BIODIDAKTIKA: Jurnal Biologi dan Pembelajarannya, Vol.20, No.1, 2025, pp. 85-95
e-ISSN 2527-4562. DOI. 10.30870/biodidaktika.v20i1.29158

Antibacterial Activity of Soursop *(Annona muricata* L.*)* Leaf and Fruit Extracts Against *Streptococcus mutans*

Submitted 4 September 2024 Revised 18 January 2025 Accepted 23 January 2025

Diva Azzahra Rizqika Putri[1], Zuraidah Zuraidah[2], Nurdin Amin[3]
[1,2,3]Department of Biology Education, Faculty of Teacher Training and Education,
Universitas Islam Negeri Ar-Raniry Banda Aceh, Aceh, Indonesia

Corresponding Email: *azzahradiva07@gmail.com

Abstract

Dental caries is a common oral health problem, with *Streptococcus mutans* playing a key role. This bacterium metabolizes sucrose into lactic acid, leading to tooth enamel demineralization and cavities. Soursop *(Annona muricata* L.*)* plants, known for their antibacterial bioactive compounds, are often used traditionally to treat microbial infections. This study investigated the inhibitory effects of soursop leaf and fruit extracts on *Streptococcus mutans* growth using a completely randomized experimental design and the Kirby-Bauer disc diffusion method. Results showed that leaf and fruit extracts at 100% concentration exhibited the strongest inhibition (14.86 mm and 14.83 mm, respectively). The weakest inhibition was observed at 40% concentration (10.60 mm for leaf and 10.13 mm for fruit extracts). However, the average inhibition diameters for both soursop extracts were smaller than the positive control's average of 27.80 mm. Based on these findings, it can be concluded that Soursop leaf and fruit extracts *(Annona muricata* L.*)* possess antibacterial activity against *Streptococcus mutans.*

Keywords: Soursop leaf, fruit extract, *Annona muricata* L., *Streptococcus mutans*, Dental caries, Inhibition zone

INTRODUCTION

The use of plants as medicine is a worldwide phenomenon, plants not only provide safe and cost-effective remedies, but they are also available and accessible at affordable prices. The use of available resources forms the basic core of any public health practice, and what is better than plants as medicine is that they are associated with fewer side effects and no known resistance to microorganisms (Phillipson, 2001). The emergence and spread of antibiotic resistance and the evolution of new strains of pathogenic agents are a great concern to community health worldwide and entail the development of new anti-microbials or potential sources of novel drugs. Commonly used medicinal plants are promising sources of biologically active and safe compounds (Manandhar *et al.,* 2019).

This provides an avenue for a newer search among plant kingdoms for alternative therapies. Annona muricata L., commonly called soursop, is gaining world wide acclaim for being a miracle tree in cancer research and can pave the way for research in many fields, including dentistry. The use of this plant in medicine has again come to fore as researchers claim it to have potential against the common pathogen (Facey, 1999). Dental caries is a common dental and oral problem. There is a strong association between oral diseases and microbial involvement (Paster, 2005).

Streptococcus mutans, a gram-positive bacterium found in the human oral cavity, plays a significant role in the metabolism of sucrose into lactic acid, leading to tooth enamel

demineralization and cavities. This bacterium is a primary cause of dental caries (Gartika, 2013). According to the 2018 National Basic Health Research Results Report for Dental and Oral Health, Riskesdas 2018 recorded a prevalence of dental and oral problems at 57.6% (Riskesdas, 2018).

Dental caries often require specialized treatments like tooth extraction, fillings, or antibiotics. While antibiotics can have side effects, traditional medicine offers an alternative approach (Nufus, 2019). In many developing countries, including Indonesia, traditional medicine has gained popularity due to its perceived lack of side effects and resistance issues compared to synthetic drugs. Traditional medicine offers a more cost-effective and accessible option (Widjijono, 2008). The soursop *(Annona muricata* L.*)* is a common example of a plant used in traditional medicine.

The tropical fruit tree soursop plant can bear fruit year-round under suitable groundwater conditions. All parts of the soursop plant have medicinal uses, including its leaves (Mardiana, 2007). Soursop leaves have been traditionally used to treat headaches, insomnia, liver disease, diabetes, and hypertension and as anti-cancer, anti-hypertensive, anti-inflammatory, and anti-seizure agents (Herwandi, 2019). Soursop leaves are also used as antibacterial, antiviral, antioxidant, and anti-fungal. As an antibacterial, soursop is known to kill both Gram-positive and Gram-negative bacteria (Utami, 2022).

Researchers have carried out phytochemical tests on soursop leaf and fruit extracts used in this research. The results of phytochemical testing show that soursop leaf extract contains alkaloids, flavonoids, quinones, polyphenols, and steroids. Meanwhile, soursop fruit extract contains alkaloids, saponins, flavonoids, quinones, polyphenols, and triterpenoids. The active substances contained in soursop leaf and fruit extracts have a different effect on destroying bacterial cells *Streptococcus mutans.*

The anti-fungal efficacy of soursop extracts has been evaluated to a much larger degree than its antibacterial properties. The anti-fungal property of Soursop extract is comparatively higher than its antibacterial efficacy, as at all concentrations, the extract showed potent anti-fungal substances against *Candida albicans,* which substantiates the previous findings of Johny *et al.,* (2011).

The literature currently has inadequate evidence of using Soursop leaf and fruit extract (*Annona muricata* L.) on systemic and oral pathogens. Hence, the present study is an archetypal report of the anti-microbial potential of soursop on a common oral pathogen using an in-vitro study model, to compare the antibacterial activity of different concentrations of a *Annona muricata* L. leaf and fruit extracts against *Streptococcus mutans.*

METHOD

This research is a laboratory experiment using a Completely Randomized Design (CRD). This research was conducted at the Microbiology Laboratory of the Biology Education Study Program, FTK UIN Ar-Raniry. The disc diffusion method was used to measure the inhibition zones of soursop leaf and fruit extracts against *Streptococcus mutans,* using a caliper with 0.05 mm accuracy. The measurements were taken around the disc, which was placed on Mueller Hinton Agar (MHA) inoculated with the test bacteria. Each sample was repeated three times in six treatments. Antibacterial strength was determined by the diameter of the inhibition zone, following the criteria established by Davis and Stout (1971):

1. Weak, if the diameter of the inhibition zone is <5 mm.
2. Medium, if the diameter of the inhibition zone is 6-10 mm.
3. Strong, if the diameter of the inhibition zone is 11-20 mm.
4. Very strong, if the diameter of the inhibition zone is <20 mm.

Tools and materials

The tools used in this research were blenders, jars, *Rotary Evaporator,* dropper pipette, test tube, tube needle, tweezers, petri dish, vortex mixer, autoclave, Bunsen, incubator, scale, filter paper, Erlenmeyer, Bunsen, sample bottle, and caliper. The materials used in this research were soursop leaves, soursop fruit, bacterial isolates *Streptococcus mutans*, ethanol 96%, paper disc, distilled water, MHA, NA, Erythromycin, cotton, NaCl 0.9%, H2SO4, and $BaCl_2$.

Work Procedures

Making Soursop Leaf Extract

Soursop leaves *(Annona muricata* L.) were washed and dried at room temperature. 300 grams of dried leaves were blended and placed in a maceration vessel with 1 liter of 96% ethanol. The mixture was left in a closed vessel for one day before being filtered. The residue was extracted twice more with fresh 96% ethanol. The filtered solution was concentrated using a rotary evaporator and then further purified by filtration and evaporation at 40°C. This process produced a thick soursop leaf extract used in the subsequent research.

Making Soursop Fruit Extract

Soursop *(Annona muricata* L.) flesh was separated from the seeds and dried in an oven at ±90°C for 6 hours. The dried fruit was stored indoors until it reached a stable temperature. 300 grams of dried soursop fruit were weighed, blended into a fine powder, and macerated twice in 96% ethanol for three days. The filtered solution was concentrated using a rotary evaporator, then further purified by filtration and evaporation at 40°C. This process produced a thick soursop fruit extract used in the subsequent research.

Antibacterial Effect Test

Antibacterial activity was tested using paper discs on Mueller Hinton Agar (MHA) media. Erythromycin, effective against beta-lactamase-producing bacteria, served as the positive control. 96% Ethanol, the solvent used for soursop extracts, was the negative control to ensure that the solvent itself did not inhibit bacterial growth.

Six petri dishes were prepared, and each was inoculated with paper discs containing soursop leaf and fruit extracts at concentrations of 40%, 60%, 80%, 100%, a negative control, and a positive control. The swab method was used to transfer the colonies into the agar plates. Using sterile tweezers, these paper discs were placed on MHA media. The petri dish was incubated in an incubator at 37ºC for 24 hours. Using a caliper, the inhibition zone formed around the paper disc was measured for its vertical and horizontal diameter in millimeters (mm).

RESULTS AND DISCUSSION

The use of new molecules from natural products becomes increasingly important. The *Annona muricata* L. could be used in tubes of toothpaste or mouthwashes to control the main pathogen, *Streptococcus mutans,* associated with the formation of dental caries (Moghadamtousi *et al.,* 2015).

This research demonstrates the formation of inhibition zones on paper discs containing soursop leaf and fruit extracts and the Erythromycin positive control. An inhibition zone indicates antibacterial activity, while the negative control (96% ethanol) showed no inhibitory power. The inhibition zones observed in soursop leaf and fruit extracts are attributed to their active compounds.

Phytochemical analysis revealed that soursop leaves and fruit contain similar active compounds, including alkaloids, flavonoids, quinones, and polyphenols. In addition to these, soursop leaves contain steroid compounds, while the fruit contains triterpenoid compounds. These findings align with previous research by Tuna (2015) and Priscila (2017), which identified soursop leaves and fruit as strong bacterial growth inhibitors.

The inhibition zones produced by each treatment varied in size. To analyze these differences, the average diameter of the inhibition zones was calculated by measuring both the horizontal and vertical diameters. Petri dishes containing MHA agar and *Streptococcus mutans*, which underwent three repetitions each, were removed from the incubator after 24 hours at 37°C (Figure 1 and Figure 2).

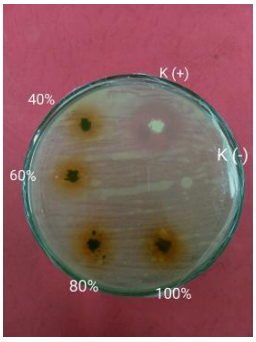

Figure 1. Soursop Leaf Extract Inhibition Zone

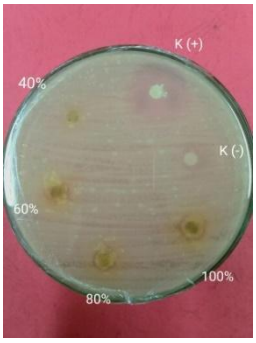

Figure 2. Soursop Fruit Extract Inhibition Zone

Figures 1 and 2 demonstrate variations in inhibition zone size across the three repetitions. The highest inhibitory power of soursop leaf and fruit extracts was observed at a 100% concentration. However, the antibiotic erythromycin, used as a positive control, exhibited a larger inhibition zone than any soursop extract concentration. The inhibition zone diameters for soursop leaf and fruit extracts are presented in Table 1.

Table 1. Duncan Test Results Diameter of Inhibitory Zone of Soursop Leaf and Fruit Extracts

No.	Treatment	Average Zone of Inhibition of Soursop Leaf Extract (mm)	Average Zone of Inhibition of Soursop Fruit Extract (mm)
1	40%	10.60 ± 0.26^{b}	10.13 ± 0.35^{b}
2	60%	11.60 ± 0.62^{c}	12.06 ± 0.15^{c}
3	80%	12.90 ± 0.30^{d}	13.60 ± 0.30^{d}
4	100%	14.86 ± 0.35^{e}	14.83 ± 0.30^{e}
5	Ethanol 96% (K-)	0.00	0.00
6	Erythromycin (K+)	26.80 ± 0.41^{f}	27.80 ± 0.26^{f}

Table 1 presents the average Duncan test results for soursop leaf and fruit extracts, demonstrating that a concentration of 40% significantly differs from 60%, 80%, and 100% in the positive control. Soursop leaf extract, which can be categorized as strong, is shown at a concentration of 60% (11.60 mm), 80% (12.90 mm), and 100% (14.86 mm), the medium category is shown at a concentration of 40% (10.60 mm). The weak category in the negative control is (0 mm). The positive control inhibition zone using the antibiotic erythromycin was declared in the very strong category (26.80 mm).

Meanwhile, the average concentration of soursop fruit extract which can be categorized as medium is shown at a concentration of 40% (10.13 mm), the strong category is shown at a concentration of 60% (12.06 mm), 80% (13.60 mm), and concentration 100% (14.83 mm). The weak category in the negative control is (0 mm). The positive control inhibition zone using the antibiotic erythromycin was declared to be in the very strong category (27.80 mm).

The positive control using Erythromycin had a larger zone of inhibition compared to the soursop leaf and fruit extract treatment and the negative control using 96% Ethanol. The positive control used was Erythromycin as an antibiotic for bacterial growth, particularly *Streptococcus mutans*. The highest inhibitory power of soursop leaf and fruit extracts was observed at a 100% concentration. The positive control used was Erythromycin, a broad-spectrum antibiotic that fights various serious infections, multidrug-resistant organisms or organisms that are resistant to two or more antibiotics so that they have been tested to inhibit bacterial growth accurately.

Soursop leaf and fruit extracts exhibited inhibitory activity against *Streptococcus mutans*, with the lowest effective concentration at 40%. Inhibitory activity generally increased with higher concentrations, reaching optimal levels at 100%. Biologically, the presence of acetogenins makes soursop potent against microorganisms. The reason for the difference in the antibacterial activity of *Annona muricata* L. leaf and fruit extracts on *Streptococcus mutans* between the findings in the present study with other studies could be, as stated by Ramalingum (2014), that the amount of acetogenins in the plant depends on the cultivation area, the variety and the climatic conditions to which it was exposed. These are bioactive compounds found in the annonacea family, these acetogenins are known to have tumoricidal, anti-malaria, anti-helminthic, anti-viral, and anti-microbial effects, suggesting many potentially useful aplication (Pai, *et al.,* 2016).

Several different classes of metabolites were reported to exist in the extract of *Annona muricata,* including tannins, alkaloids, flavonoids, polyphenols, saponins, diterpenoids, kaempferol, and acetogenin compounds (Yang *et al.,* 2015). This aligns with the phytochemical test that researchers have already conducted. Researchers have carried out phytochemical tests on soursop leaf and fruit extracts. The results of phytochemical testing show that soursop leaf extract contains alkaloids, flavonoids, quinones, polyphenols and steroids. Meanwhile, soursop fruit extract contains alkaloids, saponins, flavonoids, quinones, polyphenols, and triterpenoids. The active substances contained in soursop leaf and fruit extracts have a different effect on destroying bacterial cells *Streptococcus mutans.*

The active substances in soursop extracts influence bacterial growth and metabolism processes. Each active substance has a unique mechanism for destroying *Streptococcus mutans* cells. Alkaloid compounds, known for their anti-microbial properties, can disrupt the genetic balance of DNA, leading to bacterial DNA damage (Fibonacci, 2015). Saponin compounds exhibit antibacterial activity by reducing surface tension, causing cell leakage and death (Sulistyowati, 2017). The ability of the extract to prevent the adherence of *Streptococcus mutans* could be related to the effect of saponins and flavonoid components.

Saponins are known to have hydrophobic and hydrophilic action. The hydrophobicity of saponin allows binding to the hydrophobic end of the bacteria cell membrane. In contrast, the hydrophilic end is free and will bring a complex detergent protein, resulting in bacterial lysis (Aswal, 2010).

Flavonoid compounds damage cell wall permeability by forming complexes with extracellular proteins, interfering with cellular metabolic processes, and inhibiting bacterial growth (Rinawati, 2011). Quinone compounds exert antibacterial effects by disrupting bacterial cell metabolism (Cowan, 1999). Flavonoids, through their antibacterial action, form a complex with proteins through nonspecific forces such as hydrogen bonding, hydrophobic effects, and covalent bond formation (Kumar, 2013).

Polyphenols have an antibacterial mechanism that involves modifying the cell membrane's permeability, resulting in nutrient loss, cell lysis, and ultimately, the demise of bacterial cells (Sulistyowati, 2017). Steroids can also disrupt membrane integrity, causing cell brittleness and lysis (Samejo, 2013). Triterpenoid compounds damage bacterial cell wall proteins (Rini, 2017).

This research demonstrates that soursop leaf and fruit extracts *(Annona muricata* L.) can inhibit *Streptococcus mutans* growth. According to Davis and Stout, the level of inhibition is classified as strong. Inhibitory activity was observed at the lowest concentration of 40% and increased with higher concentrations, reaching optimal levels at 100%. Assuming that an increase in concentration improves efficacy, most of the problems encountered with using synthetic drugs and chemicals might tend to taper (Mehta, 2014). Hence, it might open new avenues for research in treating patients undergoing chemotherapy and radiotherapy for oral cancer as it shows both anti-cancer and anti-microbial activity.

CONCLUSION

The 40%, 60%, 80%, 100% concentrations of *Annona muricata* L. ethanolic leaf and fruit extracts exhibited antibacterial activity on *Streptococcus mutans*. The effects were directly proportional to the increases in concentration. All Annona muricata L. ethanolic leaf concentrations and fruit extracts showed antibacterial activity on *Streptococcus mutans* compared with 96% ethanol as a negative control. Soursop leaf and fruit extracts at a 100% concentration demonstrated the strongest inhibitory activity against *Streptococcus mutans*, forming inhibition zones of 14.86 mm and 14.83 mm, respectively. These results demonstrated that Anonna muricata L. can be used as an effective antibacterial agent and widely applied in dental materials to help fight dental caries.

REFERENCES

Aswal, D., Monica, C., & Abidin, T. (2012). Daya Antibakteri Ekstrak Buah Mahkota Dewa *(Phaleria macrocarpa)* Terhadap *Enterococcus faecalis* (In-vitro). *J Dentika Dent.* 6(35).

Cowan M M., & Clin. (1999). Plant Product as Anti-microbial Agents, *Microbiology Rev*., 12(4).

Cushnie, T. P. & Lamb A. J. (2005). Anti-microbial Activity of Flavonoids. *Int J Antimicrob Agents.* 26: 343 – 356.

Davis, W.W. & Stout, T. R. (1971). Disc Plate Method of Microbiological Antibiotic Assay. *Applied Microbiology*, 22(4), 659-665.

Facey, P. C., Pascoe, K. O., Porter, R. B., & Jones, A. D. (1999). Investigation of Plants Used In Jamaican Folk Medicine for Antibacterial Activity. *J Pharm Pharmacol.* 51(120), 60.

Gartika, M., & Satari, M. H. (2013). *Beberapa Bahan Alam Sebagai Alternatif Bahan Pencegah Karies.* Bandung: UNPAD Press.

Herwandi, H., Mahyarudin, M., & Effiana, E. (2019). Uji Aktivitas Antibakteri Ekstrak Etanol *Annona muricata* Linn. Terhadap *Vibrio cholerae* Secara *In Vitro. Majalah Kedokteran Andalas,* 42(1), 11-21.

Johny, L., Yusuf, U. K., & Nulit, R. (2011). Anti-fungal Activity of Selected Plant Leaves Crude Extracts Against a Pepper anthracnose Fungus, *Colletotrichum capsici* (Sydo) butler and bisby (Ascomycota : Phyllachorales). *African Journal of Biotechnology.* 10(20), 65.

Kumar S., & Pandey A. K. (2013). Chemistry and Biological Activities of Flavonoids: An Overview. *The Scientific World Journal.* 2013(1), 1-16.

Manandhar, S., Luitel, S., & Dahal, R. K. (2019). In vitro Anti-microbial Activity of Some Medicinal Plants Against Human Pathogenic Bacteria. *Journal of Tropical Medicine*, 2019:1895340.

Mardiana, L. & Ratnasari, J. (2011). *Ramuan dan Khasiat Sirsak.* Jakarta: Penebar Swadaya.

Masloman, A. P. (2016). Uji Daya Hambat Ekstrak Daun Sirsak *(Annona muricata* L.*)* Terhadap Pertumbuhan Jamur *Candida albicans. PHARMACON Jurnal Ilmiah Farmasi – UNSRAT,* 5(4), 61-68.

Moghadamtousi, S. Z. (2015). *Annona muricata* L. "soursop" (Annonaceae), a Fruit Used for Feeding In Ancient Peru. *Int J Mol Sci.* 16(7), 58.

Nufus LS. (2019). Tingkat Pengetahuan Masyarakat Terhadap Penggunaan Antibiotik (Amoxicilin) Berdasarkan Usia di Dusun Karang Panasan Kabupaten Lombok Utara. *Jurnal Keperawatan,* 12(2), 54-62.

Pai, B. M., Rajesh, G., & Shenoy, R. (2016). Anti-microbial Efficiacy of Soursop Leaf Extract *(Annona muricata)* on Oral Pathogens: An In-vitro Study. *Journal of Clinical and Diagnostic Research.* 10(11), 1-4.

Paster, B. J., Aas, J. A., Stokes, L. N., Olsen, I., & Dewhirst, F. E. (2005). Defining The Normal Bacterial Flora of The Oral Cavity. *J Clin Microbiol*. 43(11), 32.

Phillipson, J. D. (2001). Phytochemistry and Medicinal Plants. *Phytochemistry.* 56, 237-243.

Priscila, G. T. (2017). Uji Daya Hambat Daging Buah Sirsak *(Annona muricata* L.), terhadap Pertumbuhan Bakteri *Porphyromonas gingivalis. PHARMACON Jurnal Ilmiah Farmasi- UNSRAT*, 6(3), 2302-2493.

Ramalingum, N., & Mahomoodally, M. F. (2014). The therapeutic potential of medicinal foods. Advances in Pharmacological and Pharmaceutical Sciences, 2014(1), 354264.

Rinawati, N. (2011). *Daya Antibakteri Tumbuhan Majapahit (Crescentia cujete L.) terhadap Bakteri Vibrio alginolyticus.* Jurusan Biologi Fakultas Matematika Ilmu Pengetahuan Alam Institut Teknologi Sepuluh November Surabaya.

Rini, A.A. (2017)**.** Skrining Fitokimia dan Uji Antibateri Ekstrak Etanol Buah Kawista *(Limonia acidissima* L.) dari Daerah Kabupaten Aceh Besar terhadap Bakteri *Escherichia coli. Jurnal Ilmiah Mahasiswa Fakultas Keguruan dan Ilmu Pendidikan Unsyiah*, 2(1), 1-12.

Riset Kesehatan Dsar (Riskesdas) Nasional. (2018). Badan Penelitian dan Pengembangan Kesehatan Departemen Kesehatan RI: 2018, https://www.kemkes.go.id/article/print/18110200003/potret-sehat-indonesia-dari-riskesdas-2018.html (Diakses pada tanggal 21 Januari 2024, pukul 15.30 WIB).

Samaranayake L. (2012). *Essential Microbiology for Dentistry 4th Ed.* Churcill Livingstone Elsevier; 279–281.

Samejo, M,Q., Memon, S., Bhanger, M.I., & Khan, K. M. (2013). Isolation and Characterization of Steroids From *Calligonum polygonoides*. *J. Pharmacy Res,* 1(6), 346-349.

Sulistyowati, D. (2017). *Aktivitas Antibakteri Ekstrak Etanol Daun Sirsak (Annona muricata L.) Terhadap Methicilllin Resistant Staphylococccus aureus (MRSA)".* Universitas Setia Budi Surakarta.

Tuna, M. R., Kepel, B.J., & Leman, M. A. (2015). Uji Daya Hambat Ekstrak Daun Sirsak *(Annona muricata* L.*)* Terhadap Pertumbuhan *Staphylococcus aureus* secara *In Vitro. PHARMACON Jurnal Ilmiah Farmasi-UNSRAT*, 4(4), 2302-2493.

Utami, S. M. (2022). Studi Literatur Aktivitas Antibakteri Ekstrak Daun Sirsak Terhadap Berbagai Sampel Bakteri. *PHRASE Pharmaceutical Science Journal,* 2(1), 107-115.

Widjijono & Harsini. (2008). *Penggunaan Herbal di Bidang Kedokteran Gigi*. Majalah Kedokteran Gigi, 61-64.

Yang, C., Gundala, S. R., Mukkavilli, R., Vangala., S, Reid, M. D., & Aneja, R. (2015). Synergistic Interactions among flavonoids and acetogenins in Soursop (*Annona muricata* L.*)* Leaves Confer Protection Against Prostate Cancer. *Carcinogenesis.* 36(6), 656-665.