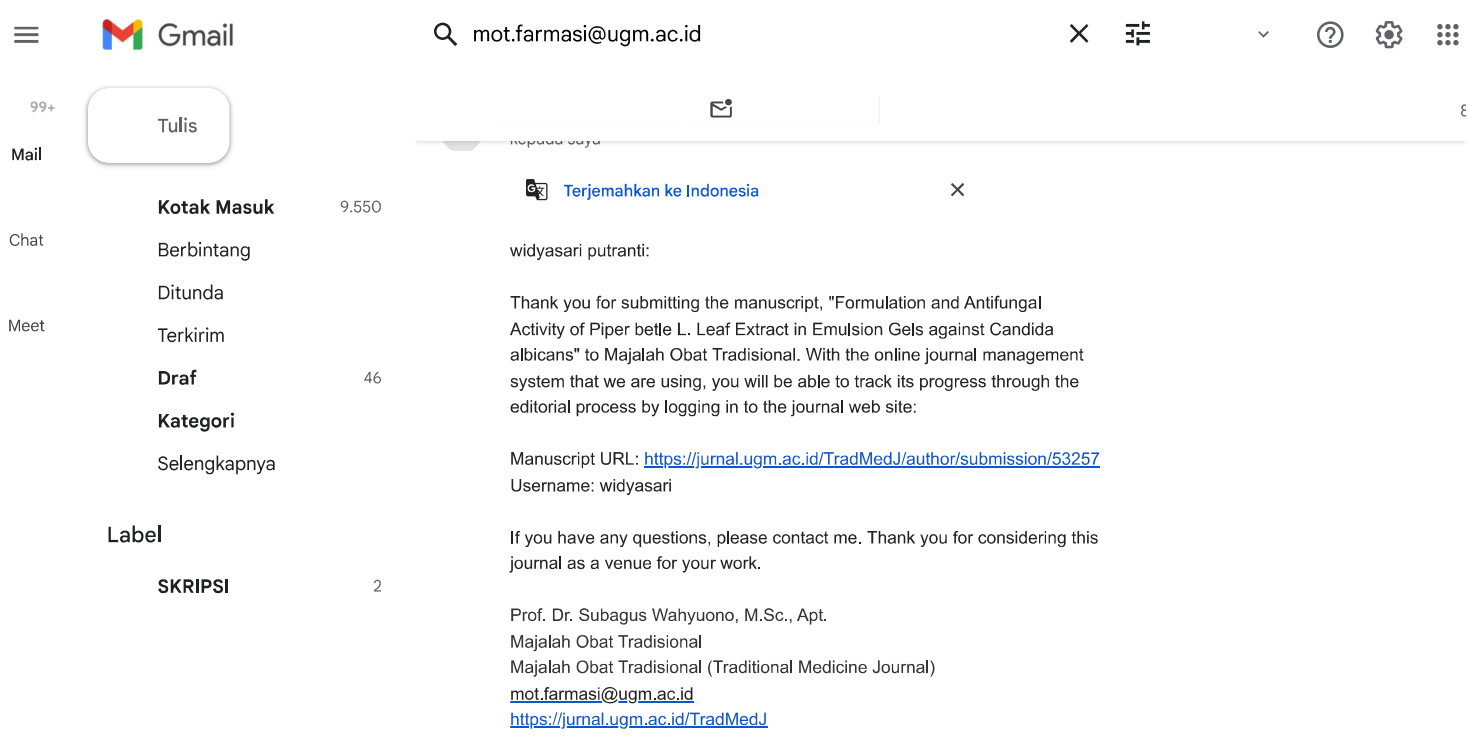
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FORMULASI DAN UJI AKTIVITAS EMULGEL EKSTRAK DAUN SIRIH (*Piper betle* L.) TERHADAP *Candida albicans*

Formulation and Antifungal Activity of *Piper betle* L. Leaf Extract in Emulsion Gels against[A1] *Candida albicans*

ABSTRACT

Candidiasis is the most common fungal infection in humans and is a form of primary and secondary infections of Candida albicans. Betel (Piper betle L.) leaf extract has been reported to exhibit efficacious antifungal effects against Candida albicans [A2] *. Emulsion gels, a type of topical dosage forms, can deliver hydrophilic and hydrophobic drugs and perform multiple and controlled releases. This research aimed to determine the antifungal activity and physical properties of emulsion gels formulated from betel leaf extract.*

The dried betel leaves were extracted by maceration with alcohol 95%. Then, with different concentrations (1, 2, and 4%), the extract was formulated into emulsion gels. These dosage forms were later subjected to antifungal activity testing against Candida albicans[A3] *using the cup plate diffusion method that involved Mycoral Cream* [A4]*for comparison. In this test, the intensity of the activity was determined from measuring the diameter of the formed inhibition zone. The second test evaluated the physical characteristics of the dosage forms, including organoleptic properties, pH, adhesion, dispersion, and viscosity. These data were analyzed using the Mann-Whitney method, and the conclusion was withdrawn from describing the results quantitatively.*

The reaction yield of the extraction was 9.702%. The analysis results showed that emulsion gels containing 1, 2, and 4% of betel leaf extract created zones of inhibition with the diameters of 5.3 ± 0.29, 6.2 ± 0.29, and 10.2 ± 0.41 mm, respectively. As for the physical properties, they differed in pH (6.39 ± 0.120, 6.17 ± 0.132, 5.66 ± 0.123), spreadability (1.849 ± 0.45, 1.816 ± 0.051, 1.771 ± 0.092 g.cm.s^{-1}), adhesion (110 ± 10.8, 126.3 ± 8.5, 142.7 ± 13.50 seconds), and viscosity (2640.35, 1992.95, 2162.12 cps) [A5]*. The betel leaf emulsion gels exhibited antifungal activity against Candida albicans ($p < 0.05$) and met the physical requirements of semi-solid dosage forms.*

Keywords: emulsion gel, betel leaf extract, antifungal activity, physical properties

INTRODUCTION

Candidiasis is the most common fungal infection in humans and is the primary and secondary infection of *Candida albicans* (Leepel, 2013[A6]). It can occur locally or systematically. Local infections can be disorders of the mouth, skin, vagina, and nail plate, while lesions due to systemic ones can grow in the kidneys, skin, eyes, and heart (Jawetz *et al.*, 2010). The standard antifungal used for the treatment of candidiasis is ketoconazole, which has several side effects from abdominal pain to nausea, vomiting, and even anorexia (Heeres et al[A7], 2010[A8]).

Candidiasis treatment has incorporated [A9]traditional antifungal medicine to reduce side effects. An example includes betel leaves that contain hydroxychavicol and have the potential for anticandida. According to Bandaranayake *et al.* (2018), the ethanol extract of betel leaves can inhibit the growth of *Candida albicans* at a concentration of 1 mg/mL. Aside from ease of administration, topical preparations can avoid the side effects, risks, and inconvenience of intravenous therapy and have various absorption conditions on the enteric route. Betel leaf extract is composed of hydrophilic and hydrophobic compounds, which can be formulated into emulsion gels to perform multiple and controlled releases (Hardenia *et al.*, 2014).

RESEARCH METHOD

TOOLS AND MATERIALS

The primary research materials were betel leaves (purchased from Beringharjo Market, Yogyakarta) and the fungus *Candida albicans*. Apart from Sterile Water for Injection, CYG medium, SDA medium, 0.9% NaCl solution, and Mycoral® Cream (Ketoconazole 2%), this experiment also used materials with pharmaceutical grades, namely alcohol 95%, alcohol 70%, distilled water, liquid paraffin, HPMC[A10], propylparaben, methylparaben, tween 80, span 80, and propylene glycol.

The research equipment included [A11]an oven, halogen moisture analyzer, rotary evaporator, water bath (Memmert), porcelain saucer, Petri dish, autoclave

(Shenan), incubator, inoculation loop, micropipette (Socorex [A12]), analytical scales, and glassware (Iwaki Pyrex).

RESEARCH PROCEDURE[A13]

Preparation of Dried Betel Leaves

Betel leaves were collected, sorted, dried, and reduced in size[A14]. These dried specimens were tested for drying losses and then identified both macroscopically and microscopically.

Extract Preparation

Extraction

Five hundred grams of the dried betel leaves were macerated with 5L of ethanol 95% (solvent) with a ratio of 1:10 for 24 hours (Hertiani and Purwantini, 2002; Depkes RI, 2008). The macerated mixture was collected and processed in a rotary evaporator at 50°C until concentrated extracts with solid green color and the peculiar smell of betel leaves were acquired (Singburaudom, 2015; Anwar *et al.*, 2014).

Phenol detection test

One gram of the extract was dissolved with 20 mL of ethanol 70%. $FeCl_3$ was dripped to 2 mL of this solution. Bluish green coloration indicates the presence of phenolic compounds (Syafitri *et al.*, 2014).

Ethanol detection test

This test aimed to ensure that the extract did not contain ethanol compounds. The extract was dissolved with H_2SO_4 in a test tube and then added with acetic acid. The tube was closed with cotton and heated to boiling point. Ethanol content is marked with the scents of esters in the cotton (Raymon *et al.*, 2016).

Emulsion Gels Preparation

The emulsion gels were prepared from the betel leaf extract with three different formulas, i.e., concentrations of 1, 2, and 4%, as determined from the orientation results. Prompting this process was the making of an oil phase by mixing span 80 and liquid paraffin at 70°C and a water phase by mixing tween 80 and some water at 70°C. Then, both phases were mixed and stirred at 70°C until

an emulsion was formed. Meanwhile, the gel was prepared by crushing HPMC and dispersing it in the water at 80°C little by little. Methyl- and propylparaben were dissolved in propylene glycol and then mixed into the gel. The formed emulsions and gels were combined with a homogenizer at a speed of 700 rpm for 45 minutes until an emulsion gel was formed. The ethanol extract was ground, added with the base of the emulsion gels (BEG), and ground[A15] again until homogenous (Yenti et al, 2014). The formulas of the betel leaf emulsion gels were modified from Yenti et al (2014), as seen in Table I.

Table I. The formula of the betel leaf emulsion gel

Ingredients	Concentrations (%)		
	F1	F2	F3[A16]
Extract	1	2	4
HPMC	2.5	2.5	2.5
Liquid Paraffin	5	5	5
Tween 80	1.08	1.08	1.08
Span 80	0.42	0.42	0.42
Propylene Glycol	10	10	10
Methylparaben	0.03	0.03	0.03
Propylparaben	0.01	0.01	0.01
Distilled Water	Ad 100	Ad 100	Ad 100

Antifungal Activity Testing

The antifungal activity test employed the cup-plate diffusion method. It began with creating the medium by suspending 650 mg of SDA medium in 100 mL of water, then pouring the mixture into Petri dish and allowing it to harden. The suspension culture of *Candida albicans* was made with 100 µl of suspension[A17] and added with physiological NaCl until the turbidity was the same as the Mc Farland standard (concentration of *C. albicans*= 1.5 x 10^8 CFU/mL). Afterward, the solution was diluted with CYG medium (1:100), forming a colony of *C. albicans* with a concentration of 10^6 CFU/mL. The surface of the solidified agar medium was spread evenly with 1 mL of the *C. albicans* suspension culture and perforated using a 5mm cork borer. The test material and 100 mg of the control[A18] were put into each hole, and then the medium was incubated at 37°C

for 24 hours. Clear rings were formed[A19], and their diameters were calculated as the diameter of the inhibition zone.

Physical Properties Evaluation

The physical properties evaluation included an organoleptic test and the measurements of pH, spreadability, adhesion, and viscosity.

Organoleptic Test

The organoleptic evaluation was performed by observing the shape, smell, and color of the preparations (Naibaho *et al.,* 2013).

pH Analysis

This test used a pH meter that had been calibrated with acetate buffer pH 4.0 and phosphate buffer pH 7.0. One gram of the emulsion gels was dissolved in 10 mL of distilled water. The electrode was dipped in the solution, and a constant pH was measured as the base pH of the emulsion gels (Yenti *et al*., 2014).

Spreadability Test

A 0.5g sample of the emulsion gel was placed between two round glass plates, and the diameter of the spread sample was measured. A 50g weight was put on top of the glass and left for one minute, then the width of the spread was measured. Afterward, another 100g weight was added, and the width of the spread was measured after one minute (Naibaho *et al.*, 2013[A20]).

Adhesive Capacity Test

A 0.25g sample of the emulsion gel was placed between two glass objects. A 1kg weight was put on top of the glass and left for five minutes. This load was lifted, and the glass objects were separated from each other with an 80g weight. The time required for the glass objects to separate was recorded (Sari *et al.*, 2015).

Viscosity Test

This test used a Rheosys Merlin VR II viscometer equipped with 25mm concentric cylinders or spindles. It processed 10 points with rotational speeds of 0.1 - 100 rpm, a delay time of 20 seconds, and at a temperature of 25°C.

Data Analysis

The results were quantitatively described, and the conclusions were drawn to see whether the betel leaf emulsion gels met the physical requirements of semisolid preparations. The antifungal capacities of betel leaf extracts against *Candida albicans* were subjected to a test of difference using the Mann Withney method[A21].[A22]

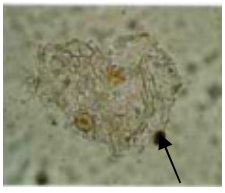
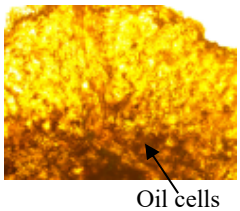
Results and Discussion

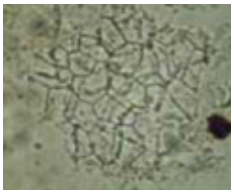
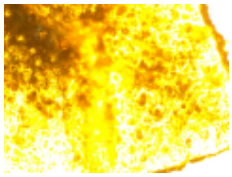
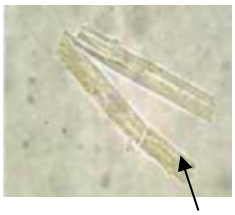
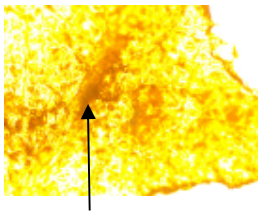
The macroscopic and microscopic tests confirmed that the dried specimens were betel leaves (*Piper betle* L.). The macroscopic and microscopic test results of the betel leaves are presented in Table II and Table III, respectively.

Table II. The results of macroscopic identification of betel leaves

Parameters	Standard Features	Results	Notes
Shape	Egg-shaped with a tapering point	Egg-shaped with a tapering point	Matched (Depkes RI, 2010)
Color	Brownish green	Green	Matched (Depkes RI, 2010)
Smell	Distinctive	Distinctive	Matched (Depkes RI, 2010)
Taste	Spicy	Spicy	Matched (Depkes RI, 2010)
Length	5-18 cm	10.5 cm	Matched (Depkes RI, 2010)
Width	3-12 cm	6 cm	Matched (Depkes RI, 2010)
Lower Surface	Rough with a lighter color	Rough with a lighter color	Matched (Depkes RI, 2010)

Table III. The results of microscopic identification of betel leaves

Parameters	Standard Features	Results	Notes
Lower epidermis with oil cells	 Oil cells	 Oil cells	(Depkes RI, 2010)

Upper epidermis			(Depkes RI, 2010)
Plant vessels with scalariform thickening			(Depkes RI, 2010)

Five hundred grams of dried betel leaves produced 48.512 g of extract, or, in other words, the yield was [A23] 9.702% w/w. Based on the organoleptic evaluation, the extract had a solid green color, thick consistency, and the typical scent of betel leaves. In the phenols test, the color of the test solution changed from green to bluish green, indicating the presence of phenolic compounds in the extract (Syafitri *et al.*, 2014). As for the ethanol test, it did not detect any smell of ester after the extract was boiled with H_2SO_4 and acetic acid, meaning that the extract does not contain ethanol (Raymon *et al.*, 2016).

The emulsion gel was made from the ingredients listed in Table I. HPMC [A24]functioned as both gelling and stabilizing agent. The combination of methyl- and propylparaben serves as a preservative, while propylene glycol dissolves methyl- and propylparaben and acts as humectants (Yenti, 2014; Rowe *et al.*, 2009; Dewi, 2015). The results of the emulsion gel formulation are presented in Figure 1.

(a) (b) (c) (d)

Figure 1. The formulation of betel leaf emulsion gels with different formulas. (a) the base of emulsion gel (BEG), (b) 1% betel leaf extract, (c) 2% betel leaf extract, (d) 4% betel leaf extract

The emulsion gels were tested for their inhibitory activity against *Candida albicans*. According to David Stout (in Hasim, 2003), the diameter of the zone of inhibition can represent the antifungal potential of test material, as summarized in Table IV.

Table IV. Antifungal potentials based on zone diameter

Zone of Inhibition (diameter)	Antibiotic Potentials
d ≥ 20 mm	Very strong
10 – 20 mm	Strong
5 – 10 mm	Moderate
d < 5 mm	Weak

[A25]

The antifungal capacity testing was performed with three-time replication. The results are presented in Table V.

Table V. The results of the antifungal capacity tests

Codes	Test Materials	Zones of Inhibition (diameter, mm)	Antifungal potentials
a.	The base of emulsion gels (BEG)	$0^{b,c,d}$	Weak
b.	Emulsion gels with 1% extract	$5.3 \pm 0.29^{a,c,d}$	Moderate
c.	Emulsion gels with 2% extract	$6.2 \pm 0.29^{a,b,d}$	Moderate
d.	Emulsion gels with 4% extract	$10.2 \pm 0.41^{a,b,c}$	Strong
e.	Mycoral® Cream	6.2 ± 0.29	Moderate

Notes:
a : significantly different from the base formula
b : significantly different from the formula of emulsion gel with 1% extract
c : significantly different from the formula of emulsion gel with 2% extract
d [A26]: significantly different from the formula of emulsion gel with 4% extract

Betel leaves contain essential oils, phenyl propane, chavicol, flavonoids, tannins, and terpenoids acting as the antifungal (Zuraidah, 2015 [A27]). Hydroxychavicol is the main phenolic compound isolated from the betel leaf extract. It can also change the structure of cell membranes, resulting in disruption of membrane permeability (Ali, 2010). Antibiotics have been proven to interact with the surface of the lipid that contains ergosterol.[A28]

The bonds between lipids and antibiotic molecules induce damage to the membranes and, thereby, disrupt the specific membrane permeability. Changes in membrane permeability can destabilize cells up to a point where molds and yeast cells die (Kusumaningtyas, 2008; Coutinho *et al.*, 2004).

Heating in the extraction process can affect the anticandidal activity of the betel leaf emulsion gels because it changes the chemical components of betel leaves (Hertiani and Purwantin, 2002).[A29]

The emulsion gels were subjected to physical properties evaluation to determine their organoleptic characteristics, pH, spreadability, adhesion, and viscosity. The results of the assessment are summarized in Table VI.

Table VI. The results of the physical properties evaluation

Analyses	Results				Notes[A30]
	BEG	F1	F2	F3[A31]	
Organoleptic	Semisolid White, distinctive	Green, typical betel leaves			Matched
pH	6.56 ± 0.104	6.39 ± 0.120	6.17 ± 0.132	5.66 ± 0.123	Matched (Riski *et al.*, 2016)
Spreadability ($g.cm.s^{-1}$)	2.292 ± 0.045	1.849 ± 0.45	1.816 ± 0.051	1.771 ± 0.092	Matched (Riski *et al.*, 2016)
Adhesive capacity (s)	85 ± 4.58	110 ± 10.8	126.3 ± 8.5	142.7 ± 13.50	Matched (Sari *et al.*, 2015)
Viscosity*[A32](cps)	1843.95	2640.35	1992.95	2162.12	Not Matched (Garg *et al.,* 2002)
Flow Types	Pseudo-plastic	Pseudo-plastic	Pseudo-plastic	Pseudo-plastic	Matched (Martin *et al.,* 1993)

Overall, the organoleptic test showed that the betel leaf emulsion gel was green and[A33] semisolid and had the smell of betel leaf extract. Meanwhile, the base of the emulsion gel was white and[A34] semisolid and smelled like HPMC. The pH levels of the emulsion gels containing 1, 2, and 4% of the betel leaf extract were 6.39 ± 0.120, 6.17 ± 0.132, and 5.66 ± 0.123, respectively, while the

base of the emulsion gel had pH= 6.56 ± 0.104. The pH measurement aims to ensure that the preparations are safe for dermal application, i.e., with pH ranging between 4.5-6.5 (Riski *et al.,* 2016). Too acidic preparations [A35]can irritate the skin, whereas too alkaline ones can cause scaly skin (Naibaho *et al.*, 2013). The pH analysis showed that emulsion gels with higher levels of betel leaf extracts had lower pH. In this study, the pH is in the allowed range of safe pH for topical use.

Spreadability is an essential aspect of topical preparations because it is related to the ease of application to the skin, removal from containers, and consumer acceptance (Yenti *et al.*, 2014). Spreadability test provides information about the ability of preparations to spread on a surface. The wider the preparation spreads on the skin, the higher the absorption of its medicinal ingredients (Naibaho *et al.*, 2013). The spreadabilities of the emulsion gels containing 1, 2, and 4% of the betel leaf extract were 1.849 ± 0.45, 1,816 ± 0.051, and 1.771 ± 0.092 g.cm.s-1, respectively, while the base of the emulsion gel had a spreadability of 2.292 ± 0.045 g.cm.s-1. The results showed that a higher level of extract in the emulsion gel was associated with lower spreadability. According to Riski (2016), favorable spreadability is 3-5 cm. Since the betel leaf emulsion gel spread up to 5.75 - 6.5 cm, it can be concluded as having good spreadability and is therefore easy to apply to the skin. [A36]

Adhesive capacity evaluation can determine the ability of preparations to stick to the skin. The duration of adherence affects the absorption level of the drug. The longer the time of contact, the more the dermal absorption of the drugs (Naibaho, 2013). Previous research has proven [A37]that the acceptable adhesion of topical preparations is not less than 4 seconds (Sari *et al.*, 2015). The adhesive capacities of the emulsion gels containing 1, 2, and 4% of the betel leaf extract were 110 ± 10.8, 126.3 ± 8.5, and 142.7 ± 13.50 seconds, respectively, while the base of the emulsion gel had an adhesive capacity of 85 ± 4.58 seconds. Based on this capacity, the emulsion gels have met the requirements for topical dosage forms. Moreover, increasing the concentration of the extract appears to strengthen adherence.

The viscosity test aimed to identify how thick preparations are. The thicker they are, the higher the viscosity. Viscosity is inversely proportional to spreadability (i.e., the greater the viscosity, the lower the spreadability)[A38] but directly proportional to adhesive capacity (i.e., the greater the viscosity, the stronger the preparation sticks to the skin). The viscosity test was carried out in a Rheosys Merlin VR II Viscometer. The built-in spindles were 25mm concentric cylinders with 10 points, a rotational speed of 0.1 - 100 rpm, and a delay time of 20 seconds.[A39] Based on the reading of the viscometer at 100 rpm, the viscosities of the emulsion gels containing 1, 2, and 4% of the betel leaf extract were 2640.35, 1992.95, and 2162.12 centipoises (cps), respectively. Acceptable viscosity for semisolid preparations is 2000-4000 cps (Garg *et al.*, 2002). The results showed that emulsion gels with 1% and 4% of the extract met the requirements for semisolid preparations, whereas the one containing 2% extract did not. [A40]

As a gelling agent in the formula, HPMC increases viscosity (Garg *et al.*, 2002; Naibaho *et al.*, 2013). HPMC is a cellulose-derived polymer whose molecules fill in the cavities formed by water molecules during dispersion, forming hydrogen bonds with the polymer molecules on the hydroxyl (–OH) group of the water molecules. The flow types of the betel leaf emulsion gel and its base were pseudoplastic. This finding is in line with Martin *et al.* (1993), which state that the flow type of parts of pharmaceutical preparations is pseudoplastic. Pseudoplastic is represented with a curve starting from (0,0) or approaching a low shear rate (Martin *et al.*, 1993). Based on the plotting of the viscosity data, the curve started from a point located close to (0,0). Furthermore, the typical non-linear curve of pseudoplastic flows was indicated by the R^2 values of the base of the emulsion gel and the emulsion gels containing 1, 2, and 4% of the betel leaf extracts, namely 0.9579, 0.4721, 0.4545, and 0.7767, respectively. This curve also showed that intensifying shearing stress was attributable to increased shearing rate (Martin *et al.*, 1993). Shearing stress causes irregular molecules to determine the direction of the flow. Bonded solvents can be detached, decrease the size of the

dispersed molecules, and reduce apparent viscosity. In other words, the larger the applied pressure, the easier the molecule to flow (Martin *et al.*, 1993).

Based on the antifungal activity testing, the emulsion gel containing 2% of the extract was not significantly different from the positive control. Its physical properties included pH 6.17 ± 0.132, an adhesive capacity of 126.3 ± 8.5 seconds, spreadability of 1.816 ± 0.051 g.cms-1, and viscosity of 1992.95 cps.[A41]

The curve obtained from the viscosity test is presented in Figure 2, where the *x*-axis is the shear stress (Pa), and the *y*-axis is the shear rate (1/s).

Figure 2. The viscosity test results of the emulsion gels containing 1, 2, and 4% of the betel leaf extract and the base of the emulsion gel

[A42]

The log curve of the viscosity test results is presented in Figure 3 with log shear stress on the *x*-axis and log shear rate on the *y*-axis. [A43]

Figure 3. The viscosity test results of the betel leaf emulsion gel in a logarithmic scale

Conclusion and Recommendation

The betel leaf emulsion gel has been proven [A44]to exhibit anticandidal activity from the formation of clear rings, marking the inhibition of *Candida albicans* growth. Besides, this emulsion gel has met the required physical properties for semisolid preparations, including organoleptic characteristics, pH, spreadability, adhesion, and viscosity. Further research is recommended to test the optimization of extract solvent, testing of chemical contents in the extract, and dissolution tests.

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Review of Traditional Medicine Journal Article

No		332. 53257
1	Title of article	**Formulation and Antifungal Activity of Piper betle L. Leaf Extract in Emulsion Gels against Candida albicans**
2	Author	Widyasari Putranti, Chairisty Asterina, Hardi Witasari
3	Journal volume number	**Volume 25 No. 2**
4	Date of submission	1/8/2020

	Review		**Response to review**
5	Title	Ok	
6	Abstracts	• The Authors need to show the conclusion more precisely, not only the results of the experiments. • Which one of the three formulations had the most optimum antifungal activity? Since it's mentioned in the page 11 that the 2% formulation did not show antifungal activity compared to the control. • If there's word number restriction for the abstract, thus the authors need to re-paraphrase some sentences.	
7	Introduction	• In my opinion, the Introduction is too short. In the end of paragraph 1, the authors need to mention "the need of some alternative/different approach on treating candidiasis, which may come from the nature in a form of medicinal plants". • I think C. albicans has not only antifungal activity but also others and the Authors also need to mention that. • The sentence 'aside from ease ….' Appeared out from nowhere. It needs to be explained why topical preparations is the most feasible one. Why not capsule? Why emulgel?	

		• Overall, paragraph 2 can be extended by telling the background why betel was chosen by adding more references.	
8	Methodology	• I think in MOT it is called "Methodology" only. Therefore, the authors are required to change the title of the section and combine both research method and research prochedure into one single section: Methodology, followed by any sub section needed. Please kindly check the example of the published one from the MOT. • • Please also kindly double check everything that is written in this section.	
9	Results and Discussion	• In my opinion, the authors can simplify some sentences and extend the ones that need to be explained further. • There isn't any discussion regarding the microscopic and macroscopic examination of betel leaves. Please add some explanation. For example, why is that the results and standard photographs are in different colors? is the results matched with the standard? Are there any limitations in the microscope that the authors used? • One reference mentioned in the section is missing from the list (Zuraidah) • The suggestion from me is, for the next submission (other than this one), if the authors photographed something, please take the photograph in the same manner (angle, position, etc) so that the appearance will look similar among the samples. Once again, the authors can see the previous accepted manuscript from MOT for the reference.	

		• There is one reference (Hasim) which I could not find anywhere on the internet. I apologize for the low-searching skill, perhaps could the authors shed light on this by providing the reference proof next time? It was also from the newspaper section, which, reference-wise, is not scientific enough to be placed here as reference. If finding this is difficult, maybe the authors can put other references mentioning the same parameters. • • C. albicans was not discussed at all even though this is the manusricpt. I think it is still necessary to discuss, either organism-related or disease-related discussion.	
10	References	• The authors are suggested to give more attention in the reference writing, not only the list, but also the way it's placed in the paragraph, especially when the reference list is written manually. • For example, is it "et al" or "*et al*"? (in paragraph) For candidiasis and *C. albicans*, the Authors can read from this: *Virulence 6:4, 307-308* and *Microbes Infect. 2016 May; 18(5): 310–321.doi:10.1016/j.micinf.2016.01.002*.	
11	Figures and Tables	• The Figure 2 and 3 are essentially the same. One is non logarithmic and the other one is logarithmic. Please take a look of similar publications regarding how they display such information. • Table V notes are ambiguous. Which one is "a"? the codes or the other one? The authors can use different symbols to distinguish this. • Still in the same table V, if the authors mentioned that BEG has weak antifungal activity, does it	

		mean actually we can use ONLY the base of the emulgel even though it's weak?	
12	General notices		
13	Editor's decision	**Diterima dengan banyak perbaikan/Accepted with major revision**	

Editor's note

- Revise your article according to the review results
- Fix your article in accordance with the attached TradMedJ author guideline
- Please send back the revised version of your article to https://jurnal.ugm.ac.id/TradMedJ/index

Yogyakarta, June 15, 2020

Editor

Review of Traditional Medicine Journal Article

No		332. 53257	
1	Title of article	**Formulation and Antifungal Activity of Piper betle L. Leaf Extract in Emulsion Gels against Candida albicans**	
2	Author	Widyasari Putranti, Chairisty Asterina, Hardi Witasari	
3	Journal volume number	**Volume 25 No. 2**	
4	Date of submission	1/8/2020	
Review			**Response to review**
5	Title	Ok	
6	Abstracts	Please revise in accordance with the improvements that occur in the text	
7	Introduction	it is not very clear what problems will be resolved in this study extract formulations into dosage forms are of course intended for the use of extracts to be more easily applied. In this study, it is not yet seen what problems exist in the extract as raw material so it needs to be formulated into a gel	
8	Methodology	The active ingredient used in the formulation is betel leaf extract, therefore it is necessary to mention the characterization of the extract produced from the extraction process including parameters that may affect the physical properties and pharmacological activities of the extract. For example water content, chromatogram profiles etc.	
9	Results and Discussion	The three formulations are only different in extract percentage. In this case, all differences in physical properties and antifungal activity that occur are only due to the amount of extract that exists, not the type of excipients that are in the formula Therefore, the discussion should be related to the nature of the extract and its interaction with the excipients and to be compared with the results of other studies that use similar raw	

		materials (extracts) even if they are not from the same species.	
10	References	We recommend using a newer published textbook. For example, the book MARTIN'S PHYSICAL PHARMACY AND PHARMACEUTICAL SCIENCES Physical Chemical and Biopharmaceutical Principles in the Pharmaceutical Sciences SIXTH EDITION published in 2011	
11	Figures and Tables	Characterization of raw materials is done through microscopy and macroscopy, it is better if the betel leaf photo is included	
12	General notices		
13	Editor's decision	**Diterima dengan banyak perbaikan/Accepted with major revision**	

Editor's note

- Revise your article according to the review results
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Author's declaration

With the submission of this manuscript, I would like to undertake that the above mentioned manuscript is original and has not been published nor accepted for publication elsewhere, and herewith be willing to hand in the right of first publication of the manuscript to Majalah Obat Tradisional (Trad. Med. J).

Authors' contributions

Authors	Contribution
Author's name	
Co-author's name 1	
Co-author's name 2	
Co-author's name 3	
As follows	

Conflict of interest:

The authors declare that they have no conflict of interest.

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[MOT] Editor Decision (Reminder)

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widyasari putranti:

Just a gentle reminder of our request for your revision of the submission, "Formulation and Antifungal Activity of Piper betle L. Leaf Extract in Emulsion Gels against Candida albicans" for Majalah Obat Tradisional. We were hoping to have this revision by July 29, 2020, and would be pleased to receive it before November 27, 2020.

Please confirm your ability to complete this vital contribution to the work of the journal. I look forward to hearing from you.

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We have reached a decision regarding your submission to Majalah Obat Tradisional, "Formulation and Antifungal Activity of Piper betle L. Leaf Extract in Emulsion Gels against Candida albicans".

Our decision is to: Accept Submission

Your manuscript has been chosen to be published in Majalah Obat Tradisional Volume 26 No. 1. The full-text in PDF format would be available to be accessed in our website by April 30, 2021.

Thank you for considering our journal as the venue for your work.

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Formulation and Antifungal Activity of *Piper betle* L. Leaf Extract in Emulsion Gels Against *Candida albicans*

Widyasari Putranti*, Chairisty Asterina, Hardi Astuti Witasari
Faculty of Pharmacy, Universitas Ahmad Dahlan, Indonesia.

ABSTRACT

Candidiasis is the most common fungal infection in humans and is a form of primary and secondary infections of *C. albicans*. Betel (*Piper betle L.*) leaf extract has been reported to exhibit efficacious antifungal effects against *C. albicans*. Emulsion gels, a type of topical dosage form, can deliver hydrophilic and hydrophobic drugs and perform multiple and controlled releases. This research aimed to determine the antifungal activity and physical properties of emulsion gels formulated from betel leaf extract. The dried betel leaves were extracted by maceration with alcohol 95%. Then, with different concentrations (1, 2, and 4%), the extract was formulated into emulsion gels. These dosage forms were later subjected to antifungal activity testing against *C. albicans* using the cup plate diffusion method that involved Mycoral Cream® for comparison. In this test, the intensity of the activity was determined by measuring the diameter of the formed inhibition zone. The second test evaluated the physical characteristics of the dosage forms, including organoleptic properties, pH, adhesion, dispersion, and viscosity. These data were analyzed using the Mann-Whitney method, and the conclusion was withdrawn from describing the results quantitatively. The reaction yield of the extraction was 9.702%. The analysis results showed that emulsion gels containing 1, 2, and 4% of betel leaf extract created zones of inhibition with diameters of 5.3 ± 0.29, 6.2 ± 0.29, and 10.2 ± 0.41 mm, respectively. As for the physical properties, they differed in pH (6.39 ± 0.120, 6.17 ± 0.132, 5.66 ± 0.123), spreadability (1.849 ± 0.45, 1.816 ± 0.051, 1.771 ± 0.092 g.cm.s^{-1}), adhesion (110 ± 10.8, 126.3 ± 8.5, 142.7 ± 13.50 seconds), and viscosity (2640.35, 1992.95, 2162.12 cps), respectively. The betel leaf emulsion gels exhibited antifungal activity against *C. albicans* ($p < 0.05$) and met the physical requirements of semi-solid dosage forms.

Keywords: emulsion gel; betel leaf extract; antifungal activity; physical properties

INTRODUCTION

Candidiasis is the most common fungal infection in humans and is the primary and secondary infection of *C. albicans* (Leepel, 2009). It can occur locally or systematically. Local infections can be disorders of the mouth, skin, vagina, and nail plate, while lesions due to systemic ones can grow in the kidneys, skin, eyes, and heart (Jawetz *et al.*, 2010). Candidiasis treatment has utilized traditional antifungal medicine to reduce side effects. An example includes betel leaves that contain hydroxychavicol and have the potential for anticandida. According to Bandaranayake *et al.,* (2018), the ethanol extract of betel leaves can inhibit the growth of *Candida albicans* at a concentration of 1 mg/mL. Aside from ease of administration, topical preparations can avoid the side effects, risks, and inconvenience of intravenous therapy and have various absorption conditions on the enteric route. Betel leaf extract is composed of hydrophilic and hydrophobic compounds, which can be formulated into emulsion gels to perform multiple and controlled releases (Hardenia *et al.*, 2014).

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METHODOLOGY

Materials

The primary research materials were betel leaves (purchased from Beringharjo Market, Yogyakarta) and the fungus *C. albicans*. Apart from Sterile Water for Injection, CYG medium, SDA medium, 0.9% NaCl solution, and Mycoral® Cream (Ketoconazole 2%), this experiment also used materials with pharmaceutical grades, namely alcohol 95%, alcohol 70%, distilled water, liquid paraffin, Hydroxyprophylmethylcellulose (HPMC), propylparaben, methylparaben, tween 80, span 80, and propylene glycol.

The research equipment included an oven, halogen moisture analyzer, rotary evaporator, water bath (Memmert®), porcelain saucer, Petri dish, autoclave (Shenan®), incubator, inoculation

Table I. The formula of the betel leaf emulsion gel

Ingredients	Concentrations (%)		
	F1	F2	F3
Extract	1	2	4
HPMC	2.5	2.5	2.5
Liquid Paraffin	5	5	5
Tween 80	1.08	1.08	1.08
Span 80	0.42	0.42	0.42
Propylene Glycol	10	10	10
Methylparaben	0.03	0.03	0.03
Propylparaben	0.01	0.01	0.01
Distilled Water	Ad 100	Ad 100	Ad 100

Note : F1 = extract ethanol betel leaf 1%; F2 = extract ethanol betel leaf 2%; F3 = extract ethanol betel leaf 4%

loop, micropipette (Socorex®), analytical scales, and glassware (Iwaki Pyrex®).

Methods

Preparation of Dried Betel Leaves

Betel leaves were collected, sorted, dried, and reduced to smaller size. These dried specimens were tested for drying losses and then identified both macroscopically and microscopically.

Extract Preparation

Extraction

Five hundred grams of the dried betel leaves were macerated with 5L of ethanol 95% (solvent) with a ratio of 1:10 for 24 hours (Hertiani and Purwantini, 2002; Depkes RI, 2008). The macerated mixture was collected and processed in a rotary evaporator at 50°C until concentrated extracts with solid green color and the peculiar smell of betel leaves were acquired (Singburaudom, 2015; Anwar *et al.*, 2014).

Phenol detection test

One gram of the extract was dissolved with 20 mL of ethanol 70%. $FeCl_3$ was dripped to 2 mL of this solution. Bluish-green coloration indicates the presence of phenolic compounds (Syafitri *et al.*, 2014).

Ethanol detection test

This test aimed to ensure that the extract did not contain ethanol compounds. The extract was dissolved with H_2SO_4 in a test tube and then added with acetic acid. The tube was closed with cotton and heated to boiling point. Ethanol content is marked with the scents of esters in the cotton (Raymon *et al.*, 2016).

Emulsion Gels Preparation

The emulsion gels were prepared from the betel leaf extract with three different formulas, i.e., concentrations of 1, 2, and 4%, as determined from the orientation results. Prompting this process was the making of an oil phase by mixing span 80 and liquid paraffin at 70°C and a water phase by mixing tween 80 and some water at 70°C. Then, both phases were mixed and stirred at 70°C until an emulsion was formed. Meanwhile, the gel was prepared by crushing HPMC and dispersing it in the water at 80°C little by little. Methyl- and propylparaben were dissolved in propylene glycol and then mixed into the gel. The formed emulsions and gels were combined with a homogenizer at a speed of 700 rpm for 45 minutes until an emulsion gel was formed. The ethanol extract was ground, added with the base of the emulsion gels (F0) until homogenous (Yenti *et al*, 2014). The formulas of the betel leaf emulsion gels were modified from Yenti *et al* (2014), as seen in Table I.

Antifungal Activity Testing

The antifungal activity test employed the cup-plate diffusion method. It began with creating the medium by suspending 650 mg of SDA medium in 100 mL of water, then pouring the mixture into Petri dish and allowing it to harden. The suspension culture of *C. albicans* was made with 100 µl and added with physiological NaCl until the turbidity was the same as the Mc Farland standard (concentration of *C. albicans*= 1.5×10^8 CFU/mL). Afterward, the solution was diluted with CYG medium (1:100), forming a colony of *C. albicans* with a concentration of 10^6 CFU/mL. The surface of the solidified agar medium was spread evenly with 1 mL of the *C. albicans* suspension culture and

Table II. The results of macroscopic identification of betel leaves

Parameters	Standard Features	Results	Notes
Shape	Egg-shaped with a tapering point	Egg-shaped with a tapering point	Matched (Depkes RI, 2010)
Color	Brownish green	Green	Matched (Depkes RI, 2010)
Smell	Distinctive	Distinctive	Matched (Depkes RI, 2010)
Taste	Spicy	Spicy	Matched (Depkes RI, 2010)
Length	5-18 cm	10.5 cm	Matched (Depkes RI, 2010)
Width	3-12 cm	6 cm	Matched (Depkes RI, 2010)
Lower Surface	Rough with a lighter color	Rough with a lighter color	Matched (Depkes RI, 2010)

perforated using a 5mm cork borer. The test material and the control (negative and positive) were put into each hole, and then the medium was incubated at 37°C for 24 hours, and their diameters were calculated as the diameter of the inhibition zone.

Physical Properties Evaluation

The physical properties evaluation included an organoleptic test and the measurements of pH, spreadability, adhesion, and viscosity.

Organoleptic Test

The organoleptic evaluation was performed by observing the shape, smell, and color of the preparations (Naibaho *et al.,* 2013).

pH Analysis

This test used a pH meter that had been calibrated with acetate buffer pH 4.0 and phosphate buffer pH 7.0. One gram of the emulsion gels was dissolved in 10 mL of distilled water. The electrode was dipped in the solution, and a constant pH was measured as the base pH of the emulsion gels (Yenti *et al*., 2014).

Spreadability Test

A half gram of emulgel is placed on a round glass scale and covered with another round glass and then left for 1 minute, after that it is added with 150 g ballast and then the distribution diameter is recorded every 1-minute interval (Garg *et al*., 2002).

Adhesive Capacity Test

A 0.25g sample of the emulsion gel was placed between two glass objects. A 1kg weight was put on top of the glass and left for five minutes. This load was lifted, and the glass objects were separated from each other with an 80g weight. The time required for the glass objects to separate was recorded (Sari *et al.*, 2015).

Viscosity Test

This test used a Rheosys Merlin VR II viscometer equipped with 25mm concentric cylinders or spindles. It processed 10 points with rotational speeds of 0.1 - 100 rpm, a delay time of 20 seconds, and at a temperature of 25°C.

RESULT AND DISCUSSION

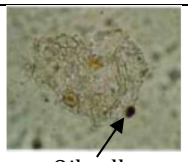
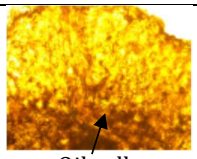
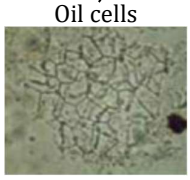
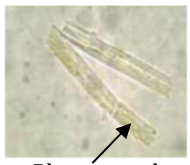
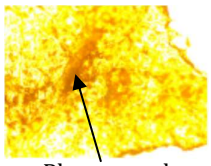
The macroscopic and microscopic tests confirmed that the dried specimens were betel leaves (*Piper betle* L.). The macroscopic and microscopic test results of the betel leaves are presented in Table II and Table III, respectively.

Five hundred grams of dried betel leaves produced 48.512 g of extract as 9.702% w/w(yield). Based on the organoleptic evaluation, the extract had a solid green color, thick consistency, and the typical scent of betel leaves. In the phenols test, the color of the test solution changed from green to bluish-green, indicating the presence of phenolic compounds in the extract (Syafitri *et al.*, 2014). As for the ethanol test, it did not detect any smell of ester after the extract was boiled with H_2SO_4 and acetic acid, meaning that the extract does not contain ethanol (Raymon *et al.*, 2016).

The emulsion gel was made from the ingredients listed in Table I. The material of HPMC functioned as both gelling and stabilizing agent. The combination of methyl- and propylparaben serves as a preservative, while propylene glycol

Figure 1. The formulation of betel leaf emulsion gels with different formulas. (F0) the base of emulsion gel (BEG), (F1) 1% betel leaf extract, (F2) 2% betel leaf extract, (F3) 4% betel leaf extract

Table III. The results of microscopic identification of betel leaves

Parameters	Standard Features	Results	Notes
Lower epidermis with oil cells	 Oil cells	 Oil cells	(Depkes RI, 2010)
Upper epidermis			(Depkes RI, 2010)
Plant vessels with scalariform thickening	 Plant vessel	 Plant vessel	(Depkes RI, 2010)

dissolves methyl- and propylparaben and acts as humectants (Yenti, 2014; Rowe *et al.*, 2009; Dewi, 2015). The results of the emulsion gel formulation are presented in Figure 1.

The emulsion gels were tested for their inhibitory activity against *Candida albicans*. The antifungal capacity testing was performed with three-time replication. The results are presented in Table IV.

Betel leaves contain essential oils, phenyl propane, chavicol, flavonoids, tannins, and terpenoids acting as the antifungal (Zuraidah, 2015). Hydroxychavicol is the main phenolic compound isolated from the betel leaf extract. It can also change the structure of cell membranes, resulting in disruption of membrane permeability (Ali, 2010). Heating in the extraction process can affect the anticandidal activity of the betel leaf emulsion gels because it changes the chemical components of betel leaves (Hertiani and Purwantin, 2002).

Antibiotics have been proven to interact with the surface of the lipid that contains ergosterol. The bonds between lipids and antibiotic molecules induce damage to the membranes and, thereby, disrupt the specific membrane permeability. Changes in membrane permeability can destabilize cells up to a point where molds and yeast cells die (Kusumaningtyas, 2008; Coutinho *et al.*, 2004).

The emulsion gels were subjected to physical properties evaluation to determine their organoleptic characteristics, pH, spreadability, adhesion, and viscosity. The results of the assessment are summarized in Table V.

Overall, the organoleptic test showed that the betel leaf emulsion gel was green, semisolid, and had the smell of betel leaf extract. Meanwhile, the base of the emulsion gel was white semisolid and smelled like HPMC. The pH levels of the emulsion gels containing 1, 2, and 4% of the betel leaf extract were 6.39 ± 0.120, 6.17 ± 0.132, and

Table IIV. The results of the antifungal capacity tests

Codes	Test Materials	Zones of Inhibition (diameter, mm)
F0	The base of emulsion gels (BEG)	$0^{b,c,d}$
F1	Emulsion gels with 1% extract	$5.3 \pm 0.29^{a,c,d}$
F2	Emulsion gels with 2% extract	$6.2 \pm 0.29^{a,b,d}$
F3	Emulsion gels with 4% extract	$10.2 \pm 0.41^{a,b,c}$
FC	Mycoral® Cream	6.2 ± 0.29

Notes: a : significantly different from the base formula (F0); b : significantly different from the formula of emulsion gel with 1% extract (F1) c : significantly different from the formula of emulsion gel with 2% extract (F2); d : significantly different from the formula of emulsion gel with 4% extract (F3)

Table V. The results of the physical properties evaluation

Analyses	Results			
	F0	F1	F2	F3
Organoleptic			Semisolid	
	White, distinctive		Green, typical betel leaves	
pH	6.56 ± 0.104	6.39 ± 0.120	6.17 ± 0.132	5.66 ± 0.123
Spreadability ($g.cm.s^{-1}$)	2.292 ± 0.045	1.849 ± 0.45	1.816 ± 0.051	1.771 ± 0.092
Adhesive capacity (s)	85 ± 4.58	110 ± 10.8	126.3 ± 8.5	142.7 ± 13.50
Viscosity (cps)	1843.95	1992.95	2162.12	2640.35
Flow Types	Pseudo-plastic	Pseudo-plastic	Pseudo-plastic	Pseudo-plastic

5.66 ± 0.123, respectively, while the base of the emulsion gel had pH= 6.56 ± 0.104. The pH measurement aims to ensure that the preparations are safe for dermal application, i.e., with pH ranging between 4.5-6.5 (Riski *et al.,* 2016). The acidic gel can irritate the skin, whereas the alkaline ones can cause scaly skin (Naibaho *et al.*, 2013). The pH analysis showed that emulsion gels with higher levels of betel leaf extracts had lower pH. In this study, the pH is in the allowed range of safe pH for topical use.

Spreadability is an essential aspect of topical preparations because it is related to the ease of application to the skin, removal from containers, and consumer acceptance (Yenti *et al.*, 2014). The spreadability test provides information about the ability of preparations to spread on a surface. The wider the preparation spreads on the skin, the higher the absorption of its medicinal ingredients (Naibaho *et al.*, 2013). The spreadabilities of the emulsion gels containing 1, 2, and 4% of the betel leaf extract were 1.849 ± 0.45, 1,816 ± 0.051, and 1.771 ± 0.092 g.cm.s-1, respectively, while the base of the emulsion gel had a spreadability of 2.292 ± 0.045 g.cm.s-1. The results showed that a higher level of extract in the emulsion gel was associated with lower spreadability. it can be concluded as having good spreadability and is therefore easy to apply to the skin.

Adhesive capacity evaluation can determine the ability of preparations to stick to the skin. The duration of adherence affects the absorption level of the drug. The longer the time of contact, the more the dermal absorption of the drugs (Naibaho, 2013). Previous research showed that the acceptable adhesion of topical preparations is not less than 4 seconds (Sari *et al.*, 2015). The adhesive capacities of the emulsion gels containing 1, 2, and 4% of the betel leaf extract were 110 ± 10.8, 126.3 ± 8.5, and 142.7 ± 13.50 seconds, respectively, while the base of the emulsion gel had an adhesive capacity of 85 ± 4.58 seconds. Based on this capacity, the emulsion gels have met the requirements for topical dosage forms. Moreover, increasing the concentration of the extract appears to strengthen adherence.

The viscosity test aimed to identify how thick preparations are. The thicker they are, the higher the viscosity. Viscosity is inversely proportional to spreadability but directly proportional to adhesive capacity (i.e., the greater the viscosity, the stronger the preparation sticks to the skin). Based on the reading of the viscometer at 100 rpm, the viscosities of the emulsion gels containing 1, 2, and 4% of the betel leaf extract were, 1992.95, 2162.12, and 2640.35 centipoises (cps), respectively. Acceptable viscosity for semisolid preparations is 2000-4000 cps

(Garg *et al.*, 2002). The results showed that emulsion gels that the higher the extract concentration the greater the viscosity.

As a gelling agent in the formula, HPMC increases the viscosity (Garg *et al.*, 2002; Naibaho *et al.*, 2013). HPMC is a cellulose-derived polymer whose molecules fill in the cavities formed by water molecules during dispersion, forming hydrogen bonds with the polymer molecules on the hydroxyl (–OH) group of the water molecules. The flow types of the betel leaf emulsion gel and its base were pseudoplastic. This finding is in line with Martin *et al.* (1993), which states that the flow type of parts of pharmaceutical preparations is pseudoplastic.

CONCLUSION

The betel leaf emulsion gel has shown to exhibit anticandidal activity from the formation of clear rings, marking the inhibition of *Candida albicans* growth. Besides, this emulsion gel has met the required physical properties for semisolid preparations, including organoleptic characteristics, pH, spreadability, adhesion, and viscosity. Further research is recommended to test the optimization of extract solvent, testing of chemical contents in the extract, and dissolution tests.

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Formulation and Antifungal Activity of *Piper betle* L. Leaf Extract in Emulsion Gels Against *Candida albicans*

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ABSTRACT

Candidiasis is the most common fungal infection in humans and is a form of primary and secondary infections of *C. albicans*. Betel (*Piper betle L.*) leaf extract has been reported to exhibit efficacious antifungal effects against *C. albicans*. Emulsion gels, a type of topical dosage form, can deliver hydrophilic and hydrophobic drugs and perform multiple and controlled releases. This research aimed to determine the antifungal activity and physical properties of emulsion gels formulated from betel leaf extract. The dried betel leaves were extracted by maceration with alcohol 95%. Then, with different concentrations (1, 2, and 4%), the extract was formulated into emulsion gels. These dosage forms were later subjected to antifungal activity testing against *C. albicans* using the cup plate diffusion method that involved Mycoral Cream® for comparison. In this test, the intensity of the activity was determined by measuring the diameter of the formed inhibition zone. The second test evaluated the physical characteristics of the dosage forms, including organoleptic properties, pH, adhesion, dispersion, and viscosity. These data were analyzed using the Mann-Whitney method, and the conclusion was withdrawn from describing the results quantitatively. The reaction yield of the extraction was 9.702%. The analysis results showed that emulsion gels containing 1, 2, and 4% of betel leaf extract created zones of inhibition with diameters of 5.3 ± 0.29, 6.2 ± 0.29, and 10.2 ± 0.41 mm, respectively. As for the physical properties, they differed in pH (6.39 ± 0.120, 6.17 ± 0.132, 5.66 ± 0.123), spreadability (1.849 ± 0.45, 1.816 ± 0.051, 1.771 ± 0.092 g.cm.s^{-1}), adhesion (110 ± 10.8, 126.3 ± 8.5, 142.7 ± 13.50 seconds), and viscosity (2640.35, 1992.95, 2162.12 cps), respectively. The betel leaf emulsion gels exhibited antifungal activity against *C. albicans* ($p < 0.05$) and met the physical requirements of semi-solid dosage forms.

Keywords: emulsion gel; betel leaf extract; antifungal activity; physical properties

INTRODUCTION

Candidiasis is the most common fungal infection in humans and is the primary and secondary infection of *C. albicans* (Leepel, 2009). It can occur locally or systematically. Local infections can be disorders of the mouth, skin, vagina, and nail plate, while lesions due to systemic ones can grow in the kidneys, skin, eyes, and heart (Jawetz *et al.*, 2010). Candidiasis treatment has utilized traditional antifungal medicine to reduce side effects. An example includes betel leaves that contain hydroxychavicol and have the potential for anticandida. According to Bandaranayake *et al.,* (2018), the ethanol extract of betel leaves can inhibit the growth of *Candida albicans* at a concentration of 1 mg/mL. Aside from ease of administration, topical preparations can avoid the side effects, risks, and inconvenience of intravenous therapy and have various absorption conditions on the enteric route. Betel leaf extract is composed of hydrophilic and hydrophobic compounds, which can be formulated into emulsion gels to perform multiple and controlled releases (Hardenia *et al.*, 2014).

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METHODOLOGY

Materials

The primary research materials were betel leaves (purchased from Beringharjo Market, Yogyakarta) and the fungus *C. albicans*. Apart from Sterile Water for Injection, CYG medium, SDA medium, 0.9% NaCl solution, and Mycoral® Cream (Ketoconazole 2%), this experiment also used materials with pharmaceutical grades, namely alcohol 95%, alcohol 70%, distilled water, liquid paraffin, Hydroxyprophylmethylcellulose (HPMC), propylparaben, methylparaben, tween 80, span 80, and propylene glycol.

The research equipment included an oven, halogen moisture analyzer, rotary evaporator, water bath (Memmert®), porcelain saucer, Petri dish, autoclave (Shenan®), incubator, inoculation

Table I. The formula of the betel leaf emulsion gel

Ingredients	Concentrations (%)		
	F1	F2	F3
Extract	1	2	4
HPMC	2.5	2.5	2.5
Liquid Paraffin	5	5	5
Tween 80	1.08	1.08	1.08
Span 80	0.42	0.42	0.42
Propylene Glycol	10	10	10
Methylparaben	0.03	0.03	0.03
Propylparaben	0.01	0.01	0.01
Distilled Water	Ad 100	Ad 100	Ad 100

Note : F1 = extract ethanol betel leaf 1%; F2 = extract ethanol betel leaf 2%; F3 = extract ethanol betel leaf 4%

loop, micropipette (Socorex®), analytical scales, and glassware (Iwaki Pyrex®).

Methods

Preparation of Dried Betel Leaves

Betel leaves were collected, sorted, dried, and reduced to smaller size. These dried specimens were tested for drying losses and then identified both macroscopically and microscopically.

Extract Preparation

Extraction

Five hundred grams of the dried betel leaves were macerated with 5L of ethanol 95% (solvent) with a ratio of 1:10 for 24 hours (Hertiani and Purwantini, 2002; Depkes RI, 2008). The macerated mixture was collected and processed in a rotary evaporator at 50°C until concentrated extracts with solid green color and the peculiar smell of betel leaves were acquired (Singburaudom, 2015; Anwar *et al.*, 2014).

Phenol detection test

One gram of the extract was dissolved with 20 mL of ethanol 70%. $FeCl_3$ was dripped to 2 mL of this solution. Bluish-green coloration indicates the presence of phenolic compounds (Syafitri *et al.*, 2014).

Ethanol detection test

This test aimed to ensure that the extract did not contain ethanol compounds. The extract was dissolved with H_2SO_4 in a test tube and then added with acetic acid. The tube was closed with cotton and heated to boiling point. Ethanol content is marked with the scents of esters in the cotton (Raymon *et al.*, 2016).

Emulsion Gels Preparation

The emulsion gels were prepared from the betel leaf extract with three different formulas, i.e., concentrations of 1, 2, and 4%, as determined from the orientation results. Prompting this process was the making of an oil phase by mixing span 80 and liquid paraffin at 70°C and a water phase by mixing tween 80 and some water at 70°C. Then, both phases were mixed and stirred at 70°C until an emulsion was formed. Meanwhile, the gel was prepared by crushing HPMC and dispersing it in the water at 80°C little by little. Methyl- and propylparaben were dissolved in propylene glycol and then mixed into the gel. The formed emulsions and gels were combined with a homogenizer at a speed of 700 rpm for 45 minutes until an emulsion gel was formed. The ethanol extract was ground, added with the base of the emulsion gels (F0) until homogenous (Yenti *et al*, 2014). The formulas of the betel leaf emulsion gels were modified from Yenti *et al* (2014), as seen in Table I.

Antifungal Activity Testing

The antifungal activity test employed the cup-plate diffusion method. It began with creating the medium by suspending 650 mg of SDA medium in 100 mL of water, then pouring the mixture into Petri dish and allowing it to harden. The suspension culture of *C. albicans* was made with 100 µl and added with physiological NaCl until the turbidity was the same as the Mc Farland standard (concentration of *C. albicans*= 1.5×10^8 CFU/mL). Afterward, the solution was diluted with CYG medium (1:100), forming a colony of *C. albicans* with a concentration of 10^6 CFU/mL. The surface of the solidified agar medium was spread evenly with 1 mL of the *C. albicans* suspension culture and

Table II. The results of macroscopic identification of betel leaves

Parameters	Standard Features	Results	Notes
Shape	Egg-shaped with a tapering point	Egg-shaped with a tapering point	Matched (Depkes RI, 2010)
Color	Brownish green	Green	Matched (Depkes RI, 2010)
Smell	Distinctive	Distinctive	Matched (Depkes RI, 2010)
Taste	Spicy	Spicy	Matched (Depkes RI, 2010)
Length	5-18 cm	10.5 cm	Matched (Depkes RI, 2010)
Width	3-12 cm	6 cm	Matched (Depkes RI, 2010)
Lower Surface	Rough with a lighter color	Rough with a lighter color	Matched (Depkes RI, 2010)

perforated using a 5mm cork borer. The test material and the control (negative and positive) were put into each hole, and then the medium was incubated at 37°C for 24 hours, and their diameters were calculated as the diameter of the inhibition zone.

Physical Properties Evaluation

The physical properties evaluation included an organoleptic test and the measurements of pH, spreadability, adhesion, and viscosity.

Organoleptic Test

The organoleptic evaluation was performed by observing the shape, smell, and color of the preparations (Naibaho *et al.,* 2013).

pH Analysis

This test used a pH meter that had been calibrated with acetate buffer pH 4.0 and phosphate buffer pH 7.0. One gram of the emulsion gels was dissolved in 10 mL of distilled water. The electrode was dipped in the solution, and a constant pH was measured as the base pH of the emulsion gels (Yenti *et al*., 2014).

Spreadability Test

A half gram of emulgel is placed on a round glass scale and covered with another round glass and then left for 1 minute, after that it is added with 150 g ballast and then the distribution diameter is recorded every 1-minute interval (Garg *et al*., 2002).

Adhesive Capacity Test

A 0.25g sample of the emulsion gel was placed between two glass objects. A 1kg weight was put on top of the glass and left for five minutes. This load was lifted, and the glass objects were separated from each other with an 80g weight. The time required for the glass objects to separate was recorded (Sari *et al.*, 2015).

Viscosity Test

This test used a Rheosys Merlin VR II viscometer equipped with 25mm concentric cylinders or spindles. It processed 10 points with rotational speeds of 0.1 - 100 rpm, a delay time of 20 seconds, and at a temperature of 25°C.

RESULT AND DISCUSSION

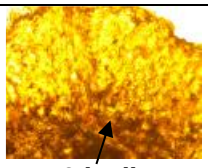
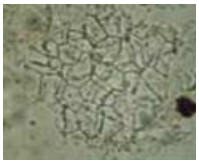
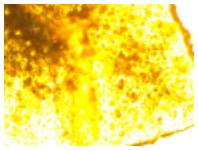
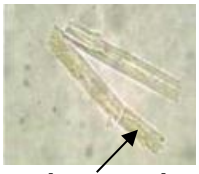
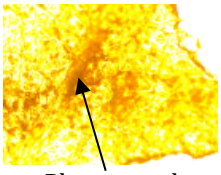
The macroscopic and microscopic tests confirmed that the dried specimens were betel leaves (*Piper betle* L.). The macroscopic and microscopic test results of the betel leaves are presented in Table II and Table III, respectively.

Five hundred grams of dried betel leaves produced 48.512 g of extract as 9.702% w/w(yield). Based on the organoleptic evaluation, the extract had a solid green color, thick consistency, and the typical scent of betel leaves. In the phenols test, the color of the test solution changed from green to bluish-green, indicating the presence of phenolic compounds in the extract (Syafitri *et al.*, 2014). As for the ethanol test, it did not detect any smell of ester after the extract was boiled with H_2SO_4 and acetic acid, meaning that the extract does not contain ethanol (Raymon *et al.*, 2016).

The emulsion gel was made from the ingredients listed in Table I. The material of HPMC functioned as both gelling and stabilizing agent. The combination of methyl- and propylparaben serves as a preservative, while propylene glycol

Figure 1. The formulation of betel leaf emulsion gels with different formulas. (F0) the base of emulsion gel (BEG), (F1) 1% betel leaf extract, (F2) 2% betel leaf extract, (F3) 4% betel leaf extract

Table III. The results of microscopic identification of betel leaves

Parameters	Standard Features	Results	Notes
Lower epidermis with oil cells	 Oil cells	 Oil cells	(Depkes RI, 2010)
Upper epidermis			(Depkes RI, 2010)
Plant vessels with scalariform thickening	 Plant vessel	 Plant vessel	(Depkes RI, 2010)

dissolves methyl- and propylparaben and acts as humectants (Yenti, 2014; Rowe *et al.*, 2009; Dewi, 2015). The results of the emulsion gel formulation are presented in Figure 1.

The emulsion gels were tested for their inhibitory activity against *Candida albicans*. The antifungal capacity testing was performed with three-time replication. The results are presented in Table IV.

Betel leaves contain essential oils, phenyl propane, chavicol, flavonoids, tannins, and terpenoids acting as the antifungal (Zuraidah, 2015). Hydroxychavicol is the main phenolic compound isolated from the betel leaf extract. It can also change the structure of cell membranes, resulting in disruption of membrane permeability (Ali, 2010). Heating in the extraction process can affect the anticandidal activity of the betel leaf emulsion gels because it changes the chemical components of betel leaves (Hertiani and Purwantin, 2002).

Antibiotics have been proven to interact with the surface of the lipid that contains ergosterol. The bonds between lipids and antibiotic molecules induce damage to the membranes and, thereby, disrupt the specific membrane permeability. Changes in membrane permeability can destabilize cells up to a point where molds and yeast cells die (Kusumaningtyas, 2008; Coutinho *et al.*, 2004).

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Overall, the organoleptic test showed that the betel leaf emulsion gel was green, semisolid, and had the smell of betel leaf extract. Meanwhile, the base of the emulsion gel was white semisolid and smelled like HPMC. The pH levels of the emulsion gels containing 1, 2, and 4% of the betel leaf extract were 6.39 ± 0.120, 6.17 ± 0.132, and

Table IIV. The results of the antifungal capacity tests

Codes	Test Materials	Zones of Inhibition (diameter, mm)
F0	The base of emulsion gels (BEG)	$0^{b,c,d}$
F1	Emulsion gels with 1% extract	$5.3 \pm 0.29^{a,c,d}$
F2	Emulsion gels with 2% extract	$6.2 \pm 0.29^{a,b,d}$
F3	Emulsion gels with 4% extract	$10.2 \pm 0.41^{a,b,c}$
FC	Mycoral® Cream	6.2 ± 0.29

Notes: a : significantly different from the base formula (F0); b : significantly different from the formula of emulsion gel with 1% extract (F1) c : significantly different from the formula of emulsion gel with 2% extract (F2); d : significantly different from the formula of emulsion gel with 4% extract (F3)

Table V. The results of the physical properties evaluation

Analyses	Results			
	F0	F1	F2	F3
Organoleptic	White, distinctive	Semisolid Green, typical betel leaves		
pH	6.56 ± 0.104	6.39 ± 0.120	6.17 ± 0.132	5.66 ± 0.123
Spreadability ($g.cm.s^{-1}$)	2.292 ± 0.045	1.849 ± 0.45	1.816 ± 0.051	1.771 ± 0.092
Adhesive capacity (s)	85 ± 4.58	110 ± 10.8	126.3 ± 8.5	142.7 ± 13.50
Viscosity (cps)	1843.95	1992.95	2162.12	2640.35
Flow Types	Pseudo-plastic	Pseudo-plastic	Pseudo-plastic	Pseudo-plastic

5.66 ± 0.123, respectively, while the base of the emulsion gel had pH= 6.56 ± 0.104. The pH measurement aims to ensure that the preparations are safe for dermal application, i.e., with pH ranging between 4.5-6.5 (Riski *et al.,* 2016). The acidic gel can irritate the skin, whereas the alkaline ones can cause scaly skin (Naibaho *et al.*, 2013). The pH analysis showed that emulsion gels with higher levels of betel leaf extracts had lower pH. In this study, the pH is in the allowed range of safe pH for topical use.

Spreadability is an essential aspect of topical preparations because it is related to the ease of application to the skin, removal from containers, and consumer acceptance (Yenti *et al.*, 2014). The spreadability test provides information about the ability of preparations to spread on a surface. The wider the preparation spreads on the skin, the higher the absorption of its medicinal ingredients (Naibaho *et al.*, 2013). The spreadabilities of the emulsion gels containing 1, 2, and 4% of the betel leaf extract were 1.849 ± 0.45, 1,816 ± 0.051, and 1.771 ± 0.092 g.cm.s-1, respectively, while the base of the emulsion gel had a spreadability of 2.292 ± 0.045 g.cm.s-1. The results showed that a higher level of extract in the emulsion gel was associated with lower spreadability. it can be concluded as having good spreadability and is therefore easy to apply to the skin.

Adhesive capacity evaluation can determine the ability of preparations to stick to the skin. The duration of adherence affects the absorption level of the drug. The longer the time of contact, the more the dermal absorption of the drugs (Naibaho, 2013). Previous research showed that the acceptable adhesion of topical preparations is not less than 4 seconds (Sari *et al.*, 2015). The adhesive capacities of the emulsion gels containing 1, 2, and 4% of the betel leaf extract were 110 ± 10.8, 126.3 ± 8.5, and 142.7 ± 13.50 seconds, respectively, while the base of the emulsion gel had an adhesive capacity of 85 ± 4.58 seconds. Based on this capacity, the emulsion gels have met the requirements for topical dosage forms. Moreover, increasing the concentration of the extract appears to strengthen adherence.

The viscosity test aimed to identify how thick preparations are. The thicker they are, the higher the viscosity. Viscosity is inversely proportional to spreadability but directly proportional to adhesive capacity (i.e., the greater the viscosity, the stronger the preparation sticks to the skin). Based on the reading of the viscometer at 100 rpm, the viscosities of the emulsion gels containing 1, 2, and 4% of the betel leaf extract were, 1992.95, 2162.12, and 2640.35 centipoises (cps), respectively. Acceptable viscosity for semisolid preparations is 2000-4000 cps

(Garg *et al.*, 2002). The results showed that emulsion gels that the higher the extract concentration the greater the viscosity.

As a gelling agent in the formula, HPMC increases the viscosity (Garg *et al.*, 2002; Naibaho *et al.*, 2013). HPMC is a cellulose-derived polymer whose molecules fill in the cavities formed by water molecules during dispersion, forming hydrogen bonds with the polymer molecules on the hydroxyl (–OH) group of the water molecules. The flow types of the betel leaf emulsion gel and its base were pseudoplastic. This finding is in line with Martin *et al.* (1993), which states that the flow type of parts of pharmaceutical preparations is pseudoplastic.

CONCLUSION

The betel leaf emulsion gel has shown to exhibit anticandidal activity from the formation of clear rings, marking the inhibition of *Candida albicans* growth. Besides, this emulsion gel has met the required physical properties for semisolid preparations, including organoleptic characteristics, pH, spreadability, adhesion, and viscosity. Further research is recommended to test the optimization of extract solvent, testing of chemical contents in the extract, and dissolution tests.

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