Meditory
(The Journal of Medical Laboratory)
Volume 13 Issue 1 Page 95-103 – June 2025
https://doi.org/10.33992/meditory.v13i1.4067

IRRITATION TEST OF LIQUID ANTIBACTERIAL SOAP COMBINING LEGUNDI AND GREEN BETEL LEAVE EXTRACTS ON WHITE RATS (Rattus norvegicus)

Ida Bagus Oka Suyasa[1*], Ni Nyoman Astika Dewi[1], Made Delia Budi Apriliapatni[1], Surya Natallia Bryan[1]

[1]Poltekkes Kemenkes Denpasar, Jalan Sanitasi No. 1 Sidakarya, Denpasar Selatan , Bali, 80224, Indonesia

Article History
Received, May 20th, 2025
Revised, May 27th, 2025
Reviewed, June 1st, 2025
Posted, June 23rd, 2025

Editor
Heri Setyo Bekti, M.Biomed

Corresponding author
Ida Bagus Oka Suyasa
e-mail:
iamgusoka@gmail.com

Keywords
legundi leave, betel leave, irritation test, rats

Abstract

Background: Soap is a cleanser that is made with a chemical reaction. Soap preparations with active ingredients derived from nature are also widely circulated. Natural ingredients that have many active substances are legundi leaves and green betel leaves. Two antimicrobial agents from these ingredients working simultaneously on a homogeneous microbial population will have a synergistic effect.
Objective: This study aims to determine the safety of using an antibacterial liquid soap formula with a combination of legundi and betel leaf extracts.
Methods: This study is an experimental research that includes physicochemical testing of the soap formula, organoleptic evaluation, and irritation testing on white rats (Rattus norvegicus) using the Draize method with a posttest-only control group design. This research was analyzed descriptively. This study adheres to the ethical principle of beneficence, prioritizing the well-being and safety of all participants.
Results: Organoleptic test, pH test, homogeneity test, and water content test meet Indonesian National Standard requirements, and there is no irritation in experimental animals.
Conclusions: An antibacterial liquid soap formula with a combination of legundi and betel leaf extracts is safe.

Cite this Article

Suyasa IBO, Dewi NNA, Apriliapatni MDB, Bryan SN. Irritation Test Of Liquid Antibacterial Soap Combining Legundi And Green Betel Leaf Extracts On White Rats (Rattus norvegicus). Meditory J Med Lab. 2025;13(1):95–103.

INTRODUCTION

Soap is produced through a chemical reaction involving sodium or potassium and fatty acids derived from either plant oils or animal fats. Compared to bar soap, liquid soap is generally preferred by consumers due to its convenience, efficiency, portability, ease of storage, and reduced risk of bacterial contamination. However, many commercially available antibacterial soaps still rely on synthetic substances such as Sodium Lauryl Sulfate (SLS) and triclosan, which may pose harmful effects on the skin. The problem that often occurs is irritant contact dermatitis, which is non-immunologic inflammation of the skin through exogenous or endogenous pathways due to direct chemical exposure to the body, which causes symptoms in the form of irritation, itching, dry skin, cracking, and redness (1). This encourages the use of soap preparations with active ingredients of natural origin.

Legundi plant or Vitex trifolia L. is known by the community as one of the concoction medicinal plants used to cure diseases such as skin disorders, post-bangau, cold fever, diarrhea, headache, asthma, and other allergic diseases (2). The phytochemical content of legundi leaves consists of phenols, flavonoids, saponins, alkaloids, and tannins. The compound is bacteriostatic, which inhibits bacterial growth and the mechanism of tannins is the inactivation of enzymes that produce and activate transport proteins in the cell wall so that it damages the bacterial cell wall. Several studies concluded that legundi plants have pharmacological activities as antibacterial, antifungal, insecticidal, anticancer, analgesic, anti-allergic and antipyretic.

Betel is a plant that grows in tropical Asia to Africa and spreads throughout Indonesia, Malaysia, India, Sri Lanka, and Thailand (3). Betel leaves are usually used by the community as a treatment to stop bleeding, itching or irritation, thrush and cure diseases due to bacterial or fungal infections (4). Betel leaf has an oil content of 4.2% which acts as an antibacterial. Betel leaves have several ingredients such as steroids, tannins, flavonoids, saponins, phenols, alkaloids, coumarins, and emodins (5). The antibacterial properties of betel leaves are attributed to the presence of various active compounds. Phenolic compounds, for example, alter bacterial cell proteins, leading to increased cell wall permeability and eventual cell lysis. Flavonoids interfere with the stability of bacterial membranes, while alkaloids affect the peptidoglycan structures within bacterial cells. The phytochemical content contained in betel leaf plants consists of antioxidants to prevent inflammation, and used to combat bacterial, protozoan, and fungal infections.

Based on this rationale, the researchers formulated a liquid soap incorporating active compounds extracted from a combination of betel leaf and legundi. The dual use of these natural ingredients aims to enhance the effectiveness of their antibacterial properties. This enhanced performance, known as a synergistic effect, arises due to the shared presence of key bioactive compounds flavonoids, saponins, and tannins in both green betel and legundi leaves. The combination of legundi leaf and betel leaf extracts as antibacterial Staphylococcus aureus reaches optimal conditions in a ratio of 2: 1 through diffusion (inhibition zone) (6). This study aimed to find out that antibacterial liquid soap from

combination of green betel and legundi leave does not cause irritation to the skin. Thus, the liquid soap is safe to be use.

.

MATERIALS AND METHODS

This research used Posttes only control design. This study using fresh legundi and betel leaves were washed with running water, and left to dry for five days while protected from direct sunlight. The criteria of the leaves are a fresh, mature, and intact leaf without any signs of insect damage or herbivory. After drying, the leaves were ground into fine powder using a blender. Extraction of Legundi leaves and betel leaves using maceration method and filtered using filter paper and compressed with a set of rotary evaporator tools to extract the liquid.

The procedure for making liquid soap suspension is done by putting 30 ml of VCO oil into a beaker. Add little by little potassium hydroxide (KOH) 40% as much as 16 ml while continuing to be heated at 50°C for 3 hours to get soap paste. Add approximately 30 ml of distilled water, then add sodium carboxyl methyl cellulose that has been developed in hot distilled water. Stir to make it homogeneous for 10 minutes. Next add stearic acid, stir again until homogeneous for about 10 minutes. Add surfactant made from tween 80, stirring until homogeneous for about 10 minutes. Then add the combination of leaf extracts, stirring until homogeneous for about 10 minutes. Liquid soap is added with distilled water until the volume is 100 ml in a clean container.

Table 1. Liquid soap formulation

Material	Unit	F0	F1	F2	F3
Combination Extract DL: DS (2:1)	%		3	4	5
VCO Oil	ml	30	30	30	30
KOH	ml	16	16	16	16
Na-CMC	gr	1	1	1	1
Tween 80	gr	1	1	1	1
Asam Stearat	gr	0,5	0,5	0,5	0,5
Aquades	ml	100	100	100	100

The organoleptical test is carried out using the hedonic test method using senses of 20 respondents such as sight, smell and touch as a tool to measure, assess or test product quality. The organoleptical tests include color, odor (aroma) and liquid soap dosage form (viscosity and texture). The favorability test aims to determine people's acceptance of a product. This test is subjective with a scale of very dislike to very like (1-7). SNI

requirements are that liquid soap preparations are homogeneous and do not change shape and have a distinctive odor.

The pH measurement is carried out on each gel preparation in the same way. pH testing is needed because liquid soap will come into direct contact with the skin, SNI requirements for the pH test are between 8.0-8.6.

The samples were first diluted using a 10^{-3} sterile diluent and thoroughly homogenized. From each dilution, 1 ml was transferred into a test tube, followed by the addition of 12–15 ml of molten Plate Count Agar (PCA) to assess the Total Microbial Contamination, in accordance with SNI 16-2897-1992. The mixture was then vortexed and gently poured into a petri dish to ensure uniform distribution. After solidification, the plates were incubated in an inverted position at 35°C for 48 hours. The number of microbial colonies of microbes in one gram or one mL (multiplying the average colony on the cup with the dilution factor used).

Irritation tests are conducted to determine the safety of using a product and are one of the requirements before a new product can be sold to the market. The irritation test uses the Draize test technique, which is a technique used to define the main local irritant as a compound that produces inflammatory reactions that are classified as skin irritants (7).

The test involved 15 male white rats, each weighing approximately 200 grams, which were divided into 5 groups. Liquid soap preparations of a combination of ethanol extracts of legundi leaves and betel leaves (3%, 4% and 5%), positive control (commercial liquid soap) and negative control (soap preparation) were applied to the back area of white rats which was shaved and left incact, as much as 0.3 g per head every 10:00 am WITA. Furthermore, observations and scoring of erythema and ederma formed based on the Draize method at 9:30 WITA the next day (7). The next application started at 10:00 a.m. on the same section and was carried out for three consecutive days.

This study received ethical committee clearance from Poltekkes Kemenkes Denpasar No. LB.02.03/EA/KEPK/ 0635 /2023.

RESULTS AND DISCUSSIONS

Results

Extract products in this study were obtained through maceration and evapovation processes on betel leaf and legundi leaf simplisia. Before being made into simplisia, each 2 kg of leaves was dried and obtained a dry sample of 320 grams of betel leaves and 340 grams of legundi leaves. Then, they were mashed with a blender so that 305 grams of betel leaf simplisia were obtained, while 315 grams of legundi leaves were obtained. To ensure that the water content of leaf simplisia does not exceed 10%, a water content test is carried out. The water content test of the simplisia obtained the results of betel leaf simplisia of 8.6% and 8.1% for legundi leaves.

The following are the results of the organoleptical test, pH test, foam height test, specific gravity test, Microbial contamination test, and irritation test.

Table 2. Physicochemical Test of Antibacterial Liquid Soap Formulation Combination of Legundi and Betel Extracts

Formula	Weigh Type	High Foam	pH	Alkali Free
	(g/mL)	cm		%
F +	1,01	10,0	8	Undetected
F0	1,02	11,0	8	Undetected
F3	1,01	11,0	8	Undetected
F4	1,01	11,5	8	Undetected
F5	1,04	11,0	8	Undetected

Table 3. Organoleptic Test Results of Antibacterial Liquid Soap Formulation Combination of Legundi and Betel Extracts

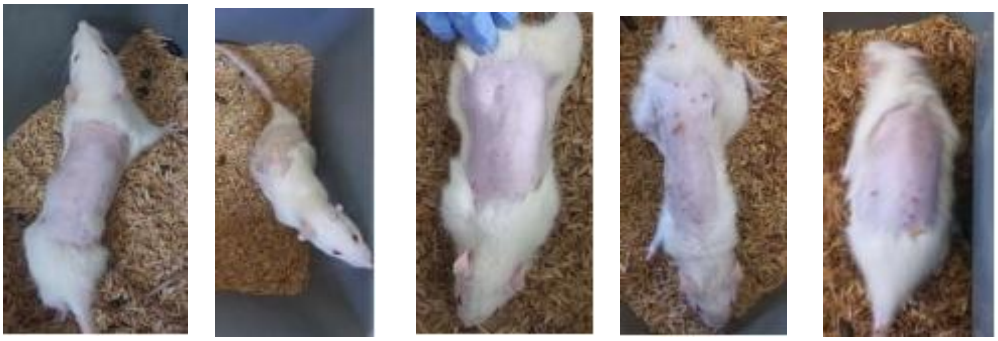
Formulation	**Form**	**Color**	**Odor**
F +	Liquid	Clear Green	There is an ascent
F0	Liquid	White	VCO oil odor
F3	Liquid	Solid Green	Typical Legundi Odor
F4	Liquid	Solid Green	Typical Legundi Odor
F5	Liquid	Solid Green	Typical Legundi Odor

Table 4. Microbial Contamination Test Results of Antibacterial Liquid Soap Formulations: Combination of Legundi and Betel Extracts

Code	Dilution					Result
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	
F+	150	110	79	66	51	$1{,}17 \times 10^{5}$
F0	41	0	0	0	0	$4{,}1 \times 10^{2}$
F3	168	0	0	0	0	$1{,}68 \times 10^{3}$
F4	79	0	0	0	0	$7{,}9 \times 10^{2}$
F5	96	0	0	0	0	$9{,}6 \times 10^{2}$

Table 5. Irritation Test Results of Antibacterial Liquid Soap Formulation Combination of Legundi and Betel Extracts in Mice

Code	Erytema		Edema		Value Index
	24 Jam	48 Jam	24 Jam	48 Jam	
F+	0	0	0	0	0
F0	0	0	0	0	0
F3	0	0	0	0	0
F4	0	0	0	0	0
F5	0	0	0	0	0



a b c d e

Picture 1. Irritation Test Results After 48 Hours (a.F+(control positif), b.F0(control negative), c.F3 (3%), d.F4 (4%), e.F5 (5%))

Discussion

The specific gravity test was carried out to assess the density of the liquid soap formulation. According to the Indonesian National Standard (SNI), the acceptable range for specific gravity is between 1.01 and 1.1 g/ml. This parameter is influenced by the physical characteristics and composition of the soap. Based on the test results, the liquid soap demonstrated a specific gravity ranging from 1.01 to 1.04 g/ml, indicating compliance with the SNI requirements. A specific gravity close to that of water suggests that the soap is likely to rinse off easily when used with running water (8).

The requirements of the Indonesian National Standard, the foam height of liquid soap is at 13-220 mm. Based on the test results of the foam height of liquid soap, the combination of Legundi Leaf and Betel Leaf extracts has a foam height of around 110-115 mm, which means that the resulting foam height is in accordance with the requirements of Indonesian National Standard. Foam is formed from the saponification reaction or soap formation reaction from bases and oils, the saponification reaction is characterized by the formation of foam (9). Aside from the saponification reaction that generates foam, surfactants such as Tween 80 also contribute to foam formation in liquid soap. Foam is considered one of the appealing characteristics of liquid soap. Its primary role is to prevent the reattachment of

dirt particles by keeping them suspended in the water, allowing them to be rinsed away effectively along with the soap solution. (10).

The pH test aims to see the pH of the combined liquid soap preparation of Legundi Leaf and Betel Leaf extracts. pH testing is needed because liquid soap will directly come into contact with the skin, Indonesian National Standard requirements for pH tests are between 8.0-8.6. Based on the results of the pH test obtained, it shows that the combined liquid soap preparation of Legundi and Betel leaf extracts meets the Indonesian National Standard requirements, which has a pH between 8.2 - 9.0.

The organoleptic test was conducted to evaluate the physical characteristics of the liquid soap formulation containing a combination of Legundi and Betel Leaf extracts. This assessment included observation of the product's shape, color, and scent. The resulting form of this liquid soap is liquid, the resulting odor has a distinctive odor from the extract. The resulting liquid soap is dark green in color, this is due to the dark green extract of Legundi Leaf and Betel Leaf. The requirements of the Indonesian National Standard are that liquid soap preparations are homogeneous and have a distinctive odor and color. The distinctive smell and color is the smell and color of the plant extracts used. Based on the results obtained, the results of the organoleptic test of liquid soap produced meet the requirements of the Indonesian National Standard.

Legundi and Betel leaves possess antimicrobial properties due to the presence of bioactive compounds such as saponins, tannins, and flavonoids. These compounds exhibit antimicrobial activity by disrupting bacterial cell membranes and inhibiting protein synthesis, ultimately impairing bacterial function and growth. The test results of F0, F3, F4, F4, F5 are still in accordance with Indonesian National Standard 06-4085-1996 which is max 1x105 gr colonies/ml. However, in F+ the value exceeds, this may be because the commercial soap used in this study had been opened or used a week before the research was carried out. When viewed from Indonesian National Standard 4085-2017, only codes F0, F4 and F5 meet the standard, which is below the maximum number of 1x103.

Observation of the irritation test with the Draize method is done based on 2 things, namely erythema and udema caused. The skin may show little or no reaction at the time of first contact with the chemical. But it can be shown after a while for example at 12-48 hours afterwards.

Irritation testing uses 15 white rats that are shaved clean on the back, then smeared with liquid soap on the shaved area and then observed after 24 hours and observed again at 48 hours. Calculations were made so that the results obtained did not find irritation in commercial soap (F+), soap base (F0), concentrations of 3%, 4% and 5%. This can be influenced by the pH which is in accordance with the Indonesian National Standard set by the Ministry of Health.

CLINICAL IMPLICATION

The findings suggest that soap formulated with Vitex trifolia and Piper betle leaf extracts may serve as a mild antiseptic agent to assist in preventing or reducing skin colonization by microorganisms, particularly in individuals with sensitive or infection-prone skin. This formulation has potential application in natural approaches for the prevention of tropical skin diseases, minimizing the reliance on synthetic chemical agents that may cause irritation. In herbal-based healthcare settings, the study provides a preliminary basis for the development of safe, plant-derived skin care products utilizing locally available medicinal plants.

LIMITATIONS

This study has several limitations. First, the effectiveness of the soap has not yet been evaluated through clinical trials in humans; therefore, its actual dermatological impact remains uncertain. Second, the optimal concentration of Vitex trifolia extract within the formulation has not been fully determined, and further studies are needed to assess the stability of active compounds and their safety in long-term use. Third, microbial contamination assessment was limited to total colony counts without evaluating the variability of specific microbial species, which may affect result generalizability. Fourth, the appearance of the liquid soap was found to be aesthetically less acceptable, suggesting the need to consider alternative forms, such as solid bar soap, to enhance user preference.

CONCLUSIONS

Liquid soap formulations containing various concentrations of Legundi and Betel leaf extracts have passed several evaluations, including organoleptic, pH, homogeneity, and water content tests, all of which conform to the standards set by the Indonesian National Standard (SNI). Furthermore, microbial contamination was not detected in any of the tested formulations. The formulations also showed no signs of irritation in experimental animals, indicating that the combined extracts are safe for topical use across different concentration levels.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with the publication of this paper. This study was carried out independently, without any commercial or financial affiliations that might be perceived as influencing the research outcomes.

AUTOR CONTRIBUTIONS

All authors contributed significantly to the preparation of this manuscript. Conceptualized the study and manuscript writing. by Suyasa. Astika conducted the data collection. Delia and Surya contributed to the analysis and interpretation of results.

ACKNOWLEDGMENTS

We acknowledge the Indonesian Republic's Ministry of Health for funding this study.

REFERENCES

1. Indrasari F, Toyo EM, Solichah AI, Suwarni S, Widiyaningrum AS. Penyuluhan Tentang Pembuatan Sabun Cuci Piring Dari Lidah Buaya. JAMAS J Abdi Masy. 2024;2(1):370–4.
2. Chan EWC, Baba S, Chan HT, Kainuma M, Tangah J. Medicinal plants of sandy shores: A short review on vitex trifolia L. and ipomoea pes-caprae (L.) R. Br. Indian J Nat Prod Resour. 2016;7(2):107–115.
3. Gultom FKB, Nababan, J ., Sinambela TM., Harizka, T., Rahmatsyah, R. Uji Daya Absorbansi Etanol pada Daun Sirih Hijau (Piper betle L.) dengan Metode Spektrofotometri UV-Vis. Einstein (e-Journal).2017;5(2):20–24.
4. Qonitah F, Abdul A. Aktivitas antioksidan dan kandungan fenolik total dari isolat polar fraksi heksana ekstrak etanol daun sirih. J Farmasetis. 2018;7(2).
5. Patil RS, Harale PM, Shivangekar KV, Kumbhar PP, Desai RR. Phytochemical potential and in vitro antimicrobial activity of Piper betle Linn. leaf extracts. J Chem Pharm Res. 2015;7(5):1095–1101.
6. Suyasa, I.B.O., T.B. Wibawa, N.M.D. Januarianti, P.D. Wahyuni. Antibacterial Activity of Legundi Leaf Extract (Vitex trifolia L.) with Betel Leaf Extract (Piper betle L.) against Staphylococcus aureus. SEAS (Sustainable Environ Agric Sci. 2022;6(2):112–118.
7. Fatmawaty A, Manggau MA, Tayeb R, Al Adawiah R. Uji Iritasi Krim Hasil Fermentasi Bunga Rosella (Hibiscus sabdariffa L.) Dengan Variasi Konsentrasi Emulgator Novemer Pada Kulit Kelinci (Oryctalagus cuniculus). J Pharm Med Sci. 2016;1(2):62–65.
8. Sari R, Ferdinan A. Pengujian Aktivitas Antibakteri Sabun Cair dari Ekstrak Kulit Daun Lidah Buaya Antibacterial. Pharm Sci Res. 2017;4(3):111–120.
9. Susanti, M. M., Guterres, A. D. A. Pengaruh Penambahan Kalium Hidroksida (KOH) Terhadap Mutu Sabun Lunak Berbahan Dasar Minyak Goreng Bekas. Medsains. 2019;4(1):25–33.
10. Sahambangung M, Datu O, Tiwow G, Potolangi N. Formulasi Sediaan Sabun Antiseptik Ekstrak Daun Pepaya Carica papaya. Biofarmasetikal Tropis. 2019;2(1):43–51.