Pharmacoscript

Yasir et al./Pharmacoscript, Volume 8, No, 2, August 2025, 378-394

P-ISSN : 2622-4941 | E-ISSN : 2685-1121

IN VITRO AND MOLECULAR DOCKING ANALYSIS OF NUTMEG EXTRACT ANTIBACTERIAL ACTIVITY AGAINST *Propionibacterium acnes*

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Received: 26/01/2025, Revised: 14/05/2025, Accepted: 16/06/2025, Published: 31/08/2025

ABSTRACT

This study investigated the antibacterial activity of nutmeg (*Myristica fragrans* Houtt.) extract against *Propionibacterium acnes* through in vitro and molecular docking approaches. The nutmeg flesh was extracted using ultrasonication with 96% ethanol, yielding 8.21% extract. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, and steroids. The antibacterial activity was evaluated using the microdilution method, determining Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of 0.5% and 1%, respectively. Molecular docking analysis was performed to understand the interaction between nutmeg's bioactive compounds and three essential *Propionibacterium Acnes* proteins: Sialidase (7LBV), lipase (5H6G), and Penicillin-Binding Protein 2 (3UPO). Among the tested compounds, myristicin showed the strongest binding affinity with 7LBV (-6.8 kcal/mol), while lignan exhibited notable interactions with 3UPO (-6.6 kcal/mol) and 5H6G (-5.9 kcal/mol). The molecular interactions were primarily stabilized through hydrophobic interactions and hydrogen bonding with specific amino acid residues. These findings suggest that nutmeg extract possesses significant antibacterial activity against *Propionibacterium Acnes*, potentially mediated through multiple molecular targets, supporting its development as a natural anti-acne ingredient.

Keywords: *nutmeg extract, Propionibacterium acnes, molecular docking, antibacterial activity, bioactive compounds.*

INTRODUCTION

Antimicrobial resistance represents one of the greatest challenges in global health, particularly in treating bacterial infections (Uddin et al., 2021). Globally, acne vulgaris affects approximately 85% of adolescents and young adults aged 12-25 years, with an increasing prevalence of antimicrobial-resistant Propionibacterium Acnes strains reported in up to 50-60% of clinical isolates (Walsh et al., 2016). In dermatology, this resistance poses a serious problem in acne treatment, largely caused by Propionibacterium acnes (Dréno et al., 2018). The economic burden of acne treatment exceeds $3 billion annually in the United States alone, with treatment failures increasingly attributed to antibiotic resistance (Tan et al., 2018). This issue drives

the need for effective and safe alternative treatments

Propionibacterium acnes is a gram-positive bacterium that plays a crucial role in acne pathogenesis (Dréno et al., 2018). The use of topical and systemic antibiotics to control this bacterium's growth often comes with the risk of resistance development. Research shows that excessive antibiotic use can cause changes in skin microflora, which can subsequently worsen acne conditions (Gozali et al., 2023).

Nutmeg (*Myristica fragrans* Houtt.) has shown promising antimicrobial potential in various studies. Methanol extract of nutmeg demonstrated antibacterial activity against *Staphylococcus aureus* with a Minimum Inhibitory Concentration (MIC) of 2.5×10^{-2} mg/mL (Arhin et al., 2024). Other studies showed that ethyl acetate extract of nutmeg flesh significantly inhibited Gram-positive bacteria including *Streptococcus mutans* ATCC 25175, *Streptococcus mitis* ATCC 6249, and *Streptococcus salivarius* ATCC 13419 with MIC values of 0.625-1.25 mg/mL (Shafiei et al., 2012). Research on nutmeg also showed inhibition of *Staphylococcus aureus* growth with a MIC value of 8% (Nurhasanah, 2016). These promising antimicrobial activities warrant further investigation through comprehensive approaches, including analysis of molecular mechanisms.

To better understand the mechanism of action of nutmeg's active compounds, molecular docking has emerged as a valuable tool. This computational approach can predict binding interactions between nutmeg's active compounds and bacterial proteins, providing insights into potential antimicrobial mechanisms. Previous studies have utilized molecular docking to identify promising antibacterial compounds from natural sources, demonstrating the value of this in-silico approach in natural product research (Zhang et al., 2013).

Although various studies have examined nutmeg's antimicrobial activity against several pathogens, significant research gaps remain unaddressed. First, no previous studies have specifically determined the MIC and MBC values of nutmeg extract against Propionibacterium Acnes, despite its clinical relevance in acne treatment (Lee et al., 2019). Second, while antimicrobial properties have been observed empirically, the molecular mechanisms underlying these effects remain unexplored, particularly regarding interactions with specific Propionibacterium Acnes virulence proteins (Nakatsuji et al., 2011). Third, previous studies have largely focused on nutmeg seed rather than flesh extract,

overlooking a potentially valuable source of bioactive compounds (Gupta et al., 2013). This research aims to determine the MIC and MBC of nutmeg extract against Propionibacterium Acnes and investigate potential protein interactions through molecular docking as fundamental steps in developing anti-acne cosmetic active ingredients. The results are expected to provide scientific foundation for effective natural-based anti-acne cosmetic formulations.

METHODS

The methodology section consists of two parts: materials and procedures.

Materials

IKA RV10 Rotary Evaporator (IKA, Germany), Ultrasonikator (Ovan, Spain), Laminar air flow (Biobase, China), Inkubator (Biobase, China).

Technical ethanol (96%), HCl, Dragendorff's reagent, acetic acid, concentrated H_2SO_4, $FeCl_3$, distilled water, Mueller Hinton Broth (MHB) medium, Mueller Hinton Agar (MHA) medium, NaCl, and *Propionibacterium Acnes* ATCC 11827 culture were used. The nutmeg fruits were collected from Paya Village, Padang Cermin District, Pesawaran Regency, Lampung Province.

Procedures

1. Extraction of nutmeg

The extraction process was conducted using ultrasonication. One hundred grams of dried nutmeg flesh simplicia was immersed in 500 mL of 96% technical ethanol. The mixture was then sonicated for 30 minutes at 50°C with 40% amplitude, followed by filtration (Baihaqi et al., 2023). The filtrate was subsequently concentrated using an IKA RV10 Rotary Evaporator at 50°C, with a rotation speed of 100 rpm and pressure of 200 mbar (Yasir et al., 2021).

2. Phytochemical screening

Phytochemical identification was conducted through several stages. For alkaloid identification, the extract was combined with 2N HCl and distributed into several test tubes, then reacted with Dragendorff's reagent, where a positive result was indicated by an orange precipitate. Flavonoid identification was performed by dissolving the extract in 1 mL of 70% ethanol, followed by the addition of Mg powder and concentrated HCl, with positive results indicated by the formation of orange, red, or yellow coloration. Saponin identification involved adding 10 mL of hot water to the extract, cooling, and shaking for 10 seconds, where a positive result was demonstrated by the formation of 1-10 cm stable foam lasting >10 minutes that persisted after the addition of 2N HCl. For

terpenoid/steroid identification, the extract was shaken with ether, and the ether layer was spotted on a plate and dried. Two drops of acetic anhydride and one drop of concentrated H_2SO_4 were added, where orange/red/yellow coloration indicated positive terpenoid results, while green coloration indicated positive steroid results. Tannin identification was conducted by shaking the extract with hot water and adding $FeCl_3$, where positive results for pyrogallol tannins were indicated by blue-black coloration, while catechol tannins showed green or blue-green coloration with precipitation (Dubale et al., 2023; Safutri et al., 2022).

3. Determination of MIC

Equipment and materials were sterilized using an autoclave at 121°C for 20 minutes. Colonies of *Propionibacterium Acnes* incubated for 18-24 hours were suspended in physiological saline solution until reaching a density corresponding to McFarland standard 0.5, or measured using UV-Vis Spectrophotometry (λ=625 nm, A=0.08-0.13). The bacterial suspension (100µL) was added to MHB media (1.9 mL) (Gajic et al., 2022; Hulankova, 2024).

Mueller-Hinton Broth media was dispensed into a 96-well microplate (100µL/well), then inoculated with the microorganism suspension (10µL; 5x105 CFU/mL). The 1% extract solution (100 µL) was added to the first well containing MHB media (100 µL) and homogenized. Serial dilution was performed by transferring 100 µL of solution from the first well to the second well sequentially until the seventh well, producing concentrations from 1% to 0.015625%. The eighth well served as a negative control (media and microbe). Incubation was carried out at 35-37°C for 18-24 hours (Hulankova, 2024).

The Minimum Inhibitory Concentration (MIC) was determined through visual observation of microorganism growth after the incubation period. This value is defined as the lowest extract concentration at which no microorganism growth was observed.

4. Determination of MBC

Mueller Hinton Agar media preparation was conducted by dissolving 38 grams of MHA in 1 L of distilled water. The mixture was heated until completely dissolved, followed by sterilization using an autoclave at 121°C for 15 minutes. The sterile media was then poured into sterile petri dishes (15-20 mL) under aseptic conditions and allowed to solidify at room temperature.

The Minimum Inhibitory Concentration (MIC) value was determined through visual observation of microorganism

growth after the incubation period, indicated by the lowest extract concentration where no microorganism growth was observed.

5. Molecular Docking

Molecular docking was performed using autodocktools GPU Version. In this method, the target protein was modeled as rigid and the ligand as flexible according to optimal conformation at its binding site on the target protein (Aloliqi, 2024).

The tested ligand molecules consisted of myristicin, sabinene, α-pinene, β-pinene, and lignan, which have been reported to be identified from nutmeg flesh. Before in-silico processing, ligands were prepared using AutoDockTools, including conversion of 2D ligands to optimized 3D structures using Open Babel software, water molecule removal, hydrogen addition, gasteiger charge addition, non-polar hydrogen merging, and rotatable bonds configuration for flexibility. Ligand files were then saved in pdbqt format.

The target proteins used were 3 important proteins of *Propionibacterium Acnes* that play critical roles in pathogenicity and virulence: Penicillin-Binding Protein 2 (PDB: 3UPO) chain A, which is essential for cell wall synthesis and a primary target for beta-lactam antibiotics; Propionibacterium Acnes surface sialidase (PDB: 7LBV), which facilitates bacterial colonization by degrading host glycans and modulating immune responses (Sharma et al., 2019); and lipase (PDB 5H6G), a key enzyme that hydrolyzes sebum triglycerides into free fatty acids that contribute to inflammation and comedone formation (Lheure et al., 2016). These proteins were selected based on their established roles in Propionibacterium Acnes pathogenesis and their potential as antimicrobial targets. Protein structure preparation was conducted using AutoDockTools software.

Docking results were evaluated based on the lowest Gibbs free energy (ΔG) in conformations placing the ligand at the protein's active site. The most stable complexes were then mapped for amino acid interactions with their ligands using LigPlus.

RESULTS AND DISCUSSION

1. Extraction of nutmeg

The ultrasonic extraction of nutmeg flesh using 96% ethanol as a solvent yielded a dark red extract with a viscous consistency. From 100 grams of dried raw plant material, 8.21 grams of extract was obtained, resulting in a yield value of 8.21%. This yield is consistent with previously reported extraction efficiencies for nutmeg using ultrasonic-assisted extraction methods.

Table 1. Phytochemical constituent

Phytochemical Component	Test	Remark
Alkaloid	Dragendorff	+
Flavonoid	HCl	+
Tannin	$FeCl_3$	+
Steroid	Ether, Acetic Acid, $H2sO_4$	+
Saponin	Distilled Water	-

The obtained yield demonstrates the effectiveness of the extraction process in isolating bioactive compounds from the sample. The selection of 96% ethanol as the solvent was based on its capability to extract various compounds ranging from polar to semi-polar, as well as its relatively safe nature and ease of evaporation during the concentration process. The ultrasonication method was chosen for its tannin advantages in enhancing extraction efficiency through cavitation, which facilitates cell wall disruption and enables better solvent access to target compounds. The generated ultrasonic waves create rapidly expanding and compressing micro-bubbles, producing mechanical energy that effectively breaks down cell walls and improves solvent penetration.

These findings align with previous research conducted by Baihaqi et al. (2023), who reported that the ultrasonication method provides optimal yield in nutmeg flesh extraction. The resulting dark red color indicates the presence of extracted phenolic and flavonoid compounds, while the viscous consistency confirms the successful concentration process using the rotary evaporator.

2. Phytochemical screening

The phytochemical screening revealed that the nutmeg flesh extract contains several secondary metabolites, including alkaloids, flavonoids, tannins, and steroids, while saponins were not detected (Table 1). The presence of alkaloids was confirmed by precipitate formation upon addition of all three reagents (Mayer, Wagner, and Dragendorff), indicating interactions between nitrogen in alkaloids and metal ions from the reagents. This precipitation occurs through the formation of coordinate covalent bonds between the reagents' metal ions and the free electron pairs on alkaloid nitrogen atoms. The precipitate intensity suggests a significant alkaloid concentration in the extract.

Flavonoid content was identified through the formation of red coloration after the addition of Mg powder and HCl, demonstrating the reduction of the benzopyrone nucleus in the flavonoid structure. This reaction involves complex formation between flavonoids and magnesium, where the resulting color intensity correlates with the sample's

flavonoid concentration. The presence of flavonoids in the extract suggests potential antioxidant and antimicrobial activities, considering their role as defense compounds in plants.

The tannin test yielded positive results with the formation of a greenish-black color after FeCl3 addition, indicating complex formation between tannins and Fe3+ ions. The color intensity suggests a relatively high tannin content in the extract. Tannins are known to inhibit microorganism growth through various mechanisms, including protein denaturation and cell membrane disruption.

Steroids were identified through the formation of green coloration, resulting from dehydration and conjugated double-bond formation. This reaction is specific to steroid structures containing cyclopentane perhydrophenanthrene rings. The non-detection of saponins in the extract may be attributed to several factors, including insufficient concentration or limitations of the extraction method used.

The obtained phytochemical profile shows similarities with research conducted by Orabi, although differences exist in saponin content. These variations may be due to various factors such as geographical growing conditions, harvest time, extraction methods, or plant parts used. The presence of these various secondary metabolites potentially provides synergistic effects in the extract's antibacterial activity (Orabi et al., 2022).

3. Antibacterial Activity

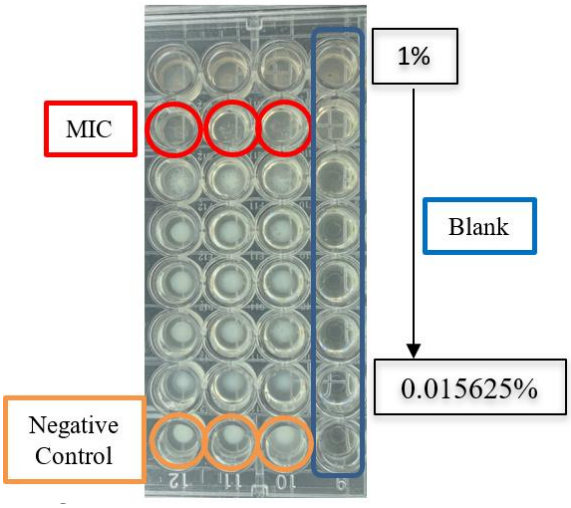


Figure 1. Minimum inhibitory concentration assay

The determination of antibacterial activity of nutmeg flesh extract against *Propionibacterium Acnes* was conducted using the microdilution method to establish MIC values, followed by MBC determination. The microdilution method was selected based on its advantages in providing more accurate results while utilizing smaller sample quantities compared to diffusion methods. The observational results revealed a MIC value of 0.5%, which was evident in well 2 as shown in **Figure 1**, where the solution appeared clear compared to the negative control that exhibited turbidity due to bacterial growth. The clarity

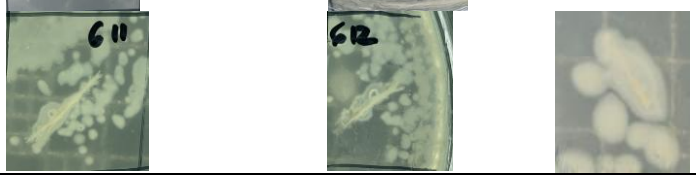
of the solution at this concentration indicates the extract's capability to inhibit *Propionibacterium Acnes* growth.

Compared with previous research, Arhin et al. (2024) reported that the antibacterial activity of nutmeg methanol extract against S. aureus yielded a MIC of 2.5 × 10^{-2} mg/mL. Although different bacterial strains were used, both studies confirm the effectiveness of nutmeg extract in inhibiting gram-positive bacterial growth. However, the antibacterial activity of nutmeg tested by Nurhasanah (2016) against S. aureus produced a MIC of 8%, a higher value compared to the previously described research results. These differences may be attributed to variations in extraction methods, plant parts used, and environmental factors affecting the sample's phytochemical profile.

The antibacterial activity of nutmeg extract can be explained through the mechanisms of action of each identified compound group. Alkaloids can inhibit bacterial cell wall synthesis, alter cell membrane permeability, inhibit bacterial metabolism, and interfere with nucleic acid and protein synthesis (Yan et al., 2021). Flavonoids work by denaturing proteins and bacterial cell phospholipid bilayers, causing irreversible damage to cell membranes (Yuan et al., 2021). Tannins can inhibit chitin synthesis required for bacterial cell wall formation and form complexes with proteins that can inactivate bacterial adhesins. Steroids can interact with cell phospholipid membranes that are permeable to lipophilic compounds, leading to decreased membrane integrity and altered cell membrane morphology, resulting in cell fragility and lysis (Farha et al., 2020).

The determination of MBC value yielded a concentration of 1% as the lowest concentration capable of killing bacteria, as evidenced by the absence of colony growth on MHA media after 24 hours of incubation (Table 2).

Table 2. Minimum Bactericidal Concentration (MBC) assay

Concentration	Picture
1%	
0.5%	

The difference between MIC and MBC values indicates that the extract exhibits bacteriostatic properties at lower concentrations and bactericidal effects at higher concentrations. These findings demonstrate promising potential compared to Nurhasanah's research, which reported a MIC value of 8% for nutmeg extract against Staphylococcus aureus (Nurhasanah, 2016). This potent antibacterial activity likely results from the synergistic effects of various secondary metabolites present in the extract, where each compound can work through different mechanisms to inhibit bacterial growth.

This research confirms the potential of nutmeg flesh extract as a natural antibacterial agent against *Propionibacterium Acnes*. The combination of effective extraction methods and the presence of various bioactive secondary metabolites contributes to the observed antibacterial activity. Further research is needed to isolate and identify specific compounds responsible for the antibacterial activity, as well as to optimize formulations for the development of effective and safe antiacne products.

14. Molecular docking

The molecular docking analysis revealed varying binding affinities between nutmeg metabolites and *Propionibacterium Acnes* target proteins, as shown in Table 3. Among the five metabolites examined, myristicin demonstrated the strongest binding affinity with sialidase (7LBV) at -6.8 kcal/mol, while lignan exhibited notable interactions with PBP2 (3UPO) at -6.6 kcal/mol and lipase (5H6G) at -5.9 kcal/mol. Both alpha- and beta-pinene showed moderate binding affinities across all three proteins, ranging from -5.1 to -6.5 kcal/mol, whereas sabinene consistently displayed the weakest interactions, with binding affinities between -4.8 and -6.4 kcal/mol.

The strong interaction between myristicin and sialidase is particularly significant as sialidase serves as a critical virulence factor in *Propionibacterium Acnes* pathogenesis (Liu et al., 2015). Sialidase catalyzes the removal of terminal sialic acid residues from host glycoproteins and glycolipids, which facilitates bacterial adhesion to host cells by exposing underlying receptor sites (Aloliqi, 2024). This enzyme plays a crucial role in enabling *Propionibacterium Acnes* to establish colonization within the pilosebaceous unit, leading to biofilm formation that enhances bacterial persistence and resistance to conventional antibiotics (Liu et al., 2015). Furthermore, sialidase activity contributes to *Propionibacterium Acnes* invasion by degrading protective mucous layers and disrupting host cell barriers, allowing for

deeper tissue penetration (Aloliqi, 2024). The subsequent inflammatory response is amplified as *Propionibacterium Acnes*-derived sialidase modifies host immune recognition molecules, potentially dysregulating immune signaling pathways and contributing to the characteristic inflammatory lesions of acne vulgaris (Liu et al., 2015).

Table 3. Binding affinities of nutmeg metabolites with *Propionibacterium acnes* target proteins

Metabolite	Affinity (kcal/mol)		
	7LBV	5H6G	3UPO
Alpha – Pinene	-6.2	-5.5	-5.4
Beta – Pinene	-6.5	-5.5	-5.1
Lignan	-6.5	-5.9	-6.6
Myristicin	-6.8	-5.8	-5.9
Sabinene	-6.4	-4.8	-5.0

These results suggest that myristicin and lignan may be the primary contributors to the antibacterial activity of nutmeg extract against *Propionibacterium Acnes* through their interactions with multiple bacterial protein targets. The strong binding affinity observed between myristicin and sialidase (-6.8 kcal/mol) indicates this nutmeg metabolite may effectively inhibit sialidase activity, thereby disrupting multiple aspects of *Propionibacterium Acnes* pathogenesis - from initial adhesion and colonization to immune evasion and inflammation induction (Aloliqi, 2024). This interaction provides a molecular basis for the therapeutic potential of myristicin against *Propionibacterium Acnes*-associated conditions.

Based on the molecular interaction analysis between Myristicin ligand and the 7LBV protein receptor, as shown in Figure 2 (A), dominant hydrophobic interactions were observed without hydrogen bonding. The predominance of hydrophobic interactions is particularly significant because it indicates a favorable binding modality that contributes to myristicin's high affinity for the sialidase active site. These interactions, involving amino acid residues including Glu318(A), Ile264(A), Arg369(A), Ala265(A), Val190(A), Gly263(A), Leu90(A), Pro91(A), Pro372(A), and Val428(A), provide a stable binding conformation that may effectively block substrate access to the enzyme's catalytic pocket.

The strength of these hydrophobic interactions explains the superior binding energy observed for myristicin compared to other tested compounds, suggesting both high affinity and potential selectivity for sialidase. Research has shown that hydrophobic interactions are generally enriched in high-efficiency ligands and make significant contributions to protein-ligand binding stability (Patil et al., 2010). The hydrophobic nature of myristicin's interaction with sialidase is particularly important because it allows the compound to

penetrate and remain stable within the hydrophobic binding pocket of the enzyme, effectively blocking its catalytic activity.

This selective inhibition of sialidase by myristicin may translate to targeted antimicrobial activity against Propionibacterium Acnes with minimal disruption to other microbial species or host tissues, representing a significant advantage over broad-spectrum antibiotics currently used in acne treatment. Furthermore, since the sialidase enzyme has been validated as a key virulence factor in Propionibacterium Acnes pathogenesis and has even been targeted in experimental vaccines against acne vulgaris (Nakatsuji et al., 2008), compounds like myristicin that can effectively inhibit this enzyme through stable hydrophobic interactions offer promising potential as novel therapeutic agents for acne and other Propionibacterium Acnes-associated conditions.

These interactions differ from reference studies where xanthommatin forms hydrogen bonds with Pro195, Asp167, Gly66, and Met165, as well as hydrophobic interactions with His24, Leu268, Lys88, Trp89, His91, Pro252, Val320, and Pro321 in the protein binding pocket (Vaithyanathan, 2024).

The molecular interactions of the lignan complex with the 3UPO protein receptor demonstrate interaction patterns similar to the reference SAB-PBP2 study, as shown in **Figure 2 (B)**. Lignan forms a hydrogen bond with the Gly472(A) residue at a distance of 3.25 Å, which plays a crucial role in binding stability. This complex structure is further reinforced by hydrophobic interactions involving several amino acid residues such as Threonine (425, 427, 470), Asparagine 283, Tyrosine (261, 155), Serine (281, 222), Glycine 426, Lysine 280, and Valine 409. This pattern is similar to the SAB-PBP2 complex, which is also stabilized by hydrogen bonds and hydrophobic interactions. The difference lies in the specific residues involved, where SAB interacts with Val 250, Lys 251, Thr 396 for SA and Asn 254, Glu 400 for SB, along with several additional hydrophobic interactions. The combination of these bonds results in a stable conformation between lignan and the 3UPO protein within the binding pocket (Wei et al., 2021).

In the interaction between the Lignan ligand and the 5H6G protein receptor (lipase) shown in **Figure 2 (C)**, hydrogen bonds form between the ligand and residues Arg207(A), His244(B), and Arg56(B) with a bond distance of 3.12 Å, contributing to binding specificity. This complex is further stabilized by hydrophobic interactions involving

residues Thr256(B), Ala247(B), Asp251(B), Arg254(B), His28(B), and Tyr58(B).

This pattern differs from the reference SAB-lipase study, where SA demonstrates more extensive bonding through hydrogen bonds with Lys88, Thr90, Asp167, Glu322, His193, Leu319, Asp320, and hydrophobic interactions with Phe119 and Arg323. In contrast, SB exhibits fewer bonds, interacting with Ala116, Ala117 (hydrogen bonds) and His91, Lys88, and Arg323 (hydrophobic interactions). Although there are differences in the involved residues, both complexes rely on a combination of hydrogen bonds and hydrophobic interactions to achieve binding stability within the protein binding pocket (Wei et al., 2022).

The molecular docking results identify myristicin as the most promising compound against Propionibacterium Acnes, exhibiting the strongest binding affinity (-6.8 kcal/mol) with sialidase through stable hydrophobic interactions. These computational findings align with the in vitro results showing potent antibacterial activity of nutmeg extract (MIC 0.5%, MBC 1%). The synergistic effect observed in vitro correlates with our docking analysis, where multiple compounds in nutmeg interact with different bacterial targets—myristicin with sialidase and lignan with PBP2 and lipase. This strong computational-experimental correlation confirms that nutmeg's anti-Propionibacterium Acnes activity likely occurs through inhibition of key bacterial enzymes, particularly myristicin's targeted inhibition of sialidase, supporting its potential development as a natural anti-acne agent.

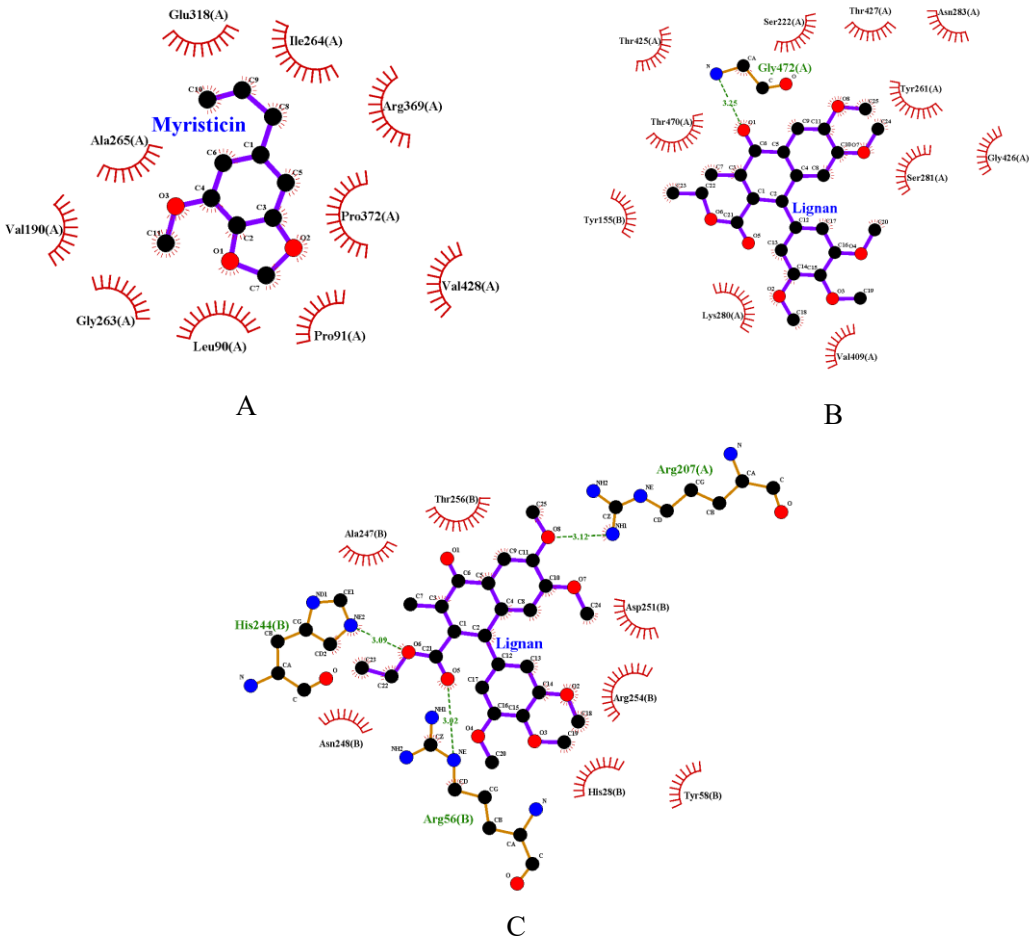


Figure 2. Molecular Interactions of Nutmeg Bioactive Compounds with *Propionibacterium Acnes* Target Proteins: (A) Myristicin-Sialidase (7LBV) Complex, (B) Lignan-PBP2 (3UPO) Complex, and (C) Lignan-Lipase (5H6G) Complex

CONCLUSIONS

This study has successfully demonstrated the antibacterial potential of nutmeg extract against *Propionibacterium Acnes* through both in vitro and molecular approaches. The extract showed significant antibacterial activity with MIC and MBC values of 0.5% and 1%, respectively, which can be attributed to the presence of bioactive compounds including alkaloids, flavonoids, tannins, and steroids. Molecular docking analysis revealed that myristicin and lignan exhibit strong binding affinities with key *Propionibacterium Acnes* proteins, particularly through hydrophobic interactions and hydrogen bonding. The strongest interaction was observed between myristicin and sialase (7LBV) with a binding affinity of -6.8 kcal/mol, followed by lignan's interaction with PBP2 (3UPO) at -6.6

kcal/mol. These findings provide a scientific foundation for the potential development of nutmeg extract as a natural anti-acne ingredient, though further studies on formulation optimization and clinical efficacy are warranted.

REFERENCES

Aloliqi, A. A. (2024). Towards identification of therapeutics against multi-infections and cancers causing *Propionibacterium acnes* : Molecular modeling and dynamics simulation investigation. Journal of Molecular Liquids, 415, 126373. https://doi.org/10.1016/j.molliq.2024.126373

Arhin, R. E., Gordon, A., Nuhu, Y., Boakye, E. E., Owusu, I., Oppong-Mensah, J., & Alhassan, S. (2024). Physicochemical and Antibacterial Properties of *Myristica fragrans* and *Syzygium aromaticum* Methanolic Extract Soap Formulations. Journal of Chemistry, 2024(1), 2457296. https://doi.org/10.1155/2024/2457296

Baihaqi, B., Nuraida, N., Fridayati, D., & Adam, K. (2023). Extraction of Nutmeg Oleoresin Using UAE (Ultrasound Assisted Extraction) Method. Jurnal Sains Pertanian (JSP), 7, 42–45. https://doi.org/10.51179/jsp.v7i2.1995

Dréno, B., Pécastaings, S., Corvec, S., Veraldi, S., Khammari, A., & Roques, C. (2018). *Cutibacterium acnes* (*Propionibacterium acnes*) and acne vulgaris: A brief look at the latest updates. Journal of the European Academy of Dermatology and Venereology, 32, 5–14. https://doi.org/10.1111/JDV.15043

Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N., & Suleman, S. (2023). Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. Journal of Experimental Pharmacology, 15, 51. https://doi.org/10.2147/JEP.S379805

Farha, A. K., Yang, Q.-Q., Kim, G., Li, H.-B., Zhu, F., Liu, H.-Y., Gan, R.-Y., & Corke, H. (2020). Tannins as an alternative to antibiotics. Food Bioscience, 38, 100751. https://doi.org/10.1016/j.fbio.2020.100751

Gajic, I., Kabic, J., Kekic, D., Jovicevic, M., Milenkovic, M., Mitic Culafic, D., Trudic, A., Ranin, L., & Opavski, N. (2022). Antimicrobial Susceptibility Testing: A Comprehensive Review of Currently Used Methods. Antibiotics,

11(4), 427. https://doi.org/10.3390/antibiotics11040427

Gozali, M. M., Kurniawati, Y., Devi, M., Bahar, E., Dewi, L., Yahya, Y. F., Rusmawardiana, Zulkarnain, S. D., & Kartowigno, S. (2023). Correlation between Antimicrobial Resistant *Propionibacterium acnes* and Severity of Acne Vulgaris: Findings from a Tertiary Hospital. Indian Journal of Dermatology, 68(4), 488. https://doi.org/10.4103/ijd.ijd_623_22

Gupta, A. D., Bansal, V. K., Babu, V., & Maithil, N. (2013). Chemistry, antioxidant and antimicrobial potential of nutmeg (*Myristica fragrans* Houtt). Journal of Genetic Engineering and Biotechnology, 11(1), 25-31. https://doi.org/10.1016/j.jgeb.2012.12.001

Hulankova, R. (2024). Methods for Determination of Antimicrobial Activity of Essential Oils In Vitro—A Review. Plants, 13(19), Article 19. https://doi.org/10.3390/plants13192784

Lee, Y. B., Byun, E. J., & Kim, H. S. (2019). Potential role of the microbiome in acne: A comprehensive review. Journal of Clinical Medicine, 8(7), 987. https://doi.org/10.3390/jcm8070987

Liu, P.F., Hsieh, Y.D., Lin, Y.C., Two, A., Shu, C.W. and Huang, C.M., 2015. Propionibacterium acnes in the pathogenesis and immunotherapy of acne vulgaris. Current Drug Metabolism, 16(4), pp.245-254. doi: 10.2174/1389200216666150812124801.

Lheure, C., Grange, P. A., Ollagnier, G., Morand, P., Désiré, N., Sayon, S., Corvec, S., Raingeaud, J., Marcelin, A. G., Calvez, V., Galliot, F., Dupin, N., & Khammari, A. (2016). TLR-2 recognizes *Propionibacterium acnes* CAMP factor 1 from highly inflammatory strains. PLoS ONE, 11(11), e0167237. https://doi.org/10.1371/journal.pone.0167237

Nakatsuji, T., Tang, D. C., Zhang, L., Gallo, R. L., & Huang, C. M. (2011). *Propionibacterium acnes* CAMP factor and host acid sphingomyelinase contribute to bacterial virulence: Potential targets for inflammatory acne treatment. PLoS ONE, 6(4), e14797. https://doi.org/10.1371/journal.pone.0014797

Nurhasanah, N. (2016). Antimicrobial Activity Of Nutmeg (*Myristica fragrans* Houtt) Fruit Methanol Extract Against Growth *Staphylococcus*

aureus and *Escherichia coli*. Jurnal Bioedukasi, 2(1). https://doi.org/10.33387/BIOEDU.V2I1.61

Orabi, M., Abdulsattar, J. O., & Nasi, Z. O. (2022). Phytochemical Profile, Antimicrobial, Antioxidant Activity and Cyclooxygenase 2 Inhibitory Properties of Nutmeg (*Myristica Fragrans*) Seeds Extract. Egyptian Journal of Chemistry, 65(1), 317–326. https://doi.org/10.21608/ejchem.2021.78192.3831

Patil, R., Das, S., Stanley, A., Yadav, L., Sudhakar, A. and Varma, A.K., 2010. Optimized hydrophobic interactions and hydrogen bonding at the target-ligand interface leads the pathways of drug-designing. PloS One, 5(8), p.e12029. https://doi.org/10.1371/journal.pone.0012029

Safutri, W., Karim, D. D. A., & Fevinia, M. (2022). Phytochemical Screening of Medicinal Plant Materials in Pringsewu Regency. Journal Pharmacy Aisyah, 1(1), Article 1.

Sharma, D., Misba, L., & Khan, A. U. (2019). Structure, function and inhibition of bacterial sialidases: A comprehensive insight. MedChemComm, 10(12), 1983-2003. https://doi.org/10.1039/c9md00206e

Shafiei, Z., Shuhairi, N. N., Yap, N. M. F. S., Sibungkil, C. A. H., & Latip, J. (2012). Antibacterial Activity of *Myristica fragrans* against Oral Pathogens. Evidence-Based Complementary and Alternative Medicine : eCAM, 2012, 825362. https://doi.org/10.1155/2012/825362

Tan, A. U., Schlosser, B. J., & Paller, A. S. (2018). A review of diagnosis and treatment of acne in adult female patients. International Journal of Women's Dermatology, 4(2), 56-71. https://doi.org/10.1016/j.ijwd.2017.10.006

Uddin, T. M., Chakraborty, A. J., Khusro, A., Zidan, B. R. M., Mitra, S., Emran, T. B., Dhama, K., Ripon, M. K. H., Gajdács, M., Sahibzada, M. U. K., Hossain, M. J., & Koirala, N. (2021). Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. Journal of Infection and Public Health, 14(12), 1750–1766. https://doi.org/10.1016/J.JIPH.2021.10.020

Vaithyanathan, P. (2024). Anti-acne Property of Octopus Skin Pigment, Xanthommatin An Insilico Evaluation.

In Review. https://doi.org/10.21203/rs.3.rs-5535357/v1

Wei, M., Yu, H., Guo, Y., Cheng, Y., Xie, Y., & Yao, W. (2021). Potent in vitro synergistic antibacterial activity of natural amphiphilic Sapindoside A and B against *Cutibacterium acnes* with destructive effect on bacterial membrane. Biochimica et Biophysica Acta (BBA) - Biomembranes, 1863(11), 183699. https://doi.org/10.1016/j.bbamem.2021.183699

Wei, M., Yu, H., Guo, Y., Cheng, Y., Xie, Y., & Yao, W. (2022). Synergistic combination of Sapindoside A and B: A novel antibiofilm agent against *Cutibacterium acnes*. Microbiological Research, 254, 126912. https://doi.org/10.1016/j.micres.2021.126912

Yan, Y., Li, X., Zhang, C., Lv, L., Gao, B., & Li, M. (2021). Research Progress on Antibacterial Activities and Mechanisms of Natural Alkaloids: A Review. Antibiotics, 10(3), 318. https://doi.org/10.3390/antibiotics10030318

Yasir, A. S., Marcellia, S., Wijaya, L. B., & Putri, T. R. (2021). Formulation and Activity Testing of Combination Gel of Aloe Vera Leaf Ethanol Extract (*Aloe vera*) and Basil Leaf (*Ocimum sanctum* L.) as Anti-Acne Agent Against Staphylococcus epidermidis Bacteria. Pharmacoscript, 4(1), 70–86.

Yuan, G., Guan, Y., Yi, H., Lai, S., Sun, Y., & Cao, S. (2021). Antibacterial activity and mechanism of plant flavonoids to gram-positive bacteria predicted from their lipophilicities. Scientific Reports, 11(1), 10471. https://doi.org/10.1038/s41598-021-90035-7

Zhang, A., Mu, H., Zhang, W., Cui, G., Zhu, J., & Duan, J. (2013). Chitosan coupling makes microbial biofilms susceptible to antibiotics. Scientific Reports, 3, 1–7. https://doi.org/10.1038/srep03364