Pharmacogn J. 2022; 14(3): 489-496
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

Azadirachta indica Hexane Extract: Potent Antibacterial Activity Against *Propionibacterium* acne and Identification of its Chemicals Content

Annysa Ellycornia Silvyana[1], Ratika Rahmasari[2], Berna Elya[1,*]

[1]Departement of Phytochemical, Faculty of Pharmacy, University of Indonesia, INDONESIA.
[2]Departement of Microbiology, Faculty of1Pharmacy, University of Indonesia, INDONESIA.

Correspondence
Berna Elya
Departement of Phytochemical, Faculty of Pharmacy, University of Indonesia, INDONESIA.
Email: berna.elya@farmasi.ui.ac.id

History
- Submission Date: 23-02-2022;
- Review completed: 18-03-2022;
- Accepted Date: 05-04-2022.

DOI : 10.5530/pj.2022.14.62

Article Available online
http://www.phcogj.com/v14/i3

ABSTRACT

Background: Acne is a skin surface disease that appears when the excessive fat deposits clogged the skin pores, causes the growth of acne-causing bacteria and stimulates inflammation. *Propionibacterium acnes* is one of common acne-causing bacteria which usually manage by synthetic chemical-based drug. However, the presence of its long- used side effects pointed the urgent need of new anti *P. acne* drug discovery. *Azadirachta indica* is a medicinal plant which empirically used as antibacterial. *A. indica* leaves has been reported to exhibit activity against *P. acne* but limited to ethanol extract. Thus, the evaluation of other extract- and identification of active compound(s) against *P. acne* is needed to be explore. **Methods**: First, the microscopic morphology of *A. indica* leaves were observed using Scanning Electron Microscope. The leaves were then extracted sequentially by hexane, ethyl acetate, and methanol solvent using the ultrasonic assisted extraction method, followed by its *in vitro* anti- *P. acne* activity evaluation. The most active extract was further evaluated for its chemical(s) content by LC-MS. **Results**: Scanning Electron Microscope identified the presence of oxalate in the leaves of *A. indica*. Evaluation of the anti-*P. acne* activity showed that the hexane extract had highest anti-*P. acne* compared to others. Further chemical identification showed that hexane extract contains three steroids, one saturated acids and one phenolic compounds. **Conclusions**: *A. indica* hexane extract leaf is prospective to be developed as an acne antibacterial.

Key words: *Azadirachta indica*, Hexane extract, Anti-*Propionibacterium acne*, Chemical content.

INTRODUCTION

Acne is a pilosebaceous disease that caused by comedones, altered sebum production, *Propionibacterium acne* invasion and inflammatory response. Eventhough the clinical presentation of acne are varying, the face-lesion development especially for women, occur frequently.[1] Currently, acne is managed by antibiotics. However, antibiotics prolong treatment may lead to bacteria resistance.[2] Thus, to overcome this situation, many researchers explore the potential traditional medicine for acne drug development and discovery.[3] *Azadirachta indica*, plant belongs to genus *Azadiractha*, is a green plant native to India which spread to Asia, Europe and Middle east area.[4] *A. indica* has been empirically used for treating bacteria-, fungi-, parasitic- and viruses diseases. To note, it has been reported that *A. indica* is clinically effective and safer than synthetic antibiotics.[5] In traditional medicine, the parts of *A. indica* which commonly used are the bark, leaves and seeds.[6] Moreover, every part of *A. indica* are known to contains compounds which affected biological activity not only *in vitro* but also *in vivo*, such as nimbolide, azadirachtin and gedunin.[7] Further phytochemical screening of *A. indica* extracts indicated the presence saponins, steroids for polar and non polar extract, moreover flavonoid and alkaloids can be found at semi polar and polar extracts.[8-10] Those metabolits has been known to affect bacterial, fungi or virus infection *in vitro* and *in vivo*.[11,12] To note, a study have shown that the ethanolic extract of *A. indica*, exhibit antibacterial activity against *P. acnes* with an inhibitory power of 7mm.[13] However, the information regarding the bioactive compound(s) responsible to its activity is still limited. Thus, in this study, we evaluated the activity of others extracts from *A. indica* leaves against *P. acne* and identified the chemical compound(s) which contained in most active extract.

MATERIALS AND METHODS

Sample and bacteria preparation

Azadirachta indica were obtained from Jatiluhur Village, West Java, Indonesia. Leaves parts were used in this study. Prior to experiment, the leaves were determined by the Indonesian Institute of Sciences, Bogor, followed by drying and grinded into powder form.

Propionibacterium acnes ATCC 11827, obtained from the Microbiology Laboratory Faculty of Medicine, University of Indonesia. The *P. acne* were maintained in blood agar (*oxoid*, UK) and incubated at 37°C anaerobic incubator.

The extraction process was carried out using the ultrasound-assisted extraction (UAE) method. Extraction was performed sequentially using hexane, ethyl acetate and methanol solvents. A total of 50 g of dried *A. indica* leaf powder was extracted by 500 mL of hexane solvent for 30 minutes at 40°C. The extraction was repeated 3 times to get the maximum yield. The filtrate was separated using filter paper, then evaporated using a rotary evaporator (Buchi Rotavator R-300, Germany) to obtain dried-hexane extract. Leave powder which extracted using hexane

Cite this article: Silvyana AE, Rahmasari R, Elya B. *Azadirachta indica* Hexane Extract: Potent Antibacterial Activity Against *Propionibacterium* acne and Identification of its Chemicals Content. Pharmacogn J. 2022;14(3): 489-496.

was dried and further extracted sequentially by ethyl acetate and Methanol using same procedures as explained above. Prior to use, dried extract was diluted by 10% DMSO and followed by 2-fold dilution by Aqua pro injection.

Microscopic Observation with light microscope and SEM

Microscopic observation of dried *A. indica* leaves was performed using a light microscope (100 × magnification) and Scanning Electron Microscope (SEM) (750 x magnification) at Indonesian Institute of Sciences, West java, Indonesia.

Minimum inhibitory concentration test

The determination of extracts minimum inhibitory concentration (MIC) was carried out by turbidity observation at 96-well microplate. 100 µl of two-fold dilution extract in Brain Heart Infusion Borth (BHIB) (oxoid, UK) medium was added to each well. Followed by addition of 100 µl of 330 × 10^4 *P. acne* suspension to a final concentration of 10%, 5%, 2.5%, 1.25%, 0.625%, 0.312%, 0.156%, 0.078%, 0.039%, and 0.019% for hexane extract; 10%, 5%, 2.5%, 1.25%, 0.625%, 0.312%, 0.156%, 0.078%, 0.039%, and 0.019% for ethyl acetate and methanol extract. The microplate was further incubated under anaerobic conditions at 37°C for 72 hours. The lowest compound concentration(s) that inhibited bacterial growth (indicated by clear-transparant yellow color) after 72 hours incubation was determined as MIC value. The experiment was performed twice, each in duplicate.

Minimum bactericidal concentration test

Determination of minimum bactericidal concentration (MBC) was performed as follow: the mixture of extract and *P. acne* which determine as MIC value labelled as P concentration. The P, P/2, 2xP concentration were further streaked on a Blood agar medium (oxoid, UK). Followed by anaerobics incubation at 37°C for 72 hours. The tested concentration that shown no bacterial growth after incubation is determined as MBC value.

Analysis of chemical compound by LCMS

The most active extracts against *P. acne* was further analyzed for its chemical compounds content using an Liquid Chromatography Mass Spectrometry (LCMS) instrument (QMicro QAA 842, a Waters Quattro Micro MS- MS detector). The evaluation was performed as follow: The *A. indica* dry hexane extract was dissolved in 5 ml of solvent and 20 µl was added to a reverse phase analysis column C18 with a particle size of150×2.1×1.9m at with flow rate of10.2 ml/min. The temperature of the column used was 50°C and the final time was 20 min. The separation of the chemical compounds took place in column with the help of1a pump using a pressure of 300 Bar. The *A. indica* was converted into a gas phase which will be ionized under vacuum. Ions are accelerated by an electric or magnetic field, by the mass to be charged (m/z) ratio. The results of the LCMS analysis were performed based on compound predictions based on the m/z profile existing secondary data and compared with the m/z profile results presented data from the sample.

RESULTS

Microscopic observation with light microscope and SEM

The light microscope and SEM observations of dried *A. indica* leaves can be seen in Figure 1.

Extraction

In order to maximize the phytochemical solution of the sample, the extraction process was performed sequentially, first by hexane solvent, to ethyl acetate solvent and last by methanol solvent. The extraction was also performed using UAE instead of temperature increasing.

Table 1: Results extraction of A. *indica* leaf using UAE method.

	H	EA	ME
Extract (g)	7.4	7	4.1
Yield (%)	3.7	3.5	2.05

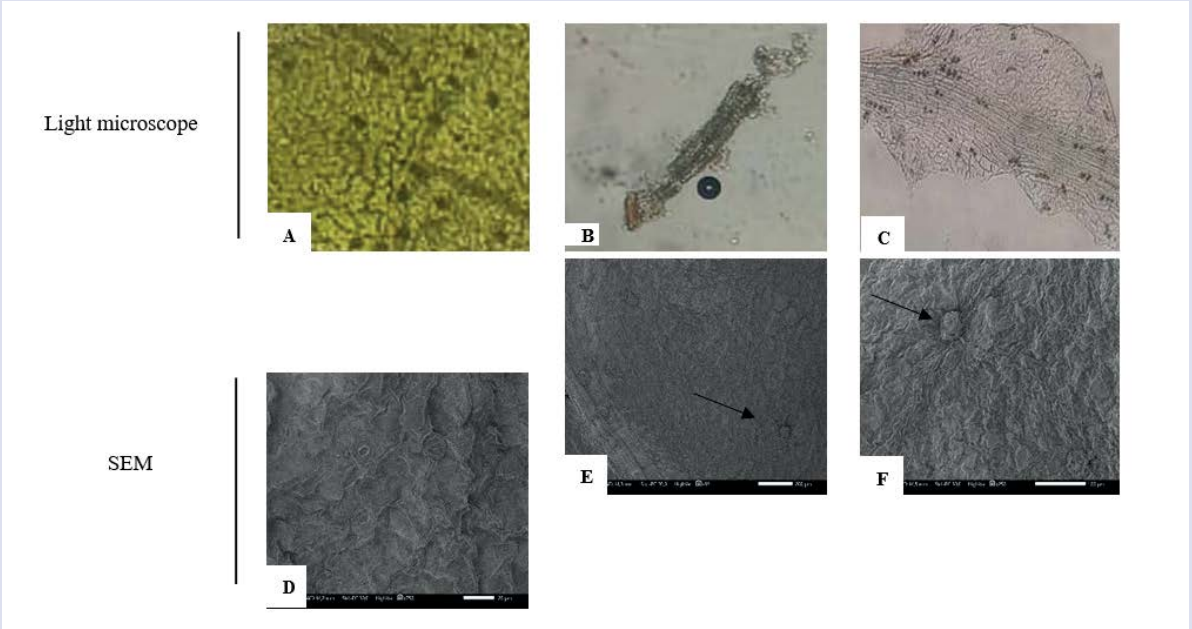


Figure 1: Light Microscopic and SEM results of *A. indica* leaves. Light microscopic observation indicated the presence of parasitic stomata (A) and carrier bundles In *A. indica* leaves (B), Calsium oxalate (C). The SEM evaluation was further confirm the presence of D) stomata, E) and F).

The polarity of the solvent will affect the type and amount of chemical component capacity and biological activity of the extract.[14] The results of the UAE sequential extraction were shown in Table 1, respectively. The result shown that hexane extract had the highest yield compare to others extract. This result indicated that *A. indica* contained more non-polar components.

MIC and MBC against *P. acne* evaluation

The MIC test was carried out using the microdilution method with two dilutions at 96-well microplate. The evaluation of the MIC value was carried out visually. The result can be seen in table 2. The MIC evaluation indicated that hexane extracts shown no bacterial growth at 5%. Meanwhile for ethyl acetate extract and methanol extract MIC value was observed at 10%. The results are shown in table 2, respectively.

In addition to the MIC study, MBC test was also carried out to observed bactericidal activity of each extract. The result shown that methanol and ethyl acetate extract exhibit MBC at concentration 10%. However, the hexane shown MBC at lower concentration 5%. The MBC evaluation figure are shown at Figure 2, respectively.

Analysis of chemical compound by LC-MS

Based on anti-*P. acne* evaluation, Hexane extract exhibit better activity compare to others extract. Thus, hexane extract was further evaluated for its compound(s) profile using LCMS. LC-MS can be used to determine the secondary metabolite profile of extract hexane by observing the ion spectrum product in the form of compound retention time and compound fragmentation based on m/z. The compound identification process was based on the literature available in the LC-MS database and other supporting literature based on m/z compounds such as Pubmed for phytochemicals. The LCMS identification result at table 3 and figure 3, respectively.

DISCUSSION/CONCLUSION

Azadirachta indica (Neem) is an evergreen tree that proliferates and known commonly in various world regions such as Africa, America and India. It has been widely used in Chinese, Ayurvedic and Unani medicine, especially in Asian countries, to prevent and treat diseases. Various parts of the *A. indica* plant contain biological compounds responsible for its antibacterial, antiviral and antifungal activities. Several studies have shown that the *A. indica* plant has antimicrobial activity. Extracts of leaves, bark and seeds of *A. indica* are showed significant antimicrobial activity against the tested pathogens. Gram-positive bacteria (*Streptococcus spp*, *Staphylococcus aureus*), Gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Salmonella spp, Coliform spp*), fungi (*Candida spp*) and viruses (*Vaccinia, Measles, Coxsackie B*).[15-17] Several other studies have also shown that oil from *A. indica* seeds can inhibit the growth of *Salmonella typos* and *Staphylococcus aureus.*[18]

Table 2: Results of MIC test on *P. acne*.

		Visual observation									
		Concentration %									
		0.019	0.039	0.078	0.156	0.312	0.625	1.25	2.5	5	10
Extract	H	+	+	+	+	+	+	+	+	-	-
		0.019	0.039	0.078	0.156	0.312	0.625	1.25	2.5	5	10
	EA	+	+	+	+	+	+	+	+	+	-
	ME	+	+	+	+	+	+	+	+	+	-
		Concentration (µg/ml)									
Clindamycin		0,0001	0,0002	0,0005	0,001	0,002	0,004	0,008	0,015	0,03	0,06
		+	+	+	+	+	+	+	+	-	-

Description: + means turbid (bacterial growth occurred); - means clear-transparant (no bacterial growth)

Table 3: LC-MS test identification results.

	Component name	Identificationstatus	Observedm/z	ObservedRT (min)	Detectorcounts
1	Alisol I	Identified	455.352	10.40	1127325
2	Azedarachin C	Identified	587.285	5.80	1608838
3	Ganoderic acid G	Identified	555.295	7.26	2222267
4	Trichosanic acid	Identified	279.232	9.70	1248462
5	Nimbolide	Identified	467.206	7.14	3532419

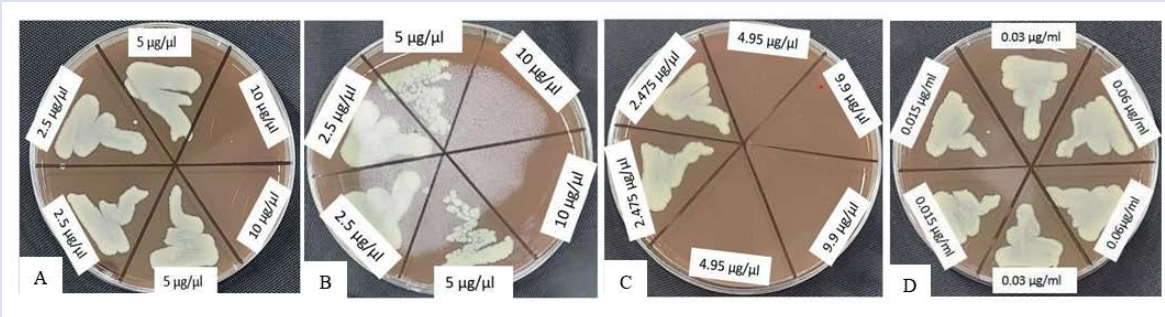
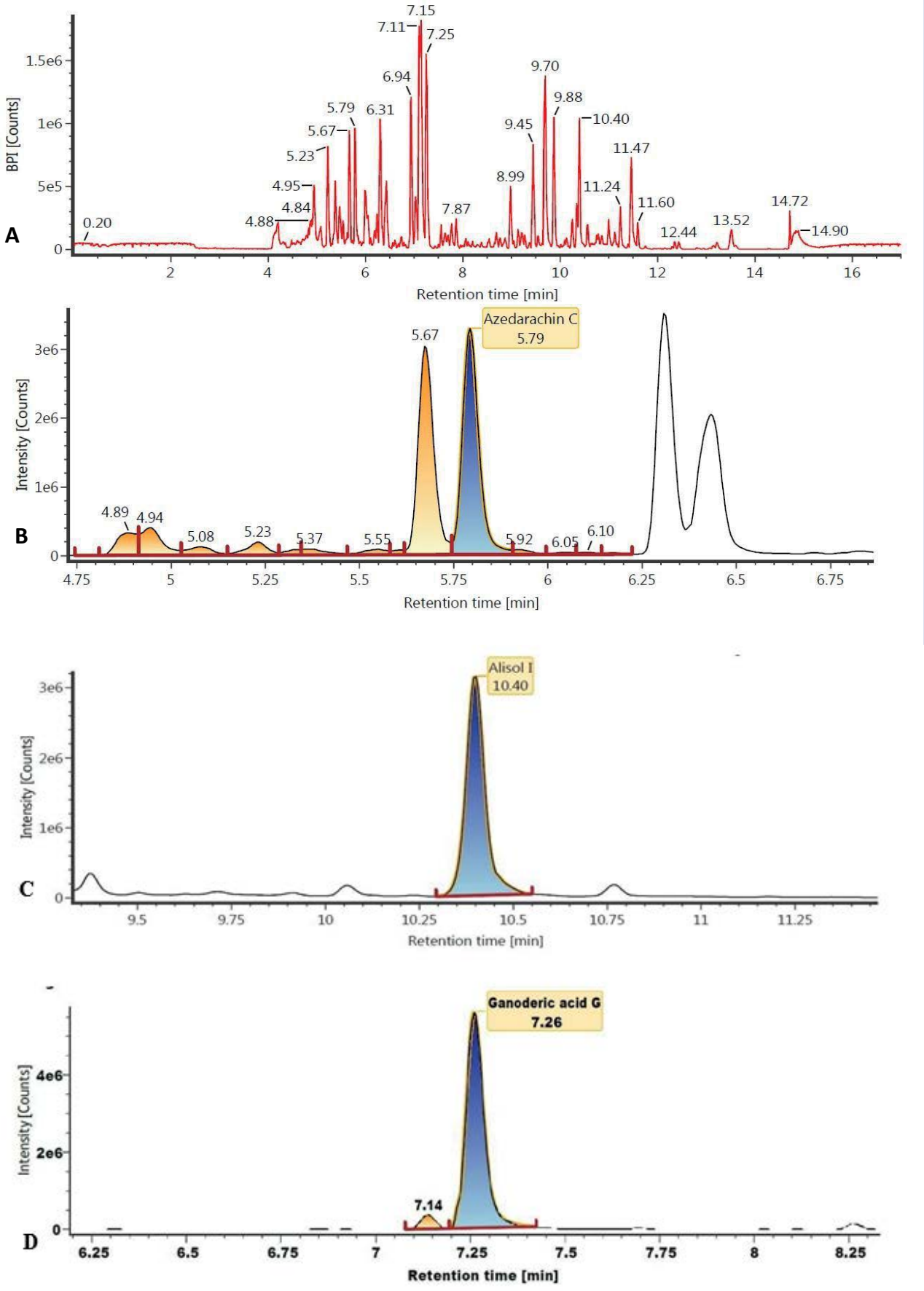


Figure 2: MBC test results of *A. indica*, (A) Methanol Extract, (B) Ethyl Acetate Extract, (C) Hexane Extract, and (D) Clindamycin at different concentration (indicated at the picture) against *P. acne*. two independent experiment each induplicated ware performed.


A
BPI [Counts]
1.5e6
1e6
5e5
0
0.20
4.88
4.84
4.95
5.23
5.67
5.79
6.31
6.94
7.11
7.15
7.25
7.87
8.99
9.45
9.70
9.88
10.40
11.24
11.47
11.60
12.44
13.52
14.72
14.90
2
4
6
8
10
12
14
16
Retention time [min]
B
Intensity [Counts]
3e6
2e6
1e6
0
Azedarachin C
5.79
5.67
4.89
4.94
5.08
5.23
5.37
5.55
5.92
6.05
6.10
4.75
5
5.25
5.5
5.75
6
6.25
6.5
6.75
Retention time [min]
C
Intensity [Counts]
3e6
2e6
1e6
0
Alisol I
10.40
9.5
9.75
10
10.25
10.5
10.75
11
11.25
Retention time [min]
D
Intensity [Counts]
4e6
2e6
0
Ganoderic acid G
7.26
7.14
6.25
6.5
6.75
7
7.25
7.5
7.75
8
8.25
Retention time [min]

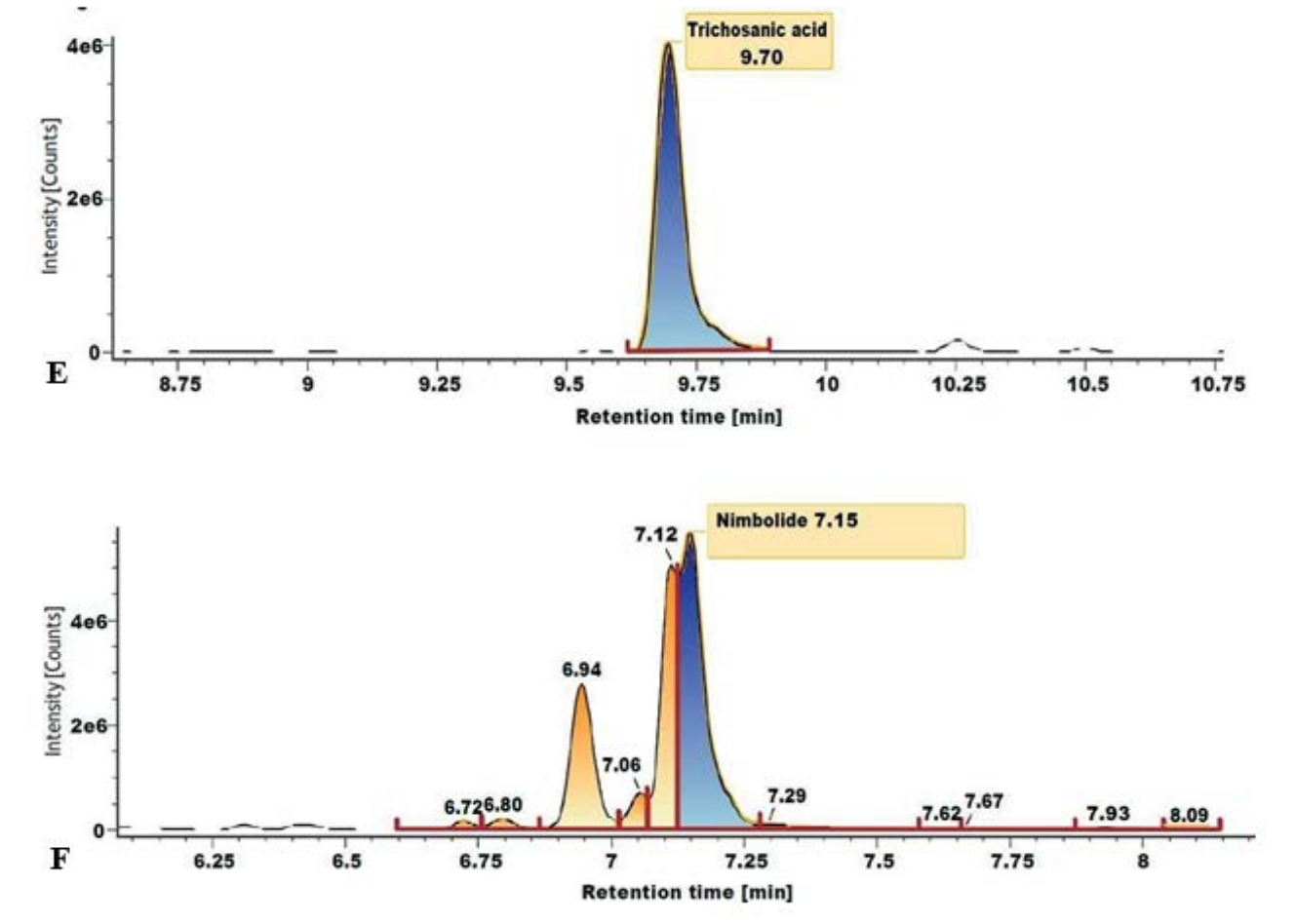


Figure 3: LC-MS graph of *A. indica,* (A) Compound Hexane Extract (B) Azedarachin C (C) Alisol I (D) Ganoderic Acid G (E) Trichosanic Acid (F) Nimbolide.

A. indica leaf extract has the best antibacterial activity, which confirms the strength of the bioactive compounds and proves the use of *A. indica* plants in primary health care.[19] However, the effectiveness of *A. indica* leaf extract against *P. acne* has not been explore yet.[20] Thus, In this study comparison of anti-*P. acne* active of *A. indica* leaf extracts using various solvents was evaluated. Our result indicated that hexane *A. indica* extract exhibit potent activity compared to others. Further, morphological identification of the leaves of *A. indica* was also carried out. The results of the light microscope and SEM, showed the presence of parasitic stomata, calcium oxalate and vascular bundles found in *A. indica* leaf plants as indicated in (Figure 1).

The ultrasonic extraction method is faster and more efficient than for extracting secondary plant metabolites than conventional methods such as maceration or soxhlation.[21] Ultra power high audio (20 to 25 kHz) can change the permeability of the cell wall and increased solvent penetrates through plant cells, increasing the solubility of phytochemicals. Extraction using UAE indicated that the extracts obtained from *A. indica* leaves H extracts had the highest yields than EA and ME extracts as shown at (Table 1). The non-polar content in *A. indica* leaves is soluble in the extraction process using N-hexane as a solvent. UAE extraction of neem leaves yielded 7.4% hexane extract, 7% ethyl acetate extract and 4.3% methanol extract. Based on the literature, *A. indica* leaves contain oil of 0.88%-9.53%. The content of *A. indica* leaf oil can vary depending on where it grows.[22] In addition, the different extraction methods used can cause differences in the oil content produced. Sonication is the most effective method of extracting natural materials. Sonication resulted in high yield, fast extraction time and good selectivity. In addition, the sonication extraction method is very effective for extracting thermolabile compounds because it can reduce exposure to high temperatures.[23]

The results of the MIC value test on the H, EA, and ME extracts as shown in (Table 3) indicated that, the turbidity was seen at a concentration 2.5%, concentration 5% and 10% for Ethyl acetate and methanol, 10%, 5% and 2.5% for hexane looks clear. Then, it was confirmed again by the MBC test and showed that the hexane extract in lower concentrations could inhibit the growth of *P. acne* compared to the EA and ME extracts of *P. acne* as shown (Figure 2). This showed that the hexane extract had better activity compared to other extracts. It was reported that several chemical compounds in the hexane extract work synergistically so that the antibacterial activity produced is more effective than the antibacterial activity of a single compound.[24] The MIC test on clindamycin was still overgrown with *P. acne* bacteria because of the bactericidal properties of clindamycin. Clindamycin is a semisynthetic antibiotic that has a mechanism of action by inhibits protein synthesis by binding to the 50S subunit of the bacterial ribosome.[25]

Based on previous reported research, it's known that the hexane extract contains terpenoid and steroid compounds.[26] Terpenoids are known to inhibit the growth or kill bacteria by interfering with forming membranes or cell walls so that they are not formed or formed imperfectly.[27] In addition, terpenoids alter porins (transmembrane proteins) on the outer membrane of the bacterial cell wall to form strong polymer bonds, destroying the porin.[28] Damage to the porin, which is

the entrance and exit of the compound, it will reduce the permeability of the bacterial cell wall. This cell wall permeability will interfere with the entry and exit of nutrients and other compounds, inhibit or dead bacterial growth. Meanwhile, the mechanism of action of terpenoids as antifungals, according to,[29] is because these terpenoid compounds are fat-soluble so that they can penetrate fungal cells membranes, affect their permeability and cause disturbances in the structure and function of cell membranes.

While the activity in ethyl-acetate and methanol extracts, it is possible that phenol compounds can form hydrogen bonds with bacterial cell proteins, which cause damage to the bacterial cell protein structure resulting in protein denaturation. Protein denaturation disrupts the permeability of the cell wall and cytoplasmic membrane, resulting in an imbalance of macromolecules and ions in the cell. As a result, the cell becomes lysed.[30]

Bacteria are the main source of inflammation in the sebaceous glands, which can lead to a variety of disorders, including acne, according to several studies. The method can take the form of microbial nuclear cell coagulation, their hydrophobicity disrupting the bacterial cytoplasmic membrane, pH gradient changes, or other causes.[31] The results of the MIC and MBC tests revealed that H extract had a higher activity in suppressing the development of *P. acne* bacteria in this investigation.

LC-MS result indicated that *A. indica* hexane extract contain steroid derivated (compound is 1,2 and 3) with R 10.40, 5.80 and 7.26. Compound 4 showed a molecular ion peak at 279.2325m/z [M+ H]+ predicted to be a saturated acid derivative. Compound 5 is thought to be a phenolic compound with R 7.15. The number of compounds suspected to be steroid derivatives based on molecular ion peaks at 455.35m/z [H + Na]+ in compound 1, 587.28 m/z [H + Na]+ in compound 2, and 555.29 m/z [Na + H]+ in compound 3. The analysis of1identification of1chemical compounds using the LC-MS method with [M+H]+ mode on extract NH from *A. indica* leaves showed that extract hexane on average had a high predictive chemical content of steroid compounds. The results of1chemical content screening based on the LC-MS approach provide an overview of the compounds contained in extract Hexane. Our LC-MS result (Figure 3) were in line with previous report which indicated the presence of steroids, sterols, triterpenoids and phenolic compounds on hexane extract.[32]

This study provides information that *A. indica* leaves have an anti-*P. acne* activity whichcauses acne, allowing this plant to be developed as traditional medicine. The *A. indica* hexane extract exhibit better activity in inhibiting the growth of *P. acne* bacteria compare to others. Furthermore, Azedarachin C, Alison I, Ganoderic Acid G, Nimbolide and Tricosanic Acid, which are found in *A. indica* leaves and are suspected of being used in LC-MS, have antibacterial properties. As a result, our research has the potential to grow.

ACKNOWLEDGEMENT

None.

CONFLICTS OF INTEREST

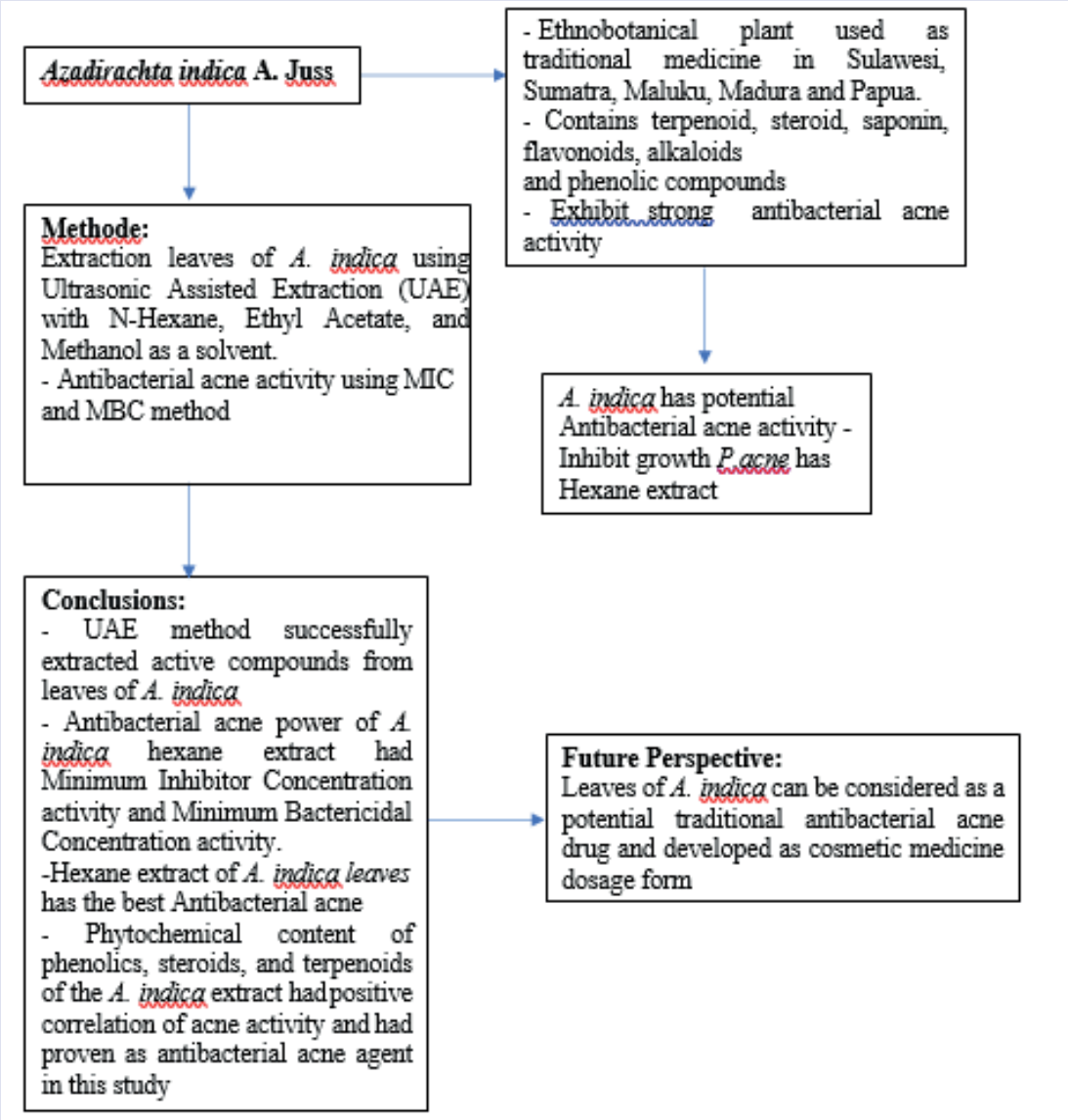
The authors declare that they have no conflicts of interest.

REFERENCES

1. Tan AU, Schlosser BJ, Paller AS. A review of diagnosis and treatment of acne in adult female patients. International Journal of Women's Dermatology. 2017;4(2):56-71.
2. Vora J, Srivastava A, Modi H. Erratum: Antibacterial and antioxidant strategies for acne treatment through plant extracts. Informatics in Medicine Unlocked. 2019.
3. Efremila, Wardenaar E, Sisillia L. Ethnobotany Study of Medicinal Plants by Ethnic Dayak Tanam Tribe, Mandor District, Landak Regency. Journal of Sustainable Forests. 2015;3:234-246.
4. Hashmat I, Azad H, Ahmed A. Neem (*Azadirachta indica* A. Juss) - A nature drugstore: An overview. International Research Journal of Biological Science. 202;1(6):76-79.
5. Dulla O, Jahan F. Ethnopharmacological survey on traditional medicinal plants at Kalaroa Upazila, Satkhira District, Khulna Division, Bangladesh. Journal of Intercultural Ethnopharmacology, 2017;6(3):316-325.
6. Koul O, Isman MB, Ketkar CM. Properties and uses of neem, *Azadirachta indica*. Canadian Journal of Botany. 1990;68(1):1-11.
7. Sarkar S, Singh RP, Bhattacharya G. Exploring the role of *Azadirachta indic*a (neem) and its active compounds in the regulation of biological pathways: an update on molecular approach. Biotech. 2021.
8. Murti RK. Study of antibacterial activity and identification of the most active fraction of neem leaves (*Azadirachta indica* A. Juss). Essay. 2011.
9. Setiawansyah A, Hakim A, Wirasisya DG. Evaluation and identification of potential antibacterial compounds in the leaves and stem skin of nemba (*Azhadirachta indica* A. Juss) on *Escherichia coli*. Indonesian Journal of Medicinal Plants. 2019.
10. Ruwandha D, Fitriyani D, Iskandar D. Test activity of tanin leaves of nemba (*Azzadirachta indica*) against *Salmonella typhi* bacteria. Journal of Research Chemistry. 2021;6(1):77.
11. Abegaz, Berhanu M, Kinfe, Henok H. Secondary metabolites, their structural diversity, bioactivity, and ecological functions: An overview. Physical Sciences Reviews. 2019;4(6):20180100.
12. Compean KL, Ynalvez RA. Antimicrobial activity of plant secondary metabolites: A review. Res J Med Plant. 2014;204-213.
13. Mas Rufah. Activity Test of Ethanol Extract of Neem Leaf (*Azadirachta indica* A. Juss) Against *Propionibacterium acnes* Bacteria. Essay. 2020.
14. Chemat, Farid. Ultrasound-assisted extraction of natural foods and products. Mechanisms, techniques, combinations, protocols and applications. A review. Ultrasonic sonochemistry. 2017;34:540-560.
15. Akpan IO, Ejele AE, Ogali RE, Achugasim O, Osuagwu CG. Bioassay-guided isolation of antimicrobial compound against (*Staphylococcus aureus*) from *Azadirachta indica* Plant. Journal of Current Chemical and Pharmaceutical Sciences. 2016;6(2):17-26.
16. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of neem (*Azadirachta indica*). Current Science. 2002;82:1336-1345.
17. Sinaga M, Ganesan K, Nair SKP, Gani SB. Preliminary phytochemical analysis and *in vitro* antibacterial activity of bark and seeds of Ethiopian neem (*Azadirachta indica* A. Juss). World Journal of Pharmacy and Pharmaceutical Sciences. 2016;5(4):1714-1723.
18. Ambarwati. The effectiveness of neem seed (*Azadirachta indica*) antibacterial substances to inhibit the growth of *Salmonella thyposa* and *Staphylococcus aureus*. Biodiversity. 2007;8(3):320-325.
19. Herrera-Calderon O, Ejaz K, Wajid M, Shehzad M, Tinco-Jayo JA, Enciso-Roca, *et al*. *Azadirachta indica*: Antibacterial activity of neem against different strains of bacteria and their active constituents as preventive in various diseases. Pharmacognosy Journal. 2019.
20. Jawetz E, Melnick JL, Adelberg EA. Medical Mycology In: Medical Microbiology (20 ed.). Jakarta: EGC. 1995.
21. Albayrak, Seville, Atasagun, Bayram, Aksoy, Ahmet. Comparison of the phenolic components and biological activities of two Centaurea sp. obtained by three extraction techniques. Asia Pacific Journal of Tropical Medicine. 2017;10(6):599-606.

22. Susila IWW. Product potential of neem (*Azadirachta indica* A. Juss) and the factors influencing the potency of neem leaves in Lombok. Faloak Journal. 2017;5(4):1714-1723.
23. Azwanida NN. A review on the extraction methods use in medicinal plants, principles, strengths and limitations. Medicinal Aromatic Plants. 2015;4(3):1-6.
24. Gupta R, Nand P, Drabu S. Insignificant anti-acne activity of *Azadirachta indica* leaves and bark. J Pharm Negat Results. 2012.
25. Cameli A, Erik J, Jack LS, Lesher R, Carol M. Antimicrobial Drugs. Dermatology. 2018;127:2215-2241.
26. Kurniati R, Saidi N, Nasution R. Antifeedant activity from neem leaf extract (*Azadirachta indica* A Juss). J Nat. 2018.
27. Ajizah A. Sensitivity of *Salmonella typhimurium* to Leaf Extract of *Psidium Guajava* L. Bioscience. 2004;1(1):31-38.
28. Amalia S, Wahdaningsih S, Untari NK. Antibacterial Activity Testing of NHexane Fraction of Red Dragon (*Hylocereus polyrhizus* Britton & Rose) Fruit Peel on *Staphylococcus aureus* ATCC 25923. Trad Med J. 2014;19(2):89-94.
29. Ahmad RZ, Gholib D. Testing of Ethanol Extract, Ethyl acetate, and Essential Oil of Beluntas Leaves (*Pluchea indica* (L) Lees.) against Trichophyton mentagrophytes and Cryptococcus neoformans *In Vitro*. Center for Veterinary Research, Bogor. 2013.
30. Pelczar M, Chan EC. Basics of microbiology. Jakarta: UI Press. 2006.
31. Afifi R, Erlin E, Rachmawati J. Anti-Bacterial Test of Belimbing Wuluh Leaf Extract (*Averrhoa bilimbi* L) Against *In Vitro* Inhibition Zone *Propionibacterium Acne* Bacteria. Quagga: Journal of Education and Biology. 2018;10(1):10-17.
32. Vashist H, Anil J. Antimicrobial activities of medicinal plants: Review. International Journal of Research .

GRAPHICAL ABSTRACT



ABOUT AUTHORS

Annysa Ellycornia S is a Pharmacist and Magister Pharmacy Student at the Faculty of Pharmacy, Universitas Indonesia, Kampus UI Depok, West Java 16424, Indonesia. She conducted research on pharmacognosy study of natural product and biotechnology of pharmacy.

Ratika Rahmasari is a Lecturer at the Faculty of Pharmacy, Universitas Indonesia, Kampus UI Depok, West Java 16424, Indonesia. She develops works in the area of Microbiology.

Berna Elya is a Professor and Lecturer at the Faculty of Pharmacy, Universitas Indonesia, Kampus UI Depok, West Java 16424, Indonesia. She develops works in the area of Pharmacognosy and Phytochemistry (Natural Product).

Cite this article: Silvyana AE, Rahmasari R, Elya B. *Azadirachta indica* Hexane Extract: Potent Antibacterial Activity Against *Propionibacterium* acne and Identification of its Chemicals Content. Pharmacogn J. 2022;14(3): 489-496.