RESEARCH ARTICLE

Nanoemulsion of Ethanolic Extract of Sungkai Leaves (*Peronema Canescens Jack*) for Anti-Acne Therapy

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ABSTRACT

This study investigates the phytochemical profile, total flavonoid content, antibacterial properties, antioxidant activities, and cytotoxicity effect of nanoemulsion ethanolic extract of Sungkai leaves. The ethanolic extract was formulated into three concentrations: 50, 100, and 150 ppm. Phytochemical screening revealed flavonoids, tannins, alkaloids, steroids, and terpenoids. The total flavonoid content was 90.542, 91.993, and 91.744% for the respective concentrations. Antibacterial activity against *Propionibacterium acnes* showed inhibition zones of 17.6, 21.2, and 24.1 mm, indicating strong to very strong activity. Antioxidant activities, evaluated using DPPH, ABTS, and FRAP assays, showed IC_{50} values categorized as very strong. The cytotoxicity test exhibited moderate activity. These findings suggest that Sungkai leaf ethanol extract nanoemulsion has rich bioactive compounds with significant antibacterial and antioxidant properties, along with moderate cytotoxic activity, highlighting its potential for pharmaceutical and cosmetic applications.

Keywords: Sungkai leaves, Nanoemulsion, Flavonoid, Antibacterial, Antioxidant, Cytotoxicity.

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INTRODUCTION

Acne is a common skin condition that is influenced by multiple factors, including genetic predisposition, hormonal changes, and environmental factors.[1] *Propionibacterium acnes* has recently been shown to play a part in acne pathogenesis, but its overgrowth is not the only cause.[2] Rather, a fine-tuned balance between the skin commensals and *P. acnes* phylotypes might be key in acne development.[3,4] Current therapies for acne consist of topical retinoids, benzoyl peroxide, and oral antibiotics or hormonal treatment.[5] However, these cures often come with downsides when it comes to antibiotic resistance and side effects.[6] Photodynamic therapy and laser/light therapies have been utilized, but there are often limitations in clinical efficacy as well as tolerability based on skin type.[7] A number of treatments may reduce lesion counts, favorably affect some patient-reported outcomes, and are typically well tolerated, according to a systematic review study.[4,8,9]

Sungkai leaves (*Peronema canescens Jack*) are a traditional medicinal plant native to Indonesia, particularly Kalimantan and Sumatra.[10] These leaves have been used in various treatments for their diverse therapeutic properties. Empirical evidence from multiple studies supports their efficacy.[11–13] For instance, GC-MS analysis revealed that Sungkai leaves contain a range of chemical compounds, including flavonoids, tannins, and saponins, which contribute to their antioxidant capacity, as demonstrated by their strong antioxidant activity with IC_{50} values of 26.389 mg/L for young leaves.[13] The presence of secondary metabolites has been linked to their anti-inflammatory activity, as seen *in-vivo* and *in-silico* studies where flavonoid and steroid fractions exhibited significant anti-inflammatory effects.[15–17]

The rationale for using a nanoemulsion of ethanolic extract from Sungkai leaves is rooted in its potential to enhance the efficacy and bioavailability of active compounds.[18,19] Nanoemulsions, characterized by submicron particle sizes, would deliver active ingredients and improve biocompatibility, making them suitable for topical applications.[20–22] Studies have shown that nanoemulsions can increase the permeability and diffusibility of active substances, thereby enhancing their therapeutic effects.[21,23] For instance, the formulation of nanoemulsions from ethanolic extracts of *Centella asiatica* has been optimized to improve blood-brain barrier permeability and exhibit significant antioxidant, anti-inflammatory, and neuroprotective activities.[24,25] Similarly, the nanoemulsification of *Petrosia* sp. ethanolic extract has been shown to produce stable and effective nanoemulsions with enhanced skin penetration and therapeutic properties.[26]

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The present study investigates the potential of a nanoemulsion formulation of the ethanolic extract of Sungkai leaves for anti-acne therapy. The study aims to characterize the phytochemical profile, total flavonoid content, antibacterial activity against *P. acnes*, antioxidant properties, and cytotoxicity of the Sungkai leaf extract nanoemulsion.

MATERIALS AND METHODS

Materials

The tools used in this research are an autoclave (All American), CO2 incubator (NU-5100E, Nuaire, USA), analytical balance (Precisa), thinky mixer, ELISA reader (Dynatech MR5000 TECAN), Tecan Group Ltd., Switzerland), inverted microscope (TC 5400, Meiji Techno, Japan), haemacytometer (Hirschmann, EM Techcolor, Germany), laminar airflow (Jouan MSC 12, Thermo Fisher Scientific, USA), flask culture 25 cm, 96 well plate, pipette tip (1000, 200 and 20 µL), 0.22 µL filter, 50 and 15 mL falcon tubes, 1.5 mL Eppendorf tube, aluminum foil, 5 mL filter syringe, filter paper, funnel (Iwaki), 100 mL infusion bottle, vial, Erlenmeyer (Iwaki), burette, dropper pipette (Iwaki), beaker (Iwaki pyrex), thermometer, aluminum foil, measuring cup, watch glass, evaporator cup, tongs, metal spatula, UV-vis spectrophotometer.

Materials used are 10 kg sungkai leaves, distilled water (Novalindo), tween 80 (Barataco), lecithin, VCO (Shankar Soya), Fibroblast cells isolated from prepuce tissue, 3D data search through the protein bank data ID: 3TZM, fetal bovine serum (FBS), phosphate-buffered saline (PBS) rich chemical CoUSA), trypsin EDTA 0.25, 3-(4,5- dimethylthiazo-phenyltetrazolium bromide (MTT) (Callbiochem, USA), dimethyl (DMSO) Fisher Chemical, USA), ethyl acetate (PT. Novalindo), DPPH.

Methods

Sungkai leaves (10 kg) were sourced from Padang Pariaman Regency, West Sumatra, and extracted using the maceration method in ethanol PA solvent.[27] Phytochemical screening of alkaloids, flavonoids, saponin, tannin, and phenolic compounds was referred to as an established method.[28–30] The total flavonoid content was determined by common protocol.[16,19,31]

To synthesize the nanoemulsion, the ethanolic extract was dissolved in the oil phase (VCO) 2.3%, lecithin 1%, and Tween-80 28.3%, then stirred with a thinky mixer at 75°C with a speed of 1500 rpm for 15 minutes. Then, the oil phase containing the ethanolic extract was mixed in the thinky mixer again, and distilled water was added little by little until homogenous.[24] The formulation of the nanoemulsion is presented in Table 1.

Table 1: Nanoemulsion formulation

Components	*Concentration*	*Function as*
Tween 80	28.3%	Co-surfactant
Lecitin	1%	Surfactant
VCO	2.3%	Oil phase
Stirring speed	1500 rpm	Stirring control
Stirring time	15 minute	Timing control

To evaluate the quality of the synthesized nanoemulsion, we employed organoleptic observation, pH test, density with picnometer, and viscosity with viscometer at spindle no. 3 and 100 rpm. The freeze and thaw at 25°C, 24 hours and -5°C, 24 hours, for three cycles.[24] The particle size analyzer (PSA) was used to measure the average size of nanoparticles with dynamic light scattering methods.[26]

The antibacterial effect of nanoemulsion (100, 150, and 200 µg/mL) against *P. acnes* (100 µL/15 mL nutrient agar) was determined in the sterile discs method.[2,32] The positive control is clindamycin, and the negative control is the basis of nanoemulsion.[33]

The DPPH method involved preparing a 0.4 mM DPPH solution, determining the maximum wavelength at 500 to 600 nm, and measuring absorbance at intervals using a 50 µg/mL vitamin C solution as standard. Vitamin C standards (2–10 ppm) and Sungkai leaf extract solutions (20–100 ppm) were tested by mixing with DPPH and measuring absorbance after 30 minutes.[34,35] For the ABTS method, ABTS and K2S2O8 stock solutions were mixed and stored in the dark. The maximum wavelength was 748 nm. Vitamin C standards (40–200 µg/mL) and sungkai leaf extract solutions were tested by mixing with ABTS and measuring absorbance after 6 minutes.[36,37] For the FRAP method, a 0.2 M phosphate buffer (pH 6.6), 1% potassium ferricyanide, 0.1% $FeCl_3$, and 10% TCA solutions were prepared. The maximum wavelength was 720 nm. Vitamin C standards (10–50 ppm) and Sungkai leaf extract were tested by reacting with FRAP reagents and measuring absorbance after 20 minutes of incubation at 50°C.[38–40] After obtaining the absorbance values from DPPH, ABTS, and FRAP tests, the percentage inhibition and the IC_{50} value are calculated using linear regression.[34,41] The nanoemulsion of ethanolic extract from sungkai leaves was tested using the microculture tetrazolium test (MTT) on fibroblast cells.[12] Equipment was sterilized, solutions were prepared, and cells were cultured in a complete medium. After incubation with various extract concentrations, the media was replaced with MTT solution and incubated again. Live cells formed formazan crystals, which were solubilized using SDS, and absorbance was recorded at 570 nm. Cell viability was then calculated, and the IC_{50} value was determined by applying probit regression analysis to the viability data against the log of the concentration. Lower IC_{50} values indicate stronger antioxidant activity of the extract.[42,43]

Data Analysis

Data obtained from each treatment calculation will be statistically processed using normality and homogeneity tests. Normality will be assessed using the Shapiro-Wilk test ($p > 0.05$), while Levene's test will check for homogeneity ($p > 0.05$). While data is normally distributed and homogeneous, a parametric One-Way ANOVA will be conducted, followed by a Post Hoc LSD test for further analysis.[34,44–46]

RESULTS AND DISCUSSION

Properties of Extract

Results indicated a percent yield of 5.852%, meeting the criterion (< 10%) for weight loss upon drying. Total ash content was found to be 4.605%, with acid-insoluble ash at 0.575%, both meeting specifications (< 5 and < 0.7%, respectively) for inorganic residue determination. These findings confirm that Sungkai leaves ethanolic extract meets the required quality standards, with minimal physiological metal content, ensuring its suitability for further applications.[19]

Phytochemical and Total Flavonoid Content

Phytochemistry screening in ethanolic extract revealed the flavonoids, terpenoids, steroids, tannins, and alkaloids contents. The maximum wavelength observed in this study was 413.70 nm. Results indicated that the flavonoid content in the extract at concentrations of 50, 100, and 150 ppm was 90.542, 91.993, and 91.744%, respectively.[47,48]

Characteristics of Nanoemulsion of Ethanolic Extract

The nanoemulsion formula is a thick liquid, clear dark green, with the characteristic aroma of sungkai leaf extract. These parameters remained unchanged during the 6-week observation. The average pH of the nanoemulsion base is 6.34, within the skin's pH range. Freeze-thaw stability testing demonstrated no color or phase separation after three cycles of storage. The base viscosity is 184 Cp, thicker than water. The density of the nanoemulsion base upon initial preparation was 1.033 g/mL. Adding the extract increases viscosity. The oil phase mixed with phospholipid-surfactant is higher than the water phase, making the base thicker with higher viscosity.[49]

Antibacterial Effects

The antibacterial activity of the 50 ppm sungkai leaf extract nanoemulsion serum showed strong activity, while concentrations of 100 and 150 ppm showed very strong activity against *P. acnes* bacteria, as presented in Table 2.

Sungkai leaf extract shows higher antibacterial potential compared to banana peel extract, especially at higher concentrations (100 and 150 ppm), where it exhibits very strong activity. Banana peel extract shows moderate activity against *P. acnes* but weak activity against *S. aureus* at comparable concentrations.[2,50] In comparison, moringa leaf extract also shows antibacterial properties, particularly against *S. epidermidis*. The inhibition zones for moringa leaf extract are 9.15 mm at 0%, 10.2 mm at 2.5%, 11.05 mm at 5%, and 12.3 mm at 7.5%, indicating moderate activity at 2.5 and 5% and strong activity at 7.5%.[51]

Table 2: Bacterial inhibition of nanoemulsion

Samples (Concentration)	*Inhibition diameter (mm)*			
	Disc 1	*Disc 2*	*Disc 3*	*Average*
50 ppm	16.9	17.8	18.2	17.6
100 ppm	21.1	22.3	20.1	21.2
150 ppm	23.9	24.1	24.2	24.1
Clindamycin	36.2	37.5	38.9	37.5

Antioxidant Activity

DPPH assay

Absorbance was detected at a peak wavelength of 517.60 nm for test solutions at concentrations of 50, 100, and 150 ppm. The antioxidant activity is presented in Table 3.

Based on the results, the IC_{50} value of the serum nanoemulsion of sungkai leaf extract is 48.870 µg/mL, indicating very strong antioxidant activity. These findings align with a previous study (IC_{50} value = 3.98 µg/mL) for red betel leaf extract, also within the very strong range.[40]

ABTS assay

ABTS was assessed by a max wavelength of 745.40 nm, as shown in Table 4.

The extract of sungkai leaves demonstrated stronger antioxidant capacity compared to black soybean extract (77.39 µg/mL) and daidzein (83.34 µg/mL), as shown in another study.[41] This indicates that sungkai leaf extract is more effective at lower concentrations in neutralizing free radicals.

FRAP assay

FRAP assay with a maximum wavelength of 413.70 nm was presented in Table 5.

The data indicate that the ethanolic extract has antioxidant activity, suggesting its ability to reduce Fe^{3+} ions and inhibit free radical formation. These findings are in line with a study of mangosteen peel ethanol extract, which demonstrated strong linearity using the FRAP colorimetric method.[52]

Table 3: Nanoemulsion's antioxidant with DPPH assay

Sample	Concentration (µg/mL)	Absorbance (nm)	Inhibition (%)	Linear regression	IC_{50} (µg/mL)
Vitamin C	100	0.244	81.888	y= 12.992+19.642 R^2=0.945	10.347
	80	0.306	75.915		
	60	0.352	71.484		
	40	0.415	65.414		
	20	0.468	60.308		
Serum nanoemulsion	150	0.381	68.6898	y=15.745x-11.235 R^2= 0.974	48.870
	100	0.475	59.633		
	50	0.565	50.963		

Table 4: Nanoemulsion's antioxidant with ABTS assay

Sample	Concentration (µg/mL)	Absorbance (nm)	Inhibition (%)	Linear regression	IC_{50} (µg/mL)
Vitamin C	100	0.113	88.070		
	80	0.156	80.526		
	60	0.229	67.719	y=21.728x+15.719 R^2=0.9306	20.586
	40	0.259	62.456		
	20	0.318	52.105		
Serum nanoemulsion	150	0.212	70.701		
	100	0.265	61.403	y=16.888x-14.868 R^2= 0.9807	46.575
	50	0.32	51.754		

Table 5: Nanoemulsion's antioxidant with FRAP assay

Sample	Concentration (µg/mL)	Absorbance (nm)	Inhibition (%)	Linear regression	IC_{50} (µg/mL)
Vitamin C	100	0.306	88.070		
	80	0.351	80.526		
	60	0.415	67.719	y=21.728x+15.719 R^2=0.9306	20.586
	40	0.455	62.456		
	20	0.510	52.105		
Serum nanoemulsion	150	0.352	70.701		
	100	0.415	61.403	y=16.888x-14.868 R^2= 0.9807	46.575
	50	0.468	51.754		

Table 6: Cytotoxicity with MTT assay

Concentration (µg/mL)	*Repetition*			*Mean*	*%Viability*	*IC_{50} (µg/mL)*
	1	*2*	*3*			
50	0.123	0.126	0.118	0.12	13.76	
100	0.151	0.167	0.175	0.16	44.72	83.2
150	0.201	0.223	0.226	0.22	83.29	

Cytotoxicity Assay

Absorbance readings from the ELISA Reader at the reaction's maximum wavelength for four controls with MTT salt indicated that as the extract concentration increased, the number of viable cells decreased, or the number of dead fibroblast cells increased. The regression equation yielded an IC_{50} value of 83.2 µg/mL for the sungkai leaf nanoemulsion serum, indicating that at this concentration, the ethanolic extract inhibits the proliferation of 50% of preputial cells, as shown in Table 6.

The lower IC_{50} value indicates greater potency at lower concentrations, resulting in lower systemic toxicity when administered to patients. According to the US National Cancer Institute, an extract is considered cytotoxic if the IC_{50} value is 30 µg/mL or lower, moderately active if the IC_{50} ranges from 30 to 100 µg/mL, and inactive if the IC_{50} exceeds 100 µg/mL.[53] The results indicate that the IC_{50} value for the sungkai leaf ethanolic extract nanoemulsion falls within the moderately active range for preputial cells, a model for acne-prone skin. This moderate IC_{50} value suggests that the formula is effective at lower concentrations, making it safer due to lower systemic toxicity.[54]

CONCLUSION

The study demonstrated bioactive compounds, including flavonoids, tannins, steroids, terpenoids, and alkaloids, in the ethanolic extract of sungkai leaves. The nanoemulsion formulated with this extract exhibited stable physical properties over an extended period, maintaining its pH compatibility with skin, and showed no significant changes, indicating its suitability for topical applications. The extract demonstrated strong antibacterial activity against *P. acnes* and exhibited very strong antioxidant activity across multiple assays. Additionally, it showed moderate cytotoxicity, suggesting that it can be safely used in pharmaceutical and cosmetic applications, highlighting its potential for effective anti-acne therapy.

REFERENCES

1. Knutsen-Larson S, Dawson AL, Dunnick CA, et al. Acne Vulgaris: Pathogenesis, Treatment, and Needs Assessment. *Dermatologic Clinics*; 30. Epub ahead of print 2012. DOI: 10.1016/j.det.2011.09.001.
2. Siregar H, Chiuman L, Girsang E. Antibacterial Effectiveness Of Banana Fruit Extract (Musa Paradisiaca Cv. Awak) Against Staphylococcus Aureus And Propionibacterium Acne Bacteria With Disc Defusion Method. *Journal Health & Science : Gorontalo Journal Health and Science Community*; 6. Epub ahead of print 2022. DOI: 10.35971/gojhes.v6i2.14494.
3. Angie E, Abdullah H, Martinus AR, et al. The Potential of Forest Honeybee on Decreasing Malondialdehyde Levels in Adults with Mild Acne Vulgaris. 2020. Epub ahead of print 2020. DOI: 10.5220/0009490803090316.
4. Castillo DE, Nanda S, Keri JE. Propionibacterium (Cutibacterium) acnes Bacteriophage Therapy in Acne: Current Evidence and Future Perspectives. *Dermatology and Therapy*; 9. Epub ahead of print 2019. DOI: 10.1007/s13555-018-0275-9.
5. Vasam M, Korutla S, Bohara RA. Acne vulgaris: A review of the pathophysiology, treatment, and recent nanotechnology-based advances. *Biochemistry and Biophysics Reports*; 36. Epub ahead of print 2023. DOI: 10.1016/j.bbrep.2023.101578.
6. Patel HA, Guo L, Feldman SR. The impact of current investigational drugs for acne on future treatment strategies. *Expert Opinion on Investigational Drugs*; 33. Epub ahead of print 2024. DOI: 10.1080/13543784.2024.2313091.
7. Kim RH, Armstrong AW. Current state of acne treatment: Highlighting lasers, photodynamic therapy, and chemical peels. *Dermatology Online Journal*; 17. Epub ahead of print 2011. DOI: 10.5070/d30t40h9px.
8. Kataria U, Chhillar D. Acne: Etiopathogenesis and its management. *International Archives of Integrated Medicine Usha Kataria IAIM*; 2.
9. Tan ST, Firmansyah Y. New Drug Formulations For Acne Vulgaris-Pathogenesis Based Treatment Of Acne Vulgaris. *Jurnal Medika Hutama*; 2.
10. Gendro Sari S, Aulya D. Morfologi Batang Dan Daun Sungkai (Peronema Canescens) Pada Lingkungan Tumbuh Yang Berbeda. *Seminar Nasional Hasil Penelitian dan Pengabdian Kepada Masyarakat 2022 LP2M UST Jogja* .
11. Ulfa E, Dollangi S. Pemanfaatan Ekstrak Daun Sungkai (Peronema canescens Jack) Untuk Menurunkan Kadar Asam Lemak Bebas Dan Bilangan Peroksida Dalam Minyak Jelantah. *Jurnal Karya Ilmiah Multidisiplin (JURKIM)*; 3. Epub ahead of print 2023. DOI: 10.31849/jurkim.v3i1.11327.
12. Dillasamola D, Aldi Y, Wahyuni FS, et al. Study of Sungkai (Peronema canescens, Jack) leaf extract activity as immunostimulators with *in-vivo* and in vitro methods. *Pharmacognosy Journal*; 13. Epub ahead of print 2021. DOI: 10.5530/PJ.2021.13.177.
13. Okfrianti Y, Irnameria D, Bertalina B. Aktivitas Antioksidan Ekstrak Etanol Daun Sungkai (Peronema canescens Jack). *Jurnal Kesehatan*; 13. Epub ahead of print 2022. DOI: 10.26630/jk.v13i2.3200.
14. Afnuhazi R, Sari FS, Hendrawati, et al. The effect of Stewed Sungkai Leaves (Peronema canescens Jack) on the decrease in Inflammatory Symptoms of Covid-19 patients. *Res J Pharm Technol*; 15. Epub ahead of print 2022. DOI: 10.52711/0974-360X.2022.00921.
15. Dillasamola D, Wahyuni FS, Rita RS, et al. Immunostimulating Activity of Sungkai Leaf Stigmasterol Isolate against Cluster Differentiated 8+ T (CD8+T) from Covid-19 Disease Exposure. *Pharmacognosy Journal*; 15. Epub ahead of print 2023. DOI: 10.5530/pj.2023.15.145.
16. Aulena DN, Yani DF, Mariyamah M, et al. Determination of Flavonoid Content and Anti-Inflammatory Activity Extract and Fraction of Sungkai Leaf (Peronema canescens Jack). *Jurnal Ilmu Kefarmasian Indonesia*; 21. Epub ahead of print 2023. DOI: 10.35814/jifi.v21i2.1437.
17. R A, P D, D G, et al. A Study on In-vitro Anti-Inflammatory and Antioxidant Activity of Aqueous Extract of Terminalia chebula. *International Journal Of Pharmaceutical Quality Assurance* 2024; 15: 907–910.
18. Rahardhian MRR, Susilawati Y, Musfiroh I, et al. *In-silico* Study Of Bioactive Compounds From Sungkai (Peronema Canescens) As Immunomodulator. *International Journal of Applied Pharmaceutics*; 14. Epub ahead of print 2022. DOI: 10.22159/ijap.2022.v14s4.PP33.
19. Ma'ruf M, Bachri MochS, Nurani LH. Phytochemical Screening Analysis and Determination of Total Flavonoids and Total Phenolics Content of Ethanol Extract of Sungkai Leaf (Penorema canescens Jack) from Samarinda City. *Jurnal Mandala Pharmacon Indonesia*; 9. Epub ahead of print 2023. DOI: 10.35311/jmpi.v9i2.360.
20. Simangunsong TL, Putra A, Ginting SF, et al. Antifungal Analysis of Papaya Seed Extracts and Biosynthesized Silver Nanoparticles. *Science and Technology Indonesia* 2022; 7: 186–194.
21. Nasution AN, Fitrisia R, Tio S, et al. Formulation and Characterization of Amomum Compactum Sol. Ex Maton Nano Emulsion and Its Antibacterial and Wound Healing Activity. *Jurnal Penelitian Pendidikan IPA*; 9. Epub ahead of print 2023. DOI: 10.29303/jppipa.v9i12.5773.
22. Meena KP, Dangi JS, Samal PK, et al. International Journal of Research in Drug Delivery Nanoparticles Technology and Recent Advances in Novel Drug Delivery Systems. *International Journal of Research in Drug Delivery*; 1.
23. Surura HN, Abdullah H, Ginting SF, et al. Green synthesis of silver nanoparticles from papaya seed extracts with alkaloid content for antibacterial application. *Jurnal Teknologi Laboratorium*; 10. Epub ahead of print 2021. DOI: 10.29238/teknolabjournal.v10i2.315.
24. Hanifah M, Jufri M. Formulation and stability testing of nanoemulsion lotion containing centella asiatica extract. *Journal of Young Pharmacists*; 10. Epub ahead of print 2018. DOI: 10.5530/jyp.2018.10.89.
25. Varalakshmi TN, Chitra V. Evaluation of Nephroprotective Activity of Hydroalcoholic Extract of Trachyspermum ammi Leaves and Citrus paradisi Fruits against Prednisolone Induced ADPKD in Experimental Rats. *International Journal of Drug Delivery Technology*; 13. Epub ahead of print 2023. DOI: 10.25258/ijddt.13.2.25.
26. Malaka MH, Indalifiany A, Sahidin S, et al. FORMULATION AND PHYSICAL STABILITY TEST OF NANOEMULGEL CONTAINING Petrosia Sp. ETHANOLIC EXTRACT. *Jurnal Farmasi Sains dan Praktis*; 7. Epub ahead of print 2022. DOI: 10.31603/pharmacy.v7i3.6080.

27. Lister INE, Amiruddin HL, Fachrial E, et al. Antiaging Effectiveness of Avocado Peel Extract Ointment (Persea americana Mill.) against Hydration, Collagen, and Elasticity Levels in Wistar Rat. *J Pharm Res Int*. Epub ahead of print 2021. DOI: 10.9734/jpri/2021/v33i32b31760.
28. Wijaya I, Ginting CN, Ginting SF, et al. Phytochemical and Antibacterial Analysis of the Formulated Cream of Black Cumin Honey. *Res J Pharm Technol*; 14. Epub ahead of print 2021. DOI: 10.52711/0974-360X.2021.00989.
29. Wardhani FM, Chiuman L, Novalinda Ginting C, et al. Efek Ekstrak Kunyit Putih (Curcuma Zedoaria) Sebagai Nefroprotektor Pada Tikus Putih Jantan Galur Wistar yang Diinduksi Tembaga. *Journal of the Indonesian Medical Association*; 69.
30. Fiska Maya Wardhani, Linda Chiuman, Chrismis Novalinda Ginting, et al. Efek Ekstrak Kunyit Putih (Curcuma Zedoaria) Sebagai Nefroprotektor Pada Tikus Putih Jantan Galur Wistar yang Diinduksi Tembaga. *Journal Of The Indonesian Medical Association*; 69. Epub ahead of print 2020. DOI: 10.47830/jinma-vol.69.8-2019-186.
31. Gulo KN, Suhartomi, Saragih AD, et al. Antioxidant Activity of Flavonoid Compounds in Ethanol and Ethyl Acetate Extract from Citrus Sinensis. In: *AIMS 2021 - International Conference on Artificial Intelligence and Mechatronics Systems*. 2021. Epub ahead of print 2021. DOI: 10.1109/AIMS52415.2021.9466078.
32. Kumar A, Sharma A. P, et al. An In-vitro Antioxidant and Antimicrobial Activity of Vitis vinifera Leaf Extract. *International Journal Of Pharmaceutical Quality Assurance* 2024; 15: 649–654.
33. Fajryana A, Ginting CN, Chiuman L, et al. Antibacterial Activity of Garlic Extract (Allium Sativum) using Fermentation and Non-Fermentation towards Propionibacterium Acne. In: *IOP Conference Series: Earth and Environmental Science*. 2022. Epub ahead of print 2022. DOI: 10.1088/1755-1315/1083/1/012070.
34. Nirwana Sembiring, Ali Napiah Nasution, Muhammad Faridz Syahrian, et al. Test of Breadfruit Leaf Extract Cream Preparation (Artocarpus Altilis) and Antioxidant Activity Test with DPPH Method. *Jurnal Multidisiplin Madani*; 3. Epub ahead of print 2023. DOI: 10.55927/mudima.v3i7.4085.
35. Ira Syaputri, Ermi Girsang, Linda Chiuman. Test of Antioxidant And Antibacterial Activity of Ethanol Extract of Andaliman Fruit (Zanthoxylum Acanthopodium Dc.) With Dpph (1.1-Diphenyl-2-Picrylhydrazil) Trapping Method And Minimum Inhibitory Concentration. *International Journal of Health and Pharmaceutical (IJHP)*; 2. Epub ahead of print 2022. DOI: 10.51601/ijhp.v2i2.36.
36. Girsang E, Napiah Nasution A, Nyoman Ehrich Liester I. Potential ABTS Reducing and Anti-collagenase Activity of Scutellarein and Apigenin. *Technology, and Sciences (ASRJETS) American Scientific Research Journal for Engineering*; 57.
37. Agung Adha Witasa Dewana, Tan Suyono, Linda Chiuman, et al. Testing Antioxidant And Antibacterial Activity Of Andaliman Fruit (Zanthoxylum Acanthopodium Dc.) Ethanol Extract With Abts Method (2,2'-Azino-Bis(3-Ethylbenz-Thiazoline-6-Sulfonic Acid) And Minimum Resistant Concentration. *International Journal of Health and Pharmaceutical (IJHP)*; 2. Epub ahead of print 2022. DOI: 10.51601/ijhp.v2i1.35.
38. Girsang E, Lister INE, Ginting CN, et al. Comparison of Antiaging and Antioxidant Activities of Protocatechuic and Ferulic Acids. *Molecular and Cellular Biomedical Sciences*; 4. Epub ahead of print 2020. DOI: 10.21705/mcbs.v4i2.90.
39. Girsang E, Lister INE, Ginting CN, et al. Antioxidant and antiaging activity of rutin and caffeic acid. *Pharmaciana*; 10. Epub ahead of print 2020. DOI: 10.12928/pharmaciana.v10i2.13010.
40. Lister INE, Ginting CN, Girsang E, et al. Antioxidant properties of red betel (Piper crocatum) leaf extract and its compounds. *Journal of Natural Remedies*; 19. Epub ahead of print 2019. DOI: 10.18311/jnr/2019/23633.
41. Juliana C, Lister INE, Girsang E, et al. Antioxidant and Elastase Inhibitor from Black Soybean (Glycine max L.) and Its Compound (Daidzein). *Journal of Biomedicine and Translational Research*; 6. Epub ahead of print 2020. DOI: 10.14710/jbtr.v6i1.5540.
42. Suwandi JF, Wijayanti MA, . M. In Vitro Antiplasmodial And Cytotoxic Activities Of A Sungkai (Peronema Canescens) Leaf Extract. *Int J Pharm Pharm Sci*; 10. Epub ahead of print 2018. DOI: 10.22159/ijpps.2018v10i10.25124.
43. Ibrahim A, Siswandono, Bambang Prajogo EW. Cytotoxic activity of peronema canescens Jack leaves on human cells: HT-29 and primary adenocarcinoma colon cancer. *Pharmacognosy Journal*; 13. Epub ahead of print 2021. DOI: 10.5530/PJ.2021.13.176.
44. Susilo RI, Nasution AN, Mutia MS. Effectiveness of mahkota dewa leaves extract as a biofilm inhibitor of *Propionibacterium acnes* growth. *Jurnal Teknologi Laboratorium*; 12. Epub ahead of print 2023. DOI: 10.29238/teknolabjournal.v12i1.393.
45. Hariyento Halim, Ermi Girsang, Ali Napiah Nasution. Hepatoprotective Effectiveness Test of Salam Leaf Extract in Anthracyclin-Induced Rats. *International Journal of Health and Pharmaceutical (IJHP)*; 2. Epub ahead of print 2022. DOI: 10.51601/ijhp.v2i1.11.
46. Welly Ria Utami Lubis, Ali Napiah Nasution, Hanjaya, et al. Antibacterial Activity Test of Carrot Tuber Ethanol Extract Hydrogel Preparation (Daucus Carota L.) in Inhibiting the Growth of Bacteria and Their Ability in the Healing Process of Grade II Burns on Wistar Rat (Rattus Norvegicus). *Jurnal Multidisiplin Madani*; 3. Epub ahead of print 2023. DOI: 10.55927/mudima.v3i6.3529.
47. Adlis Santoni, Mai Efdi, Fadhillah N. Profil Fitokimia dan Penentuan Fenolik Total, Flavonoid Total, dan Uji Aktivitas Antioksidan Ekstrak Daun Sungkai (Peronema canescens Jack) dari Daerah Kota Padang. *Jurnal Kimia Unand*; 12. Epub ahead of print 2023. DOI: 10.25077/jku.12.1.1-6.2023.
48. Emilia I, Andi Arif Setiawan, Dewi Novianti, et al. Skrining Fitokimia Ekstrak Daun Sungkai (Peronema canescens Jack.) Secara Infundasi Dan Maserasi. *Indobiosains*. Epub ahead of print 2023. DOI: 10.31851/indobiosains.v5i2.12597.
49. Dista R, Larasati C, Ayuningsih S, et al. Formulation and Characterization of Sungkai Leaf Extract Nanoemulsion. *Al-Kimia*; 10. Epub ahead of print 2022. DOI: 10.24252/al-kimia.v10i2.33482.
50. Chiuman L, Sherlyn S, Aritonang NS, et al. In Vitro Study of Antibacterial Activity of Snake Fruit Extract against Extended Spectrum Beta Lactamase (ESBL) Escherichia coli. *Jurnal Aisyah : Jurnal Ilmu Kesehatan*; 8. Epub ahead of print 2023. DOI: 10.30604/jika.v8i2.1962.
51. Nasution AN. Enhance Effectiveness of Moringa Leaves with Staphylococcus Epidermidis Bacteria. *Budapest International Research and Critics Institute (BIRCI-Journal): Humanities and Social Sciences*; 4. Epub ahead of print 2021. DOI: 10.33258/

birci.v4i2.1843.
52. Widowati W, Ginting CN, Lister INE, et al. Antiaging effects of mangosteen peel extract and its phytochemical compounds: Antioxidant activity, enzyme inhibition, and molecular docking simulation. *Trop Life Sci Res*; 31. Epub ahead of print 2020. DOI: 10.21315/tlsr2020.31.3.9.
53. Al-Sha'er MA, Basheer HA, Taha MO. Discovery of new PKN2 inhibitory chemotypes via QSAR-guided selection of docking-based pharmacophores. *Mol Divers*; 27. Epub ahead of print 2023. DOI: 10.1007/s11030-022-10434-4.
54. Ibrahim A, Siswandono S, Wardojo BPE. Potential anticancer activities of chloroform subfraction from Peronema leaf on colon cancer HT-29 cells in vitro. *J Appl Pharm Sci*; 11. Epub ahead of print 2021. DOI: 10.7324/JAPS.2021.1101206.w