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Formulation of Natural, Antiseptic Liquid from Jujube Leaf Extract for Islamic Dead Body Bathing Procedures

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Abstract.
Ziziphus spina-christi L. or Jujube, is a plant that has many health benefits, containing metabolite compounds that act as an antibacterial agent such as alkaloids, flavonoids, saponins, tannins, and polyphenols. This study aimed to make a natural antiseptic liquid from *Ziziphus spina-christi L*. leaf extract used in Islamic Bathing Procedures for a corpse. The stages of this research included extraction, an assay of the extract's antiseptic activity using the agar well diffusion method, formulating the antiseptic liquid, evaluating the antiseptic liquid, and examining the antiseptic properties of the liquid. Jujube leaf extract with a concentration of 10% was proven to have the best antibiotic properties with a very strong inhibition zone category. Antiseptic liquid formulations include extracts, DMDM hydantoin, Triethanolamine, and Aqua rose. The organoleptic evaluation results of liquid antiseptic were in the form of a homogeneous concentrate liquid, brown with a rose aroma, pH 9.048, the density of 1.020 g/mL, viscosity of 5.60 m.Pas, and 0.7 cm high foam with high foam stability of 83.33%. The antiseptic test results showed that the antiseptic solution was able to inhibit the growth of *Escherichia coli, Staphylococcus epidermidis, and Candida albicans* ranging from strong to very strong inhibition zone diameters, 17.35 mm, 21.10 mm, and 19.95 mm, respectively.

Keywords: antiseptic, antimicrobial, corpse bathing, jujube leaf (ziziphus spina christi L.)

1. INTRODUCTION

Hadith narrated by Bukhari number 1175 in the corpse chapter, Rosulullaah Muhammad SAW said "Take a bath by pouring water mixed with bidara leaves three times, five times or more if you felt necessary and make for the last one with camphor (fragrance)". Basically, all humans in this world will experience a period of death. Death is a state of missing a sign of life with a series of changes in the structure of the

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human body that are affected by decay. The decaying process was driven by at least two mechanism, namely autolysis, and putrefaction [1,2]. One of the causes of decay or decomposition is aerobic and anaerobic microorganisms that can decompose human tissues [3,4]. Several microorganisms contribute to the corpse's decomposition process, including *Escherichia coli*, *Staphylococcus epidermidis*, and *Candida albicans*, where these microbes reproduce in human blood [5]. Efforts that can be conducted to inhibit decomposing the corpse are by bathing it using natural antiseptic preparations. Commonly, Herbal extract was often used in the tradition of the mummification process to help preserve the dead bodies [6].

Jujube (*Ziziphus spina-christi*, L.) is a plant that Rasulullah used to bathe the corpse, but this sunnah is rarely applied until now. This is due to the unavailability of Jujube trees or soap products made from Jujube extract. Based on the research, Jujube was known to have an antimicrobial effect against *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella typimurum, Staphylococcus epidermidis, Proteus merabilis, Klebsiella pneumonia, Enterobacter spp., Acinetobacter, Serratia spp., Staphylococcus aureus, Staphylococcus, and Streptococcus pneumonia* [7]. Leaf extract of Jujube was also known to be active as an antifungal against candida albican [8]. Jujube leaf extract contains secondary metabolite compounds that could inhibit microbial growth. It includes alkaloids, flavonoids, polyphenols, tannins and saponins, triterpenoids, lipids, proteins, and glycosides [9]. According to the antibacterial activity and the content of these secondary metabolites, Jujube can be made as an antiseptic solution.

At present, the product for antiseptic liquid for Islamic corpse bathing, which is halal and safe was scarce. This study aims to produce a natural antiseptic solution made from Jujube leaf extract.

2. METHODS

The research was conducted by experimental laboratory method from February 2020 to June 2020 at Research Laboratory Pharmacy UNISBA. Jujube leaves (*Ziziphus spina-christi* L.), aqua rose (Darjeeling, Indonesia), DMDM hydantoin (Subur Kimia Jaya, Indonesia), Amoxicillin (Kimia Farma, Indonesia), Thiamphenicol (Kimia Farma, Indonesia), Ketoconazole (Kimia Farma, Indonesia), Dettol® (Reckitt Benckiser) were a material that been used in the study. *Escherichia coli* ATCC 25922, *Staphylococcus epidermidis* ATCC 12228, and *Candida albicans* ATCC ATCC 10231 were the organisms for testing.

The leaves of Jujube were extracted by infundation method to produce miscella. miscella was concentrated using a rotary vacuum evaporator to obtain a concentrated

extract. Phytochemical screening was conducted on leaves and extracts of Arabic bidara including flavonoids, alkaloids, tannins, saponins, and polyphenols [10].

Extracts with concentrations of 2, 4, 6, 8, and 10% were tested for their activity against *Escherichia coli*, *Staphylococcus epidermidis*, and *Candida albican* with positive control respectively Amoxicillin 1%, Thiamphenicol 1%, and Ketoconazole 1%. The assays used the agar well diffusion method with incubation at 37 ˚ C for 24 hours for antibacterial activity and incubation at 25 ˚ C for 24-48 hours for antifungal activity [11–13]. The negative control used in these three tests was aqua rose. The resulting inhibition zone diameter was then measured, and then determined the best extract concentration used for the antiseptic preparation.

The formulation of natural antiseptic liquid preparations includes concentrates of Jujube leaf extract (10%) and the ingredient of excipients as shown in Table 1. Evaluation of the final preparation includes organoleptic, pH, specific gravity, a viscosity, and determination of foam height and foam stability [14].

TABLE 1: The formula of natural antiseptic liquid of Jujube leaves.

Composition of Material	Concentration (%)
Extract of Jujube leaves	10
DMDM *hydantoin*	0,6
Triethanolamine	3
Aqua rose	ad. 50 mL

The antiseptic liquid that produced was then tested for antibacterial and antifungal activity and compared against similar commercial products (Dettol®) with the same method as in the assay of extract.

3. RESULTS AND DISCUSSION

3.1. Extraction and Phytochemical Screening Jujube Leaves

Extraction using the infudation method was successful to produce an extract yield of 29.85%. The phytochemical screening of raw material and leaf extract of Jujube showed that both the raw material and the extract contained alkaloid, flavonoids, tannins, polyphenols, and saponins. This result had similarities with the data produced by Jebur et al and Asgarpanah et al [2,3]. Based on these data, the infudation method was known to be able to extract compounds from the raw material optimally. The results of phytochemical screening can be seen in Table 2.

Table 2: Results of phytochemical screening of raw material and extracts.

Compound	Extract	Raw Material
Alkaloid	√	√
Flavonoid	√	√
Saponin	√	√
Tanin	√	√
Polifenol	√	√

(√) = detected

3.2. Assay of the Antimicrobial and Antifungal Activity of Jujube Leaf Extract

The antimicrobial activity test of Jujube leaf extract against *Staphylococcus epidermidis*, *Escherichia coli*, and *Candida albicans* using the well diffusion method showed that the extracts with all concentrations were able to produce inhibition zones. This showed that the extract had the activity of inhibiting the three microorganisms.

Table 3: Result of the antimicrobial and antifungal activity assay from Jujube leaf extract.

Extract Concentration (%)	Diameter of Inhibition Zone (mm)		
	Escherichia coli	Staphylococcus epidermidis	Candida albicans
2	12,30	7,40	10,30
4	16,90	15,75	18,05
6	19,15	17,20	18,45
8	21,65	18,65	20,35
10	24,00	25,70	24,35
[a]Positive Control (1%)	34,85	16,90	7,25
[b]Negative Control	0	0	0

[a]Amoxicillin (Escherichia coli), Thiamphenicol (Staphylococcus epidermidis), Ketokonazol (Candida albicans)
[b]Aqua rose

According to Davis and Stout, antimicrobial activity was declared as a weak level if it produced an inhibition zone diameter of less than 5 mm, moderate if it produced an inhibition zone of 5-10 mm, a strong level if it produced an inhibition zone of 10-20 mm, and was very strong if it produced an inhibition zone more than 20 mm [15]. According to the criteria for the level of activity strength, the results of the antibacterial activity test of Bidara leaf extract against *Escherichia coli* with a concentration of 2-6% was in a strong inhibition zone (12.30-19.15 mm), while at a concentration of 8-10% was the very

strong inhibition zone (21.65-24.00 mm). Amoxicillin 1% as a positive control had a very strong inhibition zone (34.85 mm).

The antibacterial activity assay of Jujube leaf extract against *Staphylococcus epidermidis* with a concentration of 2% resulted in the moderate inhibition zone (7.40 mm), at a concentration of 4-8% resulted in the strong inhibition zone (15.75-18.65 mm), whereas at a concentration of 10% resulted in a very strong inhibition zone (25.70 mm). Thiamphenicol 1% as a positive control had a strong inhibition zone (16.90 mm). The antifungal activity assay of Jujube leaf extract against *Candida albicans* with a concentration of 2-6% resulted in a strong inhibition zone (10.30-

18.45 mm), while a concentration of 8-10% resulted in a very strong inhibition zone (20.35- 24.35 mm). Fluconazole 1% as a positive control had a moderate inhibition zone (7.25 mm).

Therefore, the effective concentration in inhibiting the growth of *Escherichia coli*, *Staphylococcus epidermidis*, and *Candida albicans* from Jujube leaf extract that would be used as active substances in natural antiseptic liquid formulations was 10% (very strong).

3.3. Formulation of Natural Antiseptic Liquid Jujube Leaf Extract

Antiseptic is a pharmacy product that can reduce or inhibit microorganisms' growth by damaging tissue cells without damaging the host's tissues. This antiseptic liquid formula used Jujube leaf extract as an antimicrobial substance and the excipients including aqua rose as a carrier and can be used as an alternative fragrance, DMDM hydantoin as a preservative that is effective against bacteria, fungi or yeast and was safe for use in pharmaceutical preparations and did not irritate the skin [16], and Triethanolamine as a pH adjustment agent in order to obtain a pH that was following general antiseptic preparations, pH 9.9-10.15.Evaluation of the final formula was conducted to determine the characteristics of Jujube leaf extract's natural antiseptic liquid, including organoleptic, pH, specific gravity, viscosity, the height of foaming, and foam stability. The results obtained were listed in Table 4

An organoleptic test was performed to visually characterize the formula's physical appearance, including shape, color, and scents. The color of the preparation obtained was in accordance with the original color of the Jujube leaf extract, which was brown. It has a distinctive scent of rose from aqua rose as the carrier, with a pH of 9.48, which was closer to the pH of common antiseptic liquids, namely 9.9-10.15. This natural antiseptic

Table 4: Result of Evaluation of natural antiseptic liquid formula of Jujube leaf extract.

Evaluation Parameter	Result
Organoleptic	Form: homogeneous liquid; Color: Brown; Scents: rose flower
pH	9.48
specific gravity (g/mL)	1.02
viscosity (mPa.s)	5.60
height of foaming (cm)	0.70
foam stability (%)	83.33

liquid was concentrated, which when used, should be diluted first with a certain amount of water so that it reached a neutral pH, consequently safe for the skin.

The specific gravity value was influenced by the constituent material and its physical properties. The result obtained is 1.020 g / mL. This shows that the natural antiseptic liquid preparation of Jujube leaf extract has a specific gravity approaching the specific gravity of water, namely 1.0 g / mL. The closer to the specific gravity value of water, the more solubility it is in water.

Viscosity could be expressed as the resistance of fluid flow so intermolecular friction could occurs. Viscosity measurement could determine the ease level with which a liquid was removed from its container, thus making it easier to use. A liquid preparation could be declared easy to flow if it had a low viscosity. The viscosity of a preparation influenced the pourability of a liquid antiseptic. Visually, this natural antiseptic liquid was very easy to pour because it had a low viscosity and was included in the non-newton flow properties with a viscosity value of 5.60 m.Pas.

The height of foaming assay was conducted to determine the foam power of the natural antiseptic liquid preparation of Jujube leaf extract. The results showed that the formula's foam height was 0.7 cm, then decreased to 0.6 cm after being allowed to stand for 5 minutes. This change did not exceed the required scale of 0.1 cm, so it can be concluded that the preparation has good foam stability, namely 83.33%.

3.4. Antiseptic Activity Assay of the Product

The antimicrobial activity test of natural antiseptic liquid product of Jujube leaf extract against *Escherichia coli* and *Staphylococcus epidermidis* bacteria and the fungus *Candida albicans* showed almost equivalent effectiveness comparable to commercial products, both in concentrated form and dilution in 10 times the solvent. This was indicated by the strong to very strong inhibition zone against *Escherichia coli*, *Staphylococcus*

epidermidis, and *Candida albicans*, namely 17.35 mm, 21.10 mm, and 19.95 mm respectively (concentrate) and 14.80 mm, 15.90 mm, and 10.15 mm respectively (dilution). The results obtained can be seen in Table 5.

TABLE 5: Antimicrobial activity test of natural antiseptic liquid of Jujube leaf extract.

	Inhibition Zone Diameter (mm)		
Tested Material	**Escherichia coli**	**Staphylococcus epidermidis**	**Candida albicans**
Natural antiseptic liquid (conc. 10%)	17.35	21.1	19.95
Natural antiseptic liquid (conc. 1%)	14.80	15.90	10.15
Commercial product equivalent (conc. 1%)	17.25	16.20	57.55
Commercial product equivalent (conc. 0.1%)	15.20	14.95	40.7

4. CONCLUSION

The active ingredient used in the natural antiseptic formulations for corpse bathing was the extract with a concentration of 10% because according to the assay, it had a very strong activity as an antiseptic. The antiseptic liquid showed a homogeneous concentrate liquid, has a brown color with a rose scent, pH 9.048, specific gravity of 1.020 g

/ mL, a viscosity of 5.60 m.Pas and a foam height of 0.7 cm with high foam stability of 83.33%. The antiseptic activity of the natural antiseptic liquid against Escherichia coli, Staphylococcus epidermidis, and Candida albicans showed effectiveness and it was equivalent compared to its commercial products.

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