FARMASAINS: JURNAL FARMASI DAN ILMU KESEHATAN
Volume 5, Nomor 1, 2020. p-ISSN : 2086-3373 | e-ISSN : 2620-987X
https: ejournal.umm.ac.id/index.php/farmasains

Research Article

Formulation and evaluation of hand sanitizer gel containing infused of binahong leaf (*Anredera cordifolia*) as antibacterial preparation

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ARTICLE INFO

Article History
Received July 24, 2020
Revised July 31, 2020
Accepted August 3, 2020
Published August 6, 2020

Keywords
Binahong leaf infusion
Hand sanitizer gel
Antibacterial activity
Staphylococcus aureus

Doi
10.22219/farmasains.v5i1.13008

ABSTRACT

Hand sanitizer gel is one of the alternative methods for hand hygiene when soap and water are not available. Binahong leaf (*Anredera cordifolia*) is a natural herb that has phenolic, flavonoids, saponins, and the steroid-terpenoids compound, which is potentially used as the active ingredient in the hand sanitizer gel. The primary purpose of this research was to determine the antibacterial potential and physicochemical properties of a hand-sanitizer gel containing binahong leaves' infusion against *Staphylococcus aureus*. The efficacy of this formulation was tested against a bacterial strain using the disc diffusion method and determined by measuring the inhibition zone of each preparation. The testing of physicochemical properties includes the organoleptic test, pH, viscosity, and gel spreadability. The physicochemical characteristics of the gel showed excellent properties for all formulas. The antibacterial test showed that hand sanitizer gel containing an infusion of binahong's leaves has an inhibition zone (6.02 mm; 6.24 mm; 6.55 mm), almost close to a positive control (9.15 mm). In conclusion, the formulated hand sanitizer gel possesses the antibacterial potential to inhibit *S. aureus*.

1. INTRODUCTION

Hands are regarded as a significant source of transmission of infections and microbes (Al-zahrani & Baghdadi, 2012; MadanKumud, Neha & Seema, 2012). Hand hygiene is essential and crucial to protect against the spread of disease and is one of the primary practices to reduce the transfer of bacteria, whether from person to food contact surfaces, or from person to another person (Jain, Karibasappa, Dodamani, Prashanth & Mali, 2016; Lambrechts, Human, Doughari & Lues, 2014). However, the Indonesian public awareness of the importance of hand hygiene is lacking. Society does not realize that hands are often contaminated with microbes while doing the activity (Ningsih, Zusfahair, Kartika & Fatoni, 2017). Especially during this global pandemic, the World Health Organization (WHO) recommends proper hand hygiene as one of the essential means to prevent the spread of all infections (Jing et al., 2020; Nuwagaba et al., 2020; Pickering et al., 2013).

Hand sanitizer is an alternate way to hand washing when soap and water are not available. It is an alternative hygiene method not based on water but equally effective (Thaddeus, Francis, Jane, Obumneme & Okechukwu, 2018). It does not require the use of water, which makes the application easy and uncomplicated (Babeluk, Jutz, Mertlitz, Matiasek & Klaus, 2014). Mostly, they are available in liquid or gel preparations (Hayat & Munnawar, 2016). The active ingredient(s) is/are usually antiseptic substance(s) like povidone-iodine, ethanol,

isopropanol, benzalkonium chloride, triclosan, et cetera. (Moses, Rosemary, Linda & Nsikak, 2013). However, repeated use of alcohol-based hand rub (ABHR) can be caused some harm, like irritation, dryness, and dermatitis, potentially (Lachenmeier, 2008; Menegueti et al., 2019). Because of that reason, we have to be seeking an alternative to safe and natural antibacterial ingredients. Natural herbs are readily available in nature, not toxic, and not only kill the germs on the skin but also protect the natural skin cells (Rajurkar, 2016).

One of the plants which have a potential antibacterial effect is binahong leaf (*Anredera cordifolia*). Binahong leaf infusion in 50%-100% and Binahong extract in 20%-100% has been scientifically proven to have an antimicrobial effect (Maharani, Puspitasari & Gunawan, 2018; Prasetyaningsih, Kurniati & Setiarini, 2017). Its extracts and fractions showed bacteriostatic and bactericidal activities in 256-512 µg/ml (Leliqia, Sukandar & Fidrianny, 2017). This research will be conducted to determine the physicochemical characteristics and the inhibition zone of binahong leaf infusion against *Staphylococcus aureus* as a natural and safe antibacterial active ingredient in hand sanitizer gel.

2. MATERIALS AND METHODS

Materials

Fresh binahong leaves were obtained from Materia Medika Batu, Malang, and was infused with aquadest in Laboratory of Integrated Chemistry, University of Muhammadiyah Malang. Bacterial cultures of *Staphylococcus aureus* was obtained from Laboratory of Microbiology, University of Brawijaya, Malang. carbopol, TEA, glycerine, and propylene glycol was purchased from PT. Brataco Chemical, Malang. All the chemicals used were of technical grade.

Methods

Producing the binahong leaf infusion

Fifty grams of fresh binahong leaves were cleaned and cut into small pieces and then placed on a glass tray filled with 500 ml of aquadest. The leaves were steamed in a pan of boiling water on 90 °C for 15 minutes and occasionally stirred. After steaming was finished, leaves were filtered and then heated using a water bath until it becomes parts from the initial solution or 100% concentration was reached. The infusion was inserted into an airtight glass container that was wrapped with aluminum foil (Ardianti, Guntarti & Zaenab, 2014; Maharani et al., 2018).

Phytochemical screening

Phytochemical screening was held to figure out the compounds of binahong leaf infusion. Binahong plants are known to contain saponins, terpenoids, flavonoids, and essential oils. These compounds are bioactive compounds in plants, so they have the potential to be antibacterial agents (Wardhani & Sulistyani, 2012). Phytochemical screening was carried out to prove the compounds contained in binahong leaf infusion. This examination was carried out using the Thin Layer Chromatography (TLC) test method, which is detected under UV light and confirmed by spraying a reagent on the TLC plate (Kumalasari & Sulistyani, 2011).

Preparation of binahong leaf infusion hand sanitizer gel

Carbopol was weighed and transferred slowly into distilled water taken in a beaker glass. After 24 hours, this solution was poured into the mortar and added a few drops of triethanolamine to get proper gel consistency. Glycerin was added and stirred, followed by the addition of propylene glycol. The binahong leaf infusion was added to the mixture and vigorously stirred until the ingredients were thoroughly mixed. Lastly, the mixture was transferred into a container (Gujjar, Madhavi & Karki, 2019; Yaun & Vasquez, 2017).

Physicochemical evaluation of Binahong leaf infusion hand sanitizer gel

The physicochemical evaluation involved was organoleptic, determination of pH value, viscosity, and gel spreadability. The organoleptic test was observed as its odor, color, and consistency visually. pH value was measured using pH meter and viscosity was determined by using Viscometer. Gel spreadability was measured by applying gel on the transparent glass, then was sandwiched between two glass slides and was loaded by some loads (Yuliani, Fudholi, Pramono & Marchaban, 2012).

Acceptability test

Acceptability test was carried out using a questionnaire that was provided to 10 respondents. Respondents were students of the University of Muhammadiyah Malang, who were obtained accidentally. All respondents must be in good health and do not have any skin diseases. The gel preparation was used by applying 0.5 g of gel to the respondent's palm, then allowed to stand for 1 minute. Assessment by respondents included parameters: ease of pouring, texture, traces left and stickiness of hand sanitizer gel (Saryanti & Zulfa, 2017)

Bacteria rejuvenation

Rejuvenation of bacteria was carried out by taking from the Laboratory of Microbiology, University of Brawijaya. *S. aureus* was seeded in the Nutrient Agar (NA) media and incubated for 24 hours at 37 °C. The next day, the colony obtain was identified according to the standard procedure at University of Brawijayato ensure that the colony was *S. aureus*.

Antibacterial activity test on binahong leaf infusion hand sanitizer gel

The disc diffusion method was used to determine the zone of inhibition against *S. aureus*. Antibacterial activity of binahong leaf infusion hand sanitizer gel was evaluated using the disc diffusion method on NA media. The inhibition zones were reported in millimeter (mm). *S. aureus* was used as references for the antibacterial assay. Briefly, NA plates were inoculated with bacterial strain under aseptic conditions, and 7 mm filter paper discs were impregnated with 20 mL of each gel sample. The Petri dishes were incubated in a jar at 37 °C for 24 hours. After the incubation period, the diameter of the growth inhibition zones was measured by calculating the diameter of clear zones (Jahangirian et al., 2013; Maharani et al., 2018; Valgas, De Souza, Smânia & Smânia, 2007). Then, Dettol® was used as the positive control, and hand sanitizer gel without binahong leaf infusion was used as the negative control. Each gel samples were performed in triplicate.

3. RESULTS AND DISCUSSIONS

Phytochemical screening

The results of the TLC examination of flavonoid and terpenoid compounds could be seen in **Figure 1**. In flavonoids examination, the chloroform: acetone: formic acid (6:6:1) was used as eluent, and ammonia vapor was used as a stain appearance. While in terpenoids examination, the n-hexane: ethyl acetate (4:1) was used as eluent, and sulfuric acid anisaldehyde was used as a stain appearance then heated on a hotplate until the stain is visible.

Table 1. Formulation of binahong leaf infusion hand sanitizer gel

Ingredients	Formula 1	Formula 2	Formula 3
Binahong leaf infusion	18g (10%)	36g (20%)	54g (30%)
Carbopol	0.9g	0.9g	0.9g
Triethanolamine	Qs	qs	qs
Glycerin	9g	9g	9g
Propylene glycol	27g	27g	27g
Aqua destilata	up to 200g	up to 200g	up to 200g

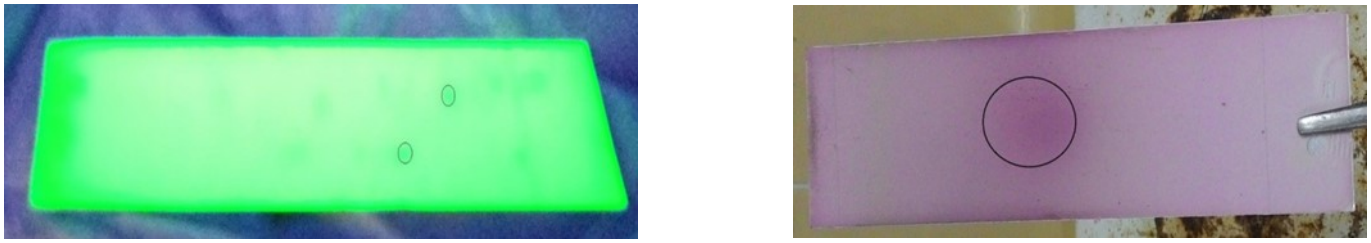

Figure 1. Thin Layer Chromatography profile of binahong leaf infusion for detecting compounds flavonoids (left) and terpenoids (right).

Table 2. Results of the physicochemical evaluation

Parameter	Formula 1	Formula 2	Formula 3
pH	6.72±0.04	6.62±0.10	6.53±0.11
Viscosity (cps)	26333.33±5008,33	22916.67±10260,16	18333,33±3055,05
Spreadability (cm)	5.27±1.06	6.17±1.04	8.10±1.90

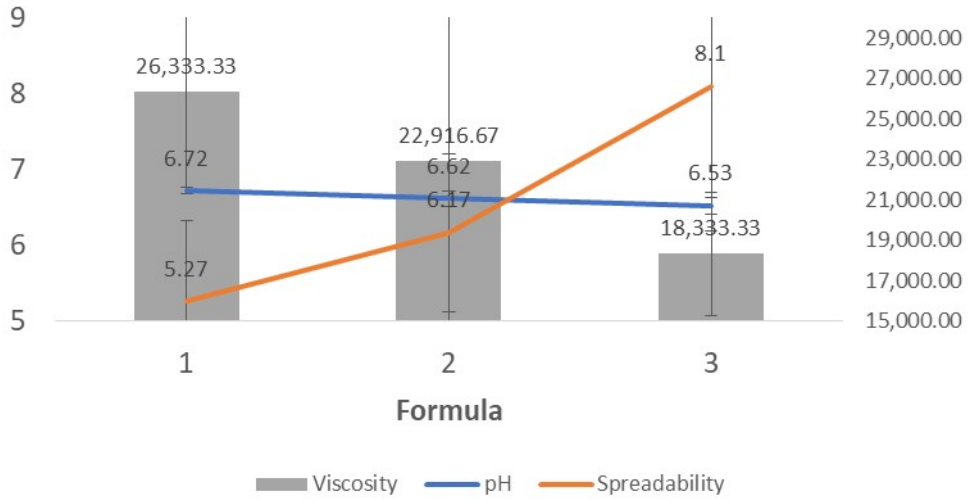

Figure 2. Results of the physicochemical (pH value, viscosity, and spreadability) evaluation.

Physicochemical evaluation of Binahong leaf infusion hand sanitizer gel

The organoleptic test was carried out to see the physical appearance by observing the color, smell, consistency, and homogeneity of the hand sanitizer gel. Color test of hand sanitizer shows that the higher the concentration of binahong leaf infusion, the darker the color of the gel produced. They had no specific smell. They had a semi-solid consistency, soft and smooth, easy to apply, light to spread, thick, and get thinner with the addition of binahong leaf infusion. Homogeneity of the hand sanitizer gels as shown by the absence of coarse particle (Direktorat Jenderal Pengawasan Obat dan Makanan, 1985) and showed no phase separation (between binahong leaf infusion and gel base) in the preparation after its application to a transparent glass.

The pH value should be in the broad range of skin pH (from 4.0 to 7.0) to avoid any irritation to the skin (Ali & Yosipovitch, 2013). The result of pH value measurement was done in triplicate and average values calculated (**Table 2**). Binahong leaf infusion has an acidic pH value of about 5.25. The variation of binahong leaf infusion concentration in hand sanitizer gel will affect the pH value of resulting preparation. The greater concentration of binahong leaf infusion, the higher the pH value of the preparation obtained. Nevertheless, the presence of triethanolamine (pH value = 10.5) makes the pH value, not too acidic (Rowe, Sheskey & Quinn, 2009).

A viscosity test was carried out to determine the thickness of preparation and was measured using Brookfield Viscometer. As shown in **Table 2**, the higher the concentration of binahong leaf infusion as active ingredients, the lower viscosity of the preparation was obtained. The viscosity of the preparation is influenced by the ingredients present in the gel. In this case, the properties of the binahong leaf infusion is very watery, which can decrease the viscosity of the gel preparation. However, the presence of carbopol, which provides viscosity/ hardness in the gel and induced the preparations to exhibit a more elastic solid behavior (A-sasutjarit, Sirivat & Vayumhasuwan, 2005; Saryanti & Zulfa, 2017), will keep the gel preparation in right semi-solid consistency.

Gel spreadability test was performed to examine the ability of binahong leaf infusion hand sanitizer gel to spread when applied on the skin. The rate of spreading depends on the time and the rate of shear and also the viscosity of the formulation (Garg, Aggarwal, Garg & Singla, 2002). In the topical preparation, spreadability is inversely proportional to its viscosity. The lower the viscosity, the higher the dispersal power (Chandira et al., 2010). The presence of binahong leaf infusion will increase the gel spreadability. However, the presence of carbopol keeps the repulsion and the cross-linking between polymer chains (Magbool, Elnima, Shayoub &

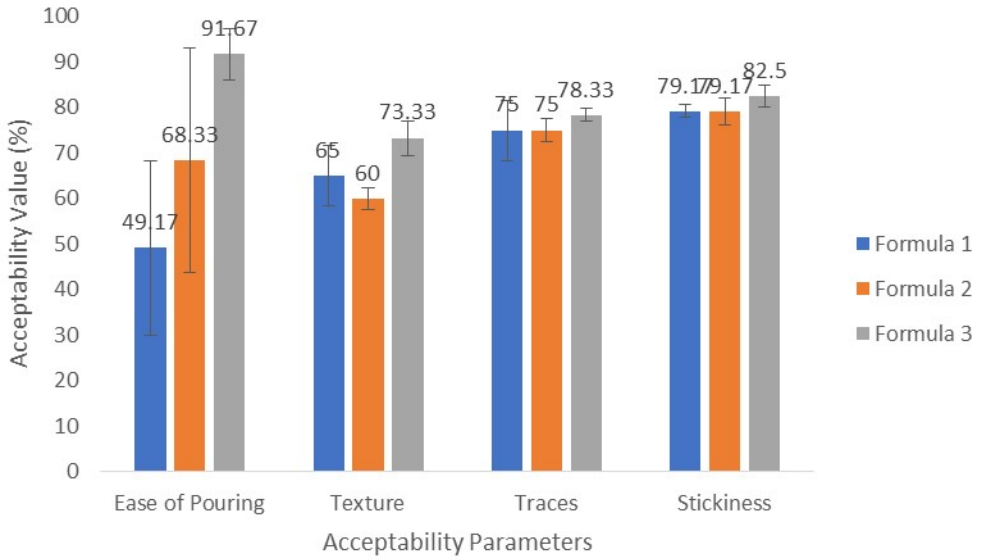

Figure 3. Results of acceptability test of binahong leaf infusion hand sanitizer gel.

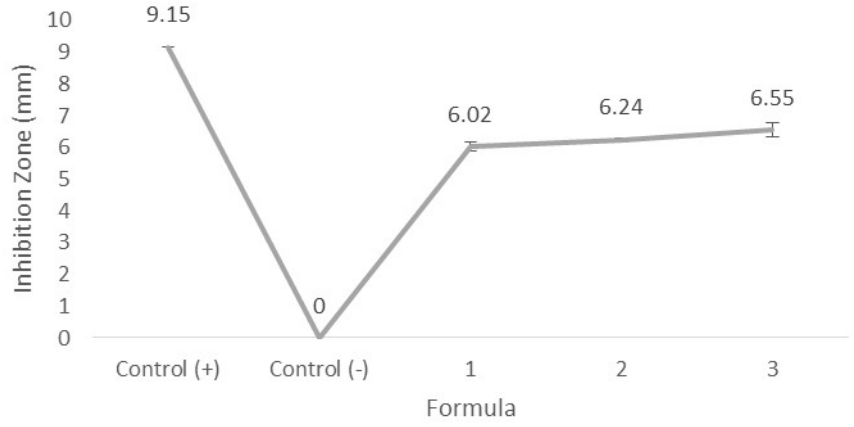

Figure 4. Results of antibacterial activity test of binahong leaf infusion hand sanitizer gel.

Hamedelniel, 2018), which keep the spreadability in good value.

Acceptability test

Acceptability test results (**Figure 3**) showed that Formula 3 is the most chosen hand sanitizer preparation in terms of ease of pouring, texture, traces left, and stickiness of the preparation. The hand sanitizer gel must be easy to pour and comfortable to use—these results related to the homogeneity and spreadability of gel preparation. Then, the texture of the preparation is affected by the consistency of the hand sanitizer gel. In this case, the variable which plays a role is the concentration of the gelling agent used. The antiseptic gel also should not leave a trace after use and are not sticky to the skin. Stickiness is a condition that can cause discomfort when used, and this can be caused because the gel preparation does not dry out quickly (Saryanti & Zulfa, 2017).

Antibacterial activity test on Binahong leaf infusion hand sanitizer gel

The result of the antibacterial activity test of binahong leaf infusion hand sanitizer gel can be seen in **Figure 3**. The results of the antibacterial activity test showed that the Binahong leaf infusion hand sanitizer gel could inhibit the growth of *S. aureus* bacteria. Binahong's leaf contains some compounds like flavonoids and terpenoids, which have antibacterial activity. Terpenoids were considered as fast-acting compounds which can inhibit bacteria mechanism by dysfunction or rupture of the cell membrane of bacteria (Guimarães et al., 2019). Then flavonoids have several mechanisms to inhibit bacteria mechanism, as follows: inhibition of cytoplasmic membrane function, inhibition of nucleic acid synthesis, inhibition of energy metabolism, and alteration of the membrane permeability (Xie, Yang, Tang, Chen & Ren, 2014). An increase of binahong leaf infusion influences

the inhibition zone in hand sanitizer gel. The test results demonstrate that the higher concentration of binahong leaf infusion as an active ingredient, the higher was the inhibition zone of the preparation. This can occur because of the higher antibacterial activity by the addition of binahong leaf infusion.

4. CONCLUSIONS

Hand sanitizer gel is one of the alternative methods for hand hygiene. The result obtained from this study provided evidence that the binahong leaf infusion hand sanitizer gel exhibited beneficial antibacterial activity against *S. aureus*. The highest antimicrobial activity was observed for Formula 3 (Binahong leaf infusion 30%), which showed the inhibition zone almost close to the positive control used. It can be concluded that the flavonoid and terpenoid compounds present in the binahong leaf infusion may be responsible for the significant antibacterial activity. Furthermore, these hand sanitizer gel has excellent physical properties for all formulas caused they showed an excellent pH value, viscosity, spreadability, and acceptability in human skin. The result also demonstrated that the various concentration of binahong leaf infusion was influenced by physicochemical characteristics, acceptability, and antibacterial activity of leaf infusion of binahong in hand sanitizer gel. Further scientific evaluation of these formulas and further characterization of gel preparation should be done to develop a better preparation of hand sanitizer gel as hand hygiene.

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