

Isolation of active compounds as anti-acne from ethanolic extract of *Artocarpus camansi* leaves

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Abstract. Acne vulgaris or acne is a chronic inflammatory skin disease in adolescents and adults affecting one self-confidence. The result of the research shows the ethanol extract, subfraction, pure isolate, and gel extract of *Artocarpus camansi* are active in inhibiting the growth of *Propionibacterium acnes*. The ethanol extract with the highest inhibition zone was at the concentration of 0.5 and 2% with 9.1 ± 0.62 mm and 8.5 ± 0.7 mm with the activity of 47.07% and 43% respectively (in comparison with the chloramphenicol $30 \mu\text{g}$ 19.33 ± 0.47 mm). The gel extract had the highest activity at 2%, with an inhibition zone of 8 ± 0.50 mm and the activity of 43.97%. Anti-acne activity for all subfractions had an activity of 32.5-33.3% while the pure isolate was at 42.10 - 45.63%. Physical properties of the gel extract were evaluated for four weeks by examining the pH test obtained with a range of 5.8-6.7, viscosity in the range of 18460-19668 cP, spreadability in the range of 5.45-7.20 cm, adhesion observation above 4 seconds, and homogeneity in a concentration of 0.5-7.5%. The pure isolate has a melting point of 147°C , presumed to be γ -sitosterol and appropriate with the screening phytochemicals results with the presence of steroid.

Keywords: *Artocarpus camansi*, Anti-acne, Gel, Medicinal plant, *Propionibacterium acnes*

INTRODUCTION

Acne vulgaris is a common chronic skin disease in the pilosebaceous unit that often affects adolescents [1] *Propionibacterium acnes* (*P. acnes*) is one of the causes involved in acne inflammation [2]. Although not life-threatening, this skin disease can affect the quality of life and produce social and economic impacts on the sufferer. It is necessary to search for appropriate and effective drugs to tackle the issue. *Artocarpus camansi* (*A. camansi*) plant is one of the plants Family Moraceae well-known to the public as a source of medicine. Scientific research has been carried out on the leaves, bark, roots, and fruit, showing that they are helpful in reduce concentrations of blood sugar [3-8]. *Artocarpus camansi* is also a good antioxidant and antibacterial activity against *Staphylococcus aureus* with a minimum inhibition concentration (MIC) of 25 mg/mL, 25 mg/mL for *Escherichia coli*, and 50 mg/mL for *Pseudomonas aeruginosa* [9]. Flavonoids in *A. camansi* are also known to have effects such as anti-inflammatory, antibacterial, antiviral [10], and

antioxidants [11]. However, research on *A. camansi* against bacteria causing acne, namely the bacterium *P. acnes*, is still lacking, even though other species from the Moraceae family are active in inhibiting the growth of other acne-causing bacteria. Various oral and topical anti-acne preparations are on the market, but gel forms are preferred. Gel forms are often better for acne treatment. Because the gel has cooling, moisturizing, and easy to use [12]. Further, gel preparation could be quickly absorbed, so it is more effective to help absorb active ingredients in the infected area [13].

METHODOLOGY

Materials

Leaves of *Artocarpus camansi* were collected from Bireuen, Aceh, Indonesia, in December 2020 and were identified in the Department of Biology of Universitas Syiah Kuala, Indonesia. Kingdom: Plantae, Sub Kingdom: Tracheobionta, Super Division: Spermatophyta, Division: Magnoliophyta, Class: Magnoliopsida, Sub Class: Hamamelididae, Order: Urtales, Family: Moraceae, Genus: *Artocarpus* JR Frost & G. I, Species: *Artocarpus camansi* Blanco. The bioindicator used in this study was the bacterium *Propionibacterium acnes*, Strain (ATCC 27853) from the Department of Biology, Universitas Sumatra Utara (USU).

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The chemical solvents used are n-hexane, ethyl acetate, and ethanol. NaCl 0.85%, 70% alcohol, lactose broth (Oxoid), Media Mueller-Hinton Agar (MHA) (Oxoid), Thin Layer Chromatography F₂₅₄ (Merck) and silica gel 60 GF₂₅₄ (Merck) and gel-making materials: Carbopol 940P, glycerin, triethanolamine were purchased at Rudang Jaya, Indonesia. Blank disk (Oxoid), antibiotic chloramphenicol disc 30µg (Oxoid) were purchased at Mitra Sejahtera Laboratorium, Indonesia.

GC-MS QP 2010 Ultra (Shimadzu, Japan), Electric balance (Mettler Toledo, Japan), Rotary evaporator (Buchi R-300), pH meter 710 A Thermo Electron Orion, Thermo Scientific Haake viscometer, scatter power test equipment, and glassware.

Extractions of *A. camansi* leaves

A 425 g of dried samples of *A. camansi* leaves cleaned from dirt and air-dried at room temperature. Thereafter were cut into small pieces and macerated using n-hexane solvent for 3 x 24 h. The filtrate was filtered; the residue was dried and then macerated again using ethanol for 3 x 24 h. The filtrate of ethanol was evaporated using a vacuum rotary evaporator and produced 34.01 g ethanol extract (8% yield of extract). The ethanol extract was used for phytochemical analysis [14], anti-acne activity assay [15] and evaluated physical properties gel extract. The ethanol extract was characterized using GC-MS and separated by gravitational column chromatography to obtain pure compounds.

Phytochemical analysis

The ethanol extract was used for phytochemical analysis such as alkaloids, terpenoids, steroids, saponins, flavonoids and phenolic [14].

Alkaloids identifications

Ethanol extract of *A. camansi* leaves was added with 0.05 N of ammonia-chloroform solutions. The liquid phase was collected into a test tube. Then, 5 mL of HCl 0.5 N was added and shaken. The mixture would be two layers. The top layer was a solution in HCL, and the bottom layer was a solution in chloroform. The solution in HCl was tested with three reagents. The formation of a yellow precipitate indicated the test with Mayer's reagent. The formation of a brown precipitate indicated the test with Wagner's reagent. Additionally, the formation of an orange or red precipitate indicated the test with Dragendorff's reagent [14].

Terpenoids and stroids identifications

Ethanol extract of *A. camansi* leaves was added with a few drops of Liebermann- Burchard. The color then changed into red, which indicated terpenoids, while green or blue color indicated steroids [14].

Saponins identifications

Ethanol extract of *A. camansi* leaves was added with the aquadest and shaken in a test tube for 5 min. A foam forming about 5 min which indicated saponins [14].

Flavonoids identifications

Ethanol extract of *A. camansi* leaves was added with 0.5 g of magnesium powder and HCl 0.5M. If the color changes into pink or purple, it indicated flavonoids [14].

Phenolics identifications

Ethanol extract of *A. camansi* leaves was added with FeCl₃. The color then changed into a deep green which indicated phenolics compounds [14].

Preparation of anti-acne gel

Composition of ethanol gel extract of *A. camansi* leaves was carried out based on (Tabel 1) in previous research conducted by Sharma [16] and then were modified.

Carbopol was developed with the help of water stirred gradually. Then, added triethanolamine and glycerin. The mixture were stirred constantly until homogeneous, next aquadest were added up to 100 mL while stirring gel base was formed. After that, the extract at various concentrations were added a stirred again until homogeneous [16].

Anti-acne activity assay

The anti-acne activity was assayed using the disk diffusion (Kirby-Bauer) method, with an incubation period of 24 hours, and three times of repetitions. As many as 30 mL of Mueller-Hinton Agar (MHA) medium were prepared and solidified onto a petri dish. The ethanol extracts of *A. camansi* leaves were made in various concentrations, namely 0.5; 2; 5; 7.5; and 12% (w/v).

Pure bacterial colonies of *P. acnes* in Nutrient Borth medium were taken with a micropipette, they were suspended in a sterile nutrient broth medium for regeneration then incubated and shaken at 150 rpm for 24 h. Right after, microorganisms were moved and suspended again in 5 mL of 0.9% NaCl solution to

Table 1. Composition of ethanol gel extract of *A. camansi* leaves

Materials	Composition of gel material (%)					
Extract	-	0.5	2	5	7.5	12
Carbopol 940	0.5	0.5	0.5	0.5	0.5	0.5
TEA	0.3	0.3	0.3	0.3	0.3	0.3
Glycerin	2.0	2.0	2.0	2.0	2.0	2.0
Alcohol	0.1	0.1	0.1	0.1	0.1	0.1
Methyl paraben	0.2	0.2	0.2	0.2	0.2	0.2
Aquades	96.9	96.4	94.9	91.9	89.4	84.9

Note: the composition of the ingredients were all made in the v/v, except for ethanol extract of *A. camansi* leaves that were using the w/v composition
TEA = Triethanolamine

Table 2. Phytochemical test results of ethanol extract of *A. camansi* leaves

Phytochemical contents	Test	Results
Alkaloids	Mayer	+
	Wagner	-
	Dragendorff	+
Terpenoids	TLC with Vanilin sulfat reagen	+
Steroids	Liebermann-Burchard	+
Flavonoids	0,5 g Mg dan hcl	+
Saponins	Water and Shaking	+
Phenolic	FeCl ₃	+

(+) presence of compounds (-) absence of compounds

reach the turbidity standard of 0.5 by McFarland or equal to 1.5×10^8 cfu mL⁻¹ [17]. Then they were swabbed into the media. Antibiotic chloramphenicol 30 µg and ethanol solvent were used as positive and negative control respectively.

Evaluation gel extract

Evaluation of the gel extract was carried out by observing its pH [18], spreadability [19], viscosity [20], adhesion [21], and homogeneity [22] for four weeks with three repetitions.

Determination of pH

2 g of ethanol gel extract was put into a test tube to measure the pH meter. The pH of good gel preparation is the range of 4.5-8 [18].

Determination of spreadability

0.5 g of ethanol gel extract was placed between round glass. Then a load of 100 g was placed on the glass and left for 60 sec to measure the constant diameter [19].

Determination of viscosity

1 g of ethanol gel extract was put into a test tube to evaluate the viscosity. The process was conducted on a Thermo Scientific Haake viscometer [20].

Determination of adhesion

0.5 g of ethanol extract gel was placed between round glass. They were pressured with a load of 50 g for 5 min. Further, the object in the glass is mounted on the test equipment that is giving a load weighing 500 g. The time required for the two glass separate was noted [21].

Determination of homogeneity

0.5 g of ethanol gel extract was placed between round glass. The extract was declared homogeneous if no coarse details were visible [22].

Table 3. Anti-acne activities of the ethanol extract and gel of *A. camansi* leaves

Sample	Zone of inhibition* (mm)	% Activity
Crude Extract	8,30±0.471	42,93%
Extract 0.5%	9,10±0.623	47,07%
Extract 2%	8,50±0.707	43,97%
Extract 5%	7,30±1.247	37,76%
Extract 7.5%	8±10.414	41,38%
Extract 12 %	7,30±1.247	37,76%
Gel Extract 0.5 %	6,33± 0.471	32,74
Gel Extract 2%	8,50± 0.50	43,97
Gel Extract 5%	7,00± 0.816	36,21
Gel Extract 7.5%	7,00± 0.816	36,21
Gel Extract 12%	7,00± 0.816	36,21
Positive control (+)	19,33 ± 0.471	-
Negative control (-)	-	-

Note: *Values are expressed as mean ± SD were calculated from three repetition

RESULTS AND DISCUSSION

Phytochemical contents

The result of phytochemical contents of ethanol extract of *A. camansi* leaves is shown in Table 2. Soegandi and Amelia [10] reported that the ethanol extract of *A. camansi* leaves contains secondary metabolites of alkaloids, triterpenoids, tannins, phenolics, flavonoids, saponins, and steroids.

Anti-acne activity

Anti-acne activities from ethanol extract and gel extract were presented in Table 3. The result showed that the concentrations of 0.5 and 2% were the optimum concentrations for inhibiting the growth of *P. acnes* bacteria. They had an inhibition zone of 9.10±0.62 and 8.50±0.70 mm, with the percent activity of 47.07 and 43.97%, compared to the positive control of chloramphenicol 30 µg with inhibition zone 19.33±0.47mm. This inhibition zone was categorized as the moderate. The classifications of inhibition zone were in four categories ;>20 mm, very strong; 10-20 mm, strong; 5-10 mm, medium; and <5 mm, no response [23].

Ethanol gel extract of *A. camansi* leaves had an inhibition zone more minor than the inhibition zone of each extract. The best gel inhibition zone was at a concentration of 2% extract with a diameter of 8.5 mm, which was considered as medium category [23]. Further, the percentage of activity was 47.07%. The difference in the concentration of the extract produced different inhibition zones for the gel. Percentage of activity was calculated by the following equation:

$$\text{Activity power \%} = \frac{\text{inhibition zone of extract} *}{\text{inhibition zone of control positive}} \times 100$$

Note * Inhibition zone of extract/sub fraction/pure isolate/gel extract.

A comparison of anti-acne activities of ethanol extract and gel extract can be seen in Figure 1. The data showed that the ethanol extract of *A. camansi* leaves, after formulated into a gel, experienced a decrease in anti-acne activity. This situation occurred because active compounds were trapped in the gel extract, which affected the active substance's diffusion process in the media [24]. Gel at a concentration of 2% produced a good consistency, not too dense and not too liquid. Presumably, it was able to release the active substance from the ethanol extract of *A. camansi* leaves into the test medium properly.

Several factors affected the size of the inhibition zone on bacteria, like plant sensitivity, reactions with the active ingredients, incubation medium, temperature [25], environmental pH, media components [26], colony density, metabolic activity of microorganisms, and impurities [27]. The results of research conducted by Vianey et al (2018) stated that the ethanol extract from the leaves of *A. camansi* had antibacterial activity against *S. aureus* and *E. coli* bacteria with inhibition zones of 11 and 10 mm. Soegandi and Amelia (2020) [10] uncovered that there were antibacterial activities of the ethanol extract of *A. camansi* leaves against *S. dysenteriae* and *B. subtilis* bacteria with a MIC of 6.25%.

The positive control used in the anti-acne test was 30 µg chloramphenicol antibiotic disc. Chloramphenicol

is an antibiotic with an action mechanism that inhibits the bacterial protein synthesis process [28]. The negative control did not produce any inhibition zone because organic solvents did not affect the bioactivity of secondary metabolites against pathogenic bacterial species [29]. Anti-acne activities resulting in this conclusion were due to the content of secondary metabolites such as, steroids [30], flavonoids [31], alkaloids [32], terpenoids [33], tannins, [34], phenolics [35].

Isolation of ethanol extract of *A. camansi* leaves and anti-acne pure isolate

Based on rechromatography, crude extract resulted in 62 fractions, later categorized into three subfractions. They were tested for their anti-acne activities (Table 4).

All subfractions had the relative same activities, so this subfraction was collected and further separated by gravity column chromatography to obtain pure compounds. The results of purity testing with three eluent systems, i.e., chloroform (100%); *n*-hexane:ethyl acetate (50:50); and (70:30). The *R_f* values of pure isolates obtained were 0.68, 0.5, and 0.72, respectively. The pure compound melted at 147°C. Based on the existing literature, it was suspected that the pure compound obtained was γ -sitosterol, which had a melting point range of 147-148 °C [36].

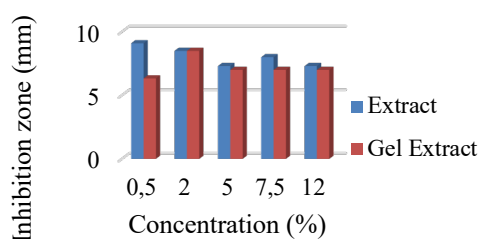


Figure 1. Comparison of inhibition zones of extract and gel extract.

Table 4. Anti-acne of sub fraction ethanol extract of *A. camansi* leaves

Sample	Zone of Inhibition* (mm)	% Activity
Subfraction I	6.50± 0.05	32.50%
Subfraction II	6.66± 0.00	33.30%
Subfraction III	6.66± 0.00	33.30%
Positive control (+)	20± 0.00	-
Negative control (-)	-	-

Note : All of subfraction were tested in 12% w/v

Note: *Values are expressed as mean ± SD were calculated from three repetition

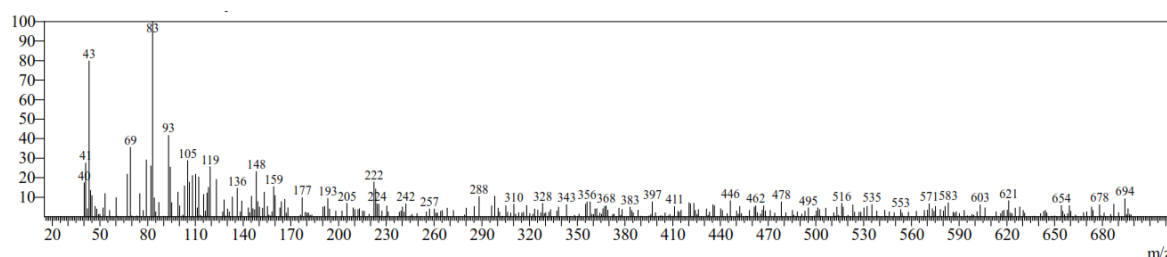


Figure 2. The spectrum of mass spectrometry was suspected γ -sitosterol.

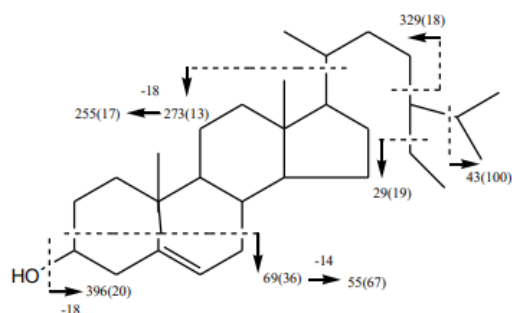


Figure 3. The fragmentation pathways of the γ -sitosterol compounds [37].

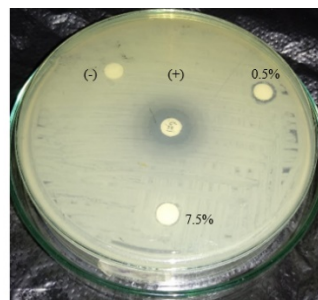


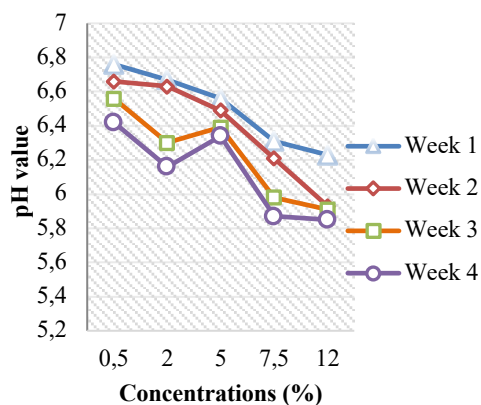
Figure 4. Inhibition zone pure isolate against *P. acnes*.

Table 5. Inhibition zones of pure isolate

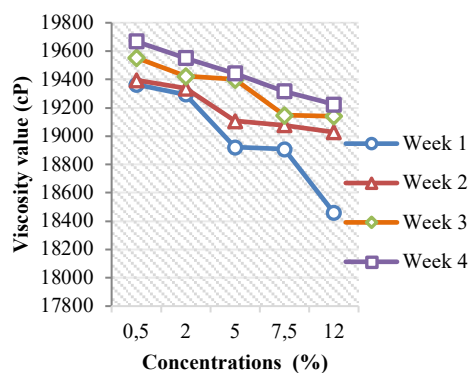
Sample	Zone of Inhibition* (mm)	% Activity
Pure isolate (0.5%)	8,67 $\pm$ 0,47	45,63
Pure isolate (7.5%)	8,00 $\pm$ 0,00	42,10
Positive control (+)	19,00 $\pm$ 0,81	-
Negative control (-)	-	-

Note: *Values are expressed as mean $\pm$ SD were calculated from three repetition

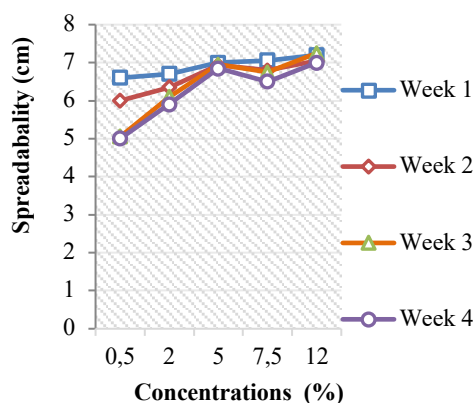
Based on the results of GCMS fragmentation pattern (Figure 2), it resulted the peaks of m/z as follows: 414, 396, 381, 354, 329, 303, 273, 255, 231, 213, 199, 185, 173, 159, 145, 133, 119, 107, 95, 81, 57, 43, and 41. This peak showed the similarity to the peak of the γ -



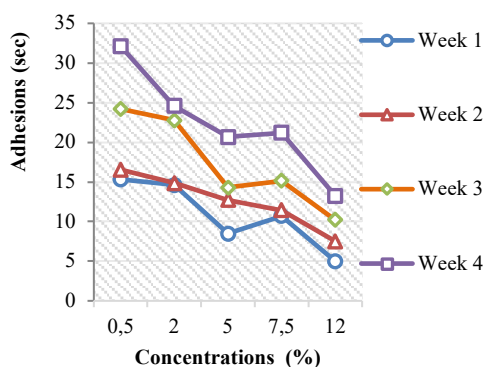
(a)



(b)



(c)



(d)

Figure 5. Graphic data for (a) pH, (b) viscosity, (c) spreadability, and (d) adhesion.

sitosterol compounds. The fragmentation pathways of the γ -sitosterol compounds were presented in Figure 3.

Anti-acne activity of pure isolate can be seen in Table 5 and Figure 4. The result of the anti-acne pure isolate was 42.10% and 45.63%, which was not much different from the activity of the crude extract. The most active concentration was 0.5%. That means the compounds that acted as anti-acne also played a significant role in the extract.

Evaluations of ethanol gel extract

Evaluations of gel extract with various concentrations had been evaluated for four weeks testing the pH, viscosity, spreadability, and adhesion (all presented in Figure 5).

The pH test on the ethanol extract of *A. camansi* leaves could be identified by the level of acidity. Good levels of pH acidity ranged from 4.5-8 [18]. Based on the data, the pH measurements ranged from 5.85 to 6.76. Meaning that ethanol gel extract was safe for use on the skin. Increasing the concentration of ethanol extract of *A. camansi* leaves in the gel caused the decreasing of the pH value (becoming more acidic). Further, the storage factor of pH value became more acidic than the previous pH. Presumably, the storage period of the gel extract evolved to be more concentrated so that the physical properties related to pH changed.

Based on viscosity data in the observation, ethanol gel extract ranged from 18460 to 19668 cP. Good topical gel preparation must align with SNI 16-4399-1996 with the viscosity value of 6000-50000 cP [38]. The viscosity value of the gel extract decreased along with the increased concentration of ethanol extract. Based on Figure 3 (b), storage time affected the viscosity. This statement was supported by Ida and Noer, 2012 [39]. One of the causes was *syneresis*, which was the release of the liquid trapped in the gel to come out to the surface [40]. Moreover, it was suspected that this occurred due to the influence of temperature and storage method of the gel preparation.

Adhesion for topical preparations was not too strong, causing skin breathing to be obstructed and it was not too weak to reduce the therapeutic effect [41]. Good topical preparation had an adhesion value of not less than 4 seconds [42]. In all concentrations, the ethanol gel extract of *A. camansi* leaves had good adhesion, above 4 seconds. Based on data, storage time made adhesion increased because the solvent used in gel extract was evaporated.

Ethanol gel extract was best homogeneity with concentrations of 0.5; 2; 5; and 7.5%. Gel extract at the concentration of 12% was not as good as gel preparation. This is presumably due to the addition of mass extract of concentration of 12% inhomogeneous.

CONCLUSION

It can be concluded that the extracts, pure isolates, and gel extracts from *A. camansi* leaves demonstrated relatively moderate inhibition power against *P. acnes*. The gel extract had an inhibition zone with a concentration of 2% and inhibition power of 43.97%, compared to the positive control of chloramphenicol 19.33mm. The pure compound had a melting point of 147-148 °C which is suggested to be γ -sitosterol.

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REFERENCES

- [1] Webster, G. F. 2002. Acne vulgaris. *Brit. Med. J.* **325** 475-479. DOI: 10.1136/bmj.325.7362.475.
- [2] Huang, W. C.; Tsai, T.H.; Chuang, L. T.; L, Y. Y.; Zouboulis, C. C.; Tsai, P. J. 2014. Anti-bacterial and anti-inflammatory properties of capric acid against *Propionibacterium acnes*: A comparative study with lauric acid. *J. Dermatol. Sci.* **73** 232-240. DOI:10.1016/j.jdermsci.2013.10.010
- [3] Nasution, R.; Muhabbah, N.; Helwati, H.; Bahi, M.; Marianne, M.; Amna, U. 2020. Isolation of lupeol acetate from fruit peels of *Artocarpus camansi*. *J. Adv. Pharm. Technol. Res.* **11** 148-156. DOI: 10.4103/japtr.japtr_6_20.
- [4] Nasution, R.; Fitrah, C. N.; Helwati, H.; Arifin, B. 2018. Antidiabetes activities extract hexane of kulu (*Artocarpus camansi*) peels. In *International Conference on Pharmaceutical and Clinical Research (ICPCR)*. **11** 12-17.
- [5] Nasution, R.; Wahyuni, S. E.; Mustanir.; Arifin, B.; Marianne. 2017. Isolation of chemical compounds from root of *Artocarpus camansi* and antidiabetic activity test. *Der. Pharma.Chemica* .**9** 60-65.
- [6] Nasution, R.; Bahi, M.; Saidi, N.; Junina, I. 2015. β -sitosterol from bark of *Artocarpus camansi* and its antidiabetic activity. In *Proceedings of The Annual International Conference, Syiah Kuala University-Life Sciences & Engineering Chapter*. **5** 118-124.
- [7] Nasution, R.; Barus, T.; Nasution, P.; Saidi, N. 2014. Isolation and structure elucidation of steroid from leaves of *Artocarpus camansi* (Kulu) as antidiabetic. *Int. J. Pharm. Tech. Res.* **6** 1279-1285.
- [8] Nasution, R.; Marianne, M. 2013. Didekilketon compounds from the leaves of *Artocarpus camansi* Blanco. In *Proceedings of The Annual*

- International Conference, Syiah Kuala University- Life Sciences & Engineering. **3** (1).
- [9] Vianney Y.M.; Amanda N.; Pieknell K.; Johan C.W.; Hardjo P.H. 2018. Evaluation of the antioxidant and antibacterial activity of breadnut (*Artocarpus camansi* Blanco) leaf extracts. *IJNPR*, **9** 151–159.
- [10] Soegandi, S.; Amelia, A. 2020. Antibacterial potency from ethanol extract leaves of kluwih (*Artocarpus camansi* Blanco) against *Shigella dysenteriae* and *Bacillus subtilis*. *JLD*, **2** 105-114. DOI: 10.19184/jld.v2i12.11568
- [11] Alcon. C. L.M.; Aimee. S. A. B.; Marie. F. N. O. 2021. Proximate composition, antioxidant capacity and functional properties of breadnut seed flour (*Artocarpus camansi*). *Turkish Jaf Sci. Tech*, **9** 1495-1499. DOI: 10.24925/turjaf.v9i8.1495-1499.4319
- [12] Nikam. S. Anti-acne gel of isotretinoin: formulation and evaluation, *Asian J. Pharm. Clin. Res.* **10** 257-266. DOI: 10.22159/ajpcr.2017.v10i11.19614
- [13] Ermawati, D. E.; Ramadhani, C. I. 2019. Formulation of anti-acne gel of *Moringa oleifera* L ethanolic extract and antibacterial test on *Staphylococcus epidermidis*. *J. Food Pharm. Sci.* **7** 34-44. <https://doi.org/10.22146/jfps.707>
- [14] Harborne, J.B. 1987. Metode Fitokimia. Terjemahan: Padmawinata, K dan Soediro, I. Institut Teknologi Bandung: Bandung.
- [15] Heatley. N. G. 1994. A method for the assay of penicillin. *Biochem J.* **38** 61-65. DOI: 10.1042/bj0380061
- [16] Sharma G.; Gadiya J.; Dhanawat M. 2018. A textbook of cosmetic formulations. Department of Pharmacy, Mewar University: Rajasthan, 312.;901.
- [17] Zapata, Adriana; Ramirez-Arcos, S. 2015. A comparative study of McFarland turbidity standards and the densimat photometer to determine bacterial cell density. *Curr. Microbiol.* **70** 907-909. DOI:10.1007/s00284-015-0801-2
- [18] Shukry, M. H.; Ghada F. 2013. Evaluation of topical gel bases formulated with various essential oils for antibacterial activity against methicillinresistant *Staphylococcus aureus*. *Trop. J. Pharm. Res.* **12** 877- 884.
- [19] Sinaga, A. A.; Luliana S.; Fahrurroji A. 2015. Antioxidant effectivity test of lotion from methanol extract of red dragon fruit (*Hylocereus polyrhizus* Britton & Rose). *Pharm Science Res.* **2** 11-20.
- [20] Djarot.P.; Isna. D.; Dwi. I. 2020. Formulasi dan uji antibakteri sediaan gel ekstrak daun mangga arumanis (*Mangira indica* L) sebagai antibakteri *Staphylococcus aureus* dan *Propionibacterium acnes*. *Fitofarmaka: Jurnal Ilmiah Farmasi.* **10** 84-96. DOI:10.33751/jf.v10i1.2072.
- [21] Agustin. R. D.; Taihuttu. Y. M. J. 2020. Formulation and physical stability test of hand sanitizer based on nutmeg oil (*Myristica fragrans* Houtt). International Conference on Sustainable Utilization of Natural Resources. 1-5
- [22] Standard Test Methods for Rheological Properties of Non-Newtonian Materials by Rotational (Brookfield type) Viscometer; D2196-05.2006
- [23] Safrida. Y. D.; Ruhiya, R. 2021. Uji daya hambat ekstrak etanol daun bandotan (*Ageratum conyzoides* L.) terhadap pertumbuhan bakteri *Escherichia coli*. *JKSD.* **1** 17-23. DOI: 10.56690/jskd.v1i1.7.
- [24] Irianto. I. D. K., Purwanto, Marwan. T. M. (2020). Aktivitas antibakteri dan uji sifat fisik sediaan gel dekokta sirih hijau (*Piper betle* L.) sebagai alternatif pengobatan mastitis sapi. *MF.* **16** 202-210 DOI:10.22146/farmaseutik.v16i2.53793
- [25] David W. W. and Stout T. R. (1971). Disc plate method of microbiological antibiotic assay. I. Factors influencing variability and error. *Appl. Microbiol.* **22** 659-665
- [26] Dali, S., Natsir, H. Usman, H. dan Ahmad, A. (2011). Bioaktivitas Antibakteri fraksi protein alga merah gelidium amansii dari perairan Cikoang Kabupaten Takalar, Sulawesi Selatan. *Majalah Farmasi dan Farmakologi*, **15** (1), 47–52/
- [27] Yahya, M.; Ginting, B.; Saidi, N. 2021 In-vitro screenings for biological and antioxidant activities of water extract from *Theobroma cacao* L. Pod Husk: Potential Utilization in Foods. *Molecules* **26** 6915. DOI: 10.3390/molecules26226915
- [28] Oong. G. C.; Prasanna. T. 2021. Chloramphenicol. StatPearls Publishing.
- [29] Khalil, A. 2012. Antimicrobial activity of ethanol leaf extracts of *Catharanthus roseus* from Saudi Arabia. 2nd International Conference on Environment Science and Biotechnology **48** 6-11.
- [30] Ouchari, L.; Boukeskase, A.; Bouizgarne, B.; Ouhdouch, Y. 2019. Antimicrobial potential of actinomycetes isolated from the unexplored hot Merzouga desert and their taxonomic diversity. *Biology Open.* **8** DOI: 10.1242/bio.035410
- [31] Xie, Y.; Weijie, Y.; Xiaoqing, C.; Licheng, R. 2015. Antibacterial activities of flavonoids: structure activity relationship and mechanism. *Curr. Med. Chem. Review.* **22** (1) 132-149
- [32] Ngapaan, T.P.; Ramasamy, P.; Wahid, M.E.A.; Segaran, T.C.; Vairappan, C.S. 2011. Biological activity of carbazdole alkaloids and essential oil of *Murraya koenigii* against antibiotic resistant

- microbes and cancer cell lines. *Molecules* **16** 9651-9664. DOI: 10.3390/molecules16119651.
- [33] Nasution, R.; Azwar, A. I.; Helwati, H.; Marianne. 2019. Antibacterial activities of perfume: combination flower *Magnolia alba*, *Cananga odorata* and *Mimusopselengi*. L, fixed with *Pogostemon cablin* Oil. *IDJPCR*. **2** 19-23. DOI: /10.32734/idjpcr.v2i1.814
- [34] Kaczmarek. B. 2020. Tannin acid with antiviral and antibacterial activities as a promising component of biomaterials- A Minireview. *Materials* **13** 1-13. DOI: 10.3390/ma13143224
- [35] Mikłasińska-Majdanik, M.; Kępa, M.; Wojtyczka R.D.; Idzik, D.; Wąsik, T.J. 2018. Phenolic compounds diminish antibiotic resistance of *Staphylococcus aureus* clinical strains. *Int. J. Environ. Res. Public Health*. **15**(10):2321. DOI: 10.3390/ijerph15102321.
- [36] Jannah, H.; I Made, S.; Yayuk, A. 2013. Analisis senyawa fitosterol dalam ekstrak buah buncis (*Phaseolus vulgaris* L.). *Chem. Prog.* **2** 70-75. DOI:10.35799/cp.6.2.2013.3497.
- [37] Asthary, A. A.; Yuharmen; Teruna, H.Y. 2014. Isolasi dan uji toksisitas senyawa metabolit sekunder dari ekstrak n-Heksana daun tumbuhan *Polyalthia rumphii* (B) Merr. (*Annonaceae*) (Karya Tulis Ilmiah). Universitas Riau: Pekanbaru.
- [38] Sediaan Tabir Surya SNI 16-3499-1996. (1996). Dewan Standarisasi Nasional: Jakarta.
- [39] Ida N.; Noer S. F. (2012). Uji stabilitas fisik gek ekstrak lidah buaya (*Aloe vera* L.). *Majalah Farmasi dan Farmakologi* **16** (2) 79-84.
- [40] Kuncari, E. S.; Iskandarsyah, I.; Praptiwi, P.. 2014. Evaluasi, uji stabilitas fisik dan sineresis sediaan gel yang mengandung minoksidil, apigenin dan perasan herba seledri (*Apium graveolens* L.). *Bul. Penelit. Kesehat.* **42** 213-222. DOI:10.22435/bpk.v42i4%20Des.3659.213-222
- [41] Surini, S.; Amirtha, N. I.; Lestari, D.C. 2018 Formulation and effectiveness of a hand sanitizer gel produced using Salam bark extract. *Int. J. App. Pharm.* **10** 216-220. DOI: 10.22159/ijap.2018.v10s1.48.
- [42] Dhase, A. S.; Khadbadi, S. S.; Saboo, S. S. 2014. Formulation and evaluation of vanishing herbal cream of crude drugs. *Am. J. Ethnomedicine*. **1** 313-318.