Soursop Leaf Extract Gel: A Promising Solution for Skin Bacterial Infections

Nuniek Nizmah Fajriyah[1], Urmatul Waznah[2], Eko Mugiyanto[2(✉)], Kusnul Khotimah[3], Eka Jamilatur Rosyidah[4], Khusnul Khotimah[4], Ahmad Samsudin[4], and Nurul Hidayati[5]

[1] Department of Nurse, Universitas Muhammadiyah Pekajangan Pekalongan, Pekalongan, Indonesia
[2] Department of Pharmacy, Universitas Muhammadiyah Pekajangan Pekalongan, Pekalongan. Indonesia
giyan77@gmail.com
[3] RSUD Kraton Pekalongan, Pekalongan, Indonesia
[4] RSI PKU Muhammadiyah Pekajangan Pekalongan, Pekalongan. Indonesia
[5] RSUD Kajen Pekalongan, Pekalongan, Indonesia

Abstract. Staphylococcus aureus and Escherichia coli are mainly two commensal and pathogenic human bacteria that cause skin infections in tropical regions. Flavonoids found in Soursop leaf's have been shown to have antibacterial effects. The aim of this research was to assess the antibacterial effectiveness of a topical gel formulation comprising an ethanol extract derived from soursop leaves against the proliferation of Escherichia coli and Staphylococcus bacteria. The soursop leaves underwent extraction through the maceration process and were subsequently formulated into a topical gel at concentrations of 1%, 5%, and 10%. Microbial testing was conducted using the well diffusion method, and the inhibitory zone was measured. A comparison was made with a negative control (no extract) and a positive control (povidone iodine) to evaluate the antibacterial efficacy. The ANOVA test was utilized to determine the statistical significance of the antibacterial activity, with a significance level set at $p < 0.05$. Analysis results indicate significant disparities in the antibacterial properties of the topical soursop leaf extract gel compared to the negative control. However, the antibacterial activity was less potent when compared to the positive control. To test its antibacterial effectiveness, more investigation must be done by transforming it into a Nano-shaped preparation.

Keywords: Soursoup Leaf, Skin Bacterial Infection, Extract Gel.

1 Introduction

In many developing countries, including Indonesia, the diagnosis and treatment of infectious diseases are characterized by the lack of strengthening of diagnostic microbiology facilities and the lack of data on the pathogen epidemiology. Staphylococcus aureus is one of the main pathogens, which mostly become the cause of infections started

Z. B. Pambuko et al. (eds.), *Proceedings of 5th Borobudur International Symposium on Humanities and Social Science (BISHSS 2023)*, Advances in Social Science, Education and Humanities Research 856,
https://doi.org/10.2991/978-2-38476-273-6_120

from superficial, deep skin, soft tissue infections (including postoperative infections) to toxic shock syndrome, severe organ or endovascular disease [1]. Its pathogenicity is the results of the armamentarium of virulence factors, enabling staphylococci to easily adapt to various environmental conditions [2].

Skin and soft tissue infections rank among the prevalent infections affecting individuals across all age brackets. While many of these infections resolve on their own or respond well to antibiotic treatment, moderate to severe cases may necessitate hospitalization and the administration of intravenous therapy [3]. The most common causative agents are Staphylococcus aureus and aerobic streptococci [4]. However, several reports correlating enterobacterium Escherichia coli to skin infections have been published including cellulitis localized to the lower or upper limbs [5], infections after surgery [6], or post-burn infections [7]. A study on monitoring skin and soft tissue infections over a 7-year period covering three continents (Europe, Latin America, and North America) showed that E. coli became an important causative agent [3].

Antibacterial is a compound capable of inhibiting or killing microorganisms in small concentrations. Soursop (Annona muricata L.) which belongs to the Anonaceae family possesses many pharmacological activities [8]. Several articles have reported the antibacterial activity of soursop [9-11]. The antibacterial properties of soursop are believed due to the content of flavonoid compounds, particularly acetogenin derivative compounds [12]. Several studies in Indonesia have also reported the activity of ethanol extract of soursop leaf as an antibacterial, especially E.coli and S.aureus bacteria [13]. The antibacterial activity of soursop leaf extract against the incision wounds of rabbit has also been reported by Wahyuni, et al. [14].

Although there have been many studies on the antibacterial activity of soursop, those related to the antibacterial activity of soursop leaf extract in topical formulation, so far, is still rare. This study, in turn, aims to test the antibacterial effectiveness of soursop leaf ethanol extract gel formulation against Escherichia coli and Staphylococcus aureus bacteria.

2 Method

2.1 Tools and Materials

Maceration was used to extract the active compounds. All extraction solvent were technical degrees. The equipment were used including: glassware, 40 mesh sieve, Bunsen and rotary evaporator (Heidolph). The soursop leaf (Annona muricata L.) used in this study was taken from Pekalongan with a determination test carried out in the Biology Laboratory of Ahmad Dahlan University. Meanwhile, bacterial culture of Escherichia coli and Staphylococcus aureus were obtained from the Pharmacology Laboratory, University of Indonesia.

2.2 Soursop Leaf Extract

The identified soursop leaves were washed, cut, dried, mashed and sieved with a 40-mesh. Soursop leaf extract (Annona muricata L.) was prepared through maceration

method using 96% ethanol solvent [15]. At room temperature, 1000 grams of powdered soursop leaves were digested in 6 liters of 96% ethanol with vigorous stirring. Then it was closed and left to stir periodically. Remaceration was performed in this extraction process by changing 3 liters of solvent. The maceration results were then collected and filtered using filter paper. To get a thick ethanolic extract of soursop leaves (Annona muricata. L), the filtrate was evaporated using a rotary evaporator at 40°C [16].

2.3 Phytochemical Screening

Phytochemical screening was carried out utilizing the method that developed by Fajriyah et al. [16]. The screening included probing for alkaloids, tannins, saponins, steroids, and flavonoids in the ethanol extract of soursop leaves.

2.4 Topical gel formulation

The topical gel formulation was made into 4 formulas with some differences in the concentration of soursop leaf extract used. The formula is presented in Table 1.

Table 1. Topical gel formulation of soursop leaf extract

Contents	Gel Concentration (%)				Function
	Negative Control	Formula I	Formula II	Formula III	
Soursop leaf extract	-	1	5	10	Active Substance
Carbopol	2	2	2	2	Base
TEA	1	1	1	1	Base
Methyl Paraben	0.1	0.1	0.1	0.1	Preservative
Propyl Paraben	0.1	0.1	0.1	0.1	Preservative
Glycerin	10	10	10	10	Humectants
Aquadest ad	100	100	100	100	Solvent

The gel preparation was made of 100 grams, the carbopol base was developed with hot aquadest in a closed beaker glass and left until being swelled, and TEA was added (Mixture 1). Propyl and methyl parabens should be dissolved in 10 cc of hot distilled water (Mixture 2). Mixture 1 and mixture 2 were then homogenized (mixture 3). Subsequently, soursop leaf extract was homogenized with glycerin and added to mixture 3 before being homogenized until a gel was formed.

2.5 Test of the effectiveness of the antibacterial preparation

The assessment of antibacterial efficacy was conducted using the well-diffusion method with two repetitions. Bacterial samples were prepared as suspensions in 0.85% NaCl

solution after growing on Mueller-Hinton Agar (MHA) media for 24 hours. This involved harvesting bacterial growth from slanted MHA media using inoculation needles and suspending them in sterile 0.85% NaCl solution.

Subsequently, solid MHA media was poured into petri dishes as the base medium, onto which a sterile backing with a 6 mm diameter was placed. A suspension of the tested bacteria was then mixed with semi-solid MHA media and poured into petri dishes containing a buffer, creating wells after solidification. Soursop leaf gel, 500 mg in quantity, was placed into each well. The test medium was then incubated at 37°C for 24 hours, and the inhibition zones were measured using a caliper for observation.

2.6 Evaluation of gel topical preparation

pH of the preparation was determined with a digital pH meter (digital pH meter, 335, systronics). The spreadability of the Nano-hydrogel formulation was checked by measuring the diameter of the spread of the Nano-hydrogel between the two glass plates after 1 minute. The Nano-hydrogel viscosity was determined using spindle number 4 at 60 rpm (Brookfield DV-II+ Pro viscometer). Fourier Transform Infra-Red/FTIR (Shimadzu IRSpirit) was used in FT-IR analysis

2.7 Data Analysis

Antibacterial activity was analyzed by measuring the inhibition zone formed on solid media with a caliper. Antibacterial activities were measured or calculated using the following formula:

$$Z = ((d1-ds)+(d2-ds))/2 \quad (1)$$

where:
Z = Clear Zone
D1 = Vertical Diameter
D2 = Horizontal Diameter
Ds = Well Diameter

3 Result and Discussion

3.1 Phytochemical Screening

Phytochemical Screening was conducted to determine the content of secondary metabolites contained in soursop leaf extract. The screening results as presented in Table 2 showed that the extract contained alkaloids, tannins, saponins, steroids and flavonoids.

Table 2. Results of Phytochemical Screening of Soursop Leaf Ethanol Extract

No.	Test	Reagen	Results
1.	Alkaloids	Dragendorf	+

2.	Tannins	FeCl3 1 %	+
3.	Saponins	Aquadest	+
4.	Steroids	HCl + H2SO4	+
5.	Flavonoids	Powder of Mg + HCl	+

The results of the phytochemical screening showed that soursop leaf extract contained flavonoid compounds. The results were found in line with previous research by Mugiyanto, et al. [17].

3.2 Test of the pharmaceutical properties of topical gel preparation

Table 3 shows the physical appearance of the soursop extract gel topical preparation. The preparation was clear greenish, homogeneous, smooth and stable without any crystals and phase separation observed. The pH value was within the range suitable for skin care without causing irritation. The spreadability of the formulation showed the phenomenon of slip and drag.

Table 3. Pharmaceutical properties of topical gel preparation of soursop leaf extract

Test	Physical Character
Appearance	Greenish
Color	Transparent
pH	7
Adhesive strength	4 seconds
Spreadability with the load of 50 g	3.1
Spreadability with the load of 100 g	3.5
Viscosity with no spindle 4 at 30 rpm	167.8

3.3 Anti-bacterial test of gel topical

The determination of the antibacterial activity of soursop leaf gel was conducted in vitro, based upon its ability to inhibit the growth of test bacteria, i.e. Escherichia coli representing Gram negative bacteria and Staphylococcus aureus representing Gram positive bacteria. Fig. 1 shows the results of testing the antibacterial activity of soursop leaf gel against E. coli and S. aureus.

A	B	C

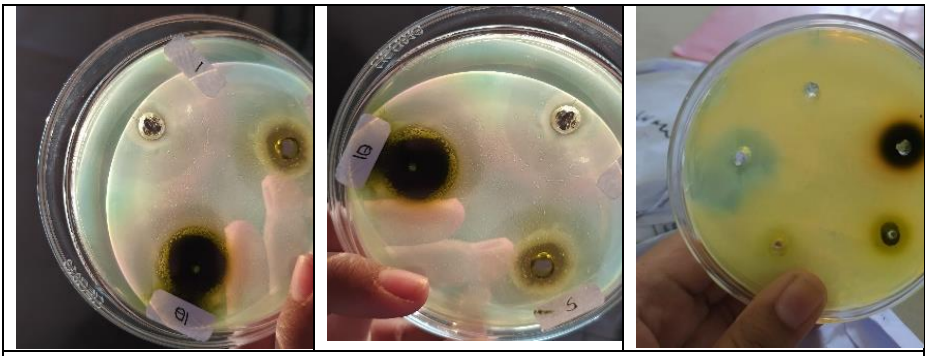

Fig. 1. a. Clear Zone of SA. b. Clear Zone of E-Coli c. Clear Zone of Negative Control and Positive Control

To determine the inhibition of bacterial growth, Fig. 2 and Fig. 3 show the antibacterial activity of gel topical preparation of soursop leaf extract against Staphylococcus aureus and Escherichia coli respectively.

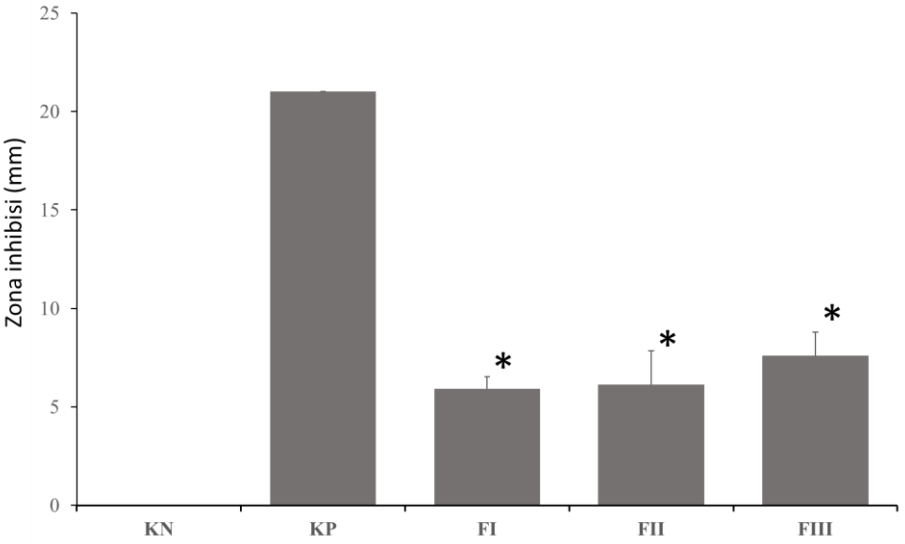

Fig. 2. The inhibitory power of soursop leaf extract against the growth of bacterium Staphylococcus aureus. Test was conducted through three repetitions. KN: Negative Control; KP: Positive Control (povidone iodine), FI: 1% soursop leaf extract; FII: 5% soursop leaf extract and FII: 10% soursop leaf extract. Y axis was in mm. *$p<0.05$ was compared to KN.

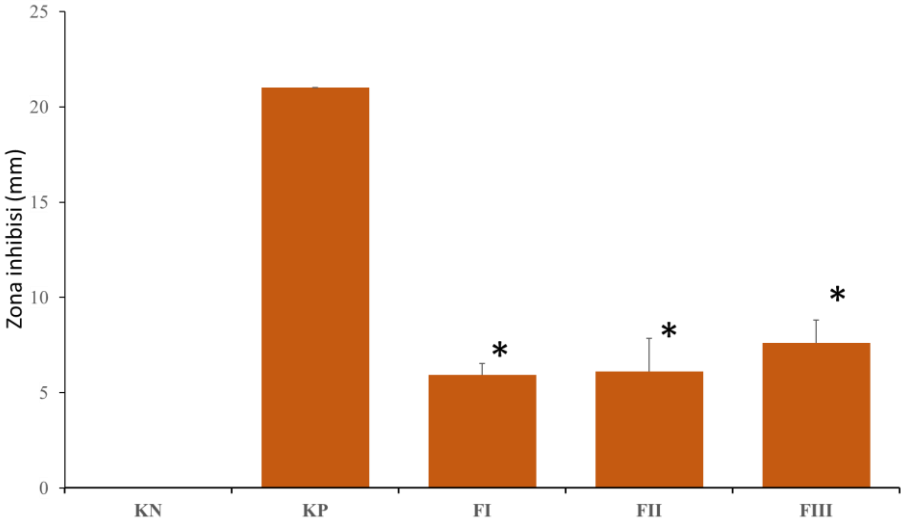

Fig. 3. The inhibitory power of soursop leaf extract against the growth of bacterium Escherichia coli. Test was conducted through three repetitions. KN: Negative Control; KP: Positive Control (povidone iodine), FI: 1% soursop leaf extract; FII: 5% soursop leaf extract; and FII: 10% soursop leaf extract. Y axis was in mm. *$p<0.05$ was compared to KN.

Based on the research results shown in Fig. 2 and Fig. 3, it can be figured out that soursop leaf gel has antibacterial activity as indicated by the formation of a clear zone (Figure 1). Research on soursop leaf extract gel preparations against Staphylococcus aureus showed the highest results in inhibiting bacteria with Formula III (10%). The average inhibition zones produced from formula III were 16.15 mm and 13.55 mm included in the medium category. The results from formula III were found much better than the average inhibition zone resulted from formula I by 8.2 mm. and 5.7 mm included in the weak category. Meanwhile, the average inhibition zones of formula II were 8.3 mm and 11.25 mm also included in the weak category. These results indicated that the higher the concentration used, the greater the inhibition zone obtained. Roslizawaty, et al. stated that the amount of a compound concentration has caused an increase in the content of an active compound that acts as an antibacterial; hence, the ability to kill bacteria increases as well [18].

The components of the soursop leaf extract, from phytochemical screening, gel affect its antibacterial properties. The screening results showed that the ethanol extract of soursop leaf (Annona muricata L.) contains several compounds: alkaloids, tannins, saponins, steroids, and flavonoids. These compounds have different mechanisms of inhibiting the bacteria. Acetogenins from the annonaceous family (soursop) are polyketide group compounds. Most acetogenins contain a long hydrocarbon chain (C32 or C34), one to three ring groups and 2.5 were substituted by tetrahydrofuran (THF) that is adjacent or non-adjacent to the center of the molecule, and a,b-unsaturated-g-lactones; the ring part at the end of the molecule as observed in Fig 4. This result is in line with the

study by Ricardo, et al. [19] comparing the FT-IR profile of acetogenin to bullatacin, which had antibacterial activity.

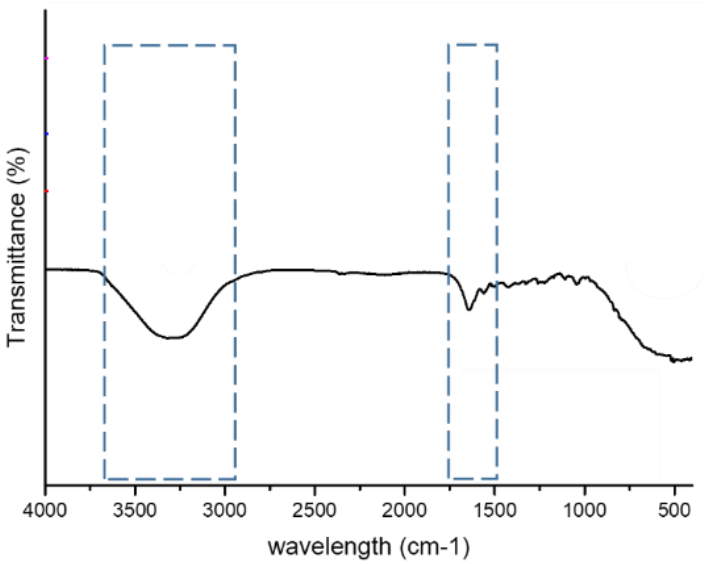

Fig. 4. FT-IR spectra of experimental results of soursop extract. FT-IR vibration spectrum obtained from the structure of acetogenin.

4 Conclusion

The topical gel preparation of soursop leaf extract contains alkaloids, tannins, saponins, steroids, and flavonoids, according to the findings of the study. The higher inhibition to both bacteria, Staphylococcus aureus and Escherichia coli, were 16.15 mm and 13.55 mm respectively that achieved by FIII. The presence of acetogenin raised suspicions of antibacterial action. These findings suggest the promising antibacterial potential of the soursop leaf extract topical gel, opening avenues for further research and development in the field of natural antimicrobial agents for pharmaceutical applications

Acknowledgement. Thank you for Indonesian Research Institute for funding this work.

References

1. Lowy FD: Staphylococcus aureus infections. N Engl J Med 1998, 339(8):520-532.
2. von Eiff C, Becker K, Machka K, Stammer H, Peters G: Nasal carriage as a

source of Staphylococcus aureus bacteremia. Study Group. N Engl J Med 2001, 344(1):11-16.
3. Moet GJ, Jones RN, Biedenbach DJ, Stilwell MG, Fritsche TR: Contemporary causes of skin and soft tissue infections in North America, Latin America, and Europe: report from the SENTRY Antimicrobial Surveillance Program (1998–2004). Diagnostic microbiology and infectious disease 2007, 57(1):7-13.
4. Petkovsek Z, Elersic K, Gubina M, Zgur-Bertok D, Starcic Erjavec M: Virulence potential of Escherichia coli isolates from skin and soft tissue infections. J Clin Microbiol 2009, 47(6):1811-1817.
5. Brzozowski D, Ross D: Upper limb Escherichia coli cellulitis in the immunocompromised. Journal of Hand Surgery 1997, 22(5):679-680.
6. Tourmousoglou C, Yiannakopoulou E, Kalapothaki V, Bramis J, Papadopoulos JS: Surgical-site infection surveillance in general surgery: a critical issue. Journal of chemotherapy 2008, 20(3):312-318.
7. Rodgers GL, Mortensen J, Fisher MC, Lo A, Cresswell A, Long SS: Predictors of infectious complications after burn injuries in children. The Pediatric infectious disease journal 2000, 19(10):990-995.
8. Mugiyanto E, Cahyanta AN, Sunadi Putra IMA, Setyahadi S, Simanjuntak P: Identifying active compounds of soursop ethanolic fraction as Î±-glucosidase inhibitor. 2019 2019, 9(2):10.
9. Pinto NdCC, Campos LM, Evangelista ACS, Lemos ASO, Silva TP, Melo RCN, de Lourenço CC, Salvador MJ, Apolônio ACM, Scio E et al: Antimicrobial Annona muricata L. (soursop) extract targets the cell membranes of Gram-positive and Gram-negative bacteria. Industrial Crops and Products 2017, 107:332-340.
10. Pai BM, Rajesh G, Shenoy R, Rao A: Anti-microbial Efficacy of Soursop Leaf Extract (Annona muricata) on Oral Pathogens: An In-vitro Study. J Clin Diagn Res 2016, 10(11):Zc01-zc04.
11. Haro G, Utami NP, Sitompul E: Study of the antibacterial activities of soursop (Annona muricata l.) leaves. International Journal of PharmTech Research 2014, 6:575-581.
12. Terezan AP, Junqueira JGM, Wakui VG, Kato L, Oliveira CMA, Martins CHG, Santiago MB, Severino VGP: Qualitative analysis of the acetogenins from Annona coriacea (Annonaceae) leaves by HPLC-Q-Orbitrap and their antibacterial potential against oral pathogens. Nat Prod Res 2022, 36(3):765-771.
13. Wirastuty RY, Lallo S, Sartini S: PENGARUH POSISI DAUN PADA TANAMAN SIRSAK (Annona muricata Linn.) DAN AKTIVITAS ANTIBAKTERI SECARA IN VITRO. Magazine of Pharmacy and Pharmacology 2019, 22(3):85-89.
14. Gusti Ayu Rai Saputri EN, Nofita Nofita, Wahyuni Wahyuni: UJI AKTIVITAS EKSTRAK ETANOL DAUN SIRSAK (Annona muricata L.) DALAM PENYEMBUHAN LUKA INSISI KULIT KELINCI (Oryctogalus cuniculus). Journal of Pharmacy Malahayati 2021, 4(1).
15. Fajriyah NN, Mugiyanto E, Irwanti M, Waznah U: PKM KELOMPOK

PETANI NANAS DALAM UPAYA DIVERSIFIKASI PRODUK DENGAN USAHA MOUTH WASH DARI KULIT NANAS. In: Prosiding Seminar Nasional Pengabdian Masyarakat LPPM UMJ: 2019.
16. Urmatul Waznah AV, Eko Mugiyanto, Nuniek Nizmah Fajriyah: Comparative Study of FT-IR Profiles Preparation of Nano-Hydrogel Combination of Soursop Extract and Zinc. Proceeding of The URECOL 2022, 16:286-292.
17. Mugiyanto E, Cahyanta AN, Putra IMAS, Setyahadi S, Simanjuntak P: Identifying active compounds of soursop ethanolic fraction as α-glucosidase inhibitor. Pharmaciana 2019, 9(2):191-200.
18. Roslizawaty - NYR, Fakhrurrazi -, Herrialfian AKTIVITAS ANTIBAKTERIAL EKSTRAK ETANOL DAN REBUSAN SARANG SEMUT (Myrmecodia sp.) TERHADAP BAKTERI Escherichia coli. Medika Veterania 2013, 7(2).
19. Aguilar-Villalva R, Molina GA, España-Sánchez BL, Díaz-Peña LF, Elizalde-Mata A, Valerio E, Azanza-Ricardo C, Estevez M: Antioxidant capacity and antibacterial activity from Annona cherimola phytochemicals by ultrasound-assisted extraction and its comparison to conventional methods. Arabian Journal of Chemistry 2021, 14(7):103239.