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Article

Phytochemical Screening and Anti-Acne Potential of Neem (*Azadirachta indica*) Leaf Extracts Using Solvents of Different Polarities

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Abstract. Neem leaves (*Azadirachta indica*) are a potential source of natural antibacterial agents against *Propionibacterium acnes,* a primary pathogen in acne vulgaris. This study investigated the effects of extraction solvent polarity on the phytochemical composition and antibacterial efficacy of *A. indica* leaf extracts against *P. acnes* ATCC 6916. Six extracts were prepared using a polarity gradient: chloroform, ethyl acetate, ethanol p.a., 70% ethanol, 50% ethanol, and aqueous. Extraction yields ranged from 6.1% to 24%, with aqueous extract yielding the highest and chloroform the lowest. Qualitative phytochemical screening revealed phenolics, flavonoids, and steroids across all extracts, whereas triterpenoids were selectively concentrated in chloroform, ethyl acetate, and ethanol p.a. extracts. Antibacterial assays demonstrated inhibition zones from 10.18 to 15.15 mm, compared to 24.48 mm for clindamycin (0.25 mg/mL). Chloroform extract exhibited a significantly higher inhibition zone than all other extracts ($p < 0.05$). However, chloroform and ethyl acetate extracts displayed identical minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) values of 1.562 mg/mL and 3.125 mg/mL, respectively. Overall, solvent polarity significantly influences extraction yield, phytochemical profile, and antibacterial potency. High potency of nonpolar and semipolar extracts indicates that active anti-*P. acnes* constituents in neem leaves are predominantly nonpolar to semipolar.

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1. Introduction

Acne vulgaris represents one of the most prevalent dermatological conditions affecting the face, marked by persistent inflammatory processes within the pilosebaceous unit. This condition presents with various types of lesions, such as comedones, papules, pustules, nodules and cysts [1]. The development of acne is multifactorial and influenced by various factors, such as genetics, stress, dietary patterns, increased sebum production, follicular hyper keratinization, and the involvement of microorganisms that can exacerbate the inflammatory response. One bacterium involved in the pathogenesis of acne is *Propionibacterium acnes* (currently classified as *Cutibacterium acnes*). This bacterium produces the enzyme lipase, which hydrolyzes triglycerides in sebum into free fatty acids. The accumulation of free fatty acids can induce an inflammatory response in the skin tissue, thereby contributing to the formation of acne lesions [2].

Acne can be treated with antibiotics, either topically or systemically, with *P. acnes* as one of the targets. However, repeated and inappropriate use of antibiotics can increase the risk of antimicrobial resistance. This condition can reduce the effectiveness of therapy and poses a significant challenge in acne management. Therefore, it is necessary to explore natural antibacterial sources that have the potential to be developed as alternatives or adjuncts to acne therapy [3-4].

One plant with potential as a source of bioactive compounds is the neem leaf (*Azadirachta indica*). Neem leaves are known to contain various secondary metabolites, including limonoids, flavonoids, phenolic compounds, tannins, saponins, alkaloids, and terpenoids. Several bioactive compounds found in neem, such as azadirachtin and nimbin, are known to possess various biological activities [5]. The presence and interactions of these secondary metabolites may contribute to the biological activity of neem leaf extract, which holds potential for further development in antibacterial research.

The content of secondary metabolites in neem leaves can be influenced by the extraction process, particularly the type of solvent used. Differences in solvent polarity can result in varying abilities to dissolve and extract specific groups of compounds from the plant matrix. This aligns with the "like dissolves like" principle, whereby compounds with relatively similar polarity characteristics tend to dissolve more easily in certain solvents. Consequently, the use of solvents with different levels of polarity can yield extracts with distinct metabolite profiles and biological activities [6].

Several studies have reported that differences in extraction solvents can affect the antibacterial activity. A study by [7] demonstrated that neem leaf extracts obtained using petroleum ether, dichloromethane, chloroform, ethanol, and water exhibited the growth of the test bacteria, with varying degrees of inhibition depending on the extraction solvent used. Among these, only the dichloromethane, ethanol, and water extracts exhibited inhibitory activity against *Escherichia coli*, *Pseudomonas denitrificans*, *Micrococcus glutamicus*, *Lactobacillus bulgaris*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Bacillus stearothermophilus*, *S. pyogenes*, *Micrococcus luteus*, and *Bacillus cereus*. This indicates that the choice of solvent strongly influences the extracted compounds and their resulting biological efficacy. However, to date, no studies have comprehensively reported the specific effect of extracting solvents across a gradient of polarities-namely chloroform, ethyl acetate, ethanol p.a., 70% ethanol, 50% ethanol, and water on the extract yield, and antibacterial activity of neem leaf (*Azadirachta indica*) against *P. acnes*. The results are expected to provide key insight into the relationship between solvent polarity, the extracted metabolites classes, and their targeted antibacterial potency.

2. Experimental Section

2.1. Materials

In this study, the materials used were Mueller Hinton Agar (Merck), Mueller Hinton Broth (Merck), test bacteria *Propionibacterium acnes* ATCC-6916, absolute ethanol (Merck), 70% ethanol, 50% ethanol, ethyl acetate (Merck), chloroform (Merck), distilled water, clindamycin, HCl 32%, Mg powder (Merck), H_2SO_4 2N, $FeCl_3$ 10%. The tools used are: Erlenmeyer flasks, volumetric flasks, petri dishes (90 × 20 mm diameter), sterile 96-well microplates, dropper pipettes, beakers, glass funnels,

inoculation loops, cork borers, Eppendorf tubes, magnetic stirrers, micropipettes, calipers (Mitutoyo), 1 mL and 20 mL syringes, analytical balance (eaADAM), oven (Philip Harris Ltd.), incubator (Froilabo), autoclave (GEA), laminar air flow cabinet (Telstar AV-100), UV-Vis spectrophotometer (Agilent Technology Cary 60 UV-Vis), and freeze dryer (Zirbus technology).

2.2. Sample Preparation and Extraction

Fresh leaves of *A. indica* (neem) were collected from the Universitas Bengkulu campus and taxonomically authenticated at the Biology Laboratory, Faculty of Mathematics and Natural Sciences. Voucher specimens (MAAI-2025) were deposited at the Organic Chemistry Laboratory of the same institution. The leaves ware fist cleaned to remove any adhering impurities and then cut into smaller pieces. The samples were subsequently dried naturally in a shaded and well ventilated are without exposure to direct sunlight. After the drying process was completed, the leaves ware ground into powder using a blender and sieved through a 50-mesh sieve to obtain a uniform and fine leaf powder.

A. indica leaf powder ware extracted using a combination by ultrasound-assisted extraction (UAE) and cold maceration. For each extraction, 10 g of the powdered simplicial was immersed in 100 mL of solvent (1:10 *w/v* ratio) using chloroform ($CHCl_3$), followed by sonication at 45°C for 30 minutes, and then macerated for a further 24 hours. This combined sonication and maceration process was repeated four times (re-sonication and re-maceration) to ensure optimal extraction. Other extracting solvents included ethyl acetate (EtOAc) and different ethanol concentrations, including EtOH p.a., 70% EtOH, and 50% EtOH using a similar method to that of the chloroform ($CHCl_3$) extraction.

For the aqueous extraction, a decoction was prepared using distilled water at 90°C under constant stirring for 30 min per cycle. The extraction process was repeated four times until the resulting aqueous solvent became colorless. All resulting extracts ware pooled, and the solvents ware evaporated using a rotary evaporator at temperatures between 45 – 60 °C, yielding a viscous paste. This concentrated extract was further processed via freeze-drying to eliminate residual water [8].

2.3. Qualitative Phytochemical Screening

2.3.1. Flavonoid Test

An amount of 0.5 mL of extract solution was added to two test tubes (labeled as test and blank). To the test tube, 50 mg of magnesium powder and 2-3 drops of concentrated HCl were added until gets bubbles formed. The appearance of red, orange, or magenta color in test tube (compared to the blank) indicates the presence of flavonoid compounds [9].

2.3.2. Steroid and Triterpenoid Test

An amount of 0.5 mL of extract solution was added to two test tubes (labeled as test and blank). Add Two drops of concreted H_2SO_4 to the test tube, followed by two drops of anhydrous acetic acid. The color change was then observed by comparing the test tube with the blank tube. The presence of steroid compounds was indicated by a color change to blue-green, whereas triterpenoids were indicated by a red, orange, or brown colors [10].

2.3.3. Phenolic Test

An amount of 0.5 mL of extract solution was added to two test tubes (labeled as test and blank). Then, 2-3 drops of 1 % $FeCl_3$ was added to the test tube. The presence of phenolic compounds was indicated by the appearance of a blackish green color [11].

2.3.4. Saponin Test

An amount of 2 mL of extract solution was mixed with 10 mL of distilled water and shaken for 1 minute, followed by the addition of two drops of 1N HCl. The formation of stable foam lasting approximately 7 minutes indicated the presence of saponins in the sample [12].

2.4 Antibacterial Assay

Antibacterial activity was tested using the well diffusion method. Sterile Mueller Hinton Agar medium (100 mL) was mixed with 2% bacterial suspension equivalent to McFarland 0.5 standard, then 15 mL was poured into each petri dish. Eight wells were made using a cork borer (6 mm) and filled with 20 µL of 50 mg/mL various extracts (chloroform, ethyl acetate, ethanol at 50%, 70%, and p.a. concentrations, and water). Clindamycin 0.25 mg/mL served as the positive control and DMSO as the negative control. Plates were incubated for 24 hours at 37 °C, and the inhibition zones (clear zones) were measured using caliper. The test was performed in four replicates [8][13].

2.5 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC of the neem leaf extracts was determined using the microdilution method in 96-well microplates. Bacterial suspensions were prepared based on OD 625 nm (0.08–0.1), equivalent to 1.5×10^8 CFU/mL, then diluted in NB to 1×10^6 CFU/mL. Wells 1–8 were filled with 100 µL sterile NB, and 100 µL of 100 mg/mL extract was added to well 1, followed by two-fold serial dilution through well 8. From well 8, 100 µL of solution was discarded, and each well then received 100 µL of bacterial suspension, giving a final volume of 200 µL at a concentration of 5×10^5 CFU/mL. Final extract concentrations ranged from 25 mg/mL to 0.192 mg/mL. Two wells served as suspension and media controls.

Microplates were incubated at 37 °C for 24 hours, and MIC was determined as the lowest concentration showing clarity [14]. All tests were performed in four replicates per extract for each solvent. MBC was determined by plating 10 µL from each well onto Mueller Hinton Agar medium and incubating at 37°C for 24 hours. Bacterial growth was then observed and compared with the suspension control. The lowest concentration showing bacterial death or no visible growth was recorded as the MBC values [15-16].

2.6. Data Analysis

Antibacterial activity assays ware conducted in four replicates, with results expressed as mean values ± standard deviation. Data ware statistically analyzed via one way ANOVA, followed by Duncan's multiple range test, to identify significant differences ($p < 0.05$) among the tested parameters at a 95% confidence level.

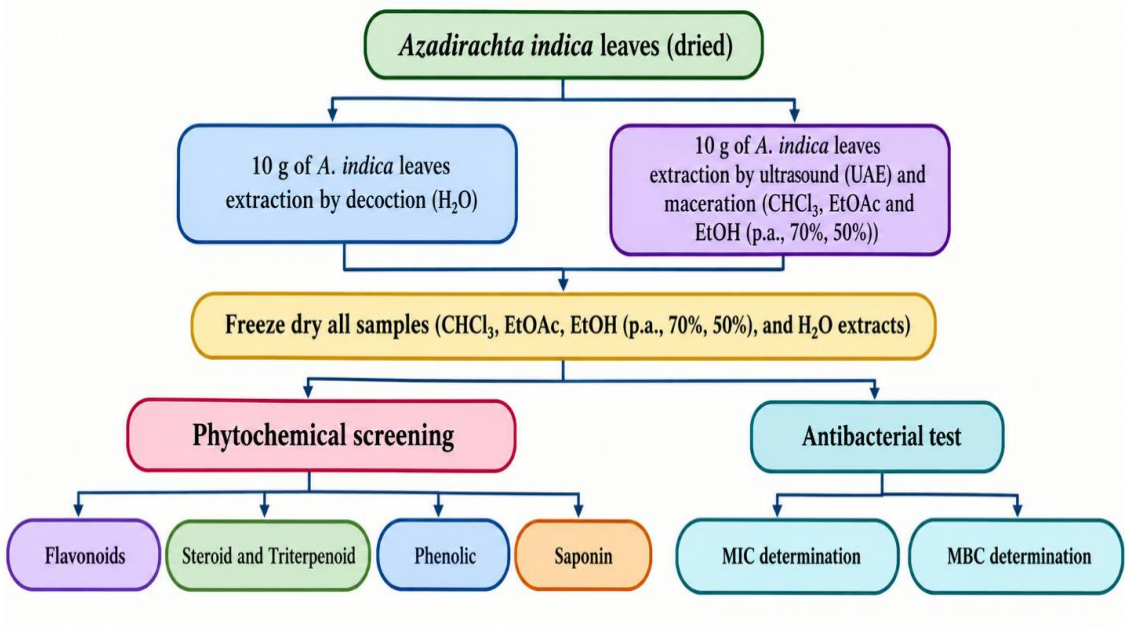


Figure 1. Experimental Flowchart

3. Results and Discussion

3.1. Extraction Yields

Leaves powder of *A. indica* was extracted using the ultrasonic assisted extraction (UAE) method and maceration was performed with five different solvents of varying polarities, including 50% ethanol, 70% ethanol, ethanol p.a, ethyl acetate, and chloroform. Meanwhile, the decoction method was used specifically form distilled water. In this study, the yield of. *A. indica* leaf extracts ranged from 6.1% to 24%, as shown in Table 1. This high yield of the distilled water extract in consistent with the study by [17] which stated that solvents with high polarity yield high extraction yields. The addition of distilled water to ethanol has been shown to increase the polarity of the solvent, thereby, optimizing the solubility of compounds [18]. In contrast, ethyl acetate and chloroform are aprotic solvents that tend to lipophilic compounds form *A. indica* leaf, resulting in yields that are much lower than those obtained with polar solvents [19].

Table 1. Yields of Neem (*Azadirachta indica*) Leaf Extracts

No	Type of Neem Extracts	Extract Mass (g)	Extract Yield (%)
1	Chloroform ($CHCl_3$)	0.61	6.1%
2	Ethyl acetate (EtOAc)	1.11	11.1%
3	Ethanol p.a (EtOH p.a)	1.59	15.9%
4	70% Ethanol (EtOH 70%)	2.02	20.2%
5	50% Ethanol (EtOH50%)	2.19	21.9%
6	Aqueous (H_2O)	2.40	24%

3.2. Qualitative Phytochemical Screening

Phytochemical identification was conducted to determine the compound composition of neem leaf extract, taking into account the influence of solvent polarity variations using reagents appropriate for five types of compounds suspected of having antibacterial activity. These phytochemical compounds include phenolics, flavonoids, saponins, and steroids/terpenoids. The results of the phytochemical screening of neem leaf extracts using various polarities of extraction solvents are presented in Table 2.

Table 2. Qualitative Phytochemical Screening of Neem (*Azadirachta indica*) Leaf Extracts

No	Phytochemical Testing	Phytochemical Screening Results	Extracts					
			H_2O	EtOH 50%	EtOH 70%	EtOH p.a	EtOAc	$CHCl_3$
1	Phenolics	Blackish-green color	+	+	+	+	+	+
2	Flavonoids	Red to orange color	+	+	+	+	+	+
3	Steroids	Green or blue color	+	+	+	+	+	+
4	Triterpenoids	Red, orange, and brown colors	-	-	-	+	+	+
5	Saponins	Foam forms 1–10 cm in 10 minutes	-	-	-	-	-	-

Note: (+): Indicates the presence of the investigated secondary metabolites
(-) : Indicates the absence of the investigated secondary metabolites

Table 2 demonstrated that all six types of neem leaf extracts contain phenolic, flavonoid and steroid compounds. These findings indicate that these compound classes exhibit a relatively wide range of polarity, enabling their extraction using various types of solvents. These results are consistent with report by [20] on the quantitative phytochemical composition of neem leaves, which states that the levels of flavonoids and phenolic compounds in neem leaves are relatively high, exceeding 24 mg/g of extract. In addition, phytochemical screening using polar (aqueous), semipolar (ethyl acetate) and nonpolar (chloroform) solvents also yielded positive results for these three classes of compounds [21-22].

Triterpenoids ware detected in neem leaf extracts prepared using $CHCl_3$, EtOAc, and EtOH p.a. as solvents, whereas saponins were absent across all extracts. This result is consistent with the characteristics of triterpenoids, which can be extracted by both polar and nonpolar solvents depending on their functional groups. Consistently, previous studies [23-24] reported that triterpenoids represented the predominant phytochemical class in neem leaves, yielding up to 13.13 mg/g. In contrast, low saponin concentrations in neem leaf extracts obtained via organic solvents have also been documented [25]. Differences in phytochemical content between this study and previous studies can arise from various factors, including the solvent to solute ration, extraction temperature and duration of the process, solvent type, leaf and fruits maturity level, cultivar variations, geographical location, edaphic conditions, harvesting season, and plant growth stage [26-27].

3.3. Antibacterial Activity of *A. indica* Leaves Extracts

Six types of *A. indica* leaf extracts were tested in-vitro to determine their antibacterial activity against *P. acnes*, a Gram-positive opportunistic bacterium that can grow under both aerobic and anaerobic conditions. Clindamycin (0.25 mg/mL) served as the positive control, generating an inhibition zone of 24.48 ± 0.12 mm, while DMSO was used as the negative control and produced no inhibition zone (0.00 mm). Test results at a concentration of 50 mg/mL showed that five of the six extracts namely $CHCl_3$, EtOAc, EtOH p.a., 70% EtOH, and 50% EtOH extracts inhibited the growth of the test bacterium (Figure 1).

As presented in Table 3, these five extracts produced inhibition zone diameters ranging from 10.18 mm to 15.15 mm against *P. acnes*. Based on the classification by [28], *A. indica* leaf extracts exhibited varying levels of antibacterial sensitivity against *P. acnes*. The aqueous extract showed no inhibitory activity (0.00 mm), whereas the 50% ethanol, 70% ethanol, ethanol p.a., and ethyl acetate extracts were categorized as sensitive. The strongest antibacterial activity was demonstrated by the chloroform extract, which was classified as highly sensitive. One-way analysis of variance (ANOVA)

revealed that solvent polarity had a statistically significant effect on the resulting antibacterial activity. Furthermore, Duncan's multiple range test ($p < 0.05$) confirmed that the inhibitory potential differed significantly among the extract groups.

Table 3. Inhibition Zone Diameters of Neem (*Azadirachta indica*) Leaf Extracts against *Propionibacterium acnes*

No	Type of Neem Extracts and Control	Diameter of Inhibition Zone (mm)				Total Average
		1	2	3	4	
1	Chloroform ($CHCl_3$)	15.09	15.12	15.19	15.20	15.15 ± 0.05 (f)
2	Ethyl acetate (EtOAc)	13.13	13.16	13.11	13.09	13.12 ± 0.03 (e)
3	Ethanol p.a (EtOH p.a)	12.00	11.96	12.15	12.00	12.03 ± 0.07 (d)
4	70% Ethanol (EtOH 70%)	11.75	11.28	11.36	11.58	11.49 ± 0.19 (c)
5	50% Ethanol (EtOH50%)	10.26	10.16	10.12	10.20	10.18 ± 0.05 (b)
6	Aqueous (H_2O)	0.00	0.00	0.00	0.00	0.00 ± 0.00 (a)
7	Control (-) (DMSO)	0.00	0.00	0.00	0.00	0.00 ± 0.00 (a)
8	Control (+) Clindamycin (0.25 mg/mL)	24.3	24.65	24.48	24.9	24.48 ± 0.12 (g)

This pattern indicates that the active antibacterial compounds in *A. indica* are predominantly nonpolar to semipolar compounds. Chloroform, a low polarity nonpolar solvent, optimally extracts these antibacterial constituents, yielding a high concentration of active substances capable of disrupting bacterial cell walls or membranes. The pronounced inhibition zones in the chloroform and ethyl acetate extracts are likely attributable to presence of limonoids. Limonoids, which belong to the tetranortriterpenoid class, represent highly modified triterpenoids characteristic of the Meliacea family. As nonpolar to semipolar secondary metabolites, limonoids are effectively extracted by these solvents, a finding supported by the positive detection of triterpenoids in both extracts.

According to literature, *A. indica* is rich in specific bioactive limonoids such as azadirachtin, nimbin, nimbidin, gedunin, and 6-desacetylnimbin, which exhibit potent growth-inhibiting activity [29] and broad-spectrum antimicrobial properties [30]. This finding is in line with the research by [31], which reported that the chloroform extract of *A. indica* leaves is more effective against MRSA bacteria compared to methanol and n-hexane. Additionally, research on a plant from the same genus, namely *A. excelsa*, using chloroform as a solvent also produced the highest inhibition zones against *Staphylococcus aureus* and *Escherichia coli* [8].

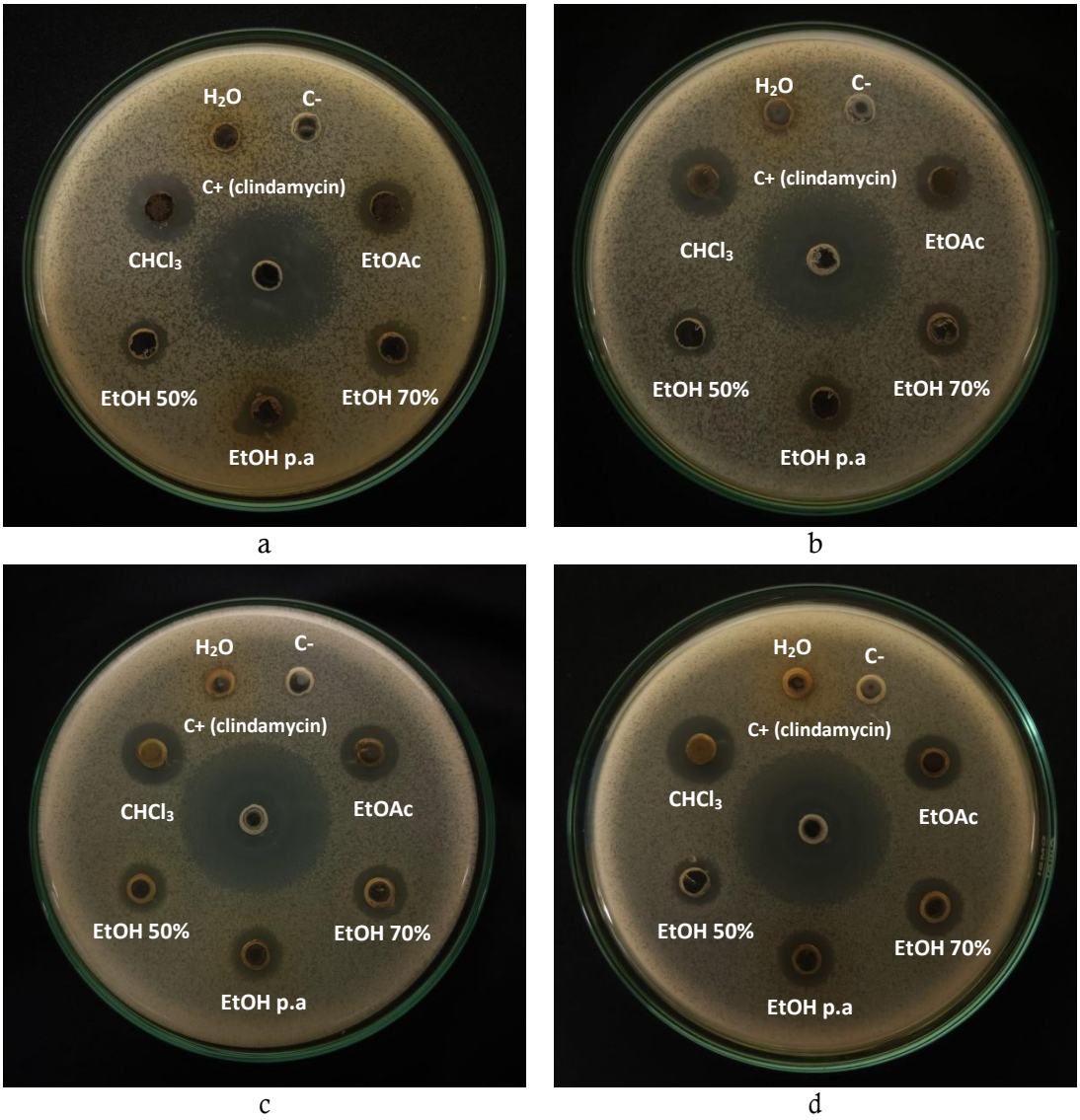


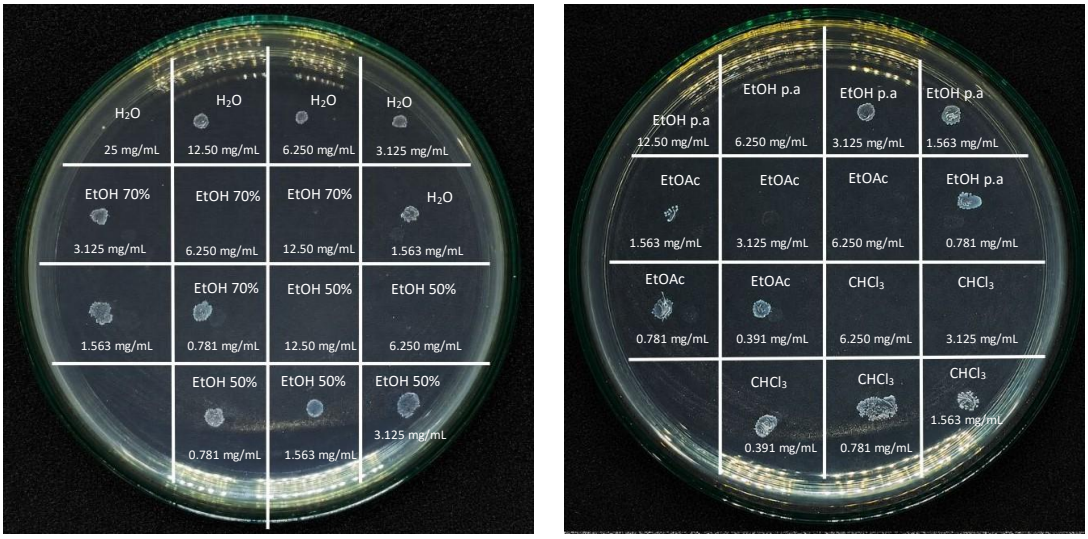
Figure 1. Antibacterial activity of *Azadirachta indica* leaf extracts ($CHCl_3$, EtOAc, EtOH p.a., 50% EtOH, 70% EtOH, and Aqueous) against *Propionibacterium acnes*: Notes (a). (b), (c), and (d) represent replicates 1, 2, 3, and 4, respectively.

3.4. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum inhibitory concentration (MIC) values were determined visually, based on the lowest extract concentration that showed no visible bacterial growth (clear broth). The six *A. indica* leaf extracts exhibited varying degree of inhibitory activity against *P. acnes*, with MIC values of 1.562 mg/mL for both chloroform and ethyl acetate extracts, 3.125 mg/mL for the 50%, 70%, and p.a. ethanol extracts, and 25 mg/mL for the aqueous extract.

The minimum bactericidal concentration (MBC), defined as the lowest concentration capable of completely eradicating bacterial viability was determined by sub culturing aliquots from the MIC wells onto agar plate (Figure 2). The ethyl acetate and chloroform extracts demonstrated the highest bactericidal potency with an MBC of 3.125 mg/mL, followed by the 50%, 70%, and p.a. ethanol

extracts at 6.250 mg/mL. In contrast, no MBC was detected for the aqueous extract within the evaluated concentration range **(**Table 4).



a b

Figure 2. Minimum bactericidal concentration (MBC) determination of *Azadirachta indica* leaf extracts against *Propionibacterium acnes*: (a) aqueous (H_2O), 50% ethanol, and 70% ethanol extracts; (b) ethanol p.a. (EtOH p.a.), ethyl acetate (EtOAc), and chloroform ($CHCl_3$) extracts

Overall, the MIC and MBC assay results demonstrated a consistent pattern across the six extracts; the ethyl acetate and chloroform extracts exhibited the strongest antibacterial activity, followed by the ethanol extract, whereas the aqueous extract showed no activity against *P. acnes.* The higher efficacy of non-polar and semi-polar extracts (such as chloroform and ethyl acetate) is attributed to the lipophilic nature of sebum matrix where *P. acnes* thrives, as well as their ability to disrupt the bacteria cytoplasmic membrane. Consequently, non-polar and semi-polar bioactive compounds can more easily permeate this lipid-rich environment. The MIC values of the chloroform and ethyl acetate neem leaf extracts in this study were lower than those reported by [32], which found an MIC of 25 mg/mL against *P. acnes* using acetone and methanol extracts of neem leaves. The MBC values of the ethanol extracts (50%, 70%, and p.a.) in this study were also comparable to the MIC value of 6 mg/mL for neem leaf ethanol extract reported by [33].

Table 4. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Values of Azadirachta indica Leaf Extracts against Propionibacterium acnes

Type of Neem Extracts	***Propionibacterium acnes***	
	MIC (mg/mL)	**MBC (mg/mL)**
Chloroform ($CHCl_3$)	1.562	3.125
Ethyl acetate (EtOAc)	1.562	3.125
Ethanol p.a (EtOH p.a)	3.125	6.250
70% Ethanol (EtOH 70%)	3.125	6.250
50% Ethanol (EtOH50%)	3.125	6.250
Aqueous (H_2O)	25	-

4. Conclusion

This study confirmed that solvent polarity determines the extraction yield, phytochemical distribution, and antibacterial efficacy of *Azadirachta indica* leaves against *Propionibacterium acnes* ATCC 6916. While the chloroform extract yielded the largest inhibition zone ($p < 0.05$), both chloroform (nonpolar) and ethyl acetate (semipolar) extracts demonstrated equivalent antibacterial potency, sharing the lowest MIC (1.562 mg/mL) and MBC (3.125 mg/mL) values. This indicates that the primary bioactive constituents responsible for anti-*P. acnes* activity in neem leaves are low-to medium-polarity compounds, particularly triterpenoids that were selectively extracted in these extracts. Consequently, nonpolar and semipolar organic solvents are recommended for targeting anti-acne compounds from *A. indica*. Further research should focus on bioactivity guided isolation of these active constituents and elucidation of their specific antibacterial mechanisms of action.

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References

[1] Vasam, M., Korutla, S., & Bohara, R. A. (2023). Acne vulgaris: A Review of the Pathophysiology, Treatment, and Recent Nanotechnology Based Advances. *Biochemistry and Biophysics Reports*, *36* (September), 101578.

[2] Pambudi, D. R., Kholilah, S., Jamalluddin, W. Bin, & Andi, M. (2023). Pengaruh Masa Inkubasi Bakteri *Propionibacterium acnes t*erhadap Aktivitas Antibakteri Ekstrak Etanol 96% Umbi Bawang Dayak (*Eleutherine americana* Merr.). *Jurnal Pharmascience*, *10*(2), 369–377.

[3] Bandyopadhyay, D. (2021). Topical Antibacterials in Dermatology Topical Antibacterial Use in Dermatology Types of Antibacterials Used Topically. *Indian Journal of Dermatology*, (66), 117–125.

[4] Salman, A. S., & Alkhuzaie, M. M. (2024). *Cutibacterium acnes (Propionibacterium acnes):* Review Article. *International Journal of Science and Research Archive*, *12*(2), 2–7.

[5] Vivek, S.,Daneyal,M. D., Khurshid, Bhupendra, K., & Amandeep, A. (2020). A Review on Phytochemistry and Pharmacological Activity of *Azadirachta indica* (Neem). *International Journal of Pharmacy and Biological Sciences*, *3*(3), 172–180.

[6] Rohaeti, E., Karunina, F., & Rafi, M. (2021). Ftir-Based Fingerprinting and Chemometrics for Rapid Investigation of Antioxidant Activity from *syzygium polyanthum* Extracts. *Indonesian Journal of Chemistry*, *21*(1), 128–136.

[7] Rani, P. B. R. & Rajakumari, DV, S. (2024). Neem Leaves Extract (*Azadirachta indica*) and Its Bactericidal Activity against Biofilm-forming Pathogenic Bacteria. *Uttar Pradesh Journal of Zoology*, 45(9), 86–93.

[8] Adfa, M., Erliana, D., Wiradimafan, K., Triawan, D. A., Salprima, Y. S., Avidlyandi, A., & Rafi, M. (2024). Application of FTIR Spectroscopy and Chemometric to Differentiat *Azadirachta excelsa* (Jack.) Jacobs Leaves Extracts Based on Solvent Polarity and Assessment of Antibacterial Activity. *Molekul*, *19*(2), 309–319.

[9] Bolaji, O., Abolade, Y. A., Aduwa, S., Isiaka, A. B., Durodola, O., Adeoye, A., & Siame, N. T. (2024). Potential Health and Environmental Benefits of the Identified Phytochemicals Screening of (*Azadirachta indica*) Neem Leaves in Bauchi Metropolis, Bauchi State, Nigeria. *GSC Biological and Pharmaceutical Sciences*, 26(03), 068-083.

[10] Turay, A., Kamara, M. D., Kamara, S., Kamara, S., & Gbabai, F. (2025b). Phytochemical Profile and In Vitro Antibacterial and Antiplasmodial Activities of *Azadirachta indica* (Neem)

Leaf and Bark Extracts. *Journal of Pharmacognosy and Phytochemistry*, *14*(5), 164–168.

[11] Yoga, I. G., Kirtanayasa, A., Agung, A., Indraningrat, G., & Candra, I. P. (2023). Phytochemical, Antibacterial and Antioxidant Activities of *Schefflera elliptica* Leaves. *Biology, Medicine, & Natural Product Chemistry*, *12*(1), 329–334.

[12] Bahri, S., Hijriani, B. I., & Widullutfi, W. (2025). Phytochemical Screening of Methanol Extract of Neem (*Azadirachta indica*) Leaves. *Jurnal Biologi Tropis*, 25(4), 6115–6121.

[13] Adfa, M., Gusatyana, N. M. C., Mudyanto, A., Avidlyandi, A., Oktiarni, D., & Salprima Yudha, S. (2021). Combined Effect of Methanol Extract of *Persea americana* Seeds and *Cassia alata* Leaves Against *Staphylococcus epidermidi* . *Proceedings of the International Seminar on Promoting Local Resources for Sustainable Agriculture and Development (ISPLRSAD 2020)*, *13*(Isplrsad 2020), 36–40.

[14] Parvekar, P., Palaskar, J., Metgud, S., Maria, R., & Dutta, S. (2020). The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Silver Nanoparticles against *Staphylococcus aureus*. *Biomaterial Investigations in Dentistry*, *7*(1), 105–109.

[15] Ousman, B. M., Mennane, Z., Boussaoudi, I., Otchom, B. B., & Saoud, Y. (2025). First Investigation of Phytochemical Screening, HPLC-MS Characterization, and Antibacterial Activity of *Azadirachta indica* A. Juss (Mim or Neem) Leaf Extracts, Grown in the Republic of Chad. *Natural Product Communications*, *20*(2).

[16] Shari, W., Erliana, D., Azizah, K., Banon, C., Avidlyandi, A., Wiradimafan, K., Yudha S, S., & Adfa, M. (2025). Effectiveness of *Cinnamomum porrectum* Roxb. Kosterm Pyroligneous Acid and Its Compounds in *Staphylococcus aureus* Growth Inhibition. *Yuzuncu Yil University Journal of Agricultural Sciences*, *35*(3), 392–402.

[17] Trivadila, Septaningsih, D. A., Karomah, A. H., Anggela, R. H., Indariani, S., Ridwan, T., Rahminiwati, M., Sa'diah, S., Iswantini, D., & Rafi, M. (2026). LC-HRMS-Based Metabolomics and Evaluation of Its Biological Activity of *Garcinia atroviridis* with Different Extracting Solvents. *Journal of Food Measurement and Characterization*, *20*, 4194–4202.

[18] Azzahra, V. O., Mardiana, S., Suharyadi, S., Sianipar Juita, R., & Ramadhan, D. S. (2025). Effect of Solvent Polarity on Extraction Yield, Phytochemical Composition, and Antioxidant Activity of *Curcuma xanthorrhiza* Roxb. *Biology, Medicine, & Natural Product Chemistry*, *14*(2), 1273–1284.

[19] Bitwell, C., Indra, S. Sen, Luke, C., & Kakoma, M. K. (2023). A Review of Modern and Conventional Extraction Techniques and Their Applications for Extracting Phytochemicals from Plants. *Scientific African*, *19*, 1–19.

[20] Ofongo, R. T. S., Ohimain, E. I., & Iyayi, E. A. (2021). Qualitative and Quantitative Phytochemical Screening of Bitter and Neem Leaves and their Potential as Antimicrobial Growth Promoter in Poultry Feed. *European Journal of Medicinal Plants*, *32*(4), 38–49.

[21] Turay, A., Kamara, M. D., Kamara, S., Kamara, S., & Gbabai, F. (2025a). Phytochemical Profile and In vitro Antibacterial and Antiplasmodial Activities of *Azadirachta indica* (neem) Leaf and Bark Extracts. *Journal of Pharmacognosy and Phytochemistry*, *14*(5), 164–168..

[22] Rahman, N. A. A., Musa, M., Yusmaidi, N., & On, S. (2020). Phytochemical Screening and Study of Antioxidant and Antimicrobial Activities of Leaf Extracts of *Azadirachta indica. ASM Science Journal*, *13*(Specialissue6), 90–95.

[23] Khanal, S. (2021). Qualitative and Quantitative Phytochemical Screening of *Azadirachta indica* Juss. Plant Parts. *International Journal of Applied Sciences and Biotechnology*, *9*(2), 122–127.

[24] Ali, J., Bangash, J. A., & Siddique, M. (2023). Comparative Bioactive Compounds and Fourier Transform Infrared Spectroscopic Evaluation of *Azadirachta indica* Extracts and Its Potential as Bio-Fungicides against Plant Pathogenic Fungi. *International Journal of Engineering, Science and Technology*, *15*(1), 1–12.

[25] Bem, A. A., Adamu, H., & Bem, L. S. (2025). Comparative Phytochemical Analysis and Medicinal Properties of Neem (*Azadirachta indica*) and Eucalyptus (*Eucalyptus globulus*) Plant. *Sahel Journal of Life Sciences FUDMA*, 3(2), 304–310.

[26] Chatepa, L. E. C., Mwamatope, B., Chikowe, I., & Masamba, K. G. (2024). Effects of Solvent Extraction on The Phytoconstituents and In Vitro Antioxidant Activity Properties of Leaf Extracts of The Two Selected medicinal plants from Malawi. *BMC Complementary Medicine and Therapies*, 24(1).

[27] Gizaw, A., Marami, L. M., Teshome, I., Sarba, E. J., Admasu, P., Babele, D. A., Dilba, G. M., Bune, W. M., Bayu, M. D., Tadesse, M., & Abdisa, K. (2022). Phytochemical Screening and in Vitro Antifungal Activity of Selected Medicinal Plants against *Candida albicans* and *Aspergillus niger* in West Shewa Zone, Ethiopia. *Advances in Pharmacological and Pharmaceutical Sciences*, *2022*.

[28] Hadi, W. R., Sipriyadi, Darwis, W., Sukmawinata, E., Masrukhin, Mashudi, Asril, M., Hidayah, T., & Trianda, A. (2024). Bioprospecting of Fragrant Ginger (*Zingiber aromaticum*) Endophytic Bacteria from Enggano Island, Indonesia as Antimicrobial Compounds Producer. *Yuzuncu Yil University Journal of Agricultural Sciences*, 34(2), 263–270.

[29] Wylie, M. R., & Merrell, D. S. (2022). The Antimicrobial Potential of the Neem Tree *Azadirachta indica*. *Frontiers in Pharmacology*, 13(891535), 1–16.

[30] Hieu, T. T., Thuy, P. T., & Duc, D. X. (2022). Isolation and Bioactivities of Limonoids from Meliaceae Family: A Review. *Current Organic Chemistry*, 26(14), 1359–1430.

[31] Benisheikh, A. A. G., Muhammad, F. M., Kelluri, H., Aliyu, Z. M., Mallam, U. B., & Jibrin, M. W. (2019). Phytochemical Extraction and Antimicrobial Studies on Crude Leaf Extract of *Azadirachta indica* (Neem) in Semi-Arid Region of Borno State, Nigeria. *International Journal of Research & Review (Www.Ijrrjournal.Com) Vol*, *6*, 12.

[32] Alnabati, N. A., Al-Hejin, A. M., Noor, S. O., Ahmed, M. M. M., Abu-Zeid, M., & Mleeh, N. T. (2021). The Antibacterial Activity of Four Saudi Medicinal Plants against Clinical Isolates of *Propionibacterium acnes*. *Biotechnology and Biotechnological Equipment*, *35*(1), 415–424.

[33] Charde, Y., Sharma, P., Choudhary, N., & Avari, J. (2014). Development and Evaluation of Herbal Formulation for the Treatment of Acne. *International Journal of Pharmaceutical Sciences and Research*, *5*(6), 2250–2260.