Antibiofilm Activity of Coconut (*Cocos nucifera*) Leaflets Against Microorganisms Causing Nosocomial Infections

Arundel Aynsley M. Alay-ay, Jewel Heart A. Abubo, Hanna Marie F. Buyacco, John Mikhad H. Liwongan, Lyka Vinia C. Mamuric, Christian M. Sanuco, RMT

Bachelor of Science in Medical Laboratory Science

ABSTRACT

This study aimed to determine the antibiofilm activity of coconut leaflets on selected biofilm-forming bacteria and fungi. Coconut leaflets were extracted and processed to obtain crude extract using aqueous, ethanol, and methanol solvents. Minimum inhibitory concentration (MIC) was determined to evaluate the effectiveness of the various solvents against *S. aureus*, *P. aeruginosa*, and *C. albicans*. After that, an eradication assay was performed by staining wells containing preformed biofilm with crystal violet, and the biofilm inhibition and eradication were observed under the microscope. Results show that among the three solvents, the ethanolic extract of coconut leaflets at a 1:2 dilution effectively inhibited the biofilms of S. aureus, P. aeruginosa, and C. albicans, with biofilm inhibition of 44%, 77%, and 50%, respectively. Results of the microscopic observation, based on the presence of bacterial cells, yeast cells, microcolonies, pseudohyphae/true hyphae, and reduction in the coverage of biofilm formed, show that the biofilm inhibition activity of ethanolic extract of coconut leaflets with a 1:2 dilution concentration was found to be significantly effective against all three microorganisms. With the same parameters, the biofilm-eradication activity of the ethanolic extract of coconut leaflets at a 1:2 dilution was found to be lacking. These findings demonstrate that coconut leaflets exhibit antibiofilm activity at a 1:2 ethanol dilution. This suggests its potential to be a biofilm inhibitor against nosocomial infection-causing microorganisms in the hospital setting.

Keywords: biofilm, biofilm eradication, biofilm formation, microcolonies, minimum inhibitory concentration

INTRODUCTION

Background of the Study

Nosocomial infections are those acquired by patients during healthcare treatment, typically developing 48 hours after hospitalization (World Health Organization, 2024). These infections can cause severe complications such as sepsis and death. Haque et al. (2018) identify several contributing factors, including invasive procedures, use of medical devices, poor hygiene, and inadequate medical practices. HAIs not only extend hospital stays but can also spread to patients' relatives. Common types include urinary tract, respiratory, bloodstream, and surgical site infections. Raoofi et al. (2023) report a global HAI rate of 8.7%, with higher prevalence in the Eastern Mediterranean and lower in the Western Pacific. Rates range from about 5% in North America and parts of Europe to 40% or more in some regions of Asia, Latin America, and Africa, largely due to poor adherence to safety protocols.

Sikora and Zahra (2023) note that nosocomial infections are caused by various microbes, including bacteria, viruses, and fungi. Common gram-positive bacteria include coagulase- negative Staphylococci, Streptococcus, and Enterococcus species. Drug-resistant strains like MRSA, VISA, and VRSA are especially concerning. Fungal infections, particularly those caused by *Candida species*, often affect immunocompromised patients or those with medical devices.

Sadly, financial constraints pose significant challenges in controlling HAI in countries like the Philippines, which is considered a developing country. Hence, there is a call to explore natural approaches to combat biofilm-forming microorganisms, which are a major cause of persistent infections in healthcare settings. By investigating the antibiofilm properties of coconut leaflets, this research contributes to the search for alternative solutions to reduce nosocomial infections. In the laboratory, this study provides a basis for further investigation into plant-based antibiofilm agents, potentially leading to the development of new antibiofilm treatments. For the medical community, the findings could have practical applications in infection prevention and treatment.

The significance of this study lies in its investigation of the antibiofilm activity of coconut leaflets against nosocomial infection-causing microorganisms. These microorganisms pose significant health risks, particularly in immunocompromised individuals. Coconut leaflets, a readily available and natural resource, have shown promise in antimicrobial activity. Exploring their potential to inhibit and eradicate these microorganisms could lead to the development of novel, eco-friendly antibiofilm therapies.

Statement of the Objectives

This study aimed to determine the antibiofilm activity of coconut leaflets on selected biofilm-forming bacteria and fungi. This study was conducted from January to May 2025.

Specifically, this sought to achieve the following objectives.

1. To determine the most effective extracting solvent for an antibiofilm activity.
    a. Aqueous
    b. Ethanol
    c. Methanol
2. To determine the most effective concentration based on the minimum inhibitory concentration of coconut leaflets crude extracts for each extracting solvent.
3. To evaluate the antibiofilm activity of coconut leaflets crude extracts against the following microorganisms in terms of:
    a. Inhibition
    b. Eradication

METHODOLOGY

Research Design

The study utilized an experimental design. The crude extract of coconut leaflets was prepared using different solvents: aqueous, ethanol, and methanol. Minimum inhibitory concentration, biofilm inhibition, and biofilm eradication assays were performed to determine the most effective extraction solvent and concentration for antibiofilm activity of coconut leaflets against nosocomial infection-causing microorganisms. Control groups were also utilized in this study. The positive control consisted of microorganisms grown in sterile growth medium without the addition of the extract, to confirm their ability to form biofilm under the experimental conditions. The negative control consisted of sterile growth medium without microorganisms and without extract.

Study Site and Sample Collection

The study was conducted at the Center for Natural Sciences of Saint Mary's University (CNS-SMU), Bayombong, Nueva Vizcaya. The coconut leaflets were collected along the Villaverde-Bagabag road in Bagabag, Nueva Vizcaya, as the area is abundant in coconut trees. *S. aureus, P. aeruginosa,* and *C. albicans* were obtained from the Center for Natural Sciences of

Saint Mary's University. Microtiter plate reading was performed at the Nueva Vizcaya Provincial Hospital (NVPH) Laboratory, located in Bambang, Nueva Vizcaya.

Plant Certification

Cocos nucifera leaves were submitted to the College of Forestry, Environment, and Resource Management of Nueva Vizcaya State University, Bayombong, Nueva Vizcaya for certification for its taxonomic identity.

Data Gathering Procedure

Dried coconut leaflets were ground into a fine powder. For crude extraction, 500 g of the powdered material was soaked in 2 L of aqueous, ethanol, or methanol solvent. After stirring for 1 hour and standing undisturbed for 24 hours, the mixtures were vortexed for 5 minutes and centrifuged at 10,000 rpm to separate the solid residues. The resulting supernatants were filtered through Whatman No. 3 filter paper and concentrated by solvent evaporation under reduced pressure in a rotary evaporator. The crude extracts were stored at 4°C until further analysis. This procedure was adapted from Alam et al. (2020).

To determine the minimum inhibitory concentration (MIC) of biofilm formation, microbial suspensions of bacteria and fungi were prepared in Mueller-Hinton Broth (MHB) and standardized using the McFarland turbidity method. A control group without extract served as the positive reference. The assay was carried out in 96-well microplates, where extract-treated cultures were incubated at 37°C for 24 hours. Following incubation, planktonic cells were removed, and wells were washed three times with phosphate-buffered saline (PBS, pH 7.4) to eliminate non-adherent cells. Plates were dried at room temperature for 24 hours, and 200 µL of 0.3% (w/v) crystal violet solution was added to each well, which was then incubated for 15 minutes. Excess dye was removed by washing with PBS, and the bound crystal violet was solubilized using 30% (v/v) glacial acetic acid. Optical density was measured at 570 nm using a microplate reader (Chamás et al., 2023). Biofilm inhibition was calculated using the following formula:

$$\text{Biofilm inhibition (\%)} = [(\text{Control OD} - \text{Treated OD}) / \text{Control OD}] \times 100$$

Biofilm-forming strains of *S. aureus*, *P. aeruginosa*, and *C. albicans* were standardized according to the McFarland standard and added to 96-well microtiter plates. Crude coconut leaflet extracts at MIC levels were applied to the wells. Plates were incubated at 37°C for 24 hours to allow biofilm formation. After incubation, each well was washed three times with PBS to remove non-adherent cells and dried for 24 hours. Biofilms were stained with 125 µL of 0.1% crystal violet solution for 30 minutes, rinsed with distilled water, and then resolubilized with 30% (v/v) glacial acetic acid (Chamás et al., 2023).

Biofilm formation was assessed by light microscopy. The resolubilized contents of the wells were mounted on glass slides and covered with cover slips. Observations under 100x magnification focused on bacterial cells and microcolonies for *S. aureus* and *P. aeruginosa*, and yeast cells along with pseudohyphae or true hyphae for *C. albicans*, based on the criteria used by Chamás et al. (2023).

For the eradication assay, 50 µL of standardized bacterial and fungal suspensions were placed into microplate wells and incubated at 37°C for 24 hours to allow biofilm formation. Afterward, the medium and planktonic cells were removed, and the wells were washed with PBS three times. MIC concentrations of the coconut leaflet extract were added, and the mixture was incubated for another 24 hours. Planktonic cells were discarded, wells were washed with PBS, and the remaining biofilm was stained with crystal violet and resolubilized using 30% glacial acetic acid (Chamás et al., 2023).

Visualization of biofilm eradication followed the same microscopy procedure. The stained and resolubilized samples were mounted and examined under a light microscope at 100x magnification. Evaluation criteria included bacterial microcolonies and cell structures for *S. aureus* and *P. aeruginosa*, and fungal elements such as yeast cells and hyphae for *C. albicans*, following the method of Chamás et al. (2023).

Ethical Considerations

The study was approved by Saint Mary's University Research Ethics Board (SMUREB) with address and contact information at 2nd Floor, Rev. John Van Bauwel Hall, SMU Main Campus, Ponce Street, Don Mariano Marcos, Bayombong, 3700 Nueva Vizcaya, Philippines (email: reb@smu.edu.ph; Cellphone No.: 09177053041).

RESULTS AND DISCUSSIONS

Section 1. Minimum Inhibitory Concentration (MIC) of the Aqueous, Ethanolic, and Methanolic Extracts

Table 1

Mean Percentage of Biofilm Inhibition Against Staphylococcus aureus using Aqueous, Ethanolic, and Methanolic Extracts of Coconut Leaflets

	Mean Percentage of Biofilm Inhibition (%)		
Dilution concentration	**Aqueous**	**Ethanol**	**Methanol**
1:2	**38.90**	**44.38**	**39.76**
1:4	11.66	21.12	6.91
1:8	10.64	27.27	15.43
1:16	11.58	14.04	11.99
1:32	13.12	25.32	3.59
1:64	17.98	29.99	15.26
1:128	2.32	36.91	8.02
1:256	12.33	33.26	18.11

Table 1 shows that the results indicate that the 1:2 dilution consistently displayed the highest percentage of inhibition across the three extract types. Specifically, the 1:2 dilution of the ethanolic extract showed the highest mean percentage of inhibition (44%), followed by the methanolic extract (39%) and aqueous extract (38%).

According to Bastos et al. (2016), this extract, when tested in vitro, demonstrates antimicrobial activity against *S. aureus*. The extract also demonstrated activity against other bacteria, including *K. pneumoniae* and *B. subtilis*. An ethanolic extract of coconut leaflets at 1:2 dilution has been found to inhibit the growth of *S. aureus* in vitro, exhibiting a similar inhibition zone to that of the positive control.

These findings suggests that the 1:2 dilution is the most effective concentration for inhibiting and eradicating biofilm formation of *S. aureus*, with the ethanolic extract yielding the best results, suggesting that the most effective extracting solvent for antibiofilm activity is ethanol. This highlights the potential of the ethanolic extract of coconut leaflets as a promising antimicrobial and antibiofilm agent, warranting further investigation into its therapeutic applications.

Table 2

Mean Percentage of Biofilm Inhibition Against Pseudomonas aeruginosa using Aqueous, Ethanolic, and Methanolic Extracts of Coconut Leaflets

Mean Percentage of Biofilm Inhibition (%)			
Dilution concentration	**Aqueous**	**Ethanol**	**Methanol**
1:2	**68.31**	**77.99**	**81.05**
1:4	42.42	36.46	68.37
1:8	31.13	14.79	31.47
1:16	30.95	35.47	50.30
1:32	23.13	41.61	38.60
1:64	16.47	35.01	19.77
1:128	36.34	44.85	44.28
1:256	43.12	34.26	33.39

Table 2 shows that the MIC assay conducted against *P. aeruginosa* showed varying inhibitory effects from different plant extracts. The 1:2 dilution of the methanolic extract exhibited the highest mean percentage of inhibition at 81%. The ethanolic extract followed with 78% inhibition, while the aqueous extract demonstrated 68% inhibition. These results indicate that the 1:2 dilution of the methanolic extract provides the most effective concentration among the tested samples for inhibiting and eradicating *P. aeruginosa* biofilm formation.

This finding is consistent with previous research. Alam et al. (2020) reported that a 1% methanolic extract of *B. ciliate* achieved more than 80% inhibition of *P. aeruginosa* biofilm formation. The similarity in biofilm inhibition percentages between the present study (81%) and the study by Alam et al. (>80%) suggests a consistent effect of methanolic extracts in *inhibiting P. aeruginosa* biofilm formation.

The similar inhibition percentages across these studies suggests a consistent antibiofilm activity of methanolic extracts against *P. aeruginosa.* Therefore, the demonstrated ability of methanolic extracts to inhibit biofilm formation presents a potential avenue for the development of alternative or supplementary strategies to mitigate *P. aeruginosa* infections.

Table 3

Mean Percentage of Biofilm Inhibition Against Candida albicans using Aqueous, Ethanolic, and Methanolic Extracts of Coconut Leaflets

Mean Percentage of Biofilm Inhibition (%)			
Dilution concentration	**Aqueous**	**Ethanol**	**Methanol**
1:2	**47.09**	**50.25**	**40.50**
1:4	22.19	16.68	2.22
1:8	16.74	2.09	1.56
1:16	15.85	3.55	10.18
1:32	20.92	16.55	12.11
1:64	8.23	30.08	20.99
1:128	18.68	29.82	22.26
1:256	10.37	12.97	32.67

Table 3 summarizes the percentage of biofilm inhibition in *Candida albicans* wells treated with three different coconut leaflet extracts. Similar to the results for *Staphylococcus aureus*, the 1:2 dilution consistently showed the highest inhibition across all extract types. The ethanolic extract achieved the greatest mean inhibition (50%), followed by the aqueous (47%)

and methanolic extracts (40%). This highlights the efficacy of the 1:2 dilution, particularly for ethanol, in inhibiting *C. albicans* biofilm formation.

These results suggest that ethanol is the most suitable solvent for biofilm inhibition assays targeting S. aureus and C. albicans, whereas methanol appears more effective for P. *aeruginosa*. This aligns with Duniq et al. (2020), who reported ethanol's efficiency in extracting antimicrobial compounds. Similarly, Bouali et al. (2024) reported antimicrobial activity of methanol extracts of *Plantago major* against both Gram-positive and Gram-negative bacteria.

Overall, ethanol-based extracts showed the highest inhibition for *S. aureus* and *C. albicans*, while methanol was most effective against *P. aeruginosa*, emphasizing the importance of solvent selection in biofilm inhibition studies.

Section 2. Optical Visualization of Biofilm Inhibition and Eradication of *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Candida albicans*

Table 4

Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans Under Microscope Subjected to Biofilm Inhibition by Ethanolic Extract of Coconut Leaflets

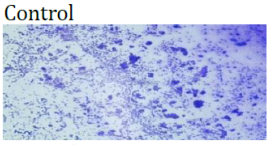
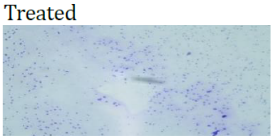
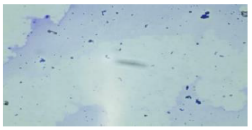
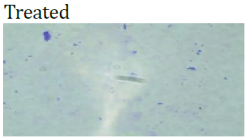
Visualized Biofilm Inhibition Under Microscope		
Microorganisms	Slides	Parameters
S. aureus	Control  Treated 	Control Bacterial cells: Present Microcolony/clusters: Present Treated Bacterial cells: Reduced Microcolony/clusters: Reduced Reduction in the coverage of biofilm formed: Evident
P. aerigunosa	Control  Treated 	Control Bacterial cells: Present Microcolony/clusters: Present Treated Bacterial cells: Reduced Microcolony/clusters: Reduced Reduction in the coverage of biofilm formed: Evident
C. albicans	Control  Treated 	Control Yeast cells: Present Pseudohyphae/true hyphae: Present Treated Yeast cells: Reduced Pseudohyphae/true hyphae: Absent Reduction in the coverage of biofilm formed: Evident

Table 2 presents the visualized biofilm inhibition activity of the ethanolic extract of coconut leaflets with a 1:2 dilution against the three microorganisms. Specifically, it presents the visualized control and treated wells of *S. aureus, P. aeruginosa, and C. albicans*. The photos were

selected from those taken during the three biofilm inhibition trials. The inhibition was evaluated by looking at the presence of major components of the biofilm, bacterial/yeast cells, microcolonies, and pseudohyphae/true hyphae, as noted by Gulati et al. (2016). Reduction in biofilm coverage is the direct comparison between the control and treated wells, indicating whether biofilm inhibition has occurred.

The MIC of the ethanolic extract of coconut leaflets can inhibit the biofilms of nosocomial-causing microorganisms, specifically *S. aureus, P. aeruginosa, and C. albicans.* The evident reduction in biofilm coverage across all tested microorganisms, as well as in bacterial cells and microcolonies, suggests the effective biofilm-inhibitory activity of the ethanolic extract of coconut leaflets.

Table 5

Staphylococcus aureus, Pseudomonas aeruginosa, and *Candida albicans Under Microscope Subjected to Biofilm Eradication by Ethanolic Extract of Coconut Leaflets*

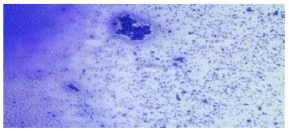
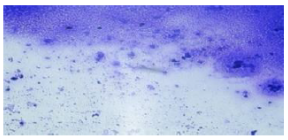
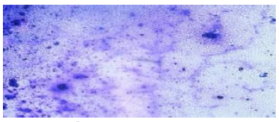
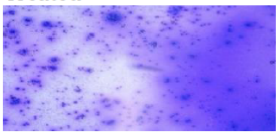
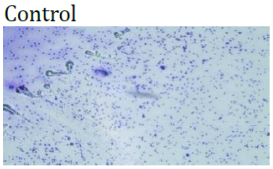
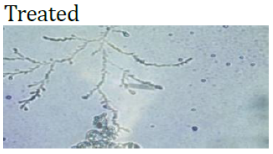
Visualized Biofilm Inhibition Under Microscope		
Microorganisms	Slides	Parameters
S. aureus	Control 	Control Bacterial cells: Present Microcolony/clusters: Present
	Treated 	Treated Bacterial cells: Present Microcolony/clusters: Present Reduction in the coverage of biofilm formed: Absent
P. aerigunosa	Control 	Control Bacterial cells: Present Microcolony/clusters: Present
	Treated 	Treated Bacterial cells: Present Microcolony/clusters: Present Reduction in the coverage of biofilm formed: Absent
C. albicans	Control 	Control Yeast cells: Present Pseudohyphae/true hyphae: Present
	Treated 	Treated Yeast cells: Present Pseudohyphae/true hyphae: Present Reduction in the coverage

Table 5 presents the visualized biofilm-eradication activity of the ethanolic extract of coconut leaflets at a 1:2 dilution against *S. aureus, P. aeruginosa, and C. albicans*. Bacterial cells and microcolonies were present in the visualized control and treated wells *of S. aureus and P. aeruginosa.* There was no reduction in biofilm coverage, further indicating that biofilm

eradication did not occur in the treated wells containing *S. aureus and P. aeruginosa*. Yeast cells and pseudohyphae/true hyphae were present in the visualized control and treated wells of *C. albicans*. There was no reduction in biofilm coverage, further indicating that biofilm eradication did not occur in the treated well containing *C. albicans*.

The results of biofilm eradication using the microtiter plate assay and visualization with crystal violet are consistent with those of Du et al. (2018). The study revealed that the concentration of epigallocatechin gallate (EGCC), a plant compound, inhibited biofilm formation by Listeria monocytogenes but could not eradicate the biofilm. According to Du et al. (2018), low concentrations of plant extract can inhibit biofilm formation, even if they are not bactericidal. According to Hall and Mah (2017), MIC is generally not enough to eradicate a mature biofilm. Even though MIC is effective at inhibiting biofilm formation in its initial stages, it is ineffective at eradicating preformed biofilm.

Hence, while the ethanolic extract of coconut leaflets demonstrated effective biofilm inhibition during the early stages of microbial development, it could not eradicate established biofilms of S*. aureus, P. aeruginosa, and C. albicans*. In terms of inhibition, the ethanolic extract of coconut leaflets successfully inhibited all the selected microorganisms. While in biofilm eradication, the ethanolic extract of coconut leaflets showed no antibiofilm activity.

CONCLUSIONS AND RECOMMENDATIONS

Conclusion

The study aimed to determine the antibiofilm activity of coconut leaflets against microorganisms causing nosocomial infections, namely Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans, using three solvents: aqueous, ethanol, and methanol. The study confirmed that ethanol is the best solvent for the crude extract of coconut leaflets among all solvents tested. The 1:2 dilution of the ethanolic extract effectively inhibits the growth of these microorganisms, suggesting its efficacy in biofilm inhibition. Although the coconut (*Cocos nucifera*) leaflet extract showed biofilm inhibition, complete biofilm eradication was not achieved at the tested concentration. This indicates that although the ethanolic extract of coconut leaflets demonstrates biofilm inhibition, further optimization is needed to maximize its efficacy in biofilm eradication. These findings demonstrate that coconut leaflets exhibit antibiofilm activity at a 1:2 ethanol dilution. This suggests its potential to be a biofilm inhibitor against nosocomial infection-causing microorganisms in the hospital setting.

Recommendations

1. While the current study demonstrates in vitro antibiofilm potential, future research should explore the efficacy of the coconut leaflet extract in in vivo models to assess its performance within a biological system and to evaluate potential toxicity.
2. To further evaluate the effectiveness of solvents in extraction, it is recommended to apply a comparative research approach. This would involve comparing different solvents under controlled conditions to determine their relative efficiencies.
3. Future research could explore the efficacy of sequential treatments, starting with the coconut leaflet extract followed by conventional antimicrobials, to see if this approach enhances biofilm eradication.
4. Investigate whether the coconut leaflet extract can enhance the susceptibility of the tested pathogens within biofilms to conventional antibiotics, potentially helping to combat antibiotic resistance.

REFERENCES

Aishwarya Vetrivel, Ramasamy, M., Preethi Vetrivel, Santhi Natchimuthu, Arunachalam, S., Kim, G.-S., & Murugesan, R. (2021). Pseudomonas aeruginosa biofilm formation and its control. *Biologics, 1*(3), 312–336. https://doi.org/10.3390/biologics1030019

Alam, K., Farraj, D. A. A., Mah-E-Fatima, S., Yameen, M. A., Elshikh, M. S., Alkufeidy, R. M., Mustafa,

A. E. M., Bhasme, P., Alshammari, M. K., Alkubaisi, N. A., Abbasi, A. M., & Naqvi, T. A. (2020). Anti-biofilm activity of plant derived extracts against infectious pathogen-*Pseudomonas aeruginosa* PAO1. *Journal of Infection and Public Health, 13*(11), 1734–1741. https://doi.org/10.1016/j.jiph.2020.07.007

B.

Ali, A., Zahra, A., Mohan K., H., F. M., T. A., Zubair, M., Roba Alatawy, A., M., & Noorani, M. S. (2023). Microbial biofilms: Applications, clinical consequences, and alternative therapies. *Microorganisms,11*(8),1934–1934. https://doi.org/10.3390/microorganisms11081934

Atriwal, T., Azeem, K., Husain, F. M., Hussain, A., Khan, M. N., Alajmi, M. F., & Abid, M. (2021). Mechanistic understanding of *Candida albicans* biofilm formation and approaches for its inhibition. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.638609

Bastos, C. A., Santos, E. M., Andrade, L. M., Rocha, D. D., Lins, R. A., Morais, D. O., ... & Silva, J. C. (2016). Qualitative assessment of the antimicrobial, antioxidant, phytochemical properties of the ethanolic extracts of the roots of *Cocos nucifera* L. *Pharmacophore*, *7*(3), 167–175.

Beveridge, F. C., Kalaipandian, S., Yang, C., & Adkins, S. W. (2022). Fruit biology of coconut (*Cocos nucifera L.*). *Plants, 11*(23), 3293. https://doi.org/10.3390/plants11233293

Chamás, J. M., Isla, M. I., & Zampini, I. C. (2023). Antibacterial and antibiofilm activity of different species of *Fabiana sp*. extract obtained via maceration and ultrasound-assisted extraction against staphylococcus epidermidis. *Plants, 12*(9),1830. https://doi.org/10.3390/plants12091830

Duniq, R., Sofya, F. C. M., & Kresnapati, K. (2020). Antibacterial activity test of ethanol extract from jackfruit leaves (*Artocarpus heterophyllus*) on Escherichia coli bacterial growth. *Jurnal USK*, *33*(1), 14-20.

Gonfa, T., Teketle, S., & Kiros, T. (2020). Effect of extraction solvent on qualitative and quantitative analysis of major phyto-constituents and in-vitro antioxidant activity evaluation of *Cadaba rotundifolia* leaf extracts. *Cogent Food & Agriculture, 6*(1), 1853867. https://doi.org/10.1080/23311932.2020.1853867

Gulati, M., & Nobile, C. J. (2016). *Candida albicans* biofilms: Development, regulation, and molecular mechanisms. *Microbes and Infection*, *18*(5), 310–321. https://doi.org/10.1016/j.micinf.2016.01.002

Hall, C. W., & Mah, T. (2017). Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiology Reviews*, *41*(3), 276–301. https://doi.org/10.1093/femsre/fux010

Han, A., Tsoi, J. K. H., Rodrigues, F. P., Leprince, J. G., & Palin, W. M. (2016). Bacterial adhesion mechanisms on dental implant surfaces and the influencing factors. *International Journal of Adhesion and Adhesives,* 69, 58–71. https://doi.org/10.1016/j.ijadhadh.2016.03.022

Haque, M., Massimo, S. , McKimm, J., & Bakar, M. A. (2018). Health care-associated infections – an overview. *Infection and Drug Resistance*, 11, 2321– 2333. https://doi.org/10.2147/idr.s177247

Idrees, M., Sawant, S., Nazira Karodia, & Rahman, A. (2021). *Staphylococcus aureus* Bbofilm: morphology, genetics, pathogenesis and treatment strategies. *International Journal of*

Environmental Research and Public Health, 18(14),7602–7602. https://doi.org/10.3390/ijerph18147602

Ignacio, I., & Miguel, T. (2021). Research opportunities on the coconut (*Cocos nucifera L.*) using new technologies. *South African Journal of Botany*, 141, 414–420. https://doi.org/10.1016/j.sajb.2021.05.030

Kalia, V. C., Patel, S. K., & Lee, J. (2023). Bacterial biofilm inhibitors: An overview. *Ecotoxicology and Environmental Safety*, 264, 115389. https://doi.org/10.1016/j.ecoenv.2023.115389

Lao, R. C. C., Yabes, A. M., Tobias-Altura, M., Panganiban, L. C. R., & Makalinao, I. R. (2022). In vitro antibacterial and antibiofilm activities of *Piper betel L*. ethanolic leaf extract on *Staphylococcus aureus*. *Acta Medica Philippina.* https://doi.org/10.47895/amp.vi0.6412

Li, X., Gu, N., Huang, T. Y., Zhong, F., & Peng, G. (2023). Pseudomonas aeruginosa: A typical biofilm forming pathogen and an emerging but underestimated pathogen in food processing. *Frontiers in Microbiology*, 13. https://doi.org/10.3389/fmicb.2022.1114199

Lima, E., Sousa, C., Meneses, L., Ximenes, N., Júnior, M. S., Vasconcelos, G., Lima, N., Patrocínio, M., Macedo, D., & Vasconcelos, S. (2015). *Cocos nucifera (L.) (Arecaceae*): A phytochemical and pharmacological review. *Brazilian Journal of Medical and Biological Research, 48*(11), 953–964. https://doi.org/10.1590/1414-431x20154773

Sharifa, A.A., Neoh, Y.L., Iswadi, M.I., Osman, K., Halim, M., Mohamed, J., Azman, A.B. & Hing, Hl. (2018). Effects of methanol, ethanol and aqueous extract of plantago major on gram positive bacteria, gram negative bacteria and yeast. *Ann Microsc.*, 8, 42-44.

Sharma, S., Mohler, J., Mahajan, S. D., Schwartz, S. A., Bruggemann, L., & Ravikumar Aalinkeel. (2023). Microbial biofilm: A review on formation, infection, antibiotic resistance, control measures, and innovative treatment. *Microorganisms, 11*(6), 1614–1614. https://doi.org/10.3390/microorganisms11061614

Sikora, A., & Zahra, F. (2023). *Nosocomial infections*. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK559312/

Sousa, S. N. B., Andrade, M. L. de, & Brito, R. D. von D. de. (2020). *Antibiofilm activity of natural products: promising strategies for combating microbial biofilms*. *4*(1). https://doi.org/10.36959/856/502

Usman, M., Yang, H., Wang, J., Tang, J., Zhang, L., & Wang, L. (2024). Formation, regulation, and eradication of bacterial biofilm in human infection. In *IntechOpen eBooks*. https://doi.org/10.5772/intechopen.114177

Uy, I. A., Maligaya, J. P. M., Quiblat, K. R. P., Demayo, C. R. S., & Demayo, C. G. (2019). Qualitative assessment of the antimicrobial, antioxidant, phytochemical properties of the ethanolic extracts of the roots of *Cocos nucifera* L. *Pharmacophore*, *10*(2), 63-69. https://doi.org/10.37024/pharmacophore.2019.10.2.63

Vani, S., Kayeen Vadakkan, & Mani, B. (2023). A narrative review on bacterial biofilm: its formation, clinical aspects, and inhibition strategies. *Future Journal of Pharmaceutical Sciences*, *9*(1). https://doi.org/10.1186/s43094-023-00499-9

Verderosa, A. D., Totsika, M., & Fairfull-Smith, K. E. (2019). Bacterial biofilm eradication agents: A current review. *Frontiers in Chemistry*, 7. https://doi.org/10.3389/fchem.2019.00824

Vetrivel, A., Ramasamy, M., Vetrivel, P., Natchimuthu, S., Arunachalam, S., Kim, G., & Murugesan, R. (2021). *Pseudomonas aeruginosa* biofilm formation and its control. *Biologics*, *1*(3), 312–336. https://doi.org/10.3390/biologics1030019

Wang, X., Liu, M., Yu, C., Li, J., & Zhou, X. (2023). Biofilm formation: Mechanistic insights and therapeutic targets. *Molecular Biomedicine,* *4*(1). https://doi.org/10.1186/s43556-023- 00164-w

World Health Organization. (2024). *Prevention of hospital-acquired infections: A practical guide*.Who.int. https://doi.org/WHO/CDS/CSR/EPH/2002.12