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Original Article

FORMULATION AND EVALUATION OF RED GINGER RHIZOME EXTRACT SOAP AS AN ANTIBACTERIAL

NOVI NURLENI*, ARIE FIRDIAWAN, AGNES RENDOWATY, RESTI KURNIASARI

Department of STIFI Bhakti Pertiwi, South Sumatera, Indonesia
***Corresponding author: Novi Nurleni; *Email: nurleni.novi29@gmail. Com**

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ABSTRACT

Objective: Current research work aimed to develop a formulation of red ginger into a stable soap preparation, effective as an antibacterial and safe for long-term use.

Methods: The maceration method was used to extract red ginger rhizome. In the formulation, there are variations in the concentration of the extract, where Formulation I (FI) 3%, Formulation II (FII) 5%, Formulation III (FIII) 7%, and Formulation IV (FIV) 0%. The evaluation of the preparation includes tests such as sensory evaluation, homogeneity, pH, viscosity and flow properties, density, foam height, and stability, as well as antibacterial activity using the agar diffusion method against *Staphylococcus aureus* ATCC 25932 and *Escherichia coli* ATCC 25922.

Results: The percentage of extract yield obtained was 2.38%. All three formulations of soap have shown good stability during the 28-day evaluation (significant>0.05), indicating no significant changes during storage, and the inhibitory power of *Staphylococcus aureus* ATCC 25923 bacteria in the three successive formulations was 9.03±0.4; 12.21±0.3; 15.26±0.4 (mm) respectively, while that of *Escherichia coli* ATCC 25922 was 6.01±0.6; 10.32±0.4; 12.58±0.6 (mm).

Conclusion: The evaluation results, all formulations have good stability during storage. The variation in concentration of red ginger extract will affect the inhibitory power against test bacteria. F III, with an extract content of 7% has better antibacterial activity compared to other formulations.

Keywords: Red ginger rhizome, Soap, Antibacterial activity, *Staphylococcus aureus, Escherichia coli*

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INTRODUCTION

Various diseases caused by microbial infections must be wary of. Some of them can cause health problems ranging from mild to severe symptoms and even the risk of death. Diseases caused by bacteria are usually treated with antibiotics. Types of pathogenic bacteria such as *Pseudomonas* sp., *Klebsiella* sp., *Escherichia coli*, *Staphylococcus haemolyticus, Staphylococcus epidermidis,* and *Staphylococcus aureus* have the highest resistance to ampicillin, amoxicillin, penicillin G, tetracycline and chloramphenicol [1]. One of the diseases caused by viruses is COVID-19. The COVID-19 cases spread rapidly between countries, including Indonesia. Indonesia has reported 377.541 confirmed cases and 12.959 deaths. Based on this data, the government has made various efforts to prevent and control diseases caused by microbial infections. One of them provides standard recommendations through regular hand washing with soap and clean water [2].

Soap is a material that can clean dirt (hydrophilic and lipophilic), and functions as a bacteriocidal and bacteriostatic if it contains active ingredients as antibacterial. However, most hand-washing soaps on the market contain synthetic active ingredients which have several drawbacks, for example, causing skin irritation, dry skin, rashes, and bacterial resistance when used continuously [3]. To overcome this, it is necessary to modify and develop hand-washing soap formulations from natural ingredients that have antibacterial properties. Where natural ingredients are a source of medicine needed in the world of health [4, 5].

One of the Indonesian plants that can be used as a raw material in the manufacture of modern and traditional medicines is red ginger (*Zingiber officinale* var. Rubrum). The benefits of red ginger are not only as a food provider but also as an antioxidant, antimicrobial, antiviral, anti-inflammatory, antiulcerogenic, anticancer, anti-hyperlipidemic, etc [6–10]. Based on previous research, ginger extract is also utilized for the treatment of COVID-19 patients to inhibit the growth of bacteria and viruses [11–13].

The test bacteria used were *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, which are the most common pathogenic bacteria that attack humans. *Staphylococcus aureus* is a Gram-positive bacterium that lives as a saprophyte in the human body's membrane channels, skin surfaces, sweat glands, and intestinal tract. *Escherichia coli* bacteria are Gram-negative bacteria which are normal flora in the human intestine that can cause Urinary Tract Infection (UTI) and diarrhea [14-17]. Based on this, the researchers were interested in modifying and developing a liquid soap formulation from red ginger extract as an antibacterial and tested it against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 bacteria *in vitro*.

MATERIALS AND METHODS

Materials used were red ginger rhizome (*Zingiber officinale* var. Rubrum of Andalas University Herbarium in Padang 175/K-ID/ANDA/III/2021) (Segamit Village, Muara Enim, South Sumatera), potassium hydroxide (KOH) olive oil (The Soap Kitchen), stearic acid (Brataco), Natrium Carboxy Methyl Cellulose (Na. CMC) (Pt. Chanshu Wealthy), Sodium Lauryl Sulfate (SLS) ((Merck, German), aquadest, distillate ethanol (Merck, German), nutrient agar (NA) (Merck), physiological NaCl 0.9%, *Staphylococcus aureus* ATCC 25923 (Universitas Indonesia) and *Escherichia coli* ATCC 25922 (Universitas Indonesia).

Red ginger rhizome (*Zingiber officinale* var. Rubrum) was cleaned and roughly chopped, then soaked in distilled ethanol for several days until the end of the soaking reached. After that, it is filtered using filter paper to separate the filtrate from the soaking. The macerate was evaporated and a concentrated extract of red ginger rhizome (*Zingiber officinale* var. Rubrum) was obtained [18, 19]. It will continue to make red ginger extract into hand soap (table 1).

Stability testing was conducted for 28 d, made for each experiment. Organoleptic test, observations include odor, color, and texture of the preparation [20]. Homogeneity is prepared by observing the mixing of all ingredients in the formulation with a microscope [20]. pH testing, measurement of preparations using a calibrated pH meter [20]. Viscosity and flowability, using a Brookfield Viscometer with a viscosity range for semisolid

preparations of 200-6000 cps [20]. The determination of density, using a pycnometer by the requirements for the specific gravity of liquid soap, is 1.01-1.10 [21, 22]. High Foam Stability and Foam Retention, The preparation was carried out by shaking in a measuring cup, where the height and retention of the foam must remain stable for at least 1 minute [23, 24].

Table 1: Formulation of red ginger rhizome extract soap

Composition	Concentration (%)			
	FI	FII	FIII	FIV
Red Ginger Extraction	3	5	7	-
Olive oil	10	10	10	10
KOH	qs	qs	qs	qs
Stearic acid	1	1	1	1
Na CMC	0.3	0.3	0.3	0.3
SLS	1.6	1.6	1.6	1.6
Aquadest ad	70 g	70 g	70 g	70 g

Antibacterial activities test red ginger rhizome extract (*Zingiber officinale* var. Rubrum) of soap *in vitro*. This test aims to determine that liquid soap containing red ginger extract can inhibit the growth of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 bacteria so that it can be used as a natural antibacterial in making liquid soap. The test bacteria used were *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, which are the most common pathogenic bacteria that attack humans. *Staphylococcus aureus* is a Gram-positive bacterium that lives as a saprophyte in the membranes of the human body, skin surface, sweat glands, and intestinal tract. *Escherichia coli* bacteria are Gram-negative bacteria that are normal flora in the human intestine and can cause Urinary Tract Infection (UTI) and diarrhea [25–27].

RESULTS AND DISCUSSION

From 5000 grams of a fresh sample of red ginger rhizome (*Zingiber officinale* var. Rubrum), which was extracted, obtained extract of 119.4 grams and obtained a percent yield of 2.38%.

The results of the organoleptic examination of FI, FII, FIII, and FIV for 28 d of soap which was light brown, with a red ginger aroma, and had a semi-solid texture. From these deviations, there are no organoleptic changes (table 2). Homogeneity testing is done to determine the distribution of ginger extract particles in soap preparations. Visually, it shows a uniform result, indicated by an even distribution of particles throughout the 28-day storage period [20].

Table 2: Organoleptic test of red ginger rhizome extract soap

Days to	Parameters	Formulation			
		FI	FII	FIII	FIV
0	Color	Light brown	Light brown	Light brown	White
	Shape	Semisolid	Semisolid	Semisolid	Semisolid
	Smell	Ginger aroma	Ginger aroma	Ginger aroma	Unscented
28	Color	Light brown	Light brown	Light brown	White
	Shape	Semisolid	Semisolid	Semisolid	Semisolid
	Smell	Ginger aroma	Ginger aroma	Ginger aroma	Unscented

pH value can be used to determine whether a solution is acidic or basic. The pH value of soap can be influenced by additional soap-forming substances such as KOH and stearic acid, as well as variations in the concentration of red ginger extract used. The pH of raw ginger is 5.6 to 5.9, which is quite acidic due to the presence of gingerol compounds. This can be seen from the higher the active substance content, the more acidic the pH of the preparation [20, 28] (table 3). During the evaluation, there were changes and a decrease in pH for each formulation. However, these changes are not significant, as indicated by a value greater than significant>0.05, it is also within the pH range of human skin it doesn't irritate the skin [29].

Table 3: pH test of red ginger rhizome extract soap

Days to	Average pH of the formulation±SD			
	FI	FII	FIII	FIV
0	6.5±0.05	6.3±0.05	6.1±0.05	6.1±0.05
14	6.4±0.05	6.2±0.05	6.0±0.05	6.1±0.05
28	6.4±0.05	6.1±0.05	5.8±0.15	6.0±0.05
Significant	0.057	0.074	0.095	1.000

Data are expressed as mean±SD (standard deviation), n=3

Table 4: Viscosity test of red ginger rhizome extract soap

Days to	Average viscosity of the formulation (cps)±SD			
	FI	FII	FIII	FIV
0	811.66±20.20	866.66±16.07	895.00±35.00	695.00±0.05
14	786.00±15.00	805.00±8.66	886.66±11.54	680.03±0.05
28	775.00±25.98	781.66±11.54	783.33±18.92	680.00±0.05
Significant	0.055	0.074	0.095	1.000

Data are expressed as mean±SD (standard deviation), n=3

The viscosity of the preparation is measured to determine the consistency of the preparation during storage. In this soap formulation, the variation in viscosity is influenced by the amount of red ginger extract used. The more extract used, the thicker the soap produced, and vice versa (table 4). During storage, there is a decrease in viscosity for each formulation, which is related to the decrease in pH that releases H^+ions, making the preparation less thick and more diluted. However, the change is not significant as indicated by significant >0.05. Flowability test of the four formulas over 28 d, there is no change in flow properties was thixotropic plastic (table 5 and fig. 1).

Table 5: Rheology test of red ginger rhizome extract soap

Speed (rpm)	Correction actor (f)	Deal reading (dr)	Viscosity (η = dr x f)	Sharing stress (F/A = dr x 7,187)	Rate of shear (dv/dr = F/A x 1/η)
1.5	200	22	4400	158.114	0.035935
3	100	40.5	4050	291.073	0.071870
6	50	62	3100	445.594	0.143740
12	25	74	1850	531.838	0.287480
30	10	83.5	835	600.114	0.718700
30	10	82	820	589.334	0.718700
12	25	71	1775	510.277	0.287480
6	50	63.5	3175	456.374	0.143740
3	100	38	3800	273.106	0.071870
1.5	200	23	4600	165.301	0.035935

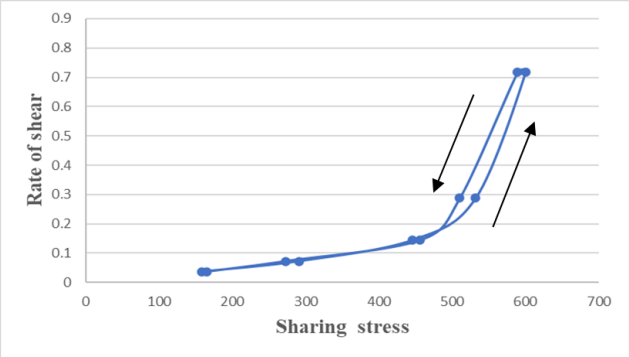

Fig. 1: Rheogram of red ginger rhizome extract soap FI

Density test of the four formulas owned preparations of red ginger rhizome extract (*Zingiber officinale* var. Rubrum) soap during 28 d of storage had the highest average density value of 1.050 grams/ml and the lowest value of 1.006 grams/ml [23] (table 6). High foam stability had the highest average high foam stability value of 4.4 cm and the lowest value of 2.5 cm (table 7). The foam retention test had the highest average foam retention value of 91.94% and the lowest value of 77.33% (table 8).

Table 6: Density test of red ginger rhizome extract soap

Days to	Average viscosity of the formulation (grams/ml)±SD			
	FI	FII	FIII	FIV
0	1.049±0.001	1.006±0.001	1.006±0.000	1.006±0.001
14	1.050±0.001	1.006±0.001	1.006±0.001	1.006±0.001
28	1.048±0.005	1.006±0.001	1.006±0.001	1.006±0.001

Data are expressed as mean±SD (standard deviation), n=3

Table 7: High foam stability of red ginger rhizome extract soap

Days to	Average viscosity of the formulation (cm)±SD			
	FI	FII	FIII	FIV
0	4.4±0.17	4.1±0.11	3.8±0.17	4.5±0.11
14	3.7±0.05	3.5±0.11	2.9±0.17	4.6±0.05
28	3.1±0.17	2.8±0.28	2.5±0.01	4.6±0.05

Data are expressed as mean±SD (standard deviation), n=3

Table 8: Foam retention test of red ginger rhizome extract soap

Days to	Average viscosity of the formulation (%)±SD			
	FI	FII	FIII	FIV
0	83.38±2.01	91.94±3.60	86.05±3.36	85.01±1.05
14	86.48±4.67	81.31±4.28	86.41±5.34	85.01±1.11
28	85.04±4.35	82.22±1.92	77.33±4.61	84.08±2.23

Data are expressed as mean±SD (standard deviation), n=3

The antibacterial activity test of soap against Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922 showed that all formulations containing red ginger extract have inhibitory effects on these bacteria (table 9). On the other hand, soap without ginger extract does not have any bacterial inhibitory effect. The higher the concentration of the extract used, the greater its ability to inhibit bacterial growth. This is due to the presence of compounds such as (zingiberene, α-farnesene, 6-gingerol, and α-curcumin) contained in ginger rhizomes that can affect the permeability and release of intracellular components by attacking bacterial cell membranes and cell walls. This can cause inhibition of bacterial growth [6,30–32].

Table 9: Inhibition against *staphylococcus aureus* ATCC 25923 and *escherichia coli* ATCC 25922 bacteria of red ginger rhizome extract soap

Average inhibition of the formulation (mm)±SD	Bacteria	
	***Staphylococcus aureus* ATCC 25923**	***Escherichia coli* ATCC 25922**
FI	9.03±0.4	6.01±0.6
FII	12.21±0.3	10.32±0.4
FIII	15.26±0.4	12.58±0.6
FIV	0.00±0.00	0.00±0.00
Clindamycin	20.78±0.1	17.34±0.2

Data are expressed as mean±SD (standard deviation), n=3

CONCLUSION

The research that has been done, shows that the three hand-washing soap preparations have been made good stability during the 28-day evaluation. Then, testing the inhibition activity of *Staphylococcus aureus* ATCC 25932 and *Escherichia coli* ATCC 25922, obtained the best inhibition activity results with an average value of *Staphylococcus aureus* 15.26±0.4 mm and *Escherichia coli* 12.58±0.6 mm in formulation III. Phytochemical screening of red ginger rhizome extract previously carried out contains gingerol, which is thought to have antibacterial activity.

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AUTHORS CONTRIBUTIONS

All authors are contributed equally

CONFLICT OF INTERESTS

Declared none

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