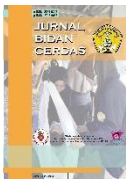
Jurnal Bidan Cerdas
e-ISSN: 2654-9352 dan p-ISSN: 2715-9965
Volume 5 Nomor 2, 2023, Halaman 58-65
DOI: 10.33860/jbc.v5i2.2167
Website:https://jurnal.poltekkespalu.ac.id/index.php/JBC
Penerbit: Poltekkes Kemenkes Palu



Antimicrobial Effects of Annona squamosa Leaf Extract on Staphylococcus aureus Growth: An In Vitro Study

Dwi Dianita Irawan, Amrina Nur Rohmah, Elia Ika Rahmawati, Risya Secha Primindari, Shinta Alifiana Rahmawati
Department of Midwifery, Universitas Muhammadiyah Lamongan, Lamongan, Indonesia
Corresponding author: irawan.dianita36@gmail.com

ARTICLE INFO

Article History:

Received: 2023-03-18
Accepted: 2023-06-06
Published: 2023-06-30

Keywords:

Staphylococcus aureus; Annona squamosa; microbe; bacteria; in vitro;

ABSTRACT

Introduction: Mastitis is inflammation of the breast tissue due to infection with the Staphylococcus aureus bacteria causing local infections. It takes the natural ingredient Annona squamosal extract as an antimicrobial. **Purpose:** to determine the effectiveness of Annona squamosa leaf extract on the inhibition and killing power of Staphylococcus. **Method:** Experimental laboratory research with antibacterial activity test. Tube dilution method by incorporating bacteria and extracts with concentrations of 20%, 22.5%, 25%, 27.5%, 30%, and 32.5% and then measuring the MIC. The diffusion method is carried out by planting bacteria in a Nutrient Agar Plate and then giving the extract with 6 concentrations to calculate the MBC. **Results**: The results of the One-Way ANOVA test obtained a significance value of ($p<0.001$), indicating that changes in the concentration of Annona squamosa made a significant difference to Staphylococcus. The correlation test showed a significant p-value of ($p<0.001$), which means that there was a significant relationship between the administration of extract and the number of Staphylococcus. **Conclusion:** Annona squamosa leaf extract is able to significantly inhibit the growth of Staphylococcus bacteria and the higher the concentration of Annona squamosa leaf extract, the fewer the number of bacterial colonies that grow.

INTRODUCTION

The Indonesian government has enacted Government Regulation Number 33 of 2012 on Exclusive Breastfeeding, which defines exclusive breastfeeding as the provision of breast milk to infants from birth up to 6 months of age, without adding any other food or drink (Kementerian Kesehatan, 2012). Exclusive breastfeeding has been found to reduce under-five mortality by approximately 13%, and 16% of neonatal deaths can be prevented if infants are breastfed from birth and receive colostrum (Ade Harshindy & Budi Raharjo, 2022). However, the coverage rate of exclusive breastfeeding in Indonesia tends to decline, with the Ministry of Health setting a target coverage rate of 80%.

Common issues that occur in breastfeeding mothers include sore nipples, breast engorgement, breast milk stasis, and mastitis. These problems can disrupt the continuity of breastfeeding and pose health risks to mothers if left untreated (Bambang Ari Purwoko, 2019). Mastitis is an inflammation or infection of the breast characterized by hardening, redness, and pain, often accompanied by a fever >38°C,

caused by a Staphylococcus aureus bacterial infection (Ika Tristanti dan Nasriyah, 2019). According to the Indonesian Demographic and Health Survey in 2015, 37.12% of postpartum mothers experienced breastfeeding issues such as breast milk stasis and mastitis (Jamaruddin S, Ferawati Taherong, & Syatirah, 2022). Staphylococcus aureus is one of the gram-positive bacteria that is part of the normal flora of the mucous membranes and skin in humans. This opportunistic bacterium can cause local infections in various parts of the body. Examples of problems that can arise from Staphylococcus aureus infection include bacteremia, pneumonia, meningitis, osteomyelitis, sepsis, nosocomial infections, and others. Staphylococcus aureus can cause both local infections on the skin and nasal mucosa and endocarditis (Rahmadani, Budiyono, 2017).

According to the Centers for Disease Control and Prevention, Staphylococcus aureus is currently a serious threat due to its resistance to various antibiotics. Hence, many studies are now focusing on the development and utilization of the antibacterial potential of natural substances (Centers For Disease Control and Prevention, 2013). Annona squamosa, commonly known as Sugar Apple, is one of the medicinal plants that show potential as an antibacterial agent. The leaves of Annona squamosa are known to possess significant health benefits due to the presence of various phytochemical compounds such as alkaloids and flavonoids (Kumar et al., 2021). Research has shown that Annona squamosa leaf extract exhibits biological activities such as anticancer, antidiabetic, antioxidant, antifungal, and antimicrobial effects (Anaya-Esparza et al., 2020). The leaf extract of Annona squamosa has demonstrated potential for pharmaceutical development (Sundaramahalingam et al., 2021).

A study by Jangnga (2018) revealed that ethanol extract of Annona squamosa leaves has inhibitory effects on the growth of Enterococcus faecalis bacteria in vitro (Jangnga & Kambaya 2018). Another study demonstrated that flavonoid compounds in sugar apple leaf extract can inhibit the growth of Staphylococcus aureus bacteria using the well diffusion method (Karunia & Supartono, 2017). These findings indicate the need for further research on the effectiveness of ethanol extract of Annona squamosa leaves in inhibiting the growth of Staphylococcus aureus bacteria using the tube dilution and diffusion methods. The aim of this study is to determine the inhibitory and bactericidal effects of Annona squamosa leaf extract on Staphylococcus aureus, thereby providing an alternative natural treatment solution for mastitis caused by Staphylococcus aureus bacteria.

METHODS

This study utilized laboratory experimental research using an in vitro approach with the tube dilution and diffusion methods for antibacterial activity testing. The research was conducted from January to February 2023 at the Biology Laboratory of Muhammadiyah University of Lamongan. The research sample consisted of Staphylococcus aureus bacteria on Nutrient Agar (NA).

The Annona squamosa leaf sample, obtained from Palang District, Tuban Regency, was extracted using the maceration method. The leaves were thoroughly washed with running water, then cut into small pieces and dried in an oven before being blended into a powdered form. The maceration process involved soaking the powdered plant material in 96% ethanol solution for three cycles of 24 hours each, with intermittent stirring. Afterward, the extract was filtered and subjected to rotary vacuum evaporation at a temperature of 60-70°C to reduce the extraction volume. The resulting concentrated extract was then diluted with 96% ethanol.

Staphylococcus aureus bacteria were prepared in the laboratory and adjusted to a McFarland standard of 0.5. The bacteria were diluted twice to achieve a bacterial concentration of 1x106. The tube dilution method was performed by adding the ethanol extract of sugar apple leaves to each reaction tube labeled A-F, with concentrations of 20%, 22.5%, 25%, 27.5%, 30%, and 32.5%, respectively. Tube KB served as the Bacterial Control. Each tube contained different series of test substance concentrations, with sterile aquadest used as the diluent. The bacterial suspension was added to each test tube. All tubes were incubated at 37°C for 18-24 hours, and the turbidity was observed to determine the Minimum Inhibitory Concentration (MIC). Subsequently, a volume of 0.01 ml was taken from each tube and streaked onto NA medium. The plates were incubated again at 37°C for 18-24 hours. After incubation, the colonies grown on the NA plates were counted to determine the Minimum Bactericidal Concentration (MBC).

The obtained data were subjected to One-Way ANOVA statistical analysis to determine differences, and Pearson correlation analysis was conducted to assess the relationship between the administration of Annona squamosa leaf extract and Staphylococcus aureus bacteria.

RESULT

The turbidity level of the sugar apple leaf extract solution was observed to determine the Minimum Inhibitory Concentration (MIC). The Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial agent that can inhibit the growth of bacteria. The comparison of turbidity levels at each concentration is shown in Figure 1.

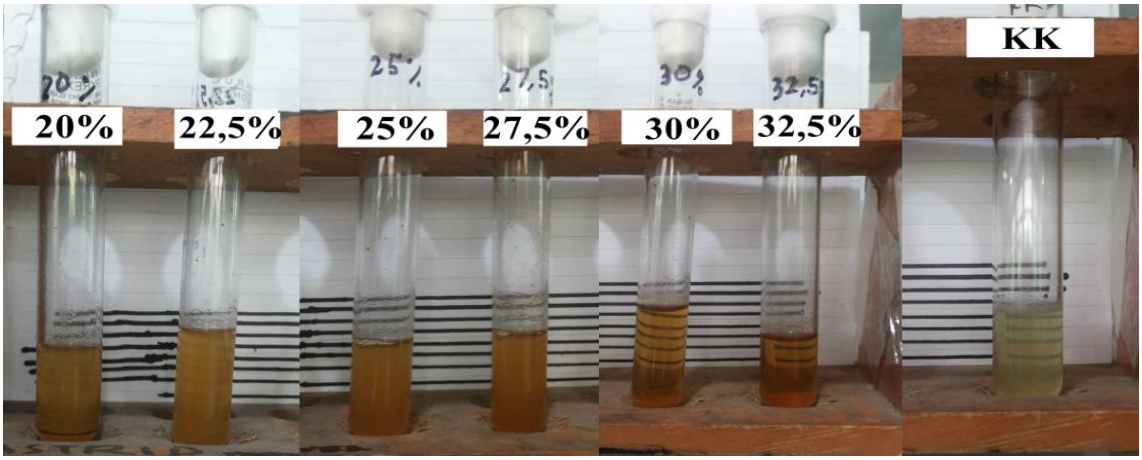


Figure 1. Results of Minimum Inhibitory Concentration (MIC) of Annona Squamosa Leaf Extract Showing the 30% Concentration

From the observations, it can be observed that the higher the treatment concentration, the lower the turbidity level in the tubes. It can be seen that the 30% concentration is the lowest concentration that does not show turbidity in the tube, indicating that the MIC in this study is at a concentration of 30% as shown in Figure 1.

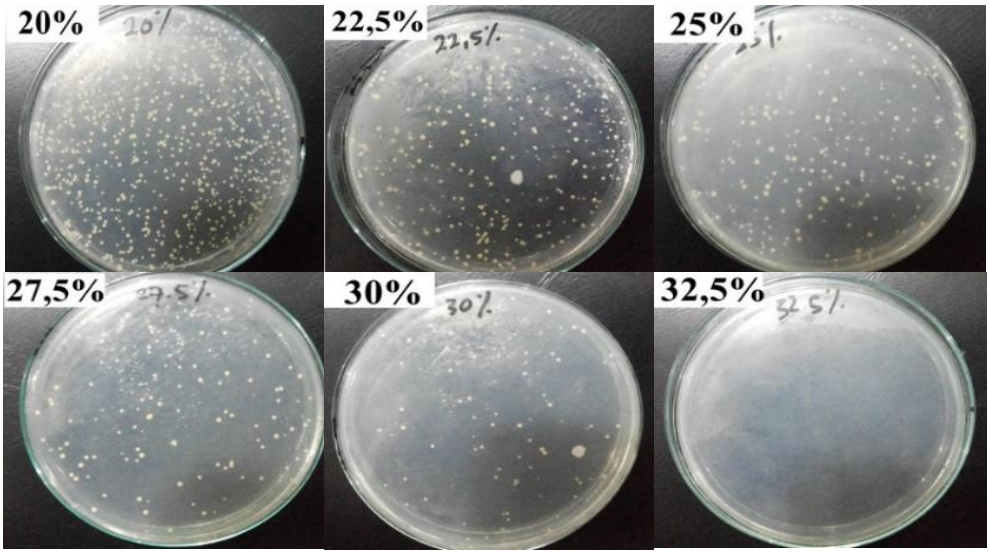


Figure 2. Results of Minimum Bactericidal Concentration (MBC) of Annona Squamosa Leaf Extract Showing the 32.5% Concentration

The Minimum Bactericidal Concentration (MBC) is the lowest concentration of an antimicrobial agent that can kill bacteria, indicated by the absence of bacterial growth on the Nutrient Agar Plate (NAP) medium. From the observations in Figure 2, it can be seen that as the concentration of sugar apple leaf extract increases, the colony count of Staphylococcus aureus decreases. The highest colony growth is observed at the KB concentration, while a decrease in bacterial growth is observed at concentrations of 32.5%, 30%, 27.5%, 25%, 22.5%, and 20%. The results show no growth of Staphylococcus aureus bacteria at a concentration of 32.5%. Thus, it can be concluded that the MBC in this study is obtained at a concentration of 32.5%. For a clearer presentation of the colony counts on each plate, Table 1 is provided.

Table 1. Number of Bacterial Colonies in Each Replication

Number	Concentration	Number of bacterial colonies per replication				Mean	Standard Deviation
		P 1	P 2	P 3	P 4		
1	32.5%	0	0	0	0	0	0
2	30.0%	89	68	45	77	69.75	±18.608
3	27.5%	122	156	168	175	155.25	±23.514
4	25.0%	315	231	256	270	268	±35.242
5	22.5%	432	407	414	400	413.25	±13.745
6	20.0%	633	580	473	510	549	±71.447
7	KB (0%)	291000	283000	301000	311000	296500	±12151.817

The One-Way ANOVA test yielded a significance value of ($p<0.001$) as shown in Table 2. This indicates that the change in sugar apple leaf extract concentration has a significant effect on Staphylococcus aureus. The correlation test, as shown in Table 3, yielded a significance value of ($p<0.001$), indicating a significant relationship between the administration of sugar apple leaves and the number of S. aureus colonies. The Pearson correlation coefficient is $r=-0.919$. The negative sign indicates an inverse relationship, where a higher concentration of sugar apple leaf extract results in a lower number of bacterial colonies, and vice versa.

Table 2. Results of One-Way ANOVA Statistical Test

Data Name	Sum of Square	DF	Mean square	F	Sig
Number of colonies	3.01+11	6	5.015+10	2377.363	.000
	4.43+08	21	21096300		
	3.01+11	27			

Table 3. Results of Pearson Correlation Statistical Test

Data Name		Concentration	Number of Colonies
Concentration	Pearson Cor	1	-.919
	Sig. (2-tailed)	.	.000
	N	28	28
Number of Colonies	Pearson Cor	-.919	1
	Sig. (2-tailed)	.000	.
	N	28	28

DISCUSSION

This study aims to determine the effectiveness of Annona squamosa leaf extract against the inhibitory and bactericidal activity on Staphylococcus aureus. The results of the study showed that Annona squamosa leaf extract was able to inhibit the growth of Staphylococcus aureus bacteria, as indicated by the obtained Minimum Inhibitory Concentration (MIC) value at a concentration of 30%. The results also showed that Annona squamosa leaf extract was able to kill Staphylococcus aureus bacteria, as indicated by the obtained Minimum Bactericidal Concentration (MBC) value at a concentration of 32.5%.

Plants contain various metabolite compounds with different chemical properties such as polarity and solubility. Several solvents have been developed for the extraction of plant metabolites, including methanol, ethanol, ethyl acetate, and other solvents (Kamoda, Lelyana, & Sugiaman, 2020). In this study, Annona squamosa extract was prepared using 96% ethanol as the solvent, as ethanol is relatively non-destructive to active chemical compounds. Additionally, 96% ethanol contains a small amount of water, allowing it to absorb more active substances. Ethanol is also a universal solvent that can dissolve both polar and nonpolar compounds, ensuring that the required active compounds are fully extracted (Al-Judaibi, Al-Zahrani, Altammar, Ismail, & Darweesh, 2014).

The different concentrations of Annona squamosa leaf extract showed a significant difference in the inhibitory activity against Staphylococcus aureus bacteria. This result is consistent with the study conducted by Santhoshkumar & Kumar (2016) on the phytochemical activity and antibacterial effect of Annona squamosa leaf extract, which found that higher concentrations of Annona squamosa leaf extract corresponded to higher antibacterial activity. The inhibitory activity of this extract may be attributed to the higher concentration of antibacterial compounds capable of inhibiting the growth of Staphylococcus aureus colonies (Santhoshkumar & Kumar, 2016).

As the concentration of Annona squamosa leaf extract increased, the number of bacterial colonies decreased, and vice versa. Gram-positive bacteria, such as Staphylococcus aureus, have a membrane structure that contains more peptides, fewer lipids, and a cell wall composed of polysaccharides (teichoic acids). Teichoic acids are polymers that can dissolve in water, making the cell wall of gram-positive bacteria more polar (Vijayalakshmi & Nithiya, 2015). Flavonoids present in Annona

squamosa leaf extract are polar compounds, allowing them to penetrate the cell wall more easily. Flavonoids act as antibacterial agents by disrupting cell wall synthesis, interfering with the cytoplasmic membrane, and inhibiting energy metabolism (Xiao, Zhu, & Zhang, 2014). This is in line with the research conducted by Alberta (2016) on the inhibitory effect of ethanol extract of Annona squamosa leaves on the growth of E. coli and Staphylococcus aureus bacteria, which found that Annona squamosa leaf extract exhibited higher antibacterial and inhibitory effects on Staphylococcus aureus compared to E. coli (Tansil et al., 2016).

Annona squamosa has long been recognized for its medicinal properties, and different parts of the plant, such as fruits, seeds, bark, twigs, and leaves, possess their own unique benefits. Annona squamosa leaves are known to have extensive pharmacological properties and biological activities such as antioxidant, antimicrobial, antidiabetic, antiviral, and anticancer effects (Mohammad Zahid, 2018). This is due to the presence of phytochemical compounds in Annona squamosa leaves, including glycosides, phytosterols, saponins, tannins, phenols, and the two most important compounds, alkaloids and flavonoids (Hosseinabadi, 2021). Alkaloids and flavonoids are the most significant compounds contributing to the antimicrobial activity of Annona squamosa leaves (Santhoshkumar & Kumar, 2016). Flavonoids can induce disturbances in microbial membranes, coagulate cytoplasmic components and cause leakage, as well as interfere with bacterial cell metabolism (Kumar et al., 2021). This is consistent with the research conducted by Swantara (2022), who performed phytochemical screening of Annona squamosa leaves and found the presence of flavonoid compounds, particularly flavonol, in the extract. These compounds exhibited inhibitory effects on the growth of Staphylococcus aureus bacteria (Swantara, Damayanti, & Suirta, 2022).

CONCLUSION AND RECOMMENDATION

The conclusion of this study is that Annona squamosa leaf extract at a concentration of 30% is capable of significantly inhibiting the growth of Staphylococcus aureus bacteria, and the higher the concentration of Annona squamosa leaf extract, the fewer bacterial colonies are observed. Furthermore, Annona squamosa leaf extract also exhibits significant bactericidal activity against Staphylococcus aureus at a concentration of 32.5%. A suggestion for further research is to conduct in vivo studies to investigate the pharmacological effects of Annona squamosa leaves on the human body.

REFERENCES

Ade Harshindy, N., & Budi Raharjo, B. (2022). Analisis Pelaksanaan Program ASi Eksklusif di Posyandu [Analysis of Exclusive Breastfeeding Program Implementation at Integrated Health Posts] Article Info. *Indonesian Journal of Public Health and Nutrition*, *2*(1), 60–66. Retrieved from https://journal.unnes.ac.id/sju/index.php/IJPHN/article/view/51375

Al-Judaibi, A., Al-Zahrani, A., Altammar, K. A., Ismail, S. B., & Darweesh, N. T. (2014). Comparative study of antibacterial activity of plant extracts from several regions of Asia. *American Journal of Pharmacology and Toxicology*, *9*(2), 139–147. https://doi.org/10.3844/ajptsp.2014.139.147

Anaya-Esparza, L. M., García-Magaña, M. de L., Abraham Domínguez-Ávila, J., Yahia, E. M., Salazar-López, N. J., González-Aguilar, G. A., & Montalvo-González, E. (2020). Annonas: Underutilized species as a potential source of bioactive compounds. *Food Research International*, *138*(June). https://doi.org/10.1016/j.foodres.2020.109775

Bambang Ari Purwoko, O. H. and H. (2019). Gambaran Masalah Pemberian Asi Pada Ibu Di Wilayah Kerja Puskesmas Palmatak [Overview of Problems in Breastfeeding among Mothers in the Palmatak Health Center's Work Area]. *JOM FKp*, *7*(1), 96–103. Retrieved from https://jom.unri.ac.id/index.php/JOMPSIK/article/view/29522

Centers For Disease Control and Prevention. (2013). Biggest Threats Antibiotic/Antimicrobiall Resistance. Retrieved from https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf

Hosseinabadi, T. (2021). The Medicinal Importance of Annona squamosa fruits. *Journal of Exploratory Research in Pharmacology*, *000*(000), 000–000. https://doi.org/10.14218/jerp.2020.00039

Ika Tristanti dan Nasriyah. (2019). Mastitis (Literature Review). *Jurnal Ilmu Keperawatan Dan Kebidanan*, *10*(2), 330–337. http://dx.doi.org/10.26751/jikk.v10i2.729

Jamaruddin S, R. N. A., Ferawati Taherong, & Syatirah. (2022). Manajemen Asuhan Kebidanan Berkelanjutan Post Natal Pada Ny"W" Dengan Bendungan Asi Hari Ketiga Sampai 31 Hari Masa Nifas Di Puskesmas Bara Baraya [Sustainable Postnatal Midwifery Care Management for Mrs. "W" with Exclusive Breast Milk from the Third Day to the 31st Day of Postpartum Period at Bara Baraya Health Center]. *Jurnal Midwifery*, *4*(2), 32–41. https://doi.org/10.24252/jmw.v4i2.29549

Jangnga, I. D., Kambaya, P. P., & Kosala, K. (2018). Uji Aktivitas Antibakteri Dan Analisis Bioautografi Kromatografi Lapis Tipis Ekstrak Etanol Daun Srikaya (Annona Squamosa L) Terhadap Enterococcus Faecalis Secara in Vitro [Antibacterial Activity Test and Thin Layer Chromatography Bioautography Analysis of Srikaya Leaf Ethanol Extract (Annona Squamosa L) against Enterococcus Faecalis in Vitro]. *ODONTO : Dental Journal*, *5*(2), 102. https://doi.org/10.30659/odj.5.2.102-109

Kamoda, H., Lelyana, S., & Sugiaman, V. K. (2020). Kadar hambat minimum dan kadar bunuh minimum ekstrak etanol lengkuas merah (Alpinia galanga L.) terhadap pertumbuhan Candida albicans [The minimum inhibitory concentration and a minimum lethal dose of red galangal (Alpinia galanga L.) ethanolic ex]. *Jurnal Kedokteran Gigi Universitas Padjadjaran*, *32*(1), 1. https://doi.org/10.24198/jkg.v32i1.25422

Karunia, S. D., Supartono, & Sumarni, W. (2017). Analisis Sifat Anti Bakteri Ekstrak Biji Srikaya (Annona squamosa L) dengan Pelarut Organik [Analysis of the Antibacterial Properties of Custard Apple (Annona squamosa L) Seed Extracts with Organic Solvents]. *Indonesian Journal of Chemical Science*, *6*(1), 56–60. Retrieved from https://journal.unnes.ac.id/sju/index.php/ijcs/article/view/11288

Kementerian Kesehatan. Peraturan Pemerintah Tentang Pemberian ASI Eksklusif Nomor 33 tahun 2012 [Government Regulation on Exclusive Breastfeeding No. 33 of 2012] (2012). Indonesia: Departemen Kesehatan. Retreived from http://hukor.kemkes.go.id/uploads/produk_hukum/PP%20No.%2033%20ttg%20Pemberian%20ASI%20Eksklusif.pdf

Kumar, M., Changan, S., Tomar, M., Prajapati, U., Saurabh, V., Hasan, M., … Mekhemar, M. (2021). Custard apple (Annona squamosa l.) leaves: Nutritional composition, phytochemical profile, and health-promoting biological activities. *Biomolecules*, *11*(5). https://doi.org/10.3390/biom11050614

Mohammad Zahid, E. al. (2018). Annona Squamosa Linn (Custard Apple): An Aromatic Medicinal Plant Fruit With Immense Nutraceutical And Therapeutic Potentials. *IJPSR*, *9*(5), 1745–1759. https://doi.org/10.13040/IJPSR.0975-8232.9(5).1745-59

Rahmadani, Budiyono, S. (2017). Gambaran Keberadaan Bakteri Staphylococcus Aureus, Kondisi Lingkungan Fisik, Dan Angka Lempeng Total Di Udara Ruang Rawat Inap Rsud Prof. Dr. M.a Hanafiah Sm Batusangkar [Description of the Presence of Staphylococcus aureus Bacteria, Physical Environmental Conditions, and Total Plate Count in the Air of Inpatient Rooms at Prof. Dr. M.a Hanafiah Sm Batusangkar Regional General Hospital]. *Jurnal Kesehatan Masyarakat (e-Journal)*, *5*(5), 492–501. Retrieved from https://ejournal3.undip.ac.id/index.php/jkm/article/view/19171

Santhoshkumar, R., & Kumar, N. S. (2016). Phytochemical analysis and antimicrobial activities of Annona squamosa (L.) leaf extracts. *International Journal of Pharmaceutical Sciences Review and Research*, *36*(1), 144–148. https://doi.org/10.35790/ebm.v4i2.14344

Sundaramahalingam, M. A., Karthikumar, S., Shyam Kumar, R., Samuel, K. J., Shajahan, S., Sivasubramanian, V., … Ganesh Moorthy, I. (2021). An intensified approach for transesterification of biodiesel from Annona squamosa seed oil using ultrasound-assisted homogeneous catalysis reaction and its process optimization. *Fuel*, *291*(December 2020), 1–14. https://doi.org/10.1016/j.fuel.2021.120195

Swantara, I. M. D., Damayanti, P. A., & Suirta, I. W. (2022). Identifikasi Serta Uji Aktivitas Antibakteri Senyawa Flavonoid Ekstrak Daun Srikaya (Annona Squamosa Linn) [Identification and Antibacterial Activity Testing of Flavonoid Compounds from Srikaya Leaf Extract (Annona Squamosa Linn)]. *Jurnal Kimia*, *16*(1), 45. https://doi.org/10.24843/jchem.2022.v16.i01.p06

Tansil, A. Y. M., Nangoy, E., Posangi, J., Bara, R. A., Farmakologi, B., Kedokteran, F., … Manado, R. (2016). Uji daya hambat ekstrak etanol daun srikaya (Annona squamosa) terhadap pertumbuhan bakteri Escherichia coli dan Staphylococcus aureus [Inhibitory Activity Test of Ethanol Extract of Srikaya Leaf (Annona squamosa) against Escherichia coli and Staphylococcus aureus Bacteria]. *Jurnal E-Biomedik*, *4*(2). Retreived from https://ejournal.unsrat.ac.id/index.php/ebiomedik/article/view/14344

Vijayalakshmi, S. L. R., & Nithiya, T. (2015). Antimicrobial Activity of Fruit Extract of Annona. *Nithiya et Al. World Journal of Pharmacy and Pharmaceutical Sciences*, *4*(05), 1257–1267. Retrieved from https://www.wjpps.com/wjpps_controller/abstract_id/3098

Xiao, Z.-T., Zhu, Q., & Zhang, H.-Y. (2014). Identifying antibacterial targets of flavonoids by comparative genomics and molecular modeling. *Open Journal of Genomics*, *3*, 1. https://doi.org/10.13055/ojgen_3_1_1.140317