Antibacterial Potential Of Ethanol Extract Of Tamarind Seed Bark (*Tamarindus Indica L.*) And Formulation Of Anti-Acne Nanogel

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Abstract.
Acne is a skin disease that affects a large number of sufferers. Acne occurs due to active oil glands under the skin. This activity is stimulated by androgen hormones. The active compound content of proanthocyanidin is the main compound in the ethanolic extract of tamarind seed bark, which can inhibit the growth of acne-causing bacteria, namely Propionibacterium acnes and Staphylococcus epidermidis. The purpose of this study was to formulate a nanogel from tamarind seed bark extract and test the antibacterial potential of an ethanol extract of tamarind seeds against acne-causing bacteria. The antibacterial potency test consisted of variations in concentration and was 0.5, 1, 5, 25, 50, 75, 100, 125, 150, and 300 (in mg/mL). The concentration of the formulated nanogel preparations was 2.5%, 5%, and 7.5%. The MIC results showed that the minimum inhibitory concentration (MIC) was at a concentration of 1 mg/mL with a zone of inhibition of 6.50 ± 0.44 mm (Propionibacterium acnes) and 6.40 ± 0.10 mm (Staphylococcus epidermidis). The minimum bactericidal concentration (MBC) was at a concentration of 25 mg/mL with a percentage reduction of 98.18% (Propionibacterium acnes) and 98.06% (Staphylococcus epidermidis). The results of the nanogel formulations showed that the particle sizes were 49.88 ± 0.11 nm (Formula I), 51.92 ± 0.09 nm (Formula II), and 59.13 ± 0.10 nm (Formula III). Conclusion The ethanolic extract of tamarind seed bark has effectiveness in inhibiting the growth of acne-causing bacteria and can be formulated as an anti-acne nanogel.

Keyword *: nanogel, antibacterial, tamarind seed bark, Propionibacterium acnes, Staphylococcus epidermidis*

I. INTRODUCTION

Skin is the outermost layer of the human body that is in direct contact with the environment outside the body. One way to make skin healthy and well-groomed is by using skin care products [1]. Facial skin is different from the skin of other parts of the human body because on the facial skin there are more sebaceous glands that produce a fatty acid called "cebum." [2]. Acne is a skin ailment in which the pores get clogged, resulting in an inflammatory pus pocket. Acne is a skin condition marked by the emergence of spots in various regions of the body, including the face, neck, back, and chest [3]. Acne occurs due to active oil glands under the skin. This activity is stimulated by the androgen hormone, which increases when a person is in puberty, and his oil glands are also increasing in height [4].*Propibacterium acnes* and *Staphylococcus epidermidis* are commonly found in acne lesions. *P. acnes* and *S. epidermidis* strains can hydrolyze triglycerides to produce free fatty acids and glycerol. Comedone lesions are made possible by these free fatty acids. [5]. Acne has long been treated with topical and systemic medicines, including antibiotics and retinoids. In dermatology medicine, however, antibiotic resistance, retinoid side effects, and medication allergies are on the rise. Alternative medicine based on medicinal plants has been researched as a solution to this problem for a variety of ailments. [6]. One of the herbal plants used for acne therapy is tamarind (Tamarindus indica L.) [7]. Proanthocyanidin is the main ingredient in the ethanolic extract of tamarind seed skin, which can inhibit the growth of Propionibacterium acnes bacteria.

In addition, ethanol extract of tamarind seed coat has been studied to have an antibacterial effect against Propionibacterium acnes and showed MIC results at a concentration of 250 mg/mL and MBC at a concentration of 500 mg/mL [6].In the pharmaceutical area, nanotechnology has a number of advantages, including enhancing drug solubility, lowering treatment dosages, and increasing absorption. As a result, nanoparticles are increasingly being employed in innovative medication delivery methods for a variety of cosmetic and dermatological dosage forms. [8]. Nanogels contain active substances that are lipophilic,

thereby increasing penetration into sebaceous tissue, which is affected by nano-sized particles [9]. Nanogel preparations can also increase the contact surface area so that the delivery of bioactives becomes faster and easier. The smaller particle size is expected to increase the contact area of the particles with the membrane and make it easier for particles to enter through the membrane [10]. So researchers are interested in investigating the antibacterial potential of ethanol extract of tamarind seed coat against Propionibacterium acnes and Staphylococcus epidermidis bacteria and formulating it into nanogel preparations.

II. METHOD

2.1 Sample

Sampling of tamarind seeds was carried out purposively and obtained from Jl. Tri Dharma, Medan, Sumatera Utara. The sample was dried to obtain a dry powder of tamarind seed bark.

2.2 Characterization of thamarind seed bark dry powder

Macroscopic examination was carried out [11], water content, water-soluble content, ethanol-soluble content [12], total ash content, and acid-insoluble ash content [12].

2.3 Preparation of Tamarind Seed Bark Ethanol Extract

The dry powder of thamarind seed bark is put into a container with a solvent (ethanol 70%) as much as 10 times the amount of dry powder. Stir well in the container for 6 hours, then leave it for up to 18 hours. The marinade is filtered and separated from the macerate. The filtrate is soaked again, let stand for 24 hours, and filtered. All the maserates were collected and evaporated until the consistency of the extract became thick. [13].

2.4 Phytochemical Screening

Phytochemical screening includes determination of alkaloids, flavonoids, tannins, saponins, and terpenoids or steroids. [11].

2.5 Antibacterial Activity Potential

Preparation of Various Concentration

3 grams of thick extract were weighed and dissolved using 10 mL of dimethyl sulfoxide to obtain a concentration of 300 mg/mL. Then the concentration series was made with dilution so that the concentration series was 150 mg/mL, 125 mg/mL, 100 mg/mL, 75 mg/mL, 50 mg/mL, 25 mg/mL, 5 mg/mL, 1 mg/mL, and 0.5 mg/mL.

Determination of Minimum Inhibitory Concentration (MIC)

Antibacterial activity potential for determination of minimum inhibitory concentration based on the agar diffusion method (Kirby-Bauer). 0.1 mL of the test bacterial inoculum was put into a sterile petri dish, then 15 mL of sterile Nutrient Agar medium was added and homogenized until solid. On the surface of the medium is placed a paper cover that has been soaked with the test solution for each concentration. Incubated at 36 oC for 24 hours, the treatment was repeated three times. Then the zone of inhibition was measured with a caliper (mm) [14], [15].

Determination of Minimum Bactericidal Concentration (MBC)

Antibacterial activity to determine the minimum bactericidal concentration (MBC) by the streaking method from the inhibition zone formed at each concentration, then swab on Plate Count Agar medium [16]. Incubated at 35 2oC for 24 hours. Then the number of colonies was calculated using a colony counter and then the percent reduction was calculated to determine the value of MBC [17], [18].

2.6 Nanogel Formulation of Tamarind Seed Bark Ethanol Extract

A nanodispersion of an ethanolic extract of tamarind seed bark was made by a modified emulsification-diffusion method. The ethanol extract of the tamarind seed bark was weighed with various weights (Table 1). For preformulation, carbopol was developed in lumps for 24 hours. The ethanol extract of the tamarind seed coat, which was weighed according to the concentration, was stirred constantly with a magnetic stirrer at 1000 rpm for 15 minutes. Propylene glycol was progressively added, then mixed continuously for 1 hour at 1000 rpm with a magnetic stirrer (organic phase). Tween 80 was dissolved in distilled water in a separate container and swirled continuously with a magnetic stirrer at 1000 rpm for 1 hour (aqueous phase) [19].

The organic phase was added to the water phase by dripping little by little while stirring constantly with a magnetic stirrer at 2500 rpm for 2 hours. Then the mixture was sonicated for 1 hour to form a nanodispersion. In lumping with carbopol that has expanded, a mixture of methyl paraben and propyl paraben that has been dissolved is added and crushed until homogeneous. The mixture of carbopol and preservatives was transferred to a glass beaker to be stirred until homogeneous at 2500 rpm and allowed to stand for 2 hours. Then 2 drops of TEA were added so that the mixture formed a gel base. The nanodispersion was added to the gel base and the remaining TEA while stirring constantly with a homogenizer at 1000 rpm for 30 minutes. After homogenization, sonication was carried out for 1 hour to form nanogels [20].

Table 1. Formula of Ethanol Extract Nanogel Tamarind Seed Bark

Ingredients	Formula I (%)	Formula II (%)	Formula III (%)
Tamarind Seed Bark Ethanol Extract	2,5	5	7,5
Carbopol	0,5	0,5	0,5
Tween 80	3	3	3
Propilenglicol	4	4	4
Ethanol	10	10	10
Methyl paraben	0,2	0,2	0,2
Propyl paraben	0,02	0,02	0,02
TEA	0,3	0,3	0,3
Aquadest	ad. 100	ad.100	ad.100

Organoleptic Test

Organoleptic examination including shape, smell, and color was carried out visually [21].

Measurement of the pH

pH determination is done using a pH meter. The number shown by the pH meter is the pH of the preparation [22].

Particle Size Analysis

Particle measurements were carried out using a Fritsch particle size analyzer (PSA). Particle measurement is done by inserting 500 mg of preparation into the tool. Leave the tool to give results. The test was carried out three times [23].

III. RESULT AND DISSCUSSION

3.1 Characterization of thamarind seed bark dry powder

The dried powder of thmarind seed macroscopic examination of tamarind seeds obtained was brown on the outside, white on the inside, round in shape, 1-2 cm in size, without a bitter aroma and taste. Examination of dry powder characterization of tamarind seed bark can be seen in the following table:

Table 2. Results of the Characterization of Tamarind Seed Bark Dry Powder Characterization

No.	Parameter	Result* (%)	Indonesian Herbal Pharmacopoeia Requirements Edition I (Depkes RI, 2013)
1.	Moisture Content	7,27 ± 0,04	≤ 10%
2.	Water-Solube Content	14,06 ± 0,02	≥ 4,4%
3.	Ethanol-Solube Content	22,06 ± 0,08	≥ 15,4%
4.	Total Ash Content	1,76 ± 0,01	≤ 16,6%
5.	Acid insoluble ash content	0,44 ± 0,05	≤ 0,7%

Note: (*) : The average result of three measurements

To assess the amount of water content in dried powder and the quality of thamarind seed bark dry powder, an investigation of the water content in dried powder was conducted. The water content obtained is 7,27 ± 0,04 percent , which implies it complies with the herbal pharmacopoeia's standard of less than 10%. Tamarind seed bark dry powder can serve as a favorable substrate for the growth of bacteria, fungi, and insects, speeding up the thamarind seed damage [24].The amounts of polar chemical compounds present in the thamarind seed bark dry powder were determined using the water soluble extract, whereas the levels of ethanol soluble chemicals, both polar and non-polar compounds, were determined using the ethanol soluble

extract. The water soluble extract content of simplicia was found to be 14,06 ± 0,02 percent, whereas the ethanol-soluble extract level was found to be 22,06 ± 0,08 percent. This indicates that the dry powder of tamarind seed bark includes more non-polar chemicals than polar components..

Determination of ash content to determine mineral content derived from plant tissue contained in thamarind seed bark dry powder [25]. By dissolving the total ash in hydrochloric acid [25]. The amount of silicate, particularly sand, present in thamarind seed bark dry powder is indicated by the acid insoluble ash concentration. The total ash content of thamarind seed bark dry powder was 1,76 ± 0,01 percent, with an acid insoluble ash content of 0,44 ± 0,05 percent, according to a study..

3.2 Phytochemical Screening

According to phytochemical screening data, the ethanolic extract of the tamarind seed coat includes flavonoid chemicals, glycosides, saponins, tannins, and triterpenoids/steroids. be seen in the table:

Table 3. Results of Phytochemical Screening of Tamarind Seed Bark Ethanol Extract

No.	Secondary Metabolites	Reagent	Result
1.	Alkaloid	Dragendroff Bouchardat Meyer	- - -
2.	Flavonoid	Mg powder + Amil Alcohol + HCl(p)	+
3.	Glikosida	Molish + H_2SO_4	+
4.	Saponin	Hot water / shaken	+
5.	Tanin	$FeCl_3$	+
6.	Triterpenoid/Steroid	Lieberman-Bourchat	+

Antibacterial properties can be found in flavonoid compounds and tannins. Secondary metabolites, such as flavonoids and tannins, work as antibacterials by interfering with the peptidoglycan constituent components of bacterial cells, causing the cell wall layer to not fully form, resulting in bacterial cell death. [26].

3.3 Minimum Inhibitory Concentration(MIC) Results

At a dosage of 25 mg/mL with a diameter of 10.00 ± 0.20 mm (*P. acnes*) and a concentration of 50 mg/mL with a diameter of 10.83 ± 0.06 mm (*S. epidermidis*), the antibacterial activity potential of the ethanolic extract of the tamarind seed coat was successful in suppressing the growth of *P. acnes* and *S. epidermidis* bacteria. Full results can be seen in the following table:

Table 4. Measurement Results of the Inhibitory Zone Diameter of Ethanol Extract of Tamarind Seed Bark against *Propionibacterium acnes* dan *Staphylococcus aureus*

Concentration (mg/mL)	Inhibitory Zones Diameter (mm)							
	Propionibacterium acnes				*Staphylococcus epidermidis*			
	P1	P2	P3	X ± SD	P1	P2	P3	X ± SD
0,5	6,0	6,0	6,0	6,00 ± 0,00	6,0	6,0	6,0	6,00 ± 0,00
1	6,8	6,0	6,7	6,50 ± 0,44	6,4	6,5	6,3	6,40 ± 0,10
5	7,6	8,0	7,9	7,83 ± 0,21	7,3	7,6	7,7	7,53 ± 0,21
25	9,8	10,0	10,2	10,00 ± 0,20	9,9	9,8	9,9	9,87 ± 0,06
50	11,9	11,9	11,6	11,80 ± 0,17	10,9	10,8	10,8	10,83 ± 0,06
75	12,7	12,3	12,6	12,53 ± 0,21	12,0	11,7	11,0	11,57 ± 0,51
100	13,6	13,1	13,4	13,37 ± 0,25	12,5	12,3	12,0	12,27 ± 0,25
125	14,1	14,0	13,9	14,00 ± 0,10	12,8	12,7	12,6	12,70 ± 0,10
150	14,9	14,8	14,6	14,77 ± 0,15	13,1	12,9	13,0	13,00 ± 0,10
300	16,3	15,9	16,5	16,23 ± 0,31	14,7	13,8	14,8	14,43 ± 0,55

As shown in Figure 1, the antibacterial activity of the ethanolic extract of tamarind seed bark with increasing concentration, and the diameter of the inhibitory zone created grew as well. The diameter of the inhibitory zone is affected by the concentration of the extract. The diameter of the inhibition zone grows in proportion to the concentration, so the least concentration that still exhibits antibacterial activity is 0.5

mg/mL, which is regarded as a weak inhibitor. The diameter of the inhibition zone generated determines the strength of an antibiotic, with weak inhibition ranging from 0 - 5 mm, moderate at 5 – 10 mm, strong at 10 – 20 mm, and very strong above 20 mm [18], [27].

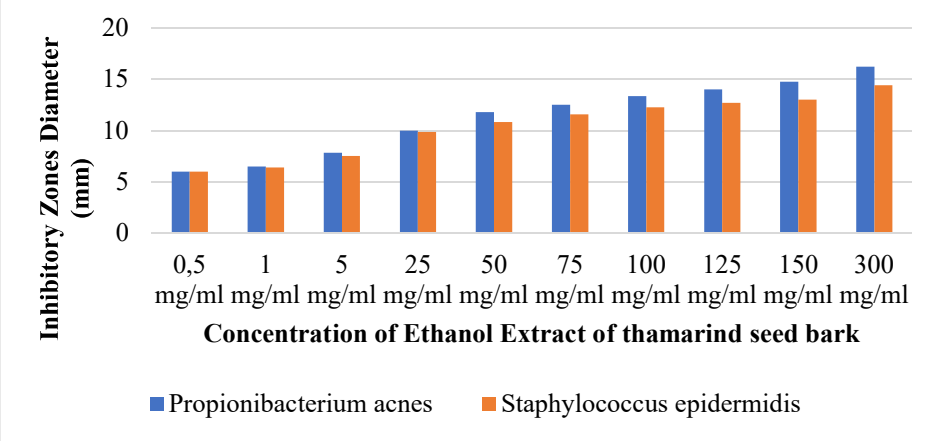

Fig 1. Graph of Inhibitory Zone Diameter of Ethanol Extract of Tamarind Seed Bark Against *Propionibacterium acnes* dan *Staphylococcus epidermidis*

3.4 Minimum Bactericidal Concentration (MBC) Results

The minimum killing rate is the concentration where there is a decrease or difference in the number of bacteria of around 98.00% to 99.99% after treatment compared to negative control (without treatment) [17].

Tabel 5. Results of the Calculation of the Number of Colonies and the Percent Reduction in *Propionibacterium acnes* dan *Staphylococcus epidermidis*

Concentration	*Propionibacterium acnes*			*Staphylococcus epidermidis*		
	Count	% Reduction	Log Reduction	Count	% Reduction	Log Reduction
Control -	1980	0,00	0,00	1802	0,00	0,00
0,5	1821	8,03	0,90	1711	5,05	0,70
1	783	60,45	1,78	731	59,43	1,77
5	405	79,55	1,90	455	74,75	1,87
25	36	98,18	1,99	35	98,06	1,99
50	20	98,99	2,00	28	98,45	1,99
75	18	99,09	2,00	23	98,72	1,99
100	7	99,65	2,00	11	99,39	2,00
125	4	99,80	2,00	8	99,56	2,00
150	1	99,95	2,00	6	99,67	2,00
300	0	100,00	2,00	0	100,00	2,00

In the table, the minimum bactericidal concentration of ethanol extract of tamarind seed bark on *P. acnes* bacteria at a concentration of 25 mg/mL with a percent reduction value of 98.18% and on *S. epidermidis* bacteria at a concentration of 25 mg/mL with a percent reduction value of 98.06%.

3.5 Formulation of Ethanol Extract Nanogel Preparation of Tamarind Seed Bark

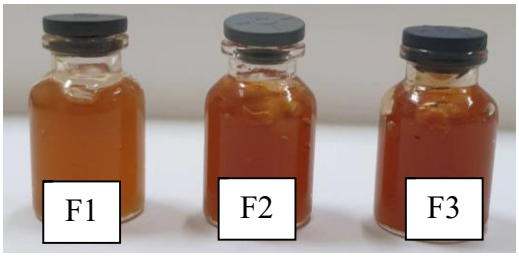

Fig 2. Ethanol Extract Nanogel Tamarind Seed Bark

Organoleptic Test

The results of the organoleptic examination were carried out on all dosage formulations and evaluated immediately after completion (Table 6)

Table 6. Results of Organoleptic Examination of Preparation of Ethanol Extract Nanogel Tamarind Seed Bark

No	Formula	Color	Smell	Appearance	Phase Separation
1	F1	Orange	Specific	Good	Not occur
2	F2	Dark Red	Specific	Good	Not occur
3	F3	Dark Red	Specific	Good	Not occur

Information :

F1 : Ethanol Extract Nanogel Tamarind Seed Bark 2.5%

F2 : Ethanol Extract Nanogel Tamarind Seed Bark 5%

F3 : Ethanol Extract Nanogel Tamarind Seed Bark 7.5%

Measurement of pH of Tamarind Seed Bark Extract Nanogel

The results of the measurement of the pH of the ethanol extract and the ethanolic extract of the tamarind seed bark were completed when the nanogel preparation was completed. The measured pH value can be seen in table 7.

Table 7. Results of Measurement of pH Ethanol Extract Nanogel Tamarind Seed Bark

No	Formula	pH value
1	F1	5,65 ± 0,05
2	F2	5,68 ± 0,08
3	F3	5,72 ± 0,02

The pH measurements revealed that all preparations met the skin pH requirements, i.e., between 4.5 and 6.5 [28]. If the pH value is too acidic, it can irritate the skin if it does not fall within the required range. Scaly skin can result from a pH value that is too alkaline [29].

Particle Size of Tamarind Seed Bark Extract Nanogel

Measurement of nanogel preparation particles was carried out using a Particle Size Analyzer (Fritsch) at room temperature when the preparation was finished. Data The results of the measurement of the nanogel preparations of the ethanol extract of the tamarind seed shell are as follows:

Table 8. Results of Measurement of Particle Size Ethanol Extract of Tamarind Seed Bark Nanogel

No	Formula	Particle size (nm)
1	F1	49,88 ± 0,11
2	F2	51,92 ± 0,09
3	F3	59,13 ± 0,010

The measurement results of nanogel preparations of tamarind seed coat extract that are formulated meet the requirements of nanometer particle size, which is not more than 1000 nm [30].

IV. CONCLUSSION

The ethanolic extract of the tamarind seed bark has antibacterial activity against *P. acnes* and *S. epidermidis*, and the nanogel formulation of the ethanol extract of the tamarind seed husk showed the particle size of the nanogel below 100 nanometers.

REFERENCES

[1] J. Prianto, *Cantik: Panduan Lengkap Merawat Kulit Wajah*. Gramedia Pustaka Utama, 2014.

[2] M. Dwikarya, “Cara Tuntas Membasmi Jerawat,” *Kawan Pustaka Jkt.*, 2007.

[3] D. Kusbianto, R. Ardiansyah, and D. A. Hamadi, “Implementasi sistem pakar forward chaining untuk identifikasi dan tindakan perawatan jerawat wajah,” ***J. Inform. Polinema***, vol. 4, no. 1, pp. 71–71, 2017.

[4] M. Dwikarya, “Merawat Kulit & Wajah,” *Jkt. Kawan Pustaka*, 2007.

[5] S. Kabau and P. Riyanto, “Hubungan antara pemakaian jenis kosmetik dengan kejadian akne vulgaris,” Fakultas Kedokteran, 2012.

[6] N. Baisaeng and S. Klayraung, “In Vitro Antibacterial Activity of Hydrogels Containing Tamarind Seed Husk Extracts against Propionibacterium acnes,” in *Key Engineering Materials*, 2019, vol. 819, pp. 85–91.

[7] A. P. Villupanoor, B. Chempakam, and T. J. Zachariah, “Chemistry of spices,” *King’s Lynn UK CAB IntlGoogle Sch.*, 2008.

[8] M. Marhamah, N. Nizar, O. Bunga, S. Kusumaningrum, and E. Rismana, "Pengujian Aktivitas Antiacne Nanopartikel Kitosan Ekstrak Kulit Buah Manggis (Garcinia Mangostana)," *Media Penelit. Dan Pengemb. Kesehat.*, vol. 24, no. 1, p. 20691, 2014.
[9] B. S. Chandrashekhar *et al.*, "Tretinoin nanogel 0.025% versus conventional gel 0.025% in patients with acne vulgaris: a randomized, active controlled, multicentre, parallel group, phase IV clinical trial," ***J. Clin. Diagn. Res. JCDR*,** vol. 9, no. 1, p. WC04, 2015.
[10] D. Thassu, M. Deleers, and Y. V. Pathak, *Nanoparticulate drug delivery systems*, vol. 166. CRC Press, 2007.
[11] Depkes R.I., "Farmakope Indonesia Edisi IV," *Jkt. Dep. Kesehat. RI*, 1995.
[12] Depkes R.I., "Farmakope Indonesia edisi ketiga," *Jkt. Dep. Kesehat. Repub. Indones.*, pp. 93–94, 1979.
[13] Depkes R.I., "Farmakope Herbal Indonesia," *Jkt. Dep. Kesehat. Repub. Indones.*, p. 531, 2017.
[14] R. Fitri, J. Reveny, U. Harahap, and H. Dharmawan, "Anti-Acne Activity From Biocellulose Mask Formula Containing (Aloe Vera (L.) Burm. F) Essence Combined With Vitamin E," ***Indones. J. Pharm. Clin.*** *Res.*, vol. 4, no. 1, pp. 1–7, 2021.
[15] U. Harahap, A. Dalimunthe, T. Hertiani, M. Muhammad, Nasri, and D. Satria, "Antioxidant and antibacterial activities of ethanol extract of Vernonia amygdalina Delile. Leaves," in *AIP Conference Proceedings*, 2021, vol. 2342, no. 1, p. 080011.
[16] A. A. Mostafa, A. A. Al-Askar, K. S. Almaary, T. M. Dawoud, E. N. Sholkamy, and M. M. Bakri, "Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases," ***Saudi J. Biol.*** *Sci.*, vol. 25, no. 2, pp. 361–366, 2018.
[17] M. Balouiri, M. Sadiki, and S. K. Ibnsouda, "Methods for in vitro evaluating antimicrobial activity: A review," ***J. Pharm. Anal***., vol. 6, no. 2, pp. 71–79, 2016.
[18] N. Nasri, U. Harahap, J. Silalahi, and D. Satria, "Antibacterial activity of lactic acid bacteria isolated from Dengke Naniura of Carp (Cyprinus carpio) against diarrhea-causing pathogenic bacteria," ***Biodiversitas J. Biol. Divers.*,** vol. 22, no. 8, 2021.
[19] A. J. Sivaram, P. Rajitha, S. Maya, R. Jayakumar, and M. Sabitha, "Nanogels for delivery, imaging and therapy," *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.*, vol. 7, no. 4, pp. 509–533, 2015.
[20] J. Ahmad, A. Gautam, S. Komath, M. Bano, A. Garg, and K. Jain, "Topical nano-emulgel for skin disorders: Formulation approach and characterization," *Recent Patents Anti-Infect. Drug Disc.*, vol. 14, no. 1, pp. 36–48, 2019.
[21] Pranuti, Susmita, Prasanna, and Vijay, "Design and Development of Nanogels Containing Carbapol as Polymer and Hydroalcoholic Extract of Pterocarpus marsupium as Herbal Medicament," ***World J. Pharm***. ***Res.***, vol. 7, no. 19, pp. 790–801, 2018.
[22] L. W. Ariani, "Formulasi Sediaan Nanogel Minyak Zaitun Sebagai Antiacne," *Repos. STIFAR*, 2020.
[23] EA. Rawlins, *Bentley's Textbook of Pharmaceuticals*. London: Bailliere Tindall, 2002.
[24] WHO, *Quality control methods for medicinal plant materials*. World Health Organization, 1998.
[25] WHO, "Quality control methods for medicinal plant materials," World Health Organization, 1992.
[26] N. Hasanah and D. R. Novian, "Daya Hambat Ekstrak Daun Belimbing Wuluh (Averrhoa bilimbi L) Terhadap Bakteri Penyebab Jerawat (Propionibacterium acnes)," ***J. Ilm. Farm.*,** vol. 9, no. 1, pp. 46–53, 2020.
[27] W. W. Davis and T. R. Stout, "Disc plate method of microbiological antibiotic assay: I. Factors influencing variability and error," *Appl. Microbiol.*, vol. 22, no. 4, pp. 659–665, 1971.
[28] R. I. Tranggono and F. Latifah, "Buku pegangan ilmu pengetahuan kosmetik," *Jkt. PT Gramedia Pustaka Utama*, vol. 3, no. 47, pp. 58–59, 2007.
[29] E. Genatrika, I. Nurkhikmah, and I. Hapsari, "Formulasi sediaan krim minyak jintan hitam (Nigella sativa L.) Sebagai antijerawat terhadap bakteri Propionibacterium acnes," ***Pharm. J. Farm. Indones. Pharm. J. Indones.*,** vol. 13, no. 02, pp. 192–201, 2016.
[30] S. Muniraj, R. Yogananda, T. S. Nagaraja, and D. R. Bharathi, "PREPARATION AND CHARACTERIZATION OF NANOGEL DRUG DELIVERY SYSTEM CONTAINING CLOTRIMAZOLE AN ANTI-FUNGAL DRUG"," 2020.