# Antibacterial-based Hand Sanitizer of Biwa Leaves' Extract (*Eriobotrya japonica* (Thunb) Lindl) from Tanah Karo

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Abstract: Biwa (*Eriobotrya japonica* (Thunb) Lindl.) is one of cultivated plant commodities in Karo regency, North Sumatera, which has been known to contain chemical compounds such as flavonoid and tannin possessing antibacteria properties. The study aimed to develop the potency of Biwa leaves' extract as hand sanitizer to inhibit the growth of *Staphylococcus aureus*. Methodologies in this study were extraction and detection of common phytochemical compounds, antibacterial test against *S. aureus* in vitro, toxicity test using *Artemia salina*, characterization of gel formulation, antibacterial test of gel formulation against *S.aureus* and antibacteria performance from formulated hand sanitizer. The phytochemical compounds detected in extract were from flavonoid and tannin groups with antibacterial activity observed at concentration of 15 and 20% with 8.0 and 9.6 mm diameter of inhibition zones respectively.The Lethal Concentration 50 ($LC_{50}$) value was obtained at 162,18 ppm while administration of hand sanitizer reduced *S.aureus* up to 17.4 colonies in average.

## 1 INTRODUCTION

Hands are the frequent mediators to microbes (virus, fungi and bacteria) in environment during our daily activities (Wijaya*.,* 2013). Practicing good sanitation is the best solution to prevent the microbial contamination to hands. Hand sanitizer is one product made from ethanol to cleanse the hands, yet preventing or reducing microbial contamination.

The use of ethanol effectively kills bacteria since of its bactericidal properties but adverse effects can be harmful from prolonged use such as burns, dry skins and irritations (Dewi., 2016). As an alternative, triclosan may be used as ethanol substitute because of its less corrosiveness although in some cases, may also induce bacterial resistance. Therefore, a solution to address this problem is sought by incorporating *Biwa* extract as active ingredient in our formulated hand sanitizer.

Biwa, also known as *loguat,* is one of the plant commodities with potential economical value yet still rarely cultivated in Indonesia. In North Sumatera, the plant has been known to be cultivated in Karo Regency. The Karonese believe that in addition to its high economical value, Biwa also efficacious as a drug. Plant parts of Biwa such as fruits have high antioxidant content, while seeds and leaves were efficacious as medicine (Morton, 2001). Other local people in North Sumatra, the Chinese, consumed Biwa drugs, especially the seeds and leaves for the treatment of diarrhea, toxin neutralization and swelling. The fruits were also used as tranquilizer, skin cosmetic, and cholestero-lowering therapy (Sembiring, 2009).

Previous study reported the phytochemicals detected in Biwa leaves containing compounds such as oleanolic, ursolic acid and megastigmane glycosides with biological properties as antiviral, antitumor, hypoglycemic, anti-inflammatory, and antibacteria (Singh., 2010). Biwa leaves are effective against bacterial and viral infections in internal bodies such as bronchitis and gastrointestinal disease (Brown, 1999). Testing of Biwa leaves externally asactive ingredient in formulated hand sanitizer is not much done yet. Based on its potential, the study will then evaluate the antibacterial properties from Biwa leaves' extract against *Staphylococcus aureus* as well as obtaining the $LC_{50}$ value from its active fraction to



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promote the use of natural and low-cost formulated hand sanitizer.

# 2 MATERIALS AND METHODS

## 2.1 Plant Materials

Fresh Biwa (*E. japonica*) were collected from Suka village, Tiga Panah district, Kabanjahe city, North Sumatera. The parts used in this study were their leaves. The sample was authenticated by Herbarium Medanese, Universitas Sumatera Utara, Medan, Indonesia.

## 2.2 Extraction Procedure

Fresh leaves were cleaned in flowing tap water, shade-dried and pulverized into coarse powder. One hundred grams (100 g) of powder were macerated with ethanol, ethyl acetate and hexane (1:1) and homogenized using shaker for 4 hr, settled down for 24 hr. Macerate were filtered using Whatman filter paper and concentrated using rotary evaporator. The crude extracts were subjected to antibacterial test in Dimethyl Sulfoxide (DMSO) solution to obtain series of concentration 5, 10, 15 and 20%.

## 2.3 Antibacterial Test

Series concentration of biwa extracts were tested against *S.aureus* using disk diffusion method. Overnight culture broth of *S.aureus* (Optical Density/ $OD_{600} = 0.1 \approx 10^8$ CFU/mL) were swabbed on Muller Hinton Agar (MHA) medium to obtain the bacteria lawn. Sterile disks impregnated with 10 µL of extracts were placed on top of medium with three replications. The plates were incubated for 24 hr in ambient condition. Halo zones around the disks expressing the inhibitory effect to tested bacteria, were measured using a caliper in millimeter unit (mm).

## 2.4 Toxicity Test

Larva of *Artemia salina* were pre-hatched using procedure from Muaja. (2013). Stock solutions of extracts in DMSO with concentration of 20000, 2000, 200 and 20 ppm were pipetted into vials containing 20 live larva with final volume in each vials reaching 5 mL. Control vials were made by replacing extract solution with saline solution. Vials were incubated for 24 hr in proper condition. Dead and live larva were counted post-incubation. Data were analyzed using standard probit analysis with 95% confidence interval (Wahyuni., 2015).

## 2.5 Gel Formulation and Antibacterial Test

The composition of three gel formulations are listed in table 1. Antibacterial test was performed according to previous procedure.

## 2.6 Antiseptic Test of Formulated and Commercial Gel

Antiseptic test was conducted by using replica-plating method [1]. Hands were washed in flowing tap water and air-dried. Thumb were gently pressed on top of Mannitol Salt Agar (MSA) to expose indigenous *S.aureus* and serve as control plate. Treatments (formulated gels 15 and 20%, blank (negative control) and commercial gel (positive control) were administered as the same procedure stated before. Plates were incubated at 28°C for 24 hr. After incubation, bacterial colonies were observed and counted. Experiments were conducted in five replications.

Table 1: Three gel formulations and their compositions.

| Materials | Formulation-1 | Formulation-2 | Formulation-3 |
|---|---|---|---|
| Biwa leaves' extract | 0% | 15% | 20% |
| Carbopol 940 | 0,5% | 0,5% | 0,5% |
| TEA | 0,5% | 0,5% | 0,5% |
| Glycerine | 1% | 1% | 1% |
| Sodium metabisulphite | 0,2% | 0,2% | 0,2% |
| Aquadest | 200 mL | 200 mL | 200 mL |

# 3 RESULTS AND DISCUSSION

## 3.1 Phytochemical Screening Results of Biwa Extracts

The phytochemical compounds detected in Biwa extracts are presented in Table 2. Flavonoid and tannin were detected only in ethanolic extract exposing that ethanol may be used in future extraction

procedure to obtain desirable compound from flavonoid and tannin groups.

Table 2: Phytochemical compound groups detected during screening.

| Solvents | Flavonoid | Tannin |
|---|---|---|
| Ethanol | + | + |
| Ethyl Acetate | - | - |
| Hexane | - | - |

Flavonoid is a group of phytochemical compounds C15 consisting of two carbon cores with three carbon units. Flavonoids are also known as bioactive compound in plants that can be used as antibacteria (Suteja., 2016). Tannins are polyphenol compounds with a high molecular weight comprising hydroxy and carboxyl groups. Tannins consisted of two types: condensed and hydrolyzed tannins. Other study reported that the tannin compound in the extract of *Averrhoa bilimbi* L. was potential as antibacteria against *Staphylococcus aureus* and *Escherichia coli* bacteria Ummah (2010). Since the desirable compounds were only detected in ethanolic extract, ethyl acetate and hexane extracts were not subjected to antibacterial test.

## 3.2 Antibacterial Activity of Biwa Ethanolic Extracts against Staphylococcus Aureus

The results of antibacterial activity of ethanolic extracts are given in Table 3. Concisely, the use of higher concentration of extracts increased the diameter of halo zones in this study, explaining the more toxicity of solutions against tested bacteria (Figure 1).

Table 3: Antibacterial activity of different concentrations of Biwa extracts against *S. Aureus.*

| | Diameter of Inhibition Zone (mm) |
|---|---|
| Chloramphenicol | 30.00 |
| Alcohol 70% | 7.00 |
| DMSO | 7.00 |
| Ethanolic extract 5% | 6.00 |
| Ethanolic extract 10% | 6.30 |
| Ethanolic extract 15% | 8.00 |
| Ethanolic extract 20% | 9.60 |

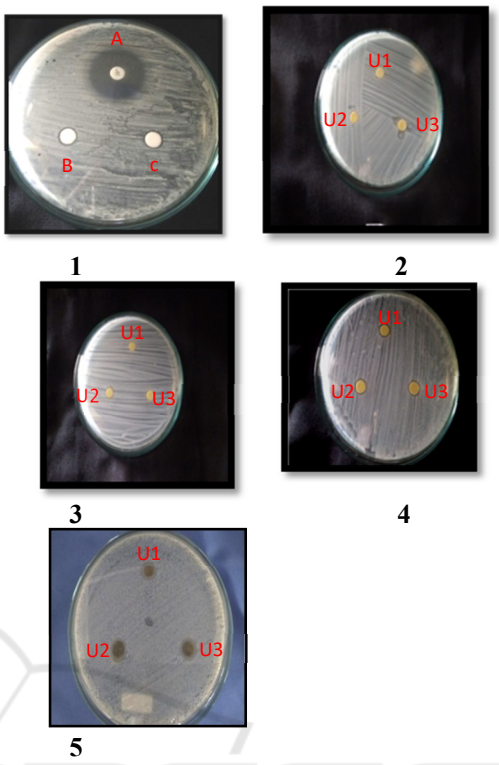



Figure 1 Representative images of inhibitory zones from plate: A. Positive (chloramphenicol) and negative control (alcohol 70% and DMSO), B. Concentration 5%, C. Concentration 10%, D. Concentration 15% and E. Concentration 20%.

Mechanism of antibacterial phenolic components e.g. steroids, alkaloids, triterpenoids, flavonoids and saponins, generally interact with intracellular protein or cytoplasmic walls via hydrogen bonding and hydrophobic interactions (Naidu and Davidson, 2000). Another mechanism is by interfering enzymatic activity of cells through the diffusion of polar extracts into cell interior.According to Siswandono and Soekardjo (2000), the concentration of certain compoundwith antibacterial properties is determined from its inhibition to bacteria growth in plate assay. In addition, the size of the inhibitory zone may be influenced by several factors: test microorganism (bacterial strain and physiological condition), culture medium, method and diffusion nature of the substances.

## 3.3 Result of Toxicity Test using Brine Shrimp Lethality Test (BSLT)

The ethanolic extract were tested for its toxicity against model organism to evaluate the safety issue as

active ingredient later for practical use as hand sanitizer. The results are presented in Table 4. Similar to previous result, the 10-fold increased concentration of extracts led to increase of dead larva in this experiment.

Table 4: Percentage of mortality during exposure to Biwa extracts.

| Concentration (ppm) | Replica 1 | Replica 2 | Replica 3 | Mean | % Mortality |
|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0% |
| 20 | 3 | 3 | 5 | 3,66 | 37% |
| 200 | 5 | 4 | 6 | 5 | 50% |
| 2000 | 6 | 5 | 8 | 6,33 | 63% |
| 20000 | 8 | 9 | 9 | 8,66 | 87% |

The result of probit analysis showed that $LC_{50}$ value of Biwa leaves' extract was 162,18 ppm.Meyer. (1982) stated that an extract to have a potential toxic activity in the BSLT toxicity if the extract caused the death of 50% of the test animals at concentrations <1000 ppm. It can be stated that leaf biwa extract is toxic with $LC_{50}$ value obtained at concentration 162,18 ppm.

## 3.4 Characterization of Gel Formulation

Antiseptic gel formulation showed good characteristics to be used as topical hand sanitizer with details in table 5.

Table 5: Characteristics of gel formulation.

| Formulation | Characters | | | | |
|---|---|---|---|---|---|
| | Flavor | Colour | Appearance | pH | Viscosity |
| Alkohol 70% | Citrus | White | Semisolid | 5 | 1737,02 |
| Formula 1 (Blanko) | None | White | Semisolid | 5 | 23828,85 |
| Formula 2 (15%) | Extract | Green | Semisolid | 6 | 4487,75 |
| Formula 3 (20%) | Extract | Green | Semisolid | 7 | 2035,67 |

From table 5, it can be seen that pH of formulated and commercial gel is below physiological skin pH. Skins are covered with acidic mantle which make the surrounding pH between 4.5–6.5 (Tifaley., 2007). The more acidic or basic condition will alter the skin conditions from drying, sensitive, and easily penetrated by microbes or infection.

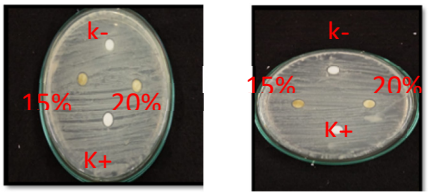
## 3.5 Antibacterial Activity of Gel Formulation

Biwa leaves' extracts in gel formulation were tested against *Staphylococcus aureus*.The gel showed inhibitory activity yet showing the retain of antibacterial properties from extracts. The results can be seen in Table 6.

Table 6: Results of antibacterial test between formulated and commercial gels.

| Sampel | Replica 1 | Replica 2 | Mean (mm) |
|---|---|---|---|
| Commercial gel (Alcohol 70%) | 6.60 | 6.60 | 6.60 |
| Blank (K-) | 0.00 | 0.00 | 0.00 |
| Gel formulation 15% | 6.20 | 6.50 | 6.30 |
| Gel formulation 20% | 7.00 | 6.60 | 7.00 |

From the results, it can be seen that antibacterial activity between formulated and commercial gels were not so different in terms of diameter of inhibition zone against *S.aureus*. Antibacterial activity produced from commercial or alcoholic gel was not quite effective since it is volatile yet reducing the effectiveness upon administration.




**1** **2**

Figure 2 Representative images of inhibitory zones from plate:K- (Blank), 15% (gel formulation), 20% (gel formulation), K+ (Commercial gel); 1 and 2 (Replica 1 and 2).

### 3.6 Antiseptic Test of Gel Formulation

The results of efficacy test using gel formulation as antiseptic can be seen in Figure 3 and 4. The average number of colonies observed from gel formulation is 17.4 colonies from concentration 20%. Although our results showed considerable decrease of *S.aureus* colonies between formulation and control, it still not compensate with the commercial gel. In case of safety use, we still promote biwa formulated gel to be used as hand sanitizer for future application.

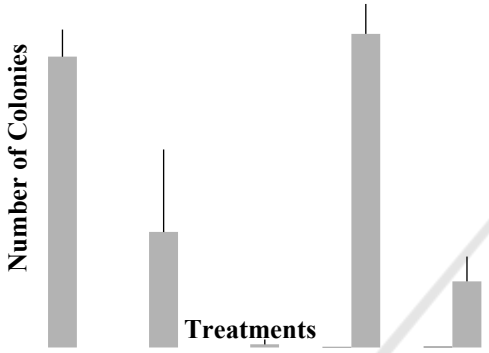



Figure 3: Result of efficacy test using gel formulation as compared with blank, control and commercial antiseptic.

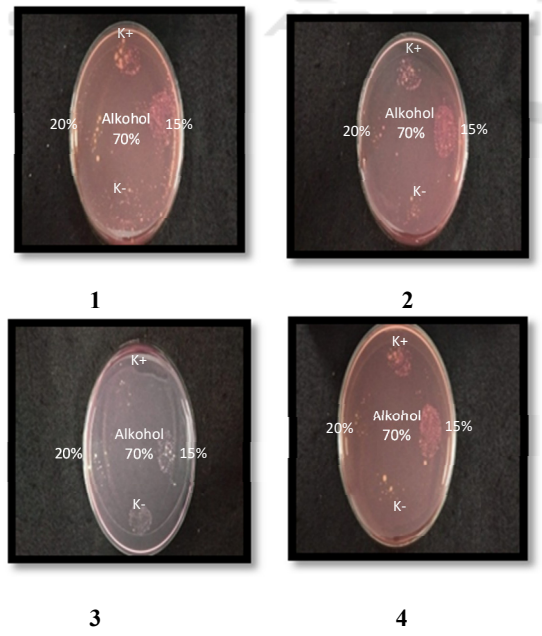



Figure 4 Antiseptic test of Biwa leaves'extract as compared with blank, control and commercial gels in Mannitol Salt Agar (MSA) with five replications.

## 4 CONCLUSIONS

Biwa leaves' ethanolic extract contained flavonoid and tannin groups in phytochemical screening with potential antibacterial activity against *S.aureus* at concentration of 15 and 20% with diameter of inhibition zone measuring 8.00 and 9.60 mm. The $LC_{50}$ of extracts obtained in this study was 162.18 ppm showing less toxicity. Gel formulation with concentration of 20% extracts decrease the population of *S.aureus* as many as 17.4 colonies.

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