of the three formulas was ranged from 135-148 dPa. But interestingly, in formula 3, found that the viscosity was increased after a period of time storage. This may be due to the effect of the highest concentration of juice on formula 3 with the highest acidity among the other two formulas. The formula 3 with 75% juice concentration was hypothesized could neutralize the pH of base gel and resulted in the increasing viscosity of the gel.

Table 6: Anti-acne gel evaluation

Day	Formula								
	1			2			3		
	Viscosity	pH	Color	Viscosity	pH	Color	Viscosity	pH	Color
1	142±0.50	6±0.50	Transparent yellow	147±0.00	6±0.00	Transparent yellow	135±0.00	6±0.00	Transparent yellow
7	138±0.50	6±0.00	Transparent yellow	145±0.00	6±0.00	Transparent yellow	143±0.00	6±0.00	Transparent yellow
14	138±0.50	6±0.00	Transparent yellow	144±0.00	6±0.00	Transparent yellow	142±0.00	6±0.00	Transparent yellow
21	136±0.50	6±0.00	Transparent yellow t	142±0.00	6±0.00	Transparent yellow	140±0.00	6±0.00	Transparent yellow
28	135±0.50	6±0.00	Transparent yellow	138±0.00	6±0.00	Transparent yellow	141±0.00	6±0.00	Transparent yellow

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Anti-acne activity

The topical treatment of infectious disorders and skin inflammations such as acne is desirable and assures advantages than other administration of treatment. The topical application can eliminate the gastrointestinal irritation and avoid first pass metabolism. The skin can lower the bioavailability of the active substance so that it can reduce the antibacterial effect to the pathological site of acne. Therefore, the topical dosage form is the most appropriate form to facilitate active moiety into the pilosebaceous skin unit. The release of the active substance is also strongly influenced by some physical characters, especially the viscosity of the gel preparation and the viscosity is mainly correlated with the concentration of the gelling agent like carbopol.

Anti-acne activity of all formula gels indicated that *C. aurantifolia* in gel preparation was more effective as anti-acne against both tested bacteria, than its pure fruit juice. The details of the inhibition were performed in table 7. All formulas resulted in higher inhibitory diameter against *P. acne* than to *S. epidermidis*. This was very interesting, considering that the main pathogen of acne was caused by *P. acne* and the *P. acne* was sensitive to all formulas gel containing *C. aurantifolia* fruit juice. But the more increasing juice concentration exhibited the more diameter of inhibition zone against both of tested bacteria. Thus, the formula 3 gave the highest anti-acne activity against *P. acne* and *S. epidermidis*. This result can be clearly seen in fig. 1.

Table 7: Anti-acne activity result

Concentration	Inhibitory diameter (mm)	
	S. epidermidis	*P. acnes*
Fruit juice not formulated (100%pure)	10.46±0.01	14.32±0.02
Formula 1	18.21±0.05	17.53±0.04
Formula 2	20.26±0.01	23.54±0.04
Formula 3	24.76±0.01	25.42±0.01

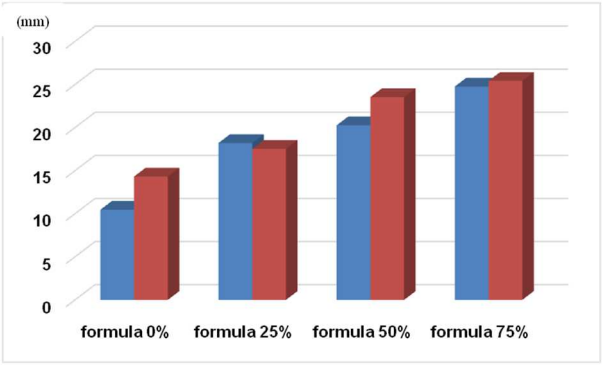

Fig. 1: Effect of juice concentration on inhibitory diameter against tested bacteria Notes: Blue (*S. epidermidis*); orange (*P. acnes*)

The above discussion regarding the scope of natural therapeutics in the form of plant extracts and various isolated secondary metabolites spells out the worth of plant-derived treatment options against acne vulgaris. These findings were proposed *C. aurantifolia* fruit juice broad relevance against acne vulgaris causing bacteria. The antiacne activity of this juice was correlated with antibacterial phytomolecules containing in the *C. aurantifolia* juice. In addition, the increasing viscosity of the formula 3 showed that the use of carbopol plays a role in the replacement of active agent that improving its bacterial inhibition.

CONCLUSION

It can be concluded that the fruit juice of *C. aurantifolia* gel formulations prepared with the concentration of 1% carbopol as gelling agents, confirm the stable physical characteristics of the base gel. In this study, the formula 3 with a concentration of 75% fruit juice presented the excellent anti-acne topical against *P. acne* and *S. epidermidis*.

AUTHORS CONTRIBUTIONS

All the author have contributed equally.

CONFLICT OF INTERESTS

Declared none

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