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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 These data were analyzed using the Mann-Whitney method, and the conclusion was withdrawn from describing the results quantitatively.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

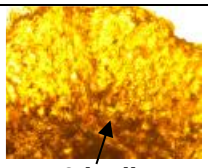
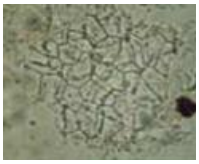
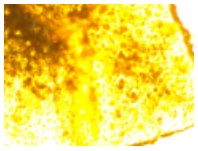
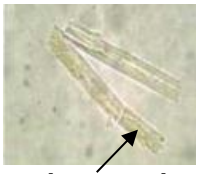
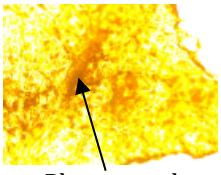
The macroscopic and microscopic tests confirmed that the dried specimens were betel leaves (*Piper betle* L.). The macroscopic and microscopic test results of the betel leaves are presented in Table II and Table III, respectively.

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).

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	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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