eISSN 2503-331X

JPSCR

JOURNAL OF PHARMACEUTICAL SCIENCE AND CLINICAL RESEARCH

Original Research Articles

Review Articles

Diterbitkan oleh:
Program Studi Farmasi
Universitas Sebelas Maret

Vol. 10 | No. 1 | Hal 1-129 | 2025

JPSCR: Journal of Pharmaceutical Science and Clinical Research

DEWAN EDITOR

- apt. Fitrawan Hernuza Pribadi, S.Farm., M.Sc., Program Studi Farmasi, Universitas Sebelas Maret, Indonesia
- apt. Rizky Dwi Larasati, M.S.Farm., Program Studi Farmasi, Universitas Sebelas Maret, Indonesia
- apt. Novita Dhewi I., S.Farm., M.Clin.Pharm., Program Studi Farmasi, Universitas Sebelas Maret, Indonesia
- apt. Lia Puspitasari, S.Farm., M.Si., Program Studi Farmasi, Universitas Sebelas Maret, Indonesia

KATA PENGANTAR

Assalamu'alaikum Wr. Wb.
Salam sejahtera untuk kita semua,

Puji syukur kepada Tuhan YME atas terbitnya *Journal of Pharmaceutical Science and Clinical Research* (JPSCR). Jurnal ini diterbitkan oleh Program Studi Farmasi FMIPA UNS, yang diharapkan menjadi media publikasi karya ilmiah terpercaya dan memberikan kontribusi bagi kemajuan khasanah kefarmasian di Indonesia.

Jurnal JPSCR lahir usaha yang sungguh-sungguh dari segenap tim redaksi dan tidak terlepas dari bantuan banyak pihak. Apresiasi dan terima kasih kami sampaikan kepada tim penyunting yang bekerja keras dalam mereview artikel demi artikel dan kepada segenap mitra bestari atas review kepakarannya. Terima kasih juga kami sampaikan kepada para akademisi dan peneliti, yang sudah menjadikan JPSCR sebagai media diseminasi hasil penelitiannya. Antusiasme dari para kontributor yang bersifat nasional tersebut merupakan modal berharga dalam kami menjaga kesinambungan dan pengembangan jurnal ini.

Akhir kata, semoga jurnal JPSCR ini dapat memberikan inspirasi keilmuan untuk lahirnya ide-ide dan temuan-temuan baru yang bermanfaat bagi komunitas ilmiah dan masyarakat secara umum. Kritik dan saran dari semua pihak senantiasa kami harapkan untuk kemajuan jurnal ini.

Wassalamu'alaikum Wr. Wb.

Surakarta, 25 April 2025

Editor JPSCR

DAFTAR ISI

JPSCR: Journal of Pharmaceutical Science and Clinical Research
Volume 10, Issue 1, Pages 1-17, March 2025
https://doi.org/10.20961/jpscr.v10i1.78958

ISSN 2503-331X (Online)

Evaluasi Pemberian Terapi pada Pasien Demam Berdarah *Dengue* di Rumah Sakit Umum Provinsi Bali

Evaluation of Therapy in Dengue Hemorrhagic Fever Patients at Bali Provincial General Hospital

Dwi Arymbhi Sanjaya[1], Herleeyana Meriyani[1]*, Rr. Asih Juanita[1], Nyoman Budiartha Siada[1], Made Elvenia Ambara Damayanti[2] dan Lusy Noviani[3]

[1]Departemen Farmakologi dan Farmasi Klinik, Fakultas Farmasi, Universitas Mahasaraswati Denpasar, Bali, Indonesia
[2]Program Studi Sarjana Farmasi, Universitas Mahasaraswati Denpasar, Bali, Indonesia
[3]Department of Pharmacy, School of Medicine and Health Sciences Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia

*Corresponding author: herleeyana.m@unmas.ac.id

Diterima: 19 September 2023; **Disetujui:** 3 Maret 2025; **Dipublikasi:** 10 April 2025

Abstrak

Sekitar 50-100 juta jiwa terjangkit demam berdarah *dengue* (DBD) setiap tahunnya dengan jumlah kematian mencapai 20.000 hingga 40.000 juta jiwa per tahun menurut *World Health Organization* (WHO). Provinsi Bali menduduki peringkat ke-3 dengan *Incidence Rate* (IR) DBD tertinggi pada tahun 2021 yaitu 59,8 per 100.000 penduduk. Penyakit DBD biasanya disertai dengan komplikasi dan penyakit penyerta yang dapat mengakibatkan peningkatan morbiditas dan mortalitas. Penelitian ini bertujuan mengetahui gambaran perbaikan klinis yang diamati dari parameter hematologi (leukosit, hemoglobin, hematokrit, dan trombosit) pada pasien DBD dengan komplikasi dan penyakit penyerta. Penelitian ini merupakan penelitian observasional dengan pendekatan *cross-sectional* yang dilakukan di rumah sakit umum Provinsi Bali. Pengambilan data dilakukan secara retrospektif yaitu data yang diperoleh dari bulan Januari 2020 hingga Desember 2021. Lima puluh tiga sampel pada penelitian diambil menggunakan teknik *total sampling*. Efektivitas penatalaksanaan DBD dengan komplikasi dan penyakit penyerta pada hari pertama, hari ketiga, dan hari kelima dianalisis menggunakan uji *Cochran* dengan *post-hoc McNemar*. Pasien DBD dengan komplikasi dan penyakit penyerta pada penelitian ini sejumlah 53 orang dengan 66% laki-laki dan 34% perempuan dengan rata-rata lama rawat inap yaitu 6,0 hari. Ringer laktat dan parasetamol merupakan jenis terapi yang paling banyak digunakan dalam penatalaksanaan DBD. Penatalaksanaan pasien DBD dengan komplikasi dan penyakit penyerta di rumah sakit umum Provinsi Bali pada tahun 2020-2021 menunjukan peningkatan trombosit dan leukosit yang signifikan sejak hari ketiga hingga hari kelima ($p<0,05$).

Kata kunci: Dengue; Hematokrit; Hemoglobin; Leukosit; Trombosit; Uji *Cochran*

Abstract

Around 50-100 million people are infected with Dengue Hemorrhagic Fever (DHF) annually, with an estimated 20,000 to 40,000 deaths per year, according to WHO. In 2021, Bali Province had the third highest incidence rate (IR) of DHF in Indonesia, recorded at 59.8 per 100,000

population. DHF is often associated with complications and comorbidities, leading to higher morbidity and mortality rates. This study aimed to determine the clinical improvement observed in hematological parameters (leukocytes, hemoglobin, hematocrit, and platelets) in dengue fever patients with complications and comorbidities. This observational cross-sectional study was conducted at General Hospital in Bali Province. Data were collected retrospectively from January 2020 to December 2021. A total of 53 patients were included using a total sampling technique. The effectiveness of DHF management with complications and comorbidities was analyzed on the first, third, and fifth days using the Cochran test with post-hoc McNemar analysis. The study included 53 DHF patients with complications and comorbidities, of whom 66% were male and 34% female, with the mean length of stay being 6.0 days. Ringer's lactate and paracetamol were the most commonly used therapies. The management of DHF patients with complications and comorbidities at General Hospital, Bali Province, in 2020-2021 showed significant clinical improvement in leukocyte and platelet levels from the third to the fifth day ($p<0.05$).

Keywords**:** *Cochran test; Dengue; Hematocrit; Hemoglobin; Leukocytes; Platelets*

1. PENDAHULUAN

Demam Berdarah *Dengue* (DBD) atau *Dengue Hemorrhagic Fever* (DHF) merupakan salah satu penyakit infeksi yang paling sering terjadi di dunia termasuk di Indonesia. *World Health Organization* (WHO) mencatat bahwa sejak pertama kali kemunculannya pada tahun 1950-an di negara Filipina dan Thailand, infeksi virus *dengue* telah menyebar dengan luas ke banyak negara lain dengan iklim tropis dan subtropis (Yoga *et al*., 2022). Demam Berdarah *Dengue* (DBD) merupakan salah satu penyakit menular yang disebabkan oleh virus dan disebarkan oleh vektor. Virus yang menyebabkan penyakit ini adalah *dengue*. Vektor penular penyakit ini berasal dari jenis nyamuk *Aedes aegypti* dan *Aedes albopictus* (Mentri Kesehatan Republik Indonesia, 2020).

Sekitar 50-100 jiwa terjangkit DBD setiap tahunnya dengan jumlah kematian mencapai 20.000 hingga 40.000 juta jiwa per tahun menurut WHO (Made *et al.*, 2022). Terdapat dua indikator utama untuk pemantauan pengendalian DBD yaitu *Incidence Rate* (IR) per 100.000 penduduk dan *Case Fatality Rate* (CFR). *Case Fatality Rate* (CFR) merupakan proporsi kematian terhadap seluruh kasus DBD yang dapat digunakan untuk menilai keberhasilan pengendalian DBD, sedangkan IR merupakan frekuensi penyakit yang terjangkit dalam masyarakat di suatu wilayah pada waktu tertentu. Berdasarkan Profil Kesehatan Indonesia dari Kementrian Kesehatan Republik Indonesia, pada tahun 2020 target nasional CFR yang ditetapkan adalah kurang dari 1%, sedangkan target nasional IR adalah kurang dari sama dengan 49 per 100.000 penduduk. Pada beberapa provinsi di Indonesia, target IR nasional yaitu kurang dari sama dengan 49 per 100.000 penduduk belum tercapai, khususnya di provinsi Bali. Pada tahun 2020 dan tahun 2021, IR pada provinsi Bali berturut-turut adalah 273,1 dan 59,8 per 100.000 penduduk. Selain itu, provinsi Bali menduduki peringkat ke-3 dengan IR tertinggi pada tahun 2021. Peningkatan tersebut dapat menjadi evaluasi bagi penatalaksanaan pasien DBD baik dari sisi ketepatan waktu penanganan maupun kualitas pelayanan kesehatan. Keterlambatan penatalaksanaan DBD dapat menyebabkan kematian (Kementerian Kesehatan Republik Indonesia, 2022).

Pada penatalaksanaan DBD tidak terdapat terapi atau pengobatan yang spesifik, pasien DBD menerima terapi suportif, yaitu terapi cairan yang merupakan kunci pada penatalaksanaan DBD (Guzman *et al*., 2016). Pada pemberian terapi cairan diperlukan pemantauan yang ketat, karena pasien DBD rentan terhadap kelebihan cairan (Dhochak & Lodha, 2017; Kularatne et al., 2015). Kelebihan maupun kekurangan cairan dapat menyebabkan berbagai komplikasi. Komplikasi yang terjadi akibat kelebihan cairan meliputi edema paru, gagal jantung, serta kebocoran kapiler yang dapat berujung pada efusi pleura dan asites (Kularatne *et al*., 2015). Pasien dengan gejala klinis yang timbul akibat komplikasi maupun patofisiologi DBD diberikan terapi simptomatik. Terapi simptomatik merupakan terapi yang diberikan untuk mengobati gejala yang timbul. Pada pasien DBD, terapi simptomatik yang biasa diberikan meliputi antipiretik, antiemetik, *antiulcer*, antasida, diuretik, dan terapi sedatif (Andriani *et al*., 2016).

Salah satu komplikasi pada pasien DBD adalah efusi pleura, yang terjadi akibat kebocoran plasma ke dalam rongga pleura (Shabbir, 2018). Selain itu, pasien DBD memiliki risiko tinggi terhadap *hypovolemic shock* yang dapat berakibat fatal (Regita Pratiwi *et al*., 2021). *Volume depletion* dan *hypovolemic shock* memiliki mortalitas sebesar 5% setiap tahunnya (Kholili & Nasronudin, 2015). Selain komplikasi, pasien DBD biasanya juga disertai dengan penyakit penyerta. Beberapa penelitian menyatakan, adanya komplikasi dan penyakit penyerta lainnya dapat mengakibatkan peningkatan morbiditas dan mortalitas (Toledo *et al*., 2016; Werneck *et al*., 2018). Evaluasi pemberian cairan dan tata laksana komplikasi dan penyakit pada pasien DBD perlu dilakukan untuk mengukur keberhasilan terapi. Penelitian mengenai efektivitas penatalaksanaan DBD dengan komplikasi dan penyakit penyerta dengan mengamati perbaikan klinis pada parameter hematologi akan bermanfaat bagi pihak rumah sakit untuk dapat melakukan evaluasi terhadap penatalaksanaan DBD dengan komplikasi dan penyakit penyerta.

2. BAHAN DAN METODE

Penelitian ini merupakan penelitian observasional dengan pendekatan *cross-sectional* yang dilakukan di rumah sakit umum Provinsi Bali dengan keterangan layak etik nomor 070/4491/RSUDW. Pengambilan data dilakukan secara retrospektif yaitu data yang diperoleh dari bulan Januari 2020 hingga Desember 2021. Data yang digunakan diperoleh dari rekam medik pasien DBD dengan komplikasi dan penyakit penyerta meliputi data usia, jenis kelamin, berat badan, tinggi badan, komplikasi, penyakit penyerta, hasil pemeriksaan hematologi (leukosit, hemoglobin, hematokrit, dan trombosit), penatalaksanaan cairan, dan pengobatan.

Teknik pengambilan sampel pada penelitian ini menggunakan teknik *total sampling*. Sampel yang digunakan dalam penelitian ini adalah seluruh pasien DBD dengan komplikasi dan penyakit penyerta dengan rentang usia rentang usia 0 tahun–65 tahun di rumah sakit umum Provinsi Bali. Pasien DBD dengan komplikasi dan penyakit penyerta dengan data yang tidak lengkap serta pasien dengan kehamilan dieksklusi dari penelitian ini.

Variabel yang diamati pada penelitian ini adalah efektivitas penatalaksanaan DBD dengan komplikasi dan penyakit penyerta berdasarkan parameter hematologi, yaitu leukosit, hemoglobin, hematokrit, dan trombosit. Nilai leukosit, hemoglobin, hematokrit, dan trombosit

diamati pada hari pertama, hari ketiga, dan hari kelima, kemudian dikategorikan menjadi normal dan tidak normal sesuai dengan nilai normal setiap parameter (Tabel 1).

Tabel 1. Nilai normal paramater hematologi pasien DBD (Demam Berdarah *Dengue*). Sumber: (Mentri Kesehatan Republik Indonesia, 2020; Pagana *et al*., 2015).

Kategori Usia	Nilai Normal
Leukosit (10^9/L)	
>2 tahun	5-10
>30 minggu hingga ≤2 tahun	6,2-17
≤30 minggu	9-30
Hemoglobin (g/dL)	
0-2 minggu	12-20
>2 minggu hingga ≤6 bulan	10-17
>6 bulan hingga ≤1 tahun	9,5-14
>1 tahun hingga ≤6 tahun	9,5-14
>6 tahun hingga ≤18 tahun	10-15,5
>18 tahun	Wanita: 12-16 Laki-laki: 14-18
Hematokrit (%)	
0-2 minggu	44-64
>2 minggu hingga ≤8 minggu	39-59
>8 minggu hingga ≤6 bulan	35-50
>6 bulan hingga ≤1 tahun	29-43
>1 tahun hingga ≤6 tahun	30-40
>6 tahun hingga ≤18 tahun	32-44
>18 tahun	Wanita: 37-47 Laki-laki: 42-52
Trombosit (10^9/L)	
Semua usia	≥ 100

Efektivitas penatalaksanaan DBD dengan komplikasi dan penyakit penyerta pada hari pertama, hari ketiga, dan hari kelima dianalisis menggunakan uji *Cochran* dengan *post-hoc McNemar*. Apabila diperoleh nilai $p<0,05$ maka penatalaksanaan DBD pada pasien rawat inap DBD dengan komplikasi dan penyakit penyerta di rumah sakit umum Provinsi Bali pada tahun 2020-2021 dinyatakan efektif.

3. HASIL DAN PEMBAHASAN

3.1. Karakteristik pasien

Sejumlah 53 rekam medis pasien DBD dengan komplikasi dan penyakit penyerta yang memenuhi kriteria inklusi dan eksklusi di rumah sakit umum Provinsi Bali digunakan dalam penelitian ini (Tabel 2). Rata-rata lama rawat inap pasien dari 53 data tersebut adalah 6 hari.

Jumlah pasien berdasarkan jenis kelamin dengan diagnosis akhir DBD dengan komplikasi dan penyakit penyerta di rumah sakit umum Provinsi Bali sebagian besar adalah laki-laki sebanyak 35 pasien (66%) dan kelompok usia terbanyak yaitu anak-anak (5-11 Tahun) sebanyak 24 pasien (45%). Penelitian yang dilakukan di Rumah Sakit Lewoleba dalam periode Januari 2019-Desember 2021, menunjukkan bahwa penderita DBD lebih banyak terjadi pada

laki-laki dengan persentase 60,5% (Tristianti *et al*., 2023). Penelitian lain di RSUD Bali Mandara periode 2019-2020 ditemukan bahwa pasien DBD didominasi oleh laki-laki dengan persentase sebesar 54,9% (Yoga *et al*., 2022). Penelitian yang dilakukan di Rumah Sakit Umum Pusat (RSUP) Dr. M. Djamil Padang dalam periode 2020-2022, juga ditemukan bahwa penderita DBD lebih banyak ditemukan pada laki-laki dengan persentase sebesar 53,6% (Birman *et al*., 2022). Hal yang menyebabkan laki-laki lebih rentan terkena infeksi virus *dengue* adalah karena pada laki-laki produksi immunoglobulin (Ig) dan antibodi sebagai sistem pertahanan tubuh dalam melawan infeksi berbeda dengan perempuan yang memiliki hormon estrogen yang mempengaruhi sintesis serta peningkatan produksi imunoglobulin (Tule, 2020). Pada laki-laki terdapat hormon testosteron yang memiliki efek imunosupresif yaitu mengurangi sekresi Interferon gamma (IFN-γ) dan Interleukin-4 (IL-4) oleh sel limfosit T serta aktivasi abnormal neutrofil, sehingga laki-laki lebih berisiko mengalami infeksi (Birman *et al*., 2022).

Tabel 2. Karakteristik pasien DBD dengan komplikasi dan penyakit penyerta di Rumah Sakit Umum Provinsi Bali.

Karakteristik	Frekuensi	Persentase (%)
Jenis Kelamin		
Laki-laki	35	66
Perempuan	18	34
Total	**53**	**100**
Usia		
Anak-anak (5-11 Tahun)	24	45
Remaja Awal (12-16 Tahun)	10	19
Remaja Akhir (17-25 Tahun)	7	13
Balita (0-5 Tahun)	6	11
Lansia Awal (46-55 Tahun)	2	4
Lansia Akhir (56-65 Tahun)	2	4
Dewasa awal (26-35 Tahun)	1	2
Dewasa akhir (36-45 Tahun)	1	2
Total	**53**	**100**

Penelitian yang dilakukan di RSUD Dr. Zainoel Abidin periode November 2016, menunjukkan mayoritas penderita DBD adalah pasien anak (usia ≤ 17 tahun) dengan rentang usia 5-11 tahun, dengan persentase sebesar 47,5% (Ikrima *et al*., 2017). Penelitian lain di RSUD Kabupaten Buleleng periode Juli-September 2013 juga menunjukkan bahwa 43,1% kasus DBD pada anak (usia maksimal 15 tahun) lebih banyak dalam rentang usia 6-11 tahun (Pranata, 2017). Secara epidemiologis, sebesar 95% kasus DBD di negara tropis menyerang anak usia dibawah 15 tahun (Pranata, 2017). Nyamuk *Aedes aegypti* mempunyai kebiasaan menggigit pada pagi hari pukul 08:00-12:00 dan sore hari pukul 15:00-17:00 bertepatan dengan waktu anak-anak beraktivitas di luar rumah sehingga meningkatkan risiko penularan (Hernawan & Afrizal, 2020; Pranata, 2017). Usia merupakan salah satu faktor yang memengaruhi kepekaan terhadap infeksi virus *dengue*, daya tahan tubuh anak-anak yang belum optimal membuat anak-anak lebih rentan terjangkit DBD dibandingkan dengan orang dewasa (Tule, 2020).

3.2. Karakteristik pemberian cairan dan pengobatan pasien dbd dengan komplikasi dan penyakit penyerta

Berdasarkan 53 data rekam medis pasien DBD di rumah sakit umum Provinsi Bali menunjukkan bahwa cairan kristaloid jenis ringer laktat merupakan terapi cairan yang paling banyak digunakan baik pada DBD derajat 1 hingga derajat IV (Tabel 3). Karakteristik pengobatan pasien DBD dengan komplikasi dan penyakit penyerta (Tabel 4) diklasifikasikan berdasarkan kondisi penyakit dan golongan obat yang diberikan. Golongan obat yang paling banyak digunakan adalah golongan antipiretik yaitu parasetamol.

Tabel 3. Karakteristik pemberian cairan pada pasien DBD dengan komplikasi dan penyakit penyerta di Rumah Sakit Umum Provinsi Bali. Keterangan: * = NaCl 0,9% diganti dengan Ringer Laktat.

Jenis Cairan	Frekuensi	Persentase (%)
DBD Derajat I		
Kristaloid		
Ringer Laktat	16	80
NaCl 0,9 %	1	5
Kristaloid dan Koloid		
Ringer Laktat + HES 6%	1	5
Ringer Laktat + Albumin 20%	1	5
Ringer Laktat + D5 ¼ NS (Dextrose 5% + NaCl 0,22%)	1	5
Total	20	**100**
DBD Derajat II		
Kristaloid		
Ringer Laktat	6	55
Futrolit	1	9
NaCl 0,9% dan Ringer Laktat*	1	9
Kristaloid dan Koloid		
Ringer Laktat + HES 6%	3	27
Total	11	**100**
DBD Derajat III		
Kristaloid		
Ringer Laktat	12	57
Kristaloid dan Koloid		
Ringer Laktat + HES 6%	3	14
Ringer Laktat + Albumin 20%	3	14
Ringer Laktat + D5 ¼ NS (Dextrose 5% + NaCl 0,22%)	1	5
Ringer Laktat + HES 6% + Albumin 20%	1	5
NaCl 0,9% + HES 6%	1	5
Total	21	**100**
DBD Derajat IV		
Kristaloid		
Ringer Laktat	1	100
Total	1	**100**

Ringer laktat merupakan jenis cairan yang paling banyak digunakan dalam penatalaksanaan DBD derajat I sampai IV di Rumah Sakit Umum Provinsi Bali. Hal ini sesuai

Pedoman Tatalaksana DBD tahun 2020 dan 2021, yang merekomendasikan penggunaan cairan kristaloid isotonis seperti NaCl 0,9% atau ringer laktat, sebagai terapi awal penderita DBD tanpa perdarahan spontan dan masif. Sesuai Pedoman Tatalaksana DBD tahun 2020 dan 2021, pasien dengan *shock* terkompensasi diberikan peningkatan dosis cairan kristaloid isotonis, lalu diturunkan secara bertahap jika status hemodinamik membaik. Namun, apabila status hemodinamik tidak membaik, dapat diberikan kristaloid isotonis (bolus kedua) atau cairan koloid. Pasien dengan *dengue shock syndrom* ¸terapi meliputi cairan kristaloid dan atau koloid. Jika terjadi perbaikan, pemberian dilanjutkan cairan kristaloid, sedangkan jika kondisi tetap *shock* dosis cairan kristaloid ditingkatkan. Pada *hypovolemic shock*, cairan kristaloid diberikan dan dipantau selama 10-15 menit, cairan kombinasi koloid dan kristaloid diberikan jika tidak mengalami perbaikan hemodinamik (Mentri Kesehatan Republik Indonesia, 2020).

Tabel 4. Karakteristik pengobatan pada pasien DBD dengan komplikasi dan penyakit penyerta di Rumah Sakit Umum Provinsi Bali.

Obat	Frekuensi	Presentase (%)
DBD + *Hypovolemic shock* + Sepsis		
Antibiotik		
Sefotaksim	1	8,3
Seftriakson	1	8,3
Antipiretik		
Parasetamol	2	16,6
Antiemetik		
Ondansetron	2	16,6
Diuretik		
Furosemid	1	8,3
Premedikasi albumin		
Furosemid	1	8,3
***Antiulcer* (*Acid Suppression Therapy*)**		
Antasida	1	8,3
Omeprazol	1	8,3
Ranitidin	1	8,3
Kortikosteroid		
Metilprednisolon	1	8,3
Total	12	100
DBD + *Volume depletion*		
Antipiretik		
Parasetamol	4	40,0
Antibiotik		
Sefotaksim	1	10,0
Kortikosteroid		
Deksametason	1	10,0
Antiemetik		
Ondansetron	2	20,0
Antiulcer (*Acid Suppression Therapy*)		
Ranitidin	2	20,0
Total	**10**	**100**

Tabel 4. Karakteristik pengobatan pada pasien DBD dengan komplikasi dan penyakit penyerta di Rumah Sakit Umum Provinsi Bali (*Lanjutan*…).

Obat	Frekuensi	Presentase (%)
DBD + *Hypovolemic shock*		
Antipiretik		
Parasetamol	24	21,4
Antibiotik		
Sefotaksim	23	20,5
Seftriakson	1	0,9
Sefoperazon	1	0,9
Azitromisin	1	0,9
Gentamisin	1	0,9
Antiemetik		
Ondansetron	20	17,9
Domperidon	1	0,9
***Antiulcer* (*Acid Suppression Therapy*)**		
Ranitidin	12	10,7
Sukralfat	4	3,6
Omeprazol	4	3,6
Antasida	4	3,6
Pantoprazol	1	0,9
Diuretik		
Furosemid	12	10,7
Premedikasi albumin		
Furosemid	3	2,7
Total	**112**	**100**
DBD + Efusi Pleura		
Antipiretik		
Parasetamol	1	16,7
Analgesik		
Morfin	1	16,7
Antibiotik		
Seftriakson	1	16,7
Antiulcer (*Acid Suppression Therapy*)		
Ranitidin	1	16,7
Antasida	1	16,7
Antiemetik		
Ondansetron	1	16,7
Total	**6**	**100**
DBD + Dyspepsia		
Antipiretik		
Parasetamol	1	20,0
Antiulcer (*Acid Suppression Therapy*)		
Sukralfat	1	20,0
Antasida	1	20,0
Omeprazol	1	20,0
Antiemetik		
Ondansetron	1	20,0
Total	**5**	**100**

Tabel 4. Karakteristik pengobatan pada pasien DBD dengan komplikasi dan penyakit penyerta di Rumah Sakit Umum Provinsi Bali (*Lanjutan*…).

Obat	Frekuensi	Presentase (%)
DBD + *Hypovolemic shock* + Efusi pleura		
Antipiretik		
Parasetamol	1	5,6
Antibiotik		
Sefotaksim	3	16,7
Seftriakson	2	11,1
Sefaleksin	1	5,6
Ampisilin	1	5,6
Mukolitik		
Ambroxol	1	5,6
Kortikosteroid		
Deksametason	2	11,1
Antiemetik		
Ondansetron	1	5,6
***Antiulcer* (*Acid Suppression Therapy*)**		
Ranitidin	2	11,1
Sukralfat	1	5,6
Diuretik		
Furosemid	2	11,1
Premedikasi albumin		
Furosemid	1	5,6
Total	**18**	**100**
DBD + *Thypoid Fever*		
Antipiretik		
Parasetamol	1	20,0
Antibiotik		
Levofloksasin	1	20,0
Antiemetik		
Ondansetron	1	20,0
Antiulcer (*Acid Suppression Therapy*)		
Ranitidin	1	20,0
Antasida	1	20,0
Total	**5**	**100**

Pasien DBD di seluruh dunia menggunakan terapi cairan untuk mengembalikan volume plasma dan memastikan perfusi organ yang optimal. Pada DBD sering terjadi kebocoran cairan melalui endotelium pada fase kritis, kemungkinan disebabkan oleh peningkatan permeabilitas vaskular yang dimediasi sitokin dan gangguan permukaan glikokalik sementara. Target terapi cairan intravena adalah untuk mengkompensasi kebocoran plasma dan menarik plasma ke dalam endotelium pembuluh darah (Seneviratne *et al*., 2018).

Cairan kristaloid adalah pilihan pertama untuk resusitasi cairan pada keadaan *hypovolemic*, perdarahan, sepsis dan dehidrasi. Cairan kristaloid merupakan larutan yang mengandung garam mineral dan molekul kecil lainnya yang larut dalam air. Sebagian besar kristaloid secara komersial bersifat isotonik terhadap plasma manusia. Cairan ini mendekati

konsentrasi berbagai zat terlarut yang ditemukan dalam plasma dan tidak memberikan efek osmotik. Cairan kristaloid berfungsi untuk memperluas volume intravaskular tanpa mengganggu konsentrasi ion atau menyebabkan perpindahan cairan yang signifikan antara ruang intraseluler, intravaskular, dan interstisial. Cairan kristaloid dapat melewati *barrier* endotel atau penetrasi melalui pori-pori dalam persimpangan antar sel pembuluh darah dalam mikrosirkulasi berbagai organ (He *et al*., 2018).

Pada Tabel 4, diketahui bahwa obat antipiretik yang paling banyak digunakan adalah parasetamol. Hal ini sesuai dengan Pedoman Tatalaksana DBD tahun 2021 yang menyatakan bahwa pasien dengan demam dapat diberikan parasetamol dengan rekomendasi dosis 10 mg/kg/dosis, dengan frekuensi tidak kurang dari 6 jam serta tidak lebih dari 4 gram/hari pada dewasa (Mentri Kesehatan Republik Indonesia, 2020).

Terapi simptomatik lainnya yang juga banyak digunakan dalam penelitian ini adalah *antiulcer*. Pada penelitian ini, obat *antiulcer* yang paling banyak digunakan oleh pasien adalah ranitidine. Pada pasien DBD cenderung terjadi mual dan muntah yang akan meningkatkan asam lambung (Khan *et al*., 2021). Penelitian yang dilakukan di Rumah Sakit Nur Hidayah Bantul menyatakan bahwa 23 pasien (50%) menggunakan ranitidin sebagai *antiulcer* yang termasuk golongan *Histamine-2 receptor antagonist* (H2RA) (Sari, 2016). *Histamine-2 receptor antagonist* (H2RA) lebih dipilih dibandingkan dengan *proton pump inhibitors* (PPI) karena, pada PPI menunjukkan korelasi positif yang signifikan dengan kejadian trombositopenia dibandingkan H2RA (Adrizain *et al*., 2021).

Pada penelitian ini, obat antiemetik yang paling banyak digunakan pasien DBD di rumah sakit umum Provinsi Bali yaitu ondansetron. Pasien DBD cenderung mengalami mual dan muntah yang berat sehingga akan mempengaruhi status gizi dan prognosis penyakit *(Khan et al*., 2021). Penelitian yang dilakukan di Rumah Sakit Nur Hidayah Bantul juga menyatakan bahwa 16 pasien DBD (35%) menggunakan ondasetron sebagai antiemetik (Sari, 2016). Ondansetron merupakan obat antiemetik yang secara umum digunakan pada pasien DBD karena lebih unggul dibandingkan dengan obat antiemetik lainnya. Ondasetron memiliki efek gastroprotektif terutama dalam melindungi mukosa lambung, penurunan substansial terhadap *ulcer* indeks dan skor perdarahan intraluminal (Kusmayati & Putri, 2022).

Sefotaksim merupakan antibiotik yang paling banyak digunakan oleh pasien DBD di Rumah Sakit Umum Provinsi Bali. Beberapa penelitian melaporkan penggunaan antibiotik golongan sefalosporin, termasuk sefotaksim merupakan antibiotik yang paling banyak digunakan pada pasien DBD yang dirawat inap (Adrizain *et al*., 2019). Namun, pemberian antibiotik tanpa indikasi infeksi bakteri pada pasien DBD dapat meningkatkan resiko resistensi antimikroba (Adrizain *et al*., 2019; Sandopa et al., 2018). Selain itu, pemberian antibiotik pada pasien DBD tidak menunjukkan hubungan yang signifikan terhadap waktu rawat inap dan perubahan suhu tubuh (Sandopa *et al*., 2018; Siregar et al., 2021). Pemantauan terhadap parameter hematologi secara berkala diperlukan selama penggunaan sefotaksim, karena terdapat penelitian yang melaporkan efek samping berupa perubahan hematologi seperti neutropenia, leukopenia dan agranulositosis (Padda & Nagalli, 2022).

Furosemid sebagai obat antidiuretik dan premedikasi albumin juga banyak digunakan pada pasien DBD di rumah sakit umum Provinsi Bali. Hal ini serupa dengan penelitian yang dilakukan di RSUP Prof. Dr.R.D. Kandou Manado, pada periode November 2013 hingga April 2014 ditemukan bahwa sebanyak 4 pasien yang mengalami *shock* disertai diuresis yang tidak mencukupi 2 ml/Kg/BB/jam saat kebutuhan cairan sudah terpenuhi (Andriani *et al*., 2016). Pemberian furosemid juga dikaitkan dengan kejadian komplikasi seperti kelebihan cairan, edema paru, kongesti wajah, peningkatan tekanan vena jugularis, efusi pleura atau asites, dimana komplikasi yang terjadi merupakan akibat dari pemberian terapi cairan yang terlalu antusias dan hidrasi yang terlalu cepat. Selain pemberian furosemid, komplikasi tersebut dapat diobati dengan pembatasan pemberian terapi cairan hingga pasien stabil (Kularatne *et al.*, 2015). Pemberian furosemid dengan albumin dapat meningkatkan efek diuresis dan natriuresis, dibandingkan dengan pengobatan furosemid tunggal. Terapi kombinasi antara furosemid dan albumin memberikan keuntungan pada keadaan pasien dengan kadar albumin awal yang lebih rendah (< 2,5 g/dL) (Lee *et al*., 2021).

Pada penelitian ini terdapat pasien DBD yang menggunakan kortikosteroid. Sebuah penelitian tentang penggunaan prednisolon selama fase akut awal menyatakan bahwa tidak ada hubungan yang signifikan antara penggunaan prednisolon dengan perpanjangan fase viremia, perkembangan *shock,* kebocoran plasma, pemulihan jumlah trombosit atau komplikasi lain dari DBD (Chan & Ooi, 2015; Kularatne, 2015). Penggunaan kortikosteroid juga tidak mengubah kinetika penanda virologi pada *dengue* atau konsentrasi sitokin plasma (Chan & Ooi, 2015). Kendati demikian, suatu penelitian pada pasien *dengue shock syndrom* sebanyak 9 dari 11 pasien yang diberikan metilprednisolon mampu bertahan, sementara pasien yang tidak diberikan kortikosteroid meninggal dunia (Bandara & Herath, 2018; Rajapakse *et al*., 2014) Namun, hingga saat ini tidak terdapat *high-quality evidence* yang mendukung mengenai efek menguntungkan pada penggunaan kortikosteroid sebagai penatalaksanaan *shock*, pencegahan komplikasi serius atau peningkatan jumlah trombosit (Rajapakse *et al*., 2014).

3.3. Gambaran perbaikan klinis pasien dbd dengan komplikasi dan penyakit penyerta

Nilai median pada parameter hematologi, meliputi leukosit, hemoglobin, hematokrit, dan trombosit diperlukan untuk mengetahui perubahan kondisi pasien dari hari pertama, hari ketiga hingga hari kelima dapat diamati pada Tabel 5. Gambaran perbaikan klinis dari 53 pasien DBD dengan komplikasi dan penyakit penyerta di rumah sakit umum Provinsi Bali (Tabel 6), dianalisis dengan menggunakan uji *McNemar* berdasarkan hasil pemeriksaan hematologi dari hari pertama, hari ketiga hingga hari kelima.

Nilai median leukosit dari hari pertama (3,93 x 10^9/L), hari ketiga (4,73 x 10^9/L), hingga hari kelima (5,98 x 10^9/L) mengalami peningkatan Tabel 5. Pada Tabel 6, menunjukkan perubahan yang signifikan pada parameter leukosit pada hari ketiga dan hari kelima ($p<0,05$), sehingga pengobatan DBD dengan komplikasi dan penyakit penyerta pada penelitian ini dapat dinyatakan efektif ditinjau dari parameter leukosit. Penelitian yang dilakukan di RSUD Prabumulih periode Januari-Mei 2017 pada pasien DBD ditemukan bahwa pada hari pertama leukosit pasien berada dibawah jumlah normal, namun pada hari ketiga hingga seterusnya nilai

leukosit akan mengalami peningkatan (Mayasari *et al*., 2019). Pada saat demam, mulai terjadi pengurangan jumlah leukosit dan neutrofil disertai limfositosis relatif. Leukopenia mencapai puncaknya sesaat sebelum demam turun dan normal kembali pada 2-3 hari setelah demam turun. Penurunan leukosit biasanya diikuti dengan penurunan trombosit dan mencapai puncaknya bersamaan dengan turunnya demam. Pada DBD jumlah leukosit biasanya normal atau menurun dengan dominasi sel neutrofil. Terjadinya leukopenia pada DBD disebabkan oleh penekanan sumsum tulang belakang akibat proses infeksi virus secara langsung atau tidak langsung melalui produksi sitokin-sitokin proinflamasi yang menekan sumsum tulang belakang (dos Santos Pires & de Oliveira, 2024; Setiawan *et al.*, 2023).

Nilai hemoglobin hari pertama (14,9 g/dL), hari ketiga (13,4 g/dL), hingga hari kelima (12,65 g/dL), mengalami penurunan (Tabel 5). Pada Tabel 6, tidak ditemukan perbedaan yang bermakna baik pada perbedaan hasil uji hemoglobin pada hari pertama hingga hari kelima ($p>0,05$). Salah satu faktor yang mempengaruhi hemoglobin adalah pola makan suatu individu. Pada pasien DBD terjadi penurunan nafsu makan dan muntah sehingga dapat mempengaruhi kadar hemoglobin (Khan *et al*., 2021). Penelitian yang dilakukan pada seluruh siswa SMP di Kecamatan Tanjung, Lombok Utara ditemukan bahwa pola makan mempengaruhi kadar hemoglobin. Semakin cukup pola makan, maka kadar hemoglobin juga akan semakin baik, karena kebutuhan zat besi yang dibutuhkan remaja 13-14 tahun sebesar 8-14 mg/hari. Kebutuhan ini bisa dicukupi dari konsumsi makanan yang mengandung zat besi tinggi, baik yang berasal dari hewani maupun nabati (Nurdiana, 2015).

Tabel 5. Nilai median untuk parameter hematologi pasien DBD dengan komplikasi dan penyakit penyerta di Rumah Sakit Umum Provinsi Bali.

Parameter Hematologi	Median (Minimum-Maksimum)		
	Hari Pertama	Hari Ketiga	Hari Kelima
Leukosit (10^9/L)	3,93 (0,94 - 10,43)	4,73 (0,91 - 16,66)	5,98 (2,73 - 10,15)
Hemoglobin (g/dL)	14,9 (4,5 - 20,2)	13,4 (8,9 - 18,4)	12,65 (10,2 - 15,7)
Hematokrit (%)	43,2 (14 - 59,2)	38,95 (24,8 - 53,3)	36,65 (15 – 47,4)
Trombosit (10^9/L)	48 (8 – 207)	43 (3 – 325)	83 (14 – 248)

Nilai median hematokrit di hari pertama (43,2%), hari ketiga (38,95%), hingga hari kelima (36,65%) mengalami penurunan (Tabel 5). Pada Tabel 6, tidak ditemukan perbedaan yang signifikan baik pada perbedaan hasil uji hematokrit pada hari pertama hingga hari kelima($p>0,05$). Penelitian yang dilakukan di RS Dr. Hasan Sadikin, menyatakan bahwa tidak terdapat perbedaan yang signifikan antara serial pengukuran hematokrit pertama dan kedua sebagai prediktor keparahan DBD. Data ini tidak relevan dengan kejadian klinis pada DBD yang menyatakan bahwa harus terdapat penurunan keparahan akibat kebocoran plasma. Hal ini dapat terjadi karena intervensi cairan awal yang mengubah perjalanan penyakit dan kasus kebocoran plasma tidak mencapai tingginya hemokonsentrasi bahkan jika pasien dalam

keadaan *shock*. Selain itu, hal yang berpengaruh pada nilai hematokrit adalah waktu antara pengukuran hematokrit pertama dan kedua disetiap individu berbeda dan memiliki banyak variasi rentang waktu antara 3-24 jam (Ramadhani *et al*., 2018). Nilai hematokrit biasanya digunakan sebagai indikator kebocoran plasma. Namun, interpretasi nilai hematokrit dapat dipengaruhi oleh faktor selain kebocoran plasma seperti demam, dehidrasi, dan pendarahan (Srikiatkhachorn, 2017).

Nilai hematokrit akan menurun saat terjadinya hemodilusi yang disebabkan oleh kadar seluler darah atau peningkatan kadar plasma darah (Hidayat *et al*., 2017). Nilai hematokrit didasarkan tidak hanya oleh plasma darah namun juga oleh jumlah eritrosit. Patofisiologi DBD menunjukkan pasien mengalami kebocoran plasma sehingga persentase hematokrit menjadi meningkat, namun apabila terjadi perdarahan atau anemia maka jumlah eritrosit yang rendah mempengaruhi nilai hematokrit sehingga menjadi rendah. Hematokrit tidak memiliki hubungan yang signifikan terhadap derajat keparahan pasien DBD (Widyanti, 2016). Hal ini sejalan dengan penelitian yang dilakukan di RSUP Sanglah pada periode September 2013-November 2014 pada pasien DBD ditemukan bahwa sebagian besar pasien DBD menunjukkan persentase hematokrit yang normal saat pertama kali dilakukan pemeriksaan (Widyanti, 2016).

Tabel 6. Gambaran perbaikan klinis pasien DBD dengan komplikasi dan penyakit penyerta di Rumah Sakit Umum Provinsi Bali berdasarkan uji *McNemar*. Keterangan: * = nilai p<0,05.

Parameter Hematologi	Hari Pengamatan (n=53)		Nilai p
Leukosit	Hari Pertama	Hari Ketiga	0,19
	Hari Pertama	Hari Kelima	0,41
	Hari Ketiga	Hari Kelima	0,02*
Hemoglobin	Hari Pertama	Hari Ketiga	0,19
	Hari Pertama	Hari Kelima	0,83
	Hari Ketiga	Hari Kelima	0,44
Hematokrit	Hari Pertama	Hari Ketiga	0,52
	Hari Pertama	Hari Kelima	0,70
	Hari Ketiga	Hari Kelima	1,00
Trombosit	Hari Pertama	Hari Ketiga	0,001*
	Hari Pertama	Hari Kelima	0,89
	Hari Ketiga	Hari Kelima	0,001*

Nilai median trombosit dari hari pertama (48 x 10^9/L) dengan hari ketiga (43 x 10^9/L) mengalami penurunan, namun pada hari ketiga (43 x 10^9/L) ke hari kelima (83 x 10^9/L) mengalami peningkatan (Tabel 5). Pada Tabel 6, terdapat perubahan yang signifikan pada hasil uji trombosit pada hari pertama dan hari ketiga serta pada hari ketiga dan pada hari kelima (p<0,05). Pada penelitian yang dilakukan di Rumah Sakit Islam Siti Rahmah Padang periode Januari-Desember 2017 pada pasien DBD, ditemukan bahwa penurunan jumlah trombosit pada pasien DBD terjadi selama demam pada hari ketiga hingga hari ketujuh yang disebabkan oleh pembentukan kompleks imun yang menandakan reaksi dari antigen virus *dengue* sehingga kuantitas dan kualitas trombosit akan terganggu (Kafrawi *et al*., 2019).

Pemberian cairan sebagai terapi suportif pada pasien DBD dengan komplikasi dan penyakit penyerta bertujuan untuk mencegah komplikasi kebocoran vaskular, *hypovolemic*

shock, dan mengkompensasi dan mengembalikan volume plasma. Pemberian cairan akan berdampak pada perubahan nilai dari parameter hematologi, khususnya leukosit dan trombosit (Chan & Ooi, 2015; Kularatne *et al*., 2015). Selain itu, terapi simptomatik yang tepat sesuai dengan gejala yang diderita pasien, juga berdampak pada perubahan nilai dari parameter hematologi (Khan *et al.*, 2021). Menurut Pedoman Tatalaksana DBD tahun 2020 dan 2021, adanya peningkatan pada trombosit >50x10^9/L, digunakan sebagai acuan untuk memulangkan pasien (Mentri Kesehatan Republik Indonesia, 2020).

Perubahan nilai parameter hematologi pada pasien DBD, seperti hemoglobin dan hematokrit tidak hanya dipengaruhi oleh pemberian cairan dan terapi simptomatik. Namun, terdapat faktor-faktor lain, seperti faktor fisiologis pasien, faktor pola makan, dan status gizi pasien yang mempengaruhi parameter hematologi tersebut (Nurdiana, 2015). Dalam penelitian ini, tidak dilakukan analisis mengenai hubungan faktor-faktor lain yang mempengaruhi nilai parameter hematologi. Oleh karena itu, dapat dilakukan analisis lebih lanjut untuk melihat hubungan antara perbaikan klinis yang diamati dari parameter hematologi dengan pemberian terapi dan faktor-faktor yang mempengaruhi nilai pada parameter hematologi tersebut.

4. KESIMPULAN

Penatalaksanaan pasien DBD dengan komplikasi dan penyakit penyerta di rumah sakit umum Provinsi Bali pada tahun 2020-2021 menunjukan perbaikan klinis yang signifikan yang diamati dari peningkatan trombosit dan leukosit yang signifikan sejak hari ketiga hingga hari kelima.

DEKLARASI KONFLIK KEPENTINGAN

Semua penulis menyatakan tidak ada konflik kepentingan terhadap naskah ini.

DAFTAR PUSTAKA

Adrizain, R., Setiabudi, D., & Chairulfatah, A. (2019). The inappropriate use of antibiotics in hospitalized dengue virus-infected children with presumed concurrent bacterial infection in teaching and private hospitals in Bandung, Indonesia. *PLoS Neglected Tropical Diseases*, *13*(6), 1–11. https://doi.org/10.1371/journal.pntd.0007438

Adrizain, R., Simanjuntak, M., & Rezano, A. (2021). Correlation of histamine-2 receptor antagonist (H2RA) and proton pump inhibitor (PPI) to the platelet count in patient with dengue viral infection. *Journal of the Medical Sciences (Berkala Ilmu Kedokteran)*, *53*(4), 343–350. https://doi.org/10.19106/jmedsci005304202104

Andriani, N. W. E., Tjitroantoso, H., & Paulina V Y Yamlean. (2016). Kajian Penatalaksanaan Terapi Pengobatan Demam Berdarah Dengue (DBD) pada Penderita Anak yang Menjalani Perawatan di RSUP Prof. Dr. R.D Kandou Tahun 2013. *Pharmacon*, *3*(2), 57–61.

Bandara, S. M. R., & Herath, H. M. M. T. B. (2018). Effectiveness of corticosteroid in the treatment of dengue – A systemic review. *Heliyon*, *4*(9), e00816. https://doi.org/10.1016/j.heliyon.2018.e00816

Birman, Y., Setiawan, P., & Hansah, R. B. (2022). Profil Demam Berdarah Dengue di RSUP Dr M. Djamil Padang Tahun 2020-2022. *Nusantara Hasana Journal*, *1*(11), 22–32.

Chan, C. Y., & Ooi, E. E. (2015). Dengue: An update on treatment options. *Future Microbiology*, *10*(12), 2017–2031. https://doi.org/10.2217/fmb.15.105

Dhochak, N., & Lodha, R. (2017). Dengue in children: Issues in critical care settings. *Journal of Pediatric Critical Care*, *4*(3), 44. https://doi.org/10.21304/2017.0403.00193

dos Santos Pires, J. P., & de Oliveira, T. R. S. (2024). Hematological complications in dengue: a literature review (2003-2023). *Revista Ciências Da Saúde CEUMA*, *2*(1), 109–116. https://doi.org/10.61695/rcs.v2i1.29

Guzman, M. G., Gubler, D. J., Izquierdo, A., Martinez, E., & Halstead, S. B. (2016). Dengue infection. *Nature Reviews Disease Primers*, *2*, 1–26. https://doi.org/10.1038/nrdp.2016.55

He, H., Liu, D., & Ince, C. (2018). Colloids and the microcirculation. *Anesthesia and Analgesia*, *126*(5), 1747–1754. https://doi.org/10.1213/ANE.0000000000002620

Hernawan, B., & Afrizal, A. R. (2020). Hubungan antara Jenis Kelamin dan Usia dengan Kejadian Dengue Syok Sindrom pada Anak di Ponorogo. *Publikasi Ilmiah*, 80–88.

Hidayat, W. A., Yaswir, R., & Murni, A. W. (2017). Hubungan Jumlah Trombosit dengan Nilai Hematokrit pada Penderita Demam Berdarah Dengue dengan Manifestasi Perdarahan Spontan di RSUP Dr. M. Djamil Padang. *Jurnal Kesehatan Andalas*, *6*(2), 446. https://doi.org/10.25077/jka.v6i2.719

Ikrima, Buchari, & Hidayat, R. (2017). Pengaruh Kadar Hematokrit Terhadap Derajat Klinis Demam Berdarah Dengue Pada Pasien Anak Ruang Rawat Inap Di Rumah Sakit Umum Daerah Zainoel Abidin Banda Aceh. *Jurnal Ilmiah Mahasiswa Kedeokteran Biomedis*, *2*(4), 1–7.

Kafrawi, V. U., Dewi, N. P., & Adelin, P. (2019). Gambaran Jumlah Trombosit dan Kadar Hematokrit Pasien Demam Berdarah Dengue di Rumah Sakit Islam Siti Rahmah Padang. *Health & Medical Journal*, *1*(1), 38–44. https://doi.org/10.33854/heme.v1i1.217

Kementerian Kesehatan Republik Indonesia. (2022). *Profil Kesehatan Indonesia Tahun 2021*. Kementerian Kesehatan Republik Indonesia.

Khan, M. A. S., Al Mosabbir, A., Raheem, E., Ahmed, A., Rouf, R. R., Hasan, M., Alam, F. B., Hannan, N., Yesmin, S., Amin, R., Ahsan, N., Anwar, S., Afroza, S., & Hossain, M. S. (2021). Clinical spectrum and predictors of severity of dengue among children in 2019 outbreak: a multicenter hospital-based study in Bangladesh. *BMC Pediatrics*, *21*(1), 1–10. https://doi.org/10.1186/s12887-021-02947-y

Kholili, U., & Nasronudin, N. (2015). A Patient Dengue Hemorrhagic Fever With Spasms. *Indonesian Journal of Tropical and Infectious Disease*, *5*(3), 72. https://doi.org/10.20473/ijtid.v5i3.239

Kularatne, S. A. M. (2015). Dengue fever. *BMJ (Online)*, *351*(September), 1–10. https://doi.org/10.1136/bmj.h4661

Kularatne, S. A. M., Weerakoon, K. G. A. D., Munasinghe, R., Ralapanawa, U. K., & Pathirage, M. (2015). Trends of fluid requirement in dengue fever and dengue haemorrhagic fever: A single centre experience in Sri Lanka. *BMC Research Notes*, *8*(1), 4–9. https://doi.org/10.1186/s13104-015-1085-0

Kusmayati, E., & Putri, N. A. (2022). Typhoid Fever dan Dengue Hemorrhagic Fever Grade II pada Anak. *GALENICAL : Jurnal Kedokteran Dan Kesehatan Mahasiswa Malikussaleh*, *1*(4), 36. https://doi.org/10.29103/jkkmm.v1i4.9043

Lee, T. H., Kuo, G., Chang, C. H., Huang, Y. T., Yen, C. L., Lee, C. C., Fan, P. C., & Chen, J. J. (2021). Diuretic effect of co-administration of furosemide and albumin in comparison to furosemide therapy alone: An updated systematic review and meta-analysis. *PLoS ONE*, *16*(12 December), 1–16. https://doi.org/10.1371/journal.pone.0260312

Made, N., Handayani, D., Putu, D., Udiyani, C., Putu, N., & Mahayani, A. (2022). *Hubungan Kadar Trombosit , Hematokrit , dan Hemoglobin dengan Derajat Demam Berdarah Dengue pada Pasien Anak Rawat Inap di BRSU Tabanan Berdasarkan data Kementrian Kesehatan Demam Berdarah Dengue dapat*. *2*(2), 130–136.

Mayasari, R., Sitorus, H., Salim, M., Oktavia, S., Supranelfy, Y., & Wurisastuti, T. (2019). Karakteristik Pasien Demam Berdarah Dengue pada Instalasi Rawat Inap RSUD Kota Prabumulih Periode Januari–Mei 2016. *Media Penelitian Dan Pengembangan Kesehatan*, *29*(1), 39–50. https://doi.org/10.22435/mpk.v29i1.271

Menteri Kesehatan Republik Indonesia. (2020). *Pedoman Nasional Pelayanan Kedokteran Tata Laksana Infeksi Dengue Dewasa*.

Nurdiana, N. (2015). Factors Affecting the Level of Hemoglobin on Junior High School Children on Coast Regional District of North Lombok. *Biota*, *8*(1), 1–18. https://doi.org/10.20414/jb.v8i1.54

Padda, I. S., & Nagalli, S. (2022). *Cefotaxime - Abstract - Europe PMC*. StatPearls Publishing.

Pagana, K. D., Pagana, T. J., & Pagana, T. N. (2015). Mosby's Diagnostic & Laboratory Test Reference. In *Journal of Chemical Information and Modeling* (Vol. 53, Issue 9).

Pranata, I. W. A. (2017). Gambaran Pola Penatalaksanaan Demam Berdarah Dengue (DBD) Pada Anak Di Instalasi Rawat Inap Rumah Sakit Umum Daerah Kabupaten Buleleng Tahun 2013. *E-Jurnal Medika*, *6*(5), 21–27.

Rajapakse, S., Rodrigo, C., Maduranga, S., & Rajapakse, A. C. (2014). Corticosteroids in the treatment of dengue shock syndrome. *Infection and Drug Resistance*, *7*, 137–143. https://doi.org/10.2147/IDR.S55380

Ramadhani, F., Ghozali, M., & Lismayanti, L. (2018). Two Serial Hematocrit Level Just After Admission to Predict Dengue Hemorrhagic Fever Severity. *Global Medical & Health Communication (GMHC)*, *6*(3), 182–187. https://doi.org/10.29313/gmhc.v6i3.3079

Regita Pratiwi, Yuniati Yuniati, & Muhammad Buchori. (2021). Hubungan Usia, Jenis Kelamin, dan Status Perdarahan terhadap terjadinya Komplikasi pada Anak dengan Infeksi Dengue di RSUD Abdul Wahab Sjaharanie Samarinda. *Jurnal Sains Dan Kesehatan*, *3*(2), 212–217. https://doi.org/10.25026/jsk.v3i2.274

Sandopa, D., Nethi, S. K., Sreeram, S. C., Vijay, N. K. G., Biradavolu, V., & Kakimani, J. V. (2018). Prescribing antibiotics to pediatric dengue: increasing risk of bacterial resistance. *Paediatrica Indonesiana*, *58*(1), 53. https://doi.org/10.14238/pi58.1.2018.53-8

Sari, D. F. (2016). Gambaran Penggunaan Parasetamol Pada Pasien Demam Berdarah Dengue Rawat Inap Di Rs Nur Hidayah Bantul Periode Oktober-Desember 2014. *Akrsfindo*, *1*(1), 25–29.

Seneviratne, H. M. T. W., Kumara, G. D. I., Ravirajah, K., Somaratne, K. M. K., & Mendis, D. (2018). Fluid management in patients with dengue hemorrhagic fever. *Asia Pac J Paediatr Child Health*, *1*, 15–23.

Setiawan, A. F., Wajib, Y. Y. P., Handono, K., & Sakti, S. P. (2023). Leucocytes, thrombocytes and immature platelets in patients with dengue hemorrhagic fever. *Bali Medical Journal*, *12*(2), 2067–2069.

Shabbir, M. (2018). Nature and Clinical Course of Pleural Effusion in Dengue Fever. *International Journal of Internal and Emergency Medicine*, *1*(1), 1006.

Siregar, A. S., Hanafi, M., Mashuri, Y. A., Indriani, T. A., Ardyanto, D. T., Suyatmi, & Hanafi, M. (2021). The relationship between antibiotics treatment with length of stay and the daily temperature fluctuation of child patients infected by dengue at UNS Hospital. *IOP Conference Series: Earth and Environmental Science*, *824*(1). https://doi.org/10.1088/1755-1315/824/1/012080

Srikiatkhachorn, A. (2017). Plasma Leakage in Dengue Hemorrhagic Fever. *Physiology & Behavior*, *176*(3), 139–148. https://doi.org/10.1053/j.gastro.2016.08.014.CagY

Toledo, J., George, L., Martinez, E., Lazaro, A., Han, W. W., Coelho, G. E., Runge Ranzinger, S., & Horstick, O. (2016). Relevance of Non-communicable Comorbidities for the Development of the Severe Forms of Dengue: A Systematic Literature Review. *PLoS Neglected Tropical Diseases*, *10*(1), 1–18. https://doi.org/10.1371/journal.pntd.0004284

Tristianti, V. R., Theodosia, R., & Beyeng, D. (2023). *The Correlation between Nutrional Status with Severity Of Dengue Infection in Pediatric Patients*. *10*(2), 14–18.

Tule, N. R. S. (2020). Systematic Review: Identifikasi Faktor Jenis Kelamin Dan Kelompok Usia Pada Pasien Demam Berdarah Dengue Dengan Pendekatan Kasus Trombositopenia Naskah Publikasi. *Universitas 'Aisyiyah*, 1–11.

Werneck, G. L., Macias, A. E., Mascarenas, C., Coudeville, L., Morley, D., Recamier, V., Guergova-Kuras, M., Puentes-Rosas, E., Baurin, N., & Toh, M. L. (2018). Comorbidities increase in-hospital mortality in dengue patients in Brazil. *Memorias Do Instituto Oswaldo Cruz*, *113*(8), 1–5. https://doi.org/10.1590/0074-02760180082

Widyanti, N. N. A. (2016). Hubungan Jumlah Hematokrit Dan Trombosit Dengan Tingkat Keparahan Pasien Demam Berdarah Dengue Di Rumah Sakit Sanglah Tahun 2013-2014. *E-Jurnal Medika*, *5*(8), 0–5.

Yoga, K., Nugraha, W., Subawa, A. A. N., Herawati, S., & Mulyantari, N. K. (2022). Karakteristik Hasil Pemeriksaan Hematologi dan Kecepatan Pemulihan. *Jurnal Medika Udayana*, *11*(10), 25–34.

JPSCR: Journal of Pharmaceutical Science and Clinical Research
Volume 10, Issue 1, Pages 18-37, March 2025
https://doi.org/10.20961/jpscr.v10i1.86516

ISSN 2503-331X (Online)

The Miracle from The Yard: *Annona muricata* as Cancer Chemo-Preventive and Co-Chemotherapy

Anwar Rovik[1,3*], Riris Istighfari Jenie[2,3] and Ratna Asmah Susidarti[2,3*]

[1]Master Program in Biotechnology, Universitas Gadjah Mada, Yogyakarta, Indonesia
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia
[3]Cancer Chemoprevention Research Center (CCRC), Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia

[*]Corresponding author: anwarrovik@mail.ugm.ac.id / ratna_asmah@ugm.ac.id

Received: June 11, 2024; **Accepted:** March 10, 2025; **Published:** April 21, 2025

Abstract
Cancer remains one of the biggest challenges to human health worldwide, with incidence and mortality rates continuing to rise. Popular cancer treatments to date include surgery, radiation-based therapy, chemotherapy, gene therapy, and hormone therapy. Chemotherapy is one of the primary treatment methods for cancer. However, it has unwanted side effects. It has prompted the search for new chemotherapeutic drugs with better efficacy, excellent selectivity, and fewer side effects. In recent years, there has been growing scientific interest in the potential of soursop leaf (*Annona muricata*) as an anticancer agent. This review paper discusses the phytochemical content of *A. muricata* and its potential as a co-chemotherapy against various types of cancer. Several bioactive compounds in soursop leaves have been reported, including annopentocin A, muricatetrocin A, annohexocin, isoannonacinone, annomuricin A, muricatin C, corossolin, and arianacin. Extracts from various parts of *A. muricata* have potential as co-chemotherapy in the treatment of breast cancer, colon cancer, prostate, lung, cervical, liver, and blood cancer. Studies concluded that *A. muricata* extracts have a good safety and tolerability profile. Its benefits as a co-chemotherapy agent will be even more significant when made into pharmaceutical dosage forms. *A. muricata* extracts or compounds packaged in pharmaceutical preparations are expected to improve patient's quality of life. However, more research is needed to determine the optimal dosage, route of administration, and potential side effects.

Keywords: *Annona muricata*; Co-chemotherapy; Metastasis; Network pharmacology

1. INTRODUCTION

Cancer is one of the major diseases that can cause death. According to the World Health Organization (WHO), cancer is the second leading cause of death globally, claiming an estimated 10 million lives in 2020. GLOBOCAN 2020, a global cancer statistics project, estimates that there were 19.3 million new cancer cases diagnosed worldwide in the same year, with lung cancer (11.4%) and female breast cancer (11.7%) being the most commonly diagnosed cancers (Sung et al., 2021).

Cancer development is a complex process influenced by genetic and environmental factors. In addition to genetic factors, several factors can increase the risk of cancer

development, including gender, age, nutrition, obesity, alcohol consumption, family history, radiation exposure, tobacco use, living environment, and economic conditions (Icanervilia et al., 2023; Rahmani et al., 2023). Cancer burden varies significantly across different regions. Developed countries tend to have higher incidence rates, likely due to increased life expectancy, screening programs, and improved diagnostic techniques(World Health Organization, 2024). However, low- and middle-income countries are often disproportionately affected by mortality rates due to limited access to early detection, diagnosis, and treatment options.

Popular cancer treatments to date include surgery, radiation-based therapy, chemotherapy, gene therapy, and hormone therapy (Rady et al., 2018). These methods can be used separately or in combination. Chemotherapy is one of the primary treatment methods for cancer. Although chemotherapy is effective in killing cancer cells and increasing the survival chance, it also provides unwanted side effects. For example, the use of doxorubicin causes side effects such as hepatotoxicity, cardiotoxicity, metastasis, and drug resistance (Damodar et al., 2014; Sun et al., 2022). It drives the search for new chemotherapeutic drugs with better efficacy, selectivity, and fewer side effects.

Researchers have long explored natural compounds as chemo-preventive agents. This enduring interest has led to developing numerous anticancer drugs derived from natural materials (Ko & Moon, 2015; Manna et al., 2023). Soursop (*Annona muricata*) is a tropical and sub-tropical fruit plant with potential bioactive compounds. Local people have used them in traditional medicine for centuries. Soursop is used to treat various conditions, such as fever, pain, respiratory and skin disorders, antiparasitic, bacterial infections, hypertension, inflammation, and diabetes (Cahyawati, 2020; Coria-Téllez et al., 2018; Damayanti et al., 2017). In recent years, there has been growing scientific interest in the potential of *A. muricata* as an anticancer agent. This review paper discusses the phytochemical content of soursop leaf and its potential as a chemo-preventive and co-chemotherapy against various types of cancer, with a particular focus on its promising antimetastatic properties.

2. METHODS

Experimental data were collected online by summarizing scientific literature articles. We obtained the article from several databases, such as Google Scholar, Scopus, and PubMed, using single and combination keywords related to the topic. The keywords used included " soursop," "*Annona muricata*," "cancer," "metastasis," and "cytotoxic." Data selection was used by looking at the year of writing between 2010 and 2024, whether the language used is Indonesian or English, and its relevance to the topic raised in the review article.

3. RESULTS AND DISCUSSION

3.1. Cancer chemotherapy and its limitations

Chemotherapy is a systemic therapy. The drugs can spread throughout the body and reach distant sites to kill cancer cells. Currently, chemotherapy is the primary treatment for cancer, especially for patients who do not respond to surgical resection (Prasad et al., 2016). It can significantly improve survival rates and reduce the risk of recurrence. Chemotherapy is less selective in killing cancer cells (Adelina et al., 2014). It can harm rapidly dividing healthy cells,

leading to various side effects. These side effects can be acute, occurring during or immediately after treatment, or chronic, persisting for months or even years after treatment.

Doxorubicin, an anthracycline antibiotic, is a potent chemotherapy drug widely used against various types of cancer. Its effectiveness comes from its ability to disrupt DNA replication and transcription in cancer cells (Sun et al., 2022). However, the use of doxorubicin causes side effects, such as hepatotoxicity, cardiotoxicity, metastasis, and resistance (Damodar et al., 2014; Sun et al., 2022). Doxorubicin increases migration and invasion of metastatic type 4T1 and MDA-MB-231 breast cancer cells (Bandyopadhyay et al., 2010). Doxorubicin boosts DCAF13 expression in breast cancer cells, promoting migration and invasion (Sun et al., 2022).

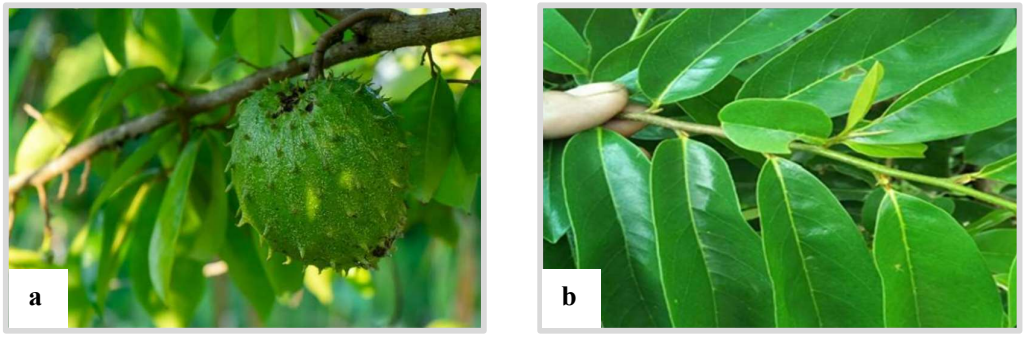


Figure 1. Anatomy of soursop. *Description*: fruit (A) and soursop leaves (B).

3.2. Cancer chemo-preventive potential of *Annona muricata*

Annona muricata is a member of the Annonaceae family with the following taxonomical description:

Kingdom: Plantae

Division: Angiosperms

Subdivision: Magnoliophyta

Class: Magnolid

Ordo: Magnoliales

Family: Annonaceae

Genus: Annona

Species: *Annona muricata* L.

A. muricata grows in the tropical and sub-tropical regions of Central and South America, West Africa, Central and East Africa, and Southeast Asia, including Indonesia. The soursop plant is about 5–10 m tall and 15–83 cm in diameter, with low branches. It tends to flower and fruit almost year-round. The fruit is an edible collective ovoid berry, dark green. The pulp is creamy white with a distinctive aroma and flavor (Lienggonegoro & Kharirie, 2020).

A. muricata extract has various pharmacological properties, including anticancer properties. In vitro testing has been widely conducted on multiple cancer cell lines. Soursop's potential as an anticancer agent is even better than other plants. The leaf extract of *A. muricata*

is reported to have excellent antitumor activity in murine models compared to curcumin, which is known as a natural chemo-preventive (Hamizah et al., 2012).

Several bioactive compounds in soursop leaves have been reported, including annopentocin A, muricatetrocin A, annohexocin, isoannonacinone, annomuricin A, muricatin C, corossolin, and arianacin (Apeh et al., 2023). There are two major classes of phytochemicals: flavonoids and acetogenins (Yang et al., 2015). The antitumor properties of *A. muricata* leaf extracts are related to the presence of several acetogenin compounds (Adelina et al., 2014; Chan et al., 2019; Moghadamtousi et al., 2015). Acetogenins have two hydroxylated tetrahydrofuran (THF) functional units and a β-unsaturated γ-lactone ring (Figure 2) (Dewayani et al., 2023). Other compounds, such as annonacin, are associated with reduced metastasis and proliferation of cancer cells at the cell membrane level. Bioactive compounds have pharmacological activities individually or in synergistic combinations (Matsushige et al., 2012; Moghadamtousi et al., 2015; Yang et al., 2015).

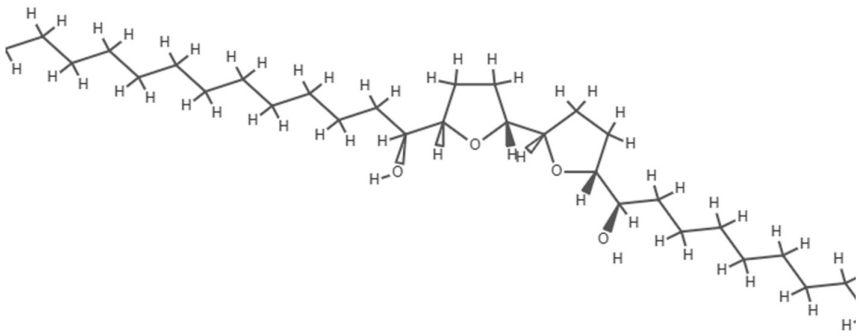


Figure 2. Structure of acetogenin compounds (Source: PubChem at https://pubchem.ncbi.nlm.nih.gov/).

3.3. *Annona muricata* as chemo preventive agent

Annona muricata bioactive compounds have demonstrated a selective cytotoxic effect, targeting cancer cells while sparing normal cells. However, in vitro studies are still limited. For example, the selectivity is exemplified by their lack of cytotoxic effects on normal spleen cells (Gavamukulya et al., 2014). This property makes these compounds promising candidates for cancer chemoprevention and as co-chemotherapy agents. By selectively targeting cancer cells, these compounds may offer a therapeutic advantage with reduced side effects compared to traditional chemotherapy.

Breast cancer is a disease in which abnormal breast cells grow out of control and form tumors (Bhanuwati & Pakpahan, 2022). More than 2.3 million women were diagnosed with breast cancer, with 685,000 deaths globally in 2020 (World Health Organization, 2024). WHO predicts the global incidence of breast cancer will increase to 3.2 million cases per year by 2050 (Momenimovahed & Salehiniya, 2019). For women, breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death (World Health Organization, 2024).

The soursop extract is toxic against breast cancer cell models, including MCF-7, 4T1, T47D, MDA-MB, MDA-MD, SK-BR, and HCC-1954 (Table 1).

Table 1. Potential use of *A. muricata* against breast cancer

Source	Cancer type	Cell line	IC_{50} (µg/ml)	Reference
Fruit	Breast	MDA-MB-468	4.8	(Dai et al., 2011)
Fruit	Breast	MDA-MD-231	>200	(Dai et al., 2011)
Fruit	Breast	MCF-7; MCF-10A	>200	(Dai et al., 2011)
Leaves	Breast	MCF-7	14.68	(Andrini et al., 2014)
Leaves	Breast	SK-BR-3	202.33	(Gavamukulya et al., 2014)
Leaves	Breast	MDA-231	248.77	(Gavamukulya et al., 2014)
Leaves	Breast	MDA-MB-231	9.37 - 100	(Moghadamtousi et al., 2014)
Leaves	Breast	MCF-7	6.39 – 85.58	(Moghadamtousi et al., 2014)
Seeds	Breast	T47D	20.36	(Arifianti et al., 2014)
Leaves	Breast	MCF-7	220	(Najmuddin et al., 2016)
Leaves	Breast	4T1	250	(Najmuddin et al., 2016)
Leaves	Breast	MDA-MD-231	350	(Najmuddin et al., 2016)
Leaves	Breast	MCF-10A	1,000	(Najmuddin et al., 2016)
Leaves	Breast	T47D	5	(Roham et al., 2016)
Leaves	Breast	MCF-7	44.94	(Fatmawati et al., 2018)
Leaves	Breast	MCF-7	9.12	(Suhendar, 2019)
Leaves	Breast	MCF-7	220	(Manshour et al., 2018)
Leaves	Breast	MCF-7	56.6	(Haryanti & Widiyastuti, 2017)
Leaves	Breast	T47D	109.91	(Fertilita et al., 2020)
Leaves	Breast	MCF-7	85.55	(Naik & Sellappan, 2020)
Leaves	Breast	MCF-7	2.86 - 48.31	(Hadisaputri et al., 2021)
Leaves	Breast	4T1	63	(Salsabila et al., 2021)
Leaves	Breast	4T1	79.2	(Merlín-Lucas et al., 2021)
Leaves	Breast	HCC-1954	125	(Lopez et al., 2023)
Leaves	Breast	MCF-7	200	(Lopez et al., 2023)

Ethyl acetate leaf extract of *A. muricata* has significant cytotoxicity activity against MCF-7 with an IC_{50} of 6.4 µg/ml and MDA-MB-31 cells with an IC_{50} of 11.4 µg/ml (Moghadamtousi et al., 2014). The soursop fruit extract is selective in reducing the viability of MDA-MB-468 breast cancer cells with an IC_{50} of 4.8 µg/ml (Dai et al., 2011). A study confirmed the ability of soursop leaf extract through apoptotic parameters (Naik & Sellappan, 2020). The study showed that soursop leaf extract treatment increased the G1phase by 30%. It induced cell cycle arrest in the G1 phase, paralleling the S phase decrease. Inaddition, there was also an increase in caspase-3 regulation. The caspase-3 in the MCF-7 cellwas upregulated compared to the control, cultured at 50 µg/ml and 100 µg/ml of soursop leaf extract. Soursop extract showed moderateto solid cytotoxicity in metastatic breast cancer, such as 4T1 and T47D (Merlín-Lucas et al., 2021; Salsabila et al., 2021). A single compound, acetogenin, showed more potent anticancer effects, with an IC_{50} of 14.69 µM on drug-resistant breast tumors (Yuan et al., 2016).

Prostate cancer is the second most commonly diagnosed cancer among men, with an estimated 1.4 million cases diagnosed worldwide in 2020. It is also the fifth leading cause of cancer death in men. Research shows that soursop is a potential anticancer agent against prostate cancer (Table 2). The soursop fruit extract was cytotoxic to prostate cancer cell lines

with varying IC_{50} values, ranging from 73 to 200 µg/ml, after 48 hours of treatment (Torres et al., 2012). Meanwhile, soursop leaf extract provided greater IC_{50} values ranging from 1.36 to 63 µg/ml (Asare et al., 2015; Foster et al., 2020; Yang et al., 2015).

Table 2. Potential use of *A. muricata* against prostate cancer

Source	Cancer type	Cell line	IC_{50} (µg/ml)	Reference
Leaves	Prostate	COLO-357	200	(Torres et al., 2012)
Leaves	Prostate	CD18/HPAF	73	(Torres et al., 2012)
Leaves	Prostate	PC-3	63	(Yang et al., 2015)
Leaves	Prostate	BPH-1	1.36	(Asare et al., 2015)
Leaves	Prostate	PC-3	80	(Manshour et al., 2018)
Leaves	Prostate	DU-145	55.501	(Foster et al., 2020)

The soursop leaf-derived compound, murihexocin C, was reported to have selective cytotoxicity against prostate adenocarcinoma cell lines (PC-3) (Wahab et al., 2018). The bioactive compound suppresses the reduction of the Δ4,5 double bond of testosterone to synthesize DHT. Inhibition of the physiological role of DHT activates transcriptional activity and androgen receptor signaling (Yu et al., 2020). This condition decreases prostate cell growth and proliferation.

Soursop bioactive compounds can inhibit the activity of P-glycoprotein (P-gp), an efflux protein. Efflux proteins play a role in drug transportation from inside the cell back to outside the target cell (König et al., 2013). Inhibition of P-gp activity increases the bioavailability of anticancer compounds. Like enzymes in drug metabolism, P-gp substrates act as inhibitors or inducers. The annopentocin A, muricatetrocin A, and annohexocin compounds from *A. muricata* have good inhibition against P-gp in prostate cancer cell lines (Apeh et al., 2023).

The bioactive compounds of *A. muricata* can also prevent and suppress metastasis in prostate cancer cell lines. The activity of soursop fruit extract is associated with decreased glucose uptake and cell ATP content (Torres et al., 2012). The study also showed the downregulation of proteins involved in cancer cell invasion and metastasis, including matrix metalloproteinase 9 (MMP-9), phosphorylated focal adhesion kinase (pFAK), and mucin 4 (MUC4) in cells.

Soursop leaf extract inhibits motility in the highly metastatic PC-3 cell line, thus preventing wound healing. In addition to significantly inhibiting extracellular vascular endothelial growth factor (VEGF) production, the extract also inhibited the formation of new blood vessels during angiogenesis (Foster et al., 2020). Angiogenesis is one of the leading mechanical steps for tumor growth, invasion, and metastasis in all cell types. In addition, *A. muricata* extract is reported to inhibit TNF-α, a transcription factor that plays a role in the expression of VEGF (Laksmitawati et al., 2016).

Colorectal cancer (CRC), also known as colon cancer, is a significant health concern worldwide. CRC is the third most common cancer and the second leading cause of cancer-related deaths globally. In 2020, estimates suggest over 1.9 million new cases and 930,000 deaths due to CRC (World Health Organization, 2024). Table 3 summarizes research on the

potential of soursop asa colon anticancer. The ethanol leaf extract of *A. muricata* is reported to inhibit the migration of colon cancer cell lines WiDr and produced an IC_{50} value of 905.77 µg/ml. The fruit extract of *A. muricata* also has good selectivity (Astuti et al., 2021).

Table 3. Potential use of *A. muricata* against colorectal cancer

Source	Cancer type	Cell line	IC_{50} (µg/ml)	Reference
Leaves	Colorectal	HT-29	11.43	(Moghadamtousi et al., 2014)
Leaves	Colorectal	HCT-116	8.98	(Moghadamtousi et al., 2014)
Leaves	Colorectal	HT-29	1.62	(Moghadamtousi et al., 2015)
Leaves	Colorectal	COLO-205	189.6	(Abdullah et al., 2017)
Leaves	Colorectal	WiDr	905.77	(Astuti et al., 2021)

Ethanol leaf extract of *A. muricata* inhibits angiogenesis and migration of colon cancer cell lines WiDr by reducing VEGF production and increasing E-cadherin. A study showed that the extract increases the amount of E-cadherin in WiDr cancer cells (Astuti et al., 2023). Decreased production of E-cadherin is one of the hallmarks of cancer cells and contributes to cancer metastasis. The loss of cell-cell adhesion and E-cadherin allows cells to separate from the primary tumor, penetrate surrounding tissues, and migrate to other sites (Astuti et al., 2023; Shamir et al., 2014).

Bioactive compounds of *A. muricata* leaves reduce the levels of molecules involvedin the cell invasion process, i.e., intercellular cell-adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in the blood (Indrawati et al., 2023). Cell migration is inhibited when acetogenin compounds attach to cell wall receptors and damage ATP in the mitochondrial wall (Gavamukulya et al., 2017); therefore, energy production in cancer cells stops. Furthermore, cancer cells are arrested in the G0/G1 phase, resulting in apoptosis (Gavamukulya et al., 2017; Moghadamtousi et al., 2014; Najmuddin et al., 2016).

Table 4. Potential use of *A. muricata* against lung cancer.

Source	Cancer type	Cell line	IC_{50} (µg/ml)	Reference
Leaves	Lung	A549	5.1	(Moghadamtousi et al., 2014)
Leaves	Lung	H1299	146	(Manshour et al., 2018)
Leaves	Lung	A549	194	(Manshour et al., 2018)
Leaves	Lung	A549	6	(Meenakshisundaram et al., 2020)
Leaves	Lung	A549	70 - 100	(Shaniba et al., 2022)

Lung cancer is the most common cancer and the leading cause of cancer death. The main risk factor for lung cancer is smoking (World Health Organization, 2024). Lung cancer rates are declining in some countries due to reduced smoking rates. However, lung cancer rates are still rising in some developing countries. Researchers explore soursop extract as a lung anticancer with various cell lines (Table 4). In vitro studies showed that soursop leaf extract has significant cytotoxicity against the A549 lung cancer cell line, with an IC_{50} value of 5.1 µg/ml (Moghadamtousi et al., 2014). High flavonoid content correlates with anticancer potential against lung cancer cells (Hasmila et al., 2019; Widyastuti & Rahayu, 2017). Meanwhile,

another study reported a higher IC_{50} value of soursop leaf extract, which was 70–100 µg/ml (Shaniba et al., 2022). Soursop leaf extract may inhibit nuclear factor-κB (NF-κB) signaling, increase ROS production, enhance the Bax/Bcl-2 ratio, and activate caspase-3in the A549 cell line (Moghadamtousi et al., 2014).

Table 5. Potential use of *A. muricata* against cervical cancer

Source	Cancer type	Cell line	IC_{50} (µg/ml)	Reference
Leaves	Cervix	HeLa	111.75	(Rachmawati et al., 2012)
Leaves	Cervix	HeLa	120	(Yuniarti et al., 2014)
Leaves	Cervix	HeLa	77.096	(Prayitno et al., 2016)
Leaves	Cervix	HeLa	25	(Jepkorir et al., 2018)
Leaves	Cervix	HeLa	200	(Manshour et al., 2018)
Leaves	Cervix	HeLa	5.91 – 35.53	(Qorina et al., 2020)

Globally, cervical cancer is the fourth most frequent cancer in women, with an estimated 660,000 new cases diagnosed in 2022. Cervical cancer is a preventable cancer that arises from the abnormal growth of cells in the cervix, the lower part of the uterus that connects to the vagina. Most cervical cancer cases correlate to infection with certain strains of human papillomavirus (HPV), a sexually transmitted virus. While most HPV infections are clear on their own, persistent infections with high-risk types can lead to cervical cancer (World Health Organization, 2024).

Current cervical cancer treatments have limitations due to significant side effects, such as altering cell metabolism, nephrotoxicity, vomiting, nausea, and anemia (Qorina et al., 2020). Some studies showed the cytotoxic activity of *A. muricata* leaf extract against cervical cancer cells (Table 5). Soursop leaf extract has good cytotoxicity against cervical cancer cells HeLa, with IC_{50} values ranging from 5.91 to 35.51 µg/ml (Qorina et al., 2020). Similarly, the cytotoxicity of soursop fruit has an IC_{50} of 25 µg/ml against HeLa cell lines (Jepkorir et al., 2018). In comparison, several other studies that used soursop leaves showed higher IC_{50} values,which ranged from 77.096 to 120 µg/ml against cervical cancer cells HeLa (Manshour et al., 2018; Prayitno et al., 2016; Rachmawati et al., 2012; Yuniarti et al., 2014). The synergistic effects of phytochemical content in plant extracts, including acetogenins, act as antiproliferation agents and significantly reduce MMP production. Acetogenin compounds inhibit the activity of enzymes or proteins, which are only found in tumor cell membranes (Manshour et al., 2018).

Table 6. Potential use of *A. muricata* against liver cancer

Source	Cancer type	Cell line	IC_{50} (µg/ml)	Reference
Leaves	Hepatocarcinoma	HepG2	9.3	(Moghadamtousi et al., 2014)
Leaves	Hepatocarcinoma	HepG2	150	(N. Liu et al., 2016)
Leaves	Hepatocarcinoma	HCT-116	150	(N. Liu et al., 2016)
Leaves	Hepatocarcinoma	Huh71T-1	<0.3	(Apriyanto et al., 2018)
Fruits	Hepatocarcinoma	Hepg2	53.7	(Thomas et al., 2019)

The most common type of liver cancer is hepatocellular carcinoma (HCC). The significant risk factors for liver cancer include chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), excessive alcohol consumption, obesity, and type 2 diabetes (World Health Organization, 2024). The current treatment regimen comprises the MAP-kinase inhibitor sorafenib, which is typically well tolerated. Sorafenib commonly causes unacceptable adverse events, such as diarrhea, skin toxicity, and hypertension (Thomas et al., 2019).

Table 6 summarizes the potential of *A. muricata* extracts against liver cancer. Ethyl acetate leaf extract of *A. muricata* showed significant cytotoxicity against the HCC cell line HepG2, with an IC_{50} value of 9.3 µg/ml (Moghadamtousi]et al., 2014). While soursop fruit extract demonstrated less potent cytotoxicity than its leaf counterpart, it exhibited significant inhibition of cell migration (Thomas et al., 2019). Another study reported a lower IC_{50} value in the Huh71T-1 cell line. The extract significantly inhibits cancer cell migration at 55 µg/ml. It also validated the induction of apoptosis and cell arrest at the G0/G1 phase of the HepG2 cell cycle (Apriyanto et al., 2018).

Table 7. Potential use of *A. muricata* against leukemia

Source	Cancer type	Cell line	IC_{50} (µg/ml)	Reference
Twigs	Leukemia	HL-60	49	(Pieme et al., 2014)
Roots	Leukemia	HL-60	9	(Pieme et al., 2014)
Leaves	Leukemia	HL-60	14	(Pieme et al., 2014)
Seeds	Leukemia	CCRF-CEM/ADR5000	0.36	(Kuete et al., 2016)
Leaves	Leukemia	CCRF-CEM/ADR5000	0.57	(Kuete et al., 2016)
Fruits	Leukemia	CCRF-CEM/ADR5000	4.58	(Kuete et al., 2016)

Leukemia is a cancer of the blood and bone marrow. Leukemia can affect people of all ages but is most common in children and adults over 55 (World Health Organization, 2024). Soursop is one of the plants that can potentially be an anticancer for leukemia (Table 7). A study analyzed the cytotoxic effects of twig, root, and leaf extracts of *A. muricata* on HL-60 leukemia cell lines (Pieme et al., 2014). Soursop extracts from different parts of the plant inhibited HL-60 cell proliferation in specific doses. The root extract had the highest cytotoxic activity, with an IC_{50} value of 9 µg/ml. Similarly, methanol extracts from soursop seeds, leaves, and fruits induce cytotoxicity in the multidrug-resistant leukemia cell line, CCRF-CEM/ADR5000 (Kuete et al., 2016). *A. muricata* has antiproliferative effects on the HL-60 leukemia cell line by inducing inhibition of cell proliferation, the formation of reactive oxygen species (ROS), and decreased MMP protein production, followed by G0/G1 phase cell cycle arrest (Pieme et al., 2014).

3.4. In vivo evaluation

In vitro studies evaluate the soursop extract using various cancer cell lines. However, cytotoxicity testing and in vivo anticancer activity are still few. In vivo studies confirmed the anticancer activity of soursop extracts and their single compounds. A study induced the carcinogenic compound 7,12 dimethylbenz-a-antracene (DMBA) in animal models (Adelina et al., 2014). DMBA metabolites are toxic and cause oxidative stress, leading to cell structure

damage and causing cell necrosis. The results showed that soursop leaf extract can significantly inhibit the development of hepatic tumors through decreased cell proliferation.

A comparative study evaluated the effect of *A. muricata* fruit and leaf extracts on DMBA-induced breast cancer in rats (Silihe et al., 2023). The study reported that *A. muricata* leaf and fruit extracts and a chemotherapeutic drug (tamoxifen) significantly reduced tumor incidence, volume, and weight. The extracts had antioxidant activity through increased glutathione levels, superoxide dismutase, and catalase activity. In addition, INF-γ, TNFα, and IL-6 levels were reduced (Silihe et al., 2023). Similarly, administering soursop leaf extract affected the histopathology of hepatocellular carcinoma in Wistar rats that received sorafenib standard therapy (Sinaga & Susilaningsih, 2019).

One study has examined the anticancer activity of soursop leaf extract in humans. A study performed an anticancer survey of human colorectal cancer cell lines (Indrawati et al., 2017). The effects of soursop extract consumption were evaluated consecutively for eight weeks in 30 patients with colorectal cancer. The extract displayed selectivity by suppressing the development of colorectal cancer cells. The results showed the correlation of extract supplementation with the patient's nutritional status, quality of life, and inflammation. In this study, several chemotherapy patients felt better after supplementing *A. muricata* leaf extract. In addition, longer extract supplementation periods may enhance patients' nutritional condition. A clinical study involved metastatic breast cancer patients (Hansra et al., 2014). The results showed that consuming boiled *A. muricata* leaves could halt the progression of chemotherapy-resistant metastatic tumors.

3.5. Unlock the new potential: antimetastatic agent

The cytotoxicity exhibited by *A. muricata* extracts and their bioactive compounds has been widely reported as an initial parameter of anticancer potential. However, few reports specifically explore its antimetastatic potential against cancer. Metastasis is the spread of cancer cells from the primary tumor to distant sites. It is also an essential factor in poor cancer prognosis. Despite advances in conventional therapies such as surgery, chemotherapy, and radiation, metastasis remains a significant challenge. Therefore, exploring new therapeutic agents that target metastasis is urgently needed.

Several factors contribute to metastasis, including the angiogenesis, invasion, and migration of cancer cells (Figure 3). The production of vascular endothelial growth factor (VEGF) and activation of matrix metalloproteinase (MMP)-2 and MMP-9 play essential roles in cancer metastasis. VEGF is a signaling protein that plays a vital role in angiogenesis and the formation of new blood vessels. VEGF is produced by tumor and surrounding stromal cells. VEGF attracts endothelial cells, cells lining blood vessels, to migrate and form new blood vessels (Bhattacharya et al., 2016, 2017). These new blood vessels supply the tumor with the oxygen and nutrients it needs to grow and thrive. The soursop fruit extract reduces the viability of MDA-MB-468 breast cancer cells that overexpress the epidermal growth factor receptor (EGFR) (Dai et al., 2011).

VEGF signaling induces endothelial cell migration in normal physiological processes and tumors. Autocrine VEGF signaling, through its receptors, mediates the migration of various tumor cells. Multiple receptor tyrosine kinases and downstream AKT signaling regulate VEGF in colorectal cancer. Loss of VEGF expression significantly decreases cell proliferation, increases apoptosis, and improves the chemotherapy sensitivity of CRC cells (Bhattacharya et al., 2016).

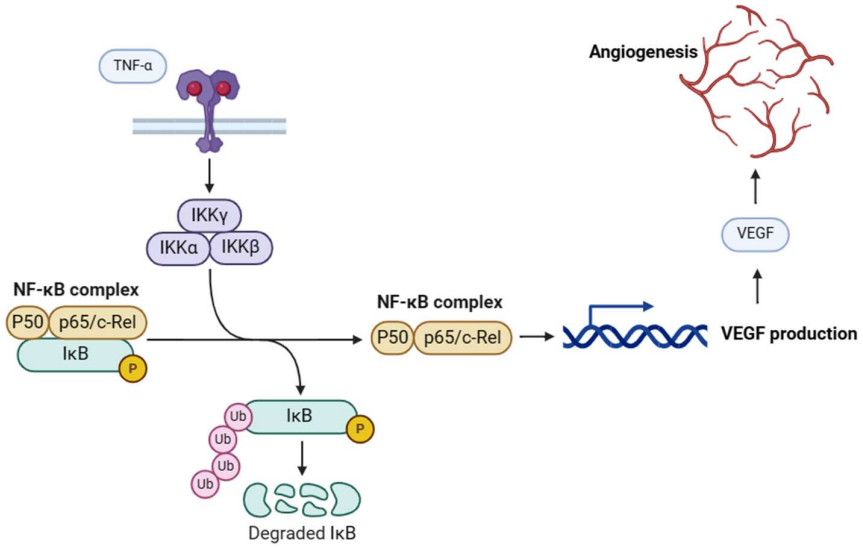


Figure 3. The schematic of TNF-α/NF-κB/VEGF pathway initial to angiogenesis. Angiogenesis contributes to the invasion and migration of cancer cells (Bhattacharya et al., 2016, 2017).

Tumor cells will produce extracellular matrix-degrading enzymes, e.g., the matrix metalloproteinase (MMP). The activity of this protein paves the way for tumor cells to expand in the metastatic process. *A. muricata* extracts, such as fruits, stems, seeds, and twigs, showed significant inhibition of MMP-2 and MMP-9 proteins in the HT1080 cell line,a highly metastatic fibrosarcoma cell (Mutakin et al., 2022). In addition, this extract also increased the expression of several endogenous inhibitors of MMP-2 and MMP-9, such as cysteine-rich protein-induced reversion with kazal motif (RECK) and tissue inhibitor of metalloproteinase-2 (TIMP-2) (Drishya et al., 2020). In addition, primary cells developed from tumor tissue obtained from patients who did not undergo chemotherapy also showed similar results. The observed inhibition of MMP-2 and MMP-9 was tumor-specific (Drishya et al., 2020). *A. muricata* extract inhibited potent migration in highly metastasized Caco and HepG2 cells (Mohammed et al., 2024). Bioactive compounds of *A. muricata* can also prevent and suppress metastasis in prostate cancer cell lines through decreasing MMP-9, phosphorylated focal adhesion kinase (Torres et al., 2012), vascular endothelial growth factor (Foster et al., 2020), and TNF-α (Laksmitawati et al., 2016). TNF-α plays a role in the expression of VEGF (Laksmitawati et al., 2016). Ethanol extract of *A. muricata* leaf inhibits angiogenesis and migration of WiDr colon cancer cell lines by increasing the amount of E-cadherin and reducing VEGF production (Astuti et al., 2023).

3.6. Future direction

Traditional herbal medicines have been used for centuries and continue to be today. Cancer drug resistance poses a significant barrier to the efficacy of conventional chemotherapy. By exploring new agents, such as *A. muricata*, that may work through different mechanisms than standard chemotherapy drugs, we can potentially overcome drug resistance and improve treatment outcomes for cancer patients. In vitro and in vivo studies show the potential of using *A. muricata* extract as a co-chemotherapeutic of existing drugs. In addition, a comprehensive literature review concluded that *A. muricata* has a good safety and tolerability profile (Chan et al., 2019).

In the community, herbal medicines are consumed separately or in combination with chemotherapy drugs. Combined use may reduce the side effects of cancer treatment. However, the use of co-chemotherapy can lead to herbal-drug interactions (HDIs). HDIs can increase toxicity, but on the other hand, they can decrease the pharmacological effect of drug components. For example, combining Lingzhi with cytotoxic drugs can improve survival and reduce the side effects of conventional chemotherapy (Lam et al., 2020). Combining soursop leaf extract with doxorubicin, the most common cancer drug, had higher cytotoxic activity against cancer than single-use (Salsabila et al., 2021). This combination also caused cell cycle arrest in the G1 phase as a single treatment and G2/M arrest in combination with doxorubicin (Salsabila et al., 2021). Although existing studies do not report harmful activity using *A. muricata* extract, developing *A. muricata* as a cancer co-chemotherapeutic requires comprehensive research.

There is still a lack of clinical trials to evaluate the safety and efficacy of *A. muricata* in cancer patients, particularly those investigating its antimetastatic effects. Future research should focus on well-designed clinical trials to assess the safety and effectiveness of *A. muricata* as an adjunctive therapy for cancer patients, particularly those with metastatic disease. In addition, its benefits as a co-chemotherapeutic agent are even more significant when made into pharmaceutical dosage forms. *A. muricata* extracts or compounds packaged in pharmaceutical preparations are expected to improve patient's quality of life. Therefore, more research is needed to determine the optimal dosage, route of administration, and potential side effects of *Annona muricata*.

Further elucidation of the molecular mechanism underlying the antimetastatic effect of *A. muricata* is needed to optimize its therapeutic potential. Researchers can use the network pharmacology approach to determine a drug's molecular mechanism of action on the target (Figure 4). Network pharmacology opens the door to drug discovery by considering the complex linkages of molecules and their interactions, resulting in more effective, personalized, and multi-targeted therapeutics for various disorders (Muhammad et al., 2018).

The in-silico study reported that acetogenin compounds could potentially bind to several cancer biomarker proteins, including EGFR, AKT1, KDR, MMP-2, MMP-9, ERBB2, IGF1R, MTOR, and HRAS (Grijaldo et al., 2023). Similarly, the bioactive compounds of soursop leaves are reportedly involved in the biological process of cell survival (Romli et al., 2022). The mechanism involves many target proteins, including CDK1, CCNB1, CCNB2, CDK6, APC,

PLK1, PPARG, PPARA, INSR, TP53, TERT, RB1, EGFR, AHR, ESR1, APEX, F2, and SYK. This study also mentions the biological processes involved and interrelated: cell cycle regulation, miRNA transcription, ATP metabolic processes, and reactive oxygen species (ROS). Target proteins regulate cancer cells' proliferation, apoptosis, invasion, and metastasis (Ilango et al., 2022; N. Liu et al., 2016).

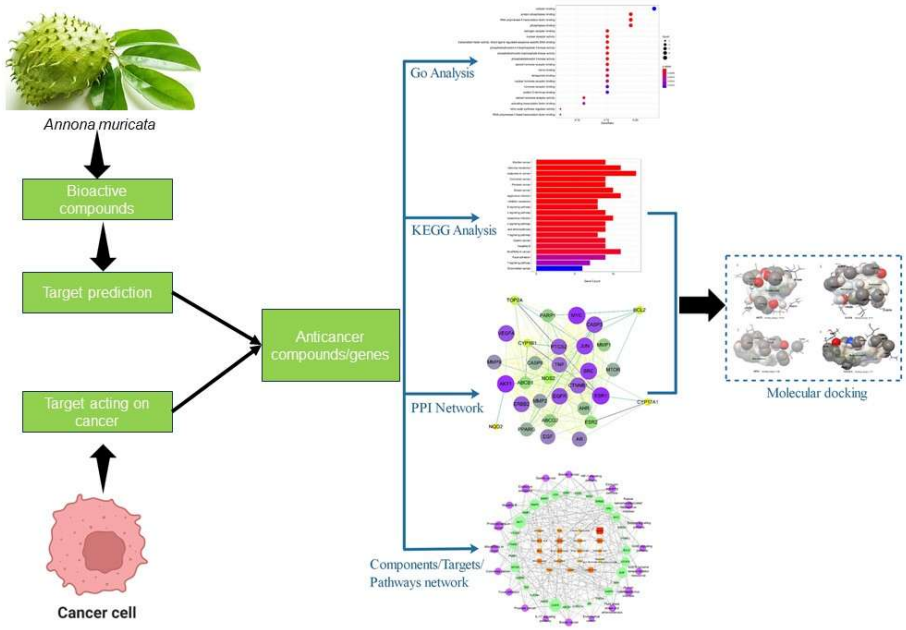


Figure 4. Schematic of network pharmacology approach in oncology (Hermawan et al., 2021; Rovik et al., 2024).

Another study revealed that *A. muricata* extract targeted six genes in colon cancer cells, namely ABCC1, ERBB2, STAT3, AR, SRC, and ABCG2 (Pandiyan et al., 2022). ABCG2 gene expression is essential in tumor progression and cell metastasis (Liu et al., 2010). In addition, overexpression of ERBB2 and STAT3 genes promotes colon cancer cell resistance to chemotherapy (Pectasides & Bass, 2015; Spitzner et al., 2014).

4. CONCLUSION

As an early parameter of anticancer potential, the cytotoxicity of *A. muricata* extracts has been widely studied on various cell lines in vitro. *A. muricata* extract has potential as a co-chemotherapy in the treatment of breast cancer, colon cancer, prostate, lung, cervical, liver, and blood cancer. Despite many studies on antiproliferation, *A. muricata* extract also has the potential to be an antimetastatic agent, overcoming drug resistance and improving treatment outcomes for cancer patients. More research is needed to determine the optimal dosage, route of administration, and potential side effects of *A. muricata*.

ACKNOWLEDGMENT

The author thanks all researchers who contributed to studying *A. muricata* and developing network pharmacology tools and databases. This research was funded by the Directorate of

Research, Technology, and Community Services, the Directorate General of Higher Education, Research, and Technology, the Indonesian Ministry of Education, Culture, Research, and Technology (No. 048/E5/PG.02.00.PL/2024) via the master thesis research scheme [Penelitian Tesis Magister] (No. 2845/UN1/DITLIT/PT.01.03/2024).

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

REFERENCES

Abdullah, M., Syam, A. F., Meilany, S., Laksono, B., Prabu, O. G., Bekti, H. S., Indrawati, L., & Makmun, D. (2017). The Value of Caspase-3 after the Application of *Annona muricata* Leaf Extract in COLO-205 Colorectal Cancer Cell Line. *Gastroenterology Research and Practice*, *2017*, 1–5. https://doi.org/10.1155/2017/4357165

Adelina, R., Febriyanti, R., Oktoberia, I. S., & Intan, P. R. (2014). Ekstrak Daun Annona muricata Linn. Sebagai Antiproliferasi terhadap Sel Hepar Tikus Terinduksi 7,12 Dimetilbenz [a] antracene (DMBA). *Jurnal Kefarmasian Indonesia*, *4*(1), 1–12.

Andrini, S., Suherman, & Widowati, W. (2014). Annona muricata leaves have strongest cytotoxic activity against breast cancer cells. *Universa Medicina*, *33*(3), 179–184.

Apeh, V. O., Adegboyega, A. E., Chukwuma, I. F., Ugwah-Oguejiofor, C. J., Aja, P. M., Ofeimun, J. O., Ale, B. A., Johnson, G. I., Ebenyi, L. N., Iwaloye, O., Ejembi, S. A., Ezugworie, F. N., & Johnson, T. O. (2023). An in-silico study of bioactive compounds of Annona muricata in the design of ani-prostate cancer agent: MM/GBSA, pharmacophore modeling and ADMET parameters. *Informatics in Medicine Unlocked*, *43*, 101377. https://doi.org/10.1016/j.imu.2023.101377

Apriyanto, D. R., Hartati, S., Dewi, B. E., Aoki-Utsuba, C., & Hotta, H. (2018). Aktivitas Sitotoksisitas Ekstrak Metanol Daun Sirsak (Annona muricata L.) terhadap Karsinoma Hepatoseluler Strain Huh71T-1 Cell Line. *Tunas Medika Jurnal Kedokteran Dan Kesehatan*, *4*(1), 1–4.

Arifianti, L., Sukardiman, Studiawan, H., Rakhmawati, & Megawati, L. (2014). Uji Aktivitas Ekstrak Biji Sirsak (Annona muricata l.) terhadap Sel Kanker Mamalia secara In Vitro. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, *1*(2), 63–66.

Asare, G. A., Afriyie, D., Ngala, R. A., Abutiate, H., Doku, D., Mahmood, S. A., & Rahman, H. (2015). Antiproliferative Activity of Aqueous Leaf Extract of *Annona muricata L.* on the Prostate, BPH-1 Cells, and Some Target Genes. *Integrative Cancer Therapies*, *14*(1), 65–74. https://doi.org/10.1177/1534735414550198

Astuti, Y., Agus Suharto, Sabtanti Harimurti, Helmi Hana Prinanda, & Wahyu Joko Priambodo. (2023). Number of e-cadherin and VEGF in WiDr cell line post-induced by extract ethanol Annona muricata leaf. *Bali Medical Journal*, *12*(1), 524–529. https://doi.org/10.15562/bmj.v12i1.3736

Astuti, Y., Priambodo, W. J., Rahmah, & Septiyana, D. A. (2021). Overview of wound assay methods extract ethanol leaves soursop (annona muricata) on widr colon cancer cells. *Bali Medical Journal*, *10*(3), 1385–1389. https://doi.org/10.15562/bmj.v10i3.2979

Bandyopadhyay, A., Wang, L., Agyin, J., Tang, Y., Lin, S., Yeh, I.-T., De, K., & Sun, L.-Z. (2010). Doxorubicin in Combination with a Small TGFβ Inhibitor: A Potential Novel Therapy for Metastatic Breast Cancer in Mouse Models. *PLoS ONE*, *5*(4), e10365. https://doi.org/10.1371/journal.pone.0010365

Bhanuwati, A. V., & Pakpahan, A. (2022). Cytotoxic test of different solvents of soursop (Annona muricata) leaf extract against HSC-3 cell line. *Dental Journal (Majalah Kedokteran Gigi)*, *55*(3), 130–136. https://doi.org/10.20473/j.djmkg.v55.i3.p130-136

Bhattacharya, R., Fan, F., Wang, R., Ye, X., Xia, L., Boulbes, D., & Ellis, L. M. (2017).

Intracrine VEGF signalling mediates colorectal cancer cell migration and invasion. *British Journal of Cancer*, *117*(6), 848–855. https://doi.org/10.1038/bjc.2017.238

Bhattacharya, R., Ye, X.-C., Wang, R., Ling, X., McManus, M., Fan, F., Boulbes, D., & Ellis, L. M. (2016). Intracrine VEGF Signaling Mediates the Activity of Prosurvival Pathways in Human Colorectal Cancer Cells. *Cancer Research*, *76*(10), 3014–3024. https://doi.org/10.1158/0008-5472.CAN-15-1605

Cahyawati, P. N. (2020). Pharmacological dan Toxical Effects of Soursop (Annona muricata): A Mini Review. *Biomedika*, *12*(2), 107–116. https://doi.org/10.23917/biomedika.v12i2.10691

Chan, W.-J. J., McLachlan, A. J., Hanrahan, J. R., & Harnett, J. E. (2019). The safety and tolerability of *Annona muricata* leaf extract: A systematic review. *Journal of Pharmacy and Pharmacology*, *72*(1), 1–16. https://doi.org/10.1111/jphp.13182

Coria-Téllez, A. V., Montalvo-Gónzalez, E., Yahia, E. M., & Obledo-Vázquez, E. N. (2018). Annona muricata: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. *Arabian Journal of Chemistry*, *11*(5), 662–691. https://doi.org/10.1016/j.arabjc.2016.01.004

Dai, Y., Hogan, S., Schmelz, E. M., Ju, Y. H., Canning, C., & Zhou, K. (2011). Selective Growth Inhibition of Human Breast Cancer Cells by Graviola Fruit Extract In Vitro and In Vivo Involving Downregulation of EGFR Expression. *Nutrition and Cancer*, *63*(5), 795–801. https://doi.org/10.1080/01635581.2011.563027

Damayanti, D. S., Utomo, D. H., & Kusuma, C. (2017). Revealing the potency of Annona muricata leaves extract as FOXO1 inhibitor for diabetes mellitus treatment through computational study. *In Silico Pharmacology*, *5*(1), 3. https://doi.org/10.1007/s40203-017-0023-3

Damodar, G., Smitha, T., Gopinath, S., Vijayakumar, S., & Rao, Y. (2014). An evaluation of hepatotoxicity in breast cancer patients receiving injection doxorubicin. *Annals of Medical and Health Sciences Research*, *4*(1), 74. https://doi.org/10.4103/2141-9248.126619

Dewayani, A. R., Ghaliya, S., Parameswari, N., Pribadi, A. P. A., Ahadi, H. M., Aulifa, D. L., Elaine, A. A., & Sitinjak, B. D. P. (2023). Studi In Silico Senyawa Daun Sirsak (Annona muricata L.) Sebagai Inhibitor BRAF V600E Pada Kanker Melanoma. *Jurnal Farmasi Udayana*, 80. https://doi.org/10.24843/JFU.2022.v11.i02.p07

Drishya, G., Nambiar, J., Shaji, S. K., Vanuopadath, M., Achuthan, A., Kumar, A., Alias, A., Sherif, A., Joseph, C., Divya, P., Kumar, D. S., Bose, C., Nair, S. V., Sudarslal, S., Kumar, G. B., Lakshmi, S., & Nair, B. G. (2020). RECK and TIMP-2 mediate inhibition of MMP-2 and MMP-9 by Annona muricata. *Journal of Biosciences*, *45*(1), 89. https://doi.org/10.1007/s12038-020-00056-z

Fatmawati, D., Suparmi, Yusuf, I., & Israhnanto. (2018). Selektivitas Antikanker Ekstrak Daun Sirsak (Annona muricata) pada Lini Sel Kanker Payudara. *Bio-Site*, *4*(2), 78–83.

Fertilita, S., Sandhika, W., & Suprabawati, D. G. A. (2020). The Cytotoxic Activity of Annona muricata Linn Leaves Ethanolic Extract (AMEE) on T47D Breast Cancer Cell Line. *Medical Laboratory Technology Journal*, *1*(1). https://doi.org/10.31964/mltj.v1i1.291

Foster, K., Oyenihi, O., Rademan, S., Erhabor, J., Matsabisa, M., Barker, J., Langat, M. K., Kendal-Smith, A., Asemota, H., & Delgoda, R. (2020). Selective cytotoxic and antimetastatic activity in DU-145 prostate cancer cells induced by Annona muricata L. bark extract and phytochemical, annonacin. *BMC Complementary Medicine and Therapies*, *20*(1), 375. https://doi.org/10.1186/s12906-020-03130-z

Gavamukulya, Y., Abou-Elella, F., Wamunyokoli, F., & AEl-Shemy, H. (2014). Phytochemical screening, antioxidant activity, and in vitro anticancer potential of ethanolic and water leaf extracts of Annona muricata (Graviola). *Asian Pacific Journal of Tropical Medicine*, *7*, S355–S363. https://doi.org/10.1016/S1995-7645(14)60258-3

Gavamukulya, Y., Wamunyokoli, F., & El-Shemy, H. A. (2017). Annona muricata: Is the natural therapy for most disease conditions, including cancer, growing in our backyard? A systematic review of its research history and future prospects. *Asian Pacific Journal of Tropical Medicine*, *10*(9), 835–848. https://doi.org/10.1016/j.apjtm.2017.08.009

Grijaldo, S. J. B., Alvarez, M. R. S., Heralde, F. M., Nacario, R. C., Lebrilla, C. B., Rabajante, J. F., & Completo, G. C. (2023). Integrating Computational Methods in Network Pharmacology and *In Silico* Screening to Uncover Multi-targeting Phytochemicals against Aberrant Protein Glycosylation in Lung Cancer. *ACS Omega*, *8*(23), 20303–20312. https://doi.org/10.1021/acsomega.2c07542

Hadisaputri, Y. E., Habibah, U., Abdullah, F. F., Halimah, E., Mutakin, M., Megantara, S., Abdulah, R., & Diantini, A. (2021). Antiproliferation Activity and Apoptotic Mechanism of Soursop (*Annona muricata* L.) Leaves Extract and Fractions on MCF-7 Breast Cancer *Cells. Breast Cancer (Dove Med Press)*, *16*(13), 447–457. https://doi.org/10.2147/BCTT.S317682

Hamizah, S., Roslida, A. H., Fezah, O., Tan, K. L., Tor, Y. S., & Tan, C. I. (2012). Chemo-preventive Potential of Annona Muricata L Leaves on Chemically-Induced Skin Papillomagenesis in Mice. *Asian Pacific Journal of Cancer Prevention*, *13*(6), 2533–2539. https://doi.org/10.7314/APJCP.2012.13.6.2533

Hansra, D. M., Silva, O., Mehta, A., & Ahn, E. (2014). Patient with Metastatic Breast Cancer Achieves Stable Disease for 5 Years on Graviola and Xeloda after Progressing on Multiple Lines of Therapy. *Advances in Breast Cancer Research*, *03*(03), 84–87. https://doi.org/10.4236/abcr.2014.33012

Haryanti, S., & Widiyastuti, Y. (2017). Aktivitas Sitotoksik pada Sel MCF-7 dari Tumbuhan Indonesia untuk Pengobatan Tradisional Kanker Payudara. *Media Penelitian Dan Pengembangan Kesehatan*, *27*(4), 247–254. https://doi.org/10.22435/mpk.v27i4.5010.247-254

Hasmila, I., Natsir, H., & Soekamto, N. H. (2019). Phytochemical analysis and antioxidant antioxidant activity of soursop leaf extract (Annona muricata Linn.). *Journal of Physics: Conference Series*, *1341*(3), 032027. https://doi.org/10.1088/1742-6596/1341/3/032027

Hermawan, A., Khumaira, A., Ikawati, M., Putri, H., Jenie, R. I., Angraini, S. M., & Muflikhasari, H. A. (2021). Identification of key genes of hesperidin in inhibition of breast cancer stem cells by functional network analysis. *Computational Biology and Chemistry*, *90*, 107427. https://doi.org/10.1016/j.compbiolchem.2020.107427

Icanervilia, A., Choridah, L., Van Asselt, A., Vervoort, J., Postma, M., Rengganis, A., & Kardinah, K. (2023). Early Detection of Breast Cancer in Indonesia: Barriers Identified in a Qualitative Study. *Asian Pacific Journal of Cancer Prevention*, *24*(8), 2749–2755. https://doi.org/10.31557/APJCP.2023.24.8.2749

Ilango, S., Sahoo, D. K., Paital, B., Kathirvel, K., Gabriel, J. I., Subramaniam, K., Jayachandran, P., Dash, R. K., Hati, A. K., Behera, T. R., Mishra, P., & Nirmaladevi, R. (2022). A Review on Annona muricata and Its Anticancer Activity. *Cancers*, *14*(18), 4539. https://doi.org/10.3390/cancers14184539

Indrawati, L., Kusmardi, & Marwito, W. (2023). The Effect of an Annona muricata Leaves Extract on Circulating Soluble Adhesion Molecules in Colin Carcinogenesis Model. *The Indonesian Journal of Gastroenterology, Hepatology, and Digestive Endoscopy*, *24*(1), 30–34.

Indrawati, L., Purwantyastuti Ascobat, Budiman Bela, Murdani Abdullah, & Ingrid S Surono. (2017). The effect of an Annona muricata leaf extract on nutritional status and cytotoxicity in colorectal cancer: A randomized controlled trial. *Asia Pacific Journal of Clinical Nutrition*, *26*(4). https://doi.org/10.6133/apjcn.062016.02

Jepkorir, M., Ambundo, T., Ngule, C., Ndungu, J., Njuguna, D., Mbugua, R., Chepngetich, J., & Mwitari, P. (2018). Phytochemical screening and in vitro Antiproliferative Activity of

the Fruit of Annona muricata and Abelmoschus esculentus Pods against Selected Cancer Cell Lines. *Journal of Complementary and Alternative Medical Research*, *5*(3), 1–11. https://doi.org/10.9734/JOCAMR/2018/41248

Ko, E.-Y., & Moon, A. (2015). Natural Products for Chemoprevention of Breast Cancer. *Journal of Cancer Prevention*, *20*(4), 223–231. https://doi.org/10.15430/JCP.2015.20.4.223

König, J., Müller, F., & Fromm, M. F. (2013). Transporters and Drug-Drug Interactions: Important Determinants of Drug Disposition and Effects. *Pharmacological Reviews*, *65*(3), 944–966. https://doi.org/10.1124/pr.113.007518

Kuete, V., Dzotam, J. K., Voukeng, I. K., Fankam, A. G., & Efferth, T. (2016). Cytotoxicity of methanol extracts of Annona muricata, Passiflora edulis and nine other Cameroonian medicinal plants towards multi-factorial drug-resistant cancer cell lines. *SpringerPlus*, *5*(1), 1666. https://doi.org/10.1186/s40064-016-3361-4

Laksmitawati, D. R., Prasanti, A. P., Larasinta, N., Syauta, G. A., Hilda, R., Ramadaniati, H. U., Widyastuti, A., Karami, N., Afni, M., Rihibiha, D. D., Kusuma, H. S. W., & Widowati, W. (2016). Anti-Inflammatory Potential of Gandarusa (<I>Gendarussa vulgaris</I> Nees) and Soursoup (<I>Annona muricata</I> L) Extracts in LPS Stimulated-Macrophage Cell (RAW264.7). *Journal of Natural Remedies*, *16*(2), 73. https://doi.org/10.18311/jnr/2016/5367

Lam, C. S., Cheng, L. P., Zhou, L. M., Cheung, Y. T., & Zuo, Z. (2020). Herb–drug interactions between the medicinal mushrooms Lingzhi and Yunzhi and cytotoxic anticancer drugs: A systematic review. *Chinese Medicine*, *15*(1), 75. https://doi.org/10.1186/s13020-020-00356-4

Lienggonegoro, L. A. & Kharirie. (2020). Daun sirsak (Annona muricata) dan potensinya sebagai antikanker. *Pros Sem Nas Masy Biodiv Indon*, *6*, 653–657. https://doi.org/10.13057/psnmbi/m060128

Liu, H. G., Pan, Y. F., You, J., Wang, O. C., Huang, K. T., & Zhang, X. H. (2010). Expression of ABCG2 and its significance in colorectal cancer. *Asian Pacific Journal of Cancer Prevention: APJCP*, *11*(4), 845–848.

Liu, N., Yang, H. L., Wang, P., Lu, Y. C., Yang, Y. J., Wang, L., & Lee, S. C. (2016). Functional proteomic analysis revels that the ethanol extract of Annona muricata L. induces liver cancer cell apoptosis through endoplasmic reticulum stress pathway. *Journal of Ethnopharmacology*, *189*, 210–217. https://doi.org/10.1016/j.jep.2016.05.045

Lopez, M. D. R. H., Casas Solis, J., Universidad de Guadalajara, Alcazar Rios, I. C., Universidad de Guadalajara, Barrientos Ramirez, L., Universidad de Guadalajara, Santerre, A., & Universidad de Guadalajara. (2023). Selective cytotoxic effect of Annona muricataL. in HCC1954 (HER2+) breast cancer cells. *Boletin Latinoamericano y Del Caribe de Plantas Medicinales y Aromaticas*, *22*(5), 688–699. https://doi.org/10.37360/blacpma.23.22.5.50

Manna, E. D. F., Serrano, D., Aurilio, G., Bonanni, B., & Lazzeroni, M. (2023). Chemoprevention and Lifestyle Modifications for Risk Reduction in Sporadic and Hereditary Breast Cancer. *Healthcare*, *11*(16), 2360. https://doi.org/10.3390/healthcare11162360

Manshour, H. H., Elkady, A. A., Elrefaei, A. H., & Hafez, H. F. (2018). Radioprotective, antioxidant antioxidant and antitumor efficacy of Annona muricata L. leaf extract. *Journal of Biochemistry & Biophysics*, *55*, 205–214.

Matsushige, A., Kotake, Y., Matsunami, K., Otsuka, H., Ohta, S., & Takeda, Y. (2012). Annonamine, a new aporphine alkaloid from the leaves of Annona muricata. *Chemical & Pharmaceutical Bulletin*, *60*(2), 257–259. https://doi.org/10.1248/cpb.60.257

Meenakshisundaram, S., Krishnamoorthy, V., Jagadeesan, Y., Vilwanathan, R., & Balaiah, A. (2020). Annona muricata assisted biogenic synthesis of silver nanoparticles regulates cell

cycle arrest in NSCLC cell lines. *Bioorganic Chemistry*, *95*, 103451. https://doi.org/10.1016/j.bioorg.2019.103451

Merlín-Lucas, V., Ordoñez-Razo, R. M., Calzada, F., Solís, A., García-Hernández, N., Barbosa, E., & Valdés, M. (2021). Antitumor Potential of Annona muricata Linn. An Edible and Medicinal Plant in Mexico: In Vitro, In Vivo, and Toxicological Studies. *Molecules*, *26*(24), 7675. https://doi.org/10.3390/molecules26247675

Moghadamtousi, S. Z., Kadir, H. A., Paydar, M., Rouhollahi, E., & Karimian, H. (2014). Annona muricata leaves induced apoptosis in A549 cells through mitochondrial-mediated pathway and involvement of NF-κB. *BMC Complementary and Alternative Medicine*, *14*(1), 299. https://doi.org/10.1186/1472-6882-14-299

Moghadamtousi, S. Z., Karimian, H., Rouhollahi, E., Paydar, M., Fadaeinasab, M., & Abdul Kadir, H. (2014). Annona muricata leaves induce G1 cell cycle arrest and apoptosis through mitochondria-mediated pathway in human HCT-116 and HT-29 colon cancer cells. *Journal of Ethnopharmacology*, *156*, 277–289. https://doi.org/10.1016/j.jep.2014.08.011

Moghadamtousi, S. Z., Rouhollahi, E., Karimian, H., Fadaeinasab, M., Firoozinia, M., Ameen Abdulla, M., & Abdul Kadir, H. (2015). The Chemopotential Effect of Annona muricata Leaves against Azoxymethane-Induced Colonic Aberrant Crypt Foci in Rats and the Apoptotic Effect of Acetogenin Annomuricin E in HT-29 Cells: A Bioassay-Guided Approach. *PLOS ONE*, *10*(4), e0122288. https://doi.org/10.1371/journal.pone.0122288

Mohammed, M. A., Elzefzafy, N., El-Khadragy, M. F., Alzahrani, A., Yehia, H. M., & Kachlicki, P. (2024). Comprehensive Tools of Alkaloid/Volatile Compounds–Metabolomics and DNA Profiles: Bioassay-Role-Guided Differentiation Process of Six Annona sp. Grown in Egypt as Anticancer Therapy. *Pharmaceuticals*, *17*(1), 103. https://doi.org/10.3390/ph17010103

Momenimovahed, Z., & Salehiniya, H. (2019). Epidemiological characteristics of and risk factors for breast cancer in the world. *Breast Cancer (Dove Medical Press)*, *11*, 151–164. https://doi.org/10.2147/BCTT.S176070

Muhammad, J., Khan, A., Ali, A., Fang, L., Yanjing, W., Xu, Q., & Wei, D.-Q. (2018). Network Pharmacology: Exploring the Resources and Methodologies. *Current Topics in Medicinal Chemistry*, *18*(12), 949–964. https://doi.org/10.2174/1568026618666180330141351

Mutakin, M., Fauziati, R., Fadhilah, F. N., Zuhrotun, A., Amalia, R., & Hadisaputri, Y. E. (2022). Pharmacological Activities of Soursop (Annona muricata Lin.). *Molecules*, *27*(4), 1201. https://doi.org/10.3390/molecules27041201

Naik, A. V., & Sellappan, K. (2020). *In vitro* evaluation of *Annona muricata* L. (Soursop) leaf methanol extracts on inhibition of tumorigenicity and metastasis of breast cancer cells. *Biomarkers*, *25*(8), 701–710. https://doi.org/10.1080/1354750X.2020.1836025

Najmuddin, S. U. F., Romli, M. F., Hamid, M., Alitheen, N. B., & Nik Abd Rahman, N. M. A. (2016). Anticancer effect of Annona Muricata Linn Leaves Crude Extract (AMCE) on breast cancer cell line. *BMC Complementary and Alternative Medicine*, *16*(1), 311. https://doi.org/10.1186/s12906-016-1290-y

Pandiyan, S. M., Shanmugaraj, P., Manoharan, J. P., & Vidyalakshmi, S. (2022). A network pharmacological approach to reveal the multidrug resistance reversal and associated mechanisms of acetogenins against colorectal cancer. *Journal of Biomolecular Structure and Dynamics*, *40*(24), 13527–13546. https://doi.org/10.1080/07391102.2021.1990130

Pectasides, E., & Bass, A. J. (2015). ERBB2 Emerges as a New Target for Colorectal Cancer. *Cancer Discovery*, *5*(8), 799–801. https://doi.org/10.1158/2159-8290.CD-15-0730

Pieme, C. A., Kumar, S. G., Dongmo, M. S., Moukette, B. M., Boyoum, F. F., Ngogang, J. Y., & Saxena, A. K. (2014). Antiproliferative activity and induction of apoptosis by Annona muricata (Annonaceae) extract on human cancer cells. *BMC Complementary and Alternative Medicine*, *14*(1), 516. https://doi.org/10.1186/1472-6882-14-516

Prasad, N., Muthusamy, G., Shanmugam, M., & Ambudkar, S. (2016). South Asian Medicinal Compounds as Modulators of Resistance to Chemotherapy and Radiotherapy. *Cancers*, *8*(3), 32. https://doi.org/10.3390/cancers8030032

Prayitno, A., Artanti, A., Dewangga, V., Abdullah, I., Hartati, M., Fitria, M., Elmanda, A., & Astirin, O. (2016). Tetrahydrofurane is a Component of Annona muricata Leaf will Induce Apoptosis Program in Cancer Cell because the Virus: A Proxy for Cancer Treatment. *Immunome Research*, *12*(2). https://doi.org/10.4172/1745-7580.10000120

Qorina, F., Arsianti, A., Fithrotunnisa, Q., Tejaputri, N., Azizah, N. N., & Putrianingsih, R. (2020). Cytotoxicity of Soursop Leaves (Annona muricata) against Cervical HeLa Cancer Cells. *Pharmacognosy Journal*, *12*(1), 20–24. https://doi.org/10.5530/pj.2020.12.4

Rachmawati, E., Karyono, S., & Suyuti, H. (2012). The effect of Annona Muricata leaf on proliferation and appoptosis of Hela cells mediated by p53. *Jurnal Kedokteraan Brawijaya*, *27*(1), 28–33.

Rady, I., Bloch, M. B., Chamcheu, R.-C. N., Banang Mbeumi, S., Anwar, M. R., Mohamed, H., Babatunde, A. S., Kuiate, J.-R., Noubissi, F. K., El Sayed, K. A., Whitfield, G. K., & Chamcheu, J. C. (2018). Anticancer Properties of Graviola (*Annona muricata*): A Comprehensive Mechanistic Review. *Oxidative Medicine and Cellular Longevity*, *2018*, 1–39. https://doi.org/10.1155/2018/1826170

Rahmani, A. H., Babiker, A. Y., & Anwar, S. (2023). Hesperidin, a Bioflavonoid in Cancer Therapy: A Review for a Mechanism of Action through the Modulation of Cell Signaling Pathways. *Molecules*, *28*(13), 5152. https://doi.org/10.3390/molecules28135152

Roham, P. H., Kharat, K. R., Mungde, P., Jadhav, M. A., & Makhija, S. J. (2016). Induction of Mitochondria Mediated Apoptosis in Human Breast Cancer Cells (T-47D) by *Annona reticulata L*. Leaves Methanolic Extracts. *Nutrition and Cancer*, *68*(2), 305–311. https://doi.org/10.1080/01635581.2016.1142583

Romli, H. K., Hartati, J., & Yuniarti, L. (2022). *Analisis In Silico Mekanisme Cell Survival Zat Aktif Daun Sirsak (Annona muricata Linn) pada Kanker Kolorektal*. *2*, 679–687. https://doi.org/10.29313/bcsms.v2i1.1316

Rovik, A., Andyra, V. U., & Afifah, L. (2024). In Silico Exploration of Suruhan Leaves (Peperomia pellucida) for Triple-Negative Breast Cancer Therapy Targeting AURKA. *Journal of Experimental and Clinical Pharmacy (JECP)*, *4*(2), 115. https://doi.org/10.52365/jecp.v4i2.1119

Salsabila, I., Nugraheni, N., Ahlina, F., Haryanti, S., & Meiyanto, E. (2021). Synergistic Cotreatment Potential of Soursop (Annona muricata L.) Leaves Extract with Doxorubicin on 4T1 Cells with Antisenescence and Anti-reactive-oxygen-species Properties. *Iranian Journal of Pharmaceutical Research*, *20*(2). https://doi.org/10.22037/ijpr.2020.112485.13788

Shamir, E. R., Pappalardo, E., Jorgens, D. M., Coutinho, K., Tsai, W.-T., Aziz, K., Auer, M., Tran, P. T., Bader, J. S., & Ewald, A. J. (2014). Twist1-induced dissemination preserves epithelial identity and requires E-cadherin. *Journal of Cell Biology*, *204*(5), 839–856. https://doi.org/10.1083/jcb.201306088

Shaniba, V. S., Aziz, A. A., Joseph, J., Jayasree, P. R., & Manish Kumar, P. R. (2022). Synthesis, Characterization and Evaluation of AntioxidantAntioxidant and Cytotoxic Potential of Annona muricata Root Extract-derived Biogenic Silver Nanoparticles. *Journal of Cluster Science*, *33*(2), 467–483. https://doi.org/10.1007/s10876-021-01981-1

Silihe, K. K., Mbou, W. D., Ngo Pambe, J. C., Kenmogne, L. V., Maptouom, L. F., Kemegne Sipping, M. T., Zingue, S., & Njamen, D. (2023). Comparative anticancer effects of Annona muricata Linn (Annonaceae) leaves and fruits on DMBA-induced breast cancer in female rats. *BMC Complementary Medicine and Therapies*, *23*(1), 234. https://doi.org/10.1186/s12906-023-04073-x

Sinaga, N. P. M., & Susilaningsih, N. (2019). Pengaruh Pemberian Ekstrak Daun Sirsak terhadap Derajat Histopatologi pada Tikus Wistar Karsinoa Hepatoseluler yang Mendapat Terapi Standar Sorafenib. *Jurnal Kedokteran Diponegoro*, *8*(4), 1197–1209.

Spitzner, M., Roesler, B., Bielfeld, C., Emons, G., Gaedcke, J., Wolff, H. A., Rave-Fränk, M., Kramer, F., Beissbarth, T., Kitz, J., Wienands, J., Ghadimi, B. M., Ebner, R., Ried, T., & Grade, M. (2014). STAT3 inhibition sensitizes colorectal cancer to chemoradiotherapy *in vitro* and *in vivo*. *International Journal of Cancer*, *134*(4), 997–1007. https://doi.org/10.1002/ijc.28429

Suhendar, U. (2019). Geographycal effect on the Cytotoxic Activity of Annona muricata L. Leaves Ethanol Extract against MCF-7 Cancer Cell. *FITOFARMAKA: Jurnal Ilmiah Farmasi*, *8*(2), 71–78. https://doi.org/10.33751/jf.v8i2.1570

Sun, Z., Zhou, D., Yang, J., & Zhang, D. (2022). Doxorubicin promotes breast cancer cell migration and invasion via DCAF13. *FEBS Open Bio*, *12*(1), 221–230. https://doi.org/10.1002/2211-5463.13330

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, *71*(3), 209–249. https://doi.org/10.3322/caac.21660

Thomas, S., Paul, J., Shrungeswara, A., Biswas, S., Shah, A., Subraya, C., & Nayak, Y. (2019). Annona muricata fruit extract protects against diethylnitrosamine-induced hepatocellular cancer in rats. *Asian Pacific Journal of Tropical Medicine*, *12*(6), 272. https://doi.org/10.4103/1995-7645.261274

Torres, M. P., Rachagani, S., Purohit, V., Pandey, P., Joshi, S., Moore, E. D., Johansson, S. L., Singh, P. K., Ganti, A. K., & Batra, S. K. (2012). Graviola: A novel promising natural-derived drug that inhibits tumorigenicity and metastasis of pancreatic cancer cells in vitro and in vivo through altering cell metabolism. *Cancer Letters*, *323*(1), 29–40. https://doi.org/10.1016/j.canlet.2012.03.031

Wahab, S. M. A., Jantan, I., Haque, Md. A., & Arshad, L. (2018). Exploring the Leaves of Annona muricata L. as a Source of Potential Anti-inflammatory and Anticancer Agents. *Frontiers in Pharmacology*, *9*, 661. https://doi.org/10.3389/fphar.2018.00661

Widyastuti, D. A., & Rahayu, P. (2017). AntioxidantAntioxidant Capacity Comparison of Ethanolic Extract of Soursop (Annona muricata Linn.) Leaves and Seeds as Cancer Prevention Candidate. *Biology, Medicine, & Natural Product Chemistry*, *6*(1), 1. https://doi.org/10.14421/biomedich.2017.61.1-4

World Health Organization. (2024). Cancer. *World Health Organization*.

Yang, C., Gundala, S. R., Mukkavilli, R., Vangala, S., Reid, M. D., & Aneja, R. (2015). Synergistic interactions among flavonoids and acetogenins in Graviola (Annona muricata) leaves confer protection against prostate cancer. *Carcinogenesis*, *36*(6), 656–665. https://doi.org/10.1093/carcin/bgv046

Yu, Z.-J., Yan, H.-L., Xu, F.-H., Chao, H.-C., Deng, L.-H., Xu, X.-D., Huang, J.-B., & Zeng, T. (2020). Efficacy and Side Effects of Drugs Commonly Used for the Treatment of Lower Urinary Tract Symptoms Associated With Benign Prostatic Hyperplasia. *Frontiers in Pharmacology*, *11*, 658. https://doi.org/10.3389/fphar.2020.00658

Yuan, F., Bai, G., Miao, Y., Chen, Y., Li, X., & Chen, J. (2016). Annosquacin B induces mitochondrial apoptosis in multidrug resistant human breast cancer cell line MCF-7/ADR through selectively modulating MAPKs pathways. *Pharmaceutical Biology*, *54*(12), 3040–3045. https://doi.org/10.1080/13880209.2016.1200634

Yuniarti, L., Sastramihardja, H., Purbaningsih, W., Tejasari, M., Respati, T., Hestu, E., & Adithya, A. (2014). Soursop effect in cervical cancer apoptosis mechanism. *Global Medical and Health Communication*, *2*(1), 6–14. https://doi.org/10.29313/gmhc.v2i1.1524

JPSCR: Journal of Pharmaceutical Science and Clinical Research
Volume 10, Issue 1, Pages 38-49, March 2025
https://doi.org/10.20961/jpscr.v10i1.90769

ISSN 2503-331X (Online)

Formulation of Calcium-Protein Capsule Supplements for Stunting Toddlers by Utilizing Leftover Milkfish Bones Production

Sarah Raisya Nurhaliza[1,2], Joseph Fide Anggi[1,2], Angel Christella[1,2], Aurelina Yunita Hess[1,2], Mazaya Salwa Nadhira[1,2] and Iyan Sopyan[1]*

[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, Indonesia
[2]Undergraduate Program, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, Indonesia

*Corresponding author: i.sopyan@unpad.ac.id

Received: July 19, 2024; **Accepted:** March 14, 2025; **Published:** April 25, 2025

Abstract

Stunting is a health problem in children due to chronic malnutrition. Stunting is characterized by failure of growth and development in children. If stunting occurs during the Golden Period (age 0-5 years), it can impact the child's brain cells, causing them not to grow optimally. Indonesia has a stunting prevalence of 36% and has not reached the WHO's expected level of below 20%. Milkfish bones (*Chanos chanos*) are the remaining processed products of the fishing industry that have not been maximally utilized and will generally be disposed of into the environment or reprocessed and used as animal feed. With the presence of calcium and protein in the milkfish's bones, the milkfish's bones can be utilized as capsule supplements to address stunting in toddlers. This research was conducted to determine milkfish bones' calcium and protein content and obtain the most suitable capsule supplement formulation for stunting. This research was carried out through several stages, such as making milkfish bone powder, formulating and making stunting capsule supplements, evaluating the preparation, and analyzing and concluding the research results. The calculation of the consumption capsule supplement dose uses the percentage of calcium and protein content determined by AAS and Kjeldahl, which is then multiplied by the total weight of the capsule. The capsule consumption frequency is determined to fulfill the calcium requirement. The dose analysis and calculation results indicate that toddlers aged 1-3 years can consume two capsules daily, which is equivalent to 264.96 mg calcium and 88.32 mg protein, while toddlers aged 4-8 can consume three capsules daily, which is equivalent to 397.44 mg calcium and 32.48 mg protein. The dosage is determined based on the daily calcium needs of toddlers, which is 700 mg for children aged 1-3 years and 1000 mg for children aged 4-8 years. Therefore, developing milkfish bones with their calcium and protein content into capsule supplements could address toddler stunting.

Keywords: Capsule; Food supplements; Milkfish bones; Stunting

1. INTRODUCTION

The COVID-19 pandemic has had extraordinary long-term effects, such as unemployment and the threat of poverty. Stunting is one of the impacts of the COVID-19 pandemic. Stunting is a state of malnutrition related to past nutritional deficiencies; hence, it is a chronic dietary problem. Stunting can occur when the fetus is still in the womb and only

appears when the child is two years old. The high level of stunting in Indonesia can threaten the country's sustainability (UNICEF Indonesia, 2021).

Indonesia is predicted to be one of the world's economic powers in the next few decades. Stunting is a big problem that must be addressed immediately because otherwise, it will impact the decline in the quality of a country's human resources, affecting its stability. The government's efforts to overcome stunting are not yet satisfactory. The assistance provided by the government only includes food once a day, so it cannot be sufficient to fulfill nutritional needs for growth, and the absence of other assistance to support children's dietary needs is a concern for various parties. Therefore, there is a need for supplements to support the nutritional needs of calcium and protein from an early age, which is expected to be able to overcome and reduce stunting in toddlers.

Calcium regulates the work of hormones and growth factors. Lack of calcium consumption is caused by low calcium intake. For young children, low calcium intake is a health issue of concern. Based on nutritional adequacy figures, calcium adequacy for children aged 1-6 is 500 mg. The recommended food intake containing calcium for children aged 1-3 years is 700 mg, and for children 4-8 years is 1000 mg (Bu et al., 2022). From the Dietary Recall data extraction, the daily intake of food containing calcium per capita is 234.46 mg (Valentina et al., 2014).

Meanwhile, the level of protein adequacy for toddlers is differentiated according to age group. Toddlers aged 12-36 months have a protein adequacy of 25 g/day, and toddlers aged 37-59 months have a protein adequacy of 39 g/day. Research shows that 14.4% of toddlers experience a protein deficit in urban areas and 23.0% in rural areas (Fuada & Hidayat, 2015). The results of this research show that the daily food intake consumed by toddlers is insufficient for the calcium and protein needs that support their growth and development, especially their height growth. One way to help fulfill these nutrients is by consuming fish rich in protein and calcium, such as milkfish.

Milkfish (*Chanos chanos*) is a brackish water commodity with high economic value and significant potential for aquaculture (Deran et al., 2023). Milkfish bones, a by-product of production, remain underutilized despite their higher calcium content compared than meat (Esma et al., 2023). Due to their high levels of calcium and protein, milkfish bones have the potential to be a nutritional supplement for managing stunting, especially in overcoming stunted height growth in toddlers, by helping meet insufficient daily food intake of calcium and protein. Based on Basic Health Research (Riskesdas) data in 2018, the national prevalence of nutritional status among children aged 0-59 months is 11.5%, classified as very short and 19.3% as short (LPB, 2018). The most dominant risk factor for stunting is daily food intake because of the lack of food diversity. Food diversity reflects the quality of food consumed. Children with a lack of diverse food intake are 3.213 times more likely to experience stunting than children with a diverse food intake (Nuurrahmawati et al., 2023). Milkfish bones contain high levels of calcium and protein, which may serve as a sustainable nutritional intervention to help reduce stunting prevalence. Factors such as poor dietary habits, nutrient absorption disorders, and lifestyle

contribute to calcium and protein deficiencies. Capsules are a suitable delivery form for such supplements due to their tasteless nature, easy to administer, and easy to fill, either directly or in large quantities commercially, making them suitable for toddlers (Gullapalli & Mazzitelli, 2017). Based on the existing background, this research was carried out to make a formulation for supplements in capsule dosage form using calcium and protein contained in milkfish bones, which have the potential to overcome stunted growth and development in children, incredibly stunted height in toddlers due to stunting.

2. MATERIAL AND METHODS

2.1. Powder production from milkfish bones (*Chanos chanos*)

Wash and boil the remaining milkfish bones from production. Boil and steam the milkfish bones sample. Soak the fresh milkfish bone sample in NaOH from Merck (Darmstadt, Germany). Wash the milkfish bone sample using a filter cloth in water. Dry, grind, sift with a 100-mesh sieve, and weigh the yield of milkfish bone powders. Mix the ingredients in the formulation into the milkfish bones powders in a mortar. Add *Saccharum lactis* from Merck (Darmstadt, Germany) to see the homogeneity of milkfish bone powders (Domili & Pertiwi, 2021; Imra et al., 2019).

2.2. Formulation of capsule supplement from milkfish bones (*Chanos chanos*)

The formulation of a capsule supplement from milkfish bones for stunting toddlers is shown in Table 1.

Table 1. A capsule supplement is formulated from Milkfish Bones (*Chanos chanos*).

Materials	Quantity	Use
Milkfish Bones Powder	600 mg	Active Compound
MCC 101	1.5 mg	Disintegrant
Aerosil	21 mg	Glidant
Talc	14 mg	Lubricant
Magnesium stearate	7 mg	Lubricant
Amylum maydis	56.5 mg	Filler
Capsule Number 00	Capsule weight 150 mg	

2.3. Capsule supplement from milkfish bones (*Chanos chanos*) production

Open the capsule and insert the capsule body into the hole in the capsule filling holder. Sprinkle and spread the milkfish bone powders into capsules with a spatula. Close the capsule by pressing the capsule body and capsule lid together. Put supplement capsules into primary and secondary packaging (Nugroho & Swanjaya, 2020).

2.4. Evaluation of capsule

2.4.1. Calcium content

Determine the amount of calcium content using the PerkinElmer AAnalyst 400 Atomic Absorption Spectrophotometer (Waltham, United States of America). The instrument will

evaporate and decompose the sample into the atomic gas. Look at the absorbance value and the appearance of a brick-red color due to the presence of calcium ions (Azis et al., 2018).

2.4.2. Protein content

Determine the amount of protein content using the Kjeldahl method in 3 stages: destruction, distillation, and titration (Purnama & Pakerti, 2022).

2.4.3. Ninhydrin test

Perform the test by preparing a sample solution of as much as 1 mL and a Ninhydrin solution from Merck (Darmstadt, Germany) of as much as 5 mL. Heat the mixture to 100^{o}C for 15 minutes. The test will have positive results if the solution changes color to blue-purple (Panjaitan et al., 2023).

2.4.4. Disintegration time

Determine the capsule disintegration time by running the VK100 Automated Disintegration Apparatus (California, United States of America) for 30 minutes, lift the basket, and observe all the capsules. The instrument will destroy the entire capsule except for part of the capsule shell (USP, 2023).

2.4.5. Flow properties

Determine the flow properties by using the Granule Flow Tester AMS-G1. Calculate the angle of repose (USP, 2023).

2.4.6. Capsule weight uniformity

Determine weight uniformity by weighing the capsule and capsule shell. The process of weighing capsules and capsule shells using Mettler Toledo Standard Level analytical balance. Calculate the difference between the capsule shell weight and the capsule weight (USP, 2023).

3. RESULTS AND DISCUSSION

3.1. Formulation of capsule supplement from milkfish bones (*Chanos chanos*)

The formulation of supplement capsules from milkfish bones consists of milkfish bone powder, MCC 101, Aerosil, Talc, Magnesium stearate, and *Amylum maydis*. Microcrystalline cellulose with formulations made from disintegrants and lubricants can complement each other to improve disintegration ability. Microcrystalline cellulose has good flow properties and a low compressibility index. Microcrystalline cellulose also has advantages such as broad compatibility when mixed with other excipients, being inert, easy to handle, and easy to obtain (Silalahi & Husni, 2018). Aerosil has powerful water absorption properties, as much as 50% of the water content of the dried sample without losing its good flow properties, and can be used as an adsorbent because it can protect the properties of the material used and increase homogeneity (Rina et al., 2023). The lubricant used in this capsule formulation is talc, which aims to reduce friction on the powder and prevent the powder from sticking together, making it easier to put the powder into the capsule (Wahyuni et al., 2023). Metal stearate is the most

efficient and commonly used lubricant. In general, this lubricant is not reactive. The most widely used metal stearate is magnesium. The hydrophobic nature of magnesium stearate will create a film coating on solid material particles to reduce friction between the particles and make it easier for them to flow (Puspadina et al., 2021). *Amylum maydis* is used as a filler because corn starch can accelerate the disintegration time of the capsules. This effect is attributed to the amylose contained in *Amylum maydis*, which absorbs water and facilitates starch swelling, thereby promoting capsule breakdown and disintegration (Kuswanti et al., 2023).

3.2. The characteristics of milkfish bone powder (*Chanos chanos*)

3.2.1. Qualitative analysis of proteins using ninhydrin test

The Ninhydrin test is a qualitative method used to detect the presence of free amino acids in a sample. In this test, Ninhydrin acts as an oxidizer, that induces oxidative decarboxylation of α amino acids, producing CO_2, NH_3, and aldehydes with shorter carbon chains than the original amino acid. The reduced form of Ninhydrin subsequently reacts with ammonia (NH_3) to form a complex that produces a characteristics blue-purple color (Prastika et al*.,* 2019). This test was carried out to determine the alpha amino acid content (Dwiningrum et al., 2023). Alpha amino acids are organic molecules with an amino group in the α position relative to carboxylic groups such as alanine, phenylalanine, and tyrosine (Zhao & Lu, 2014). Alpha amino acids are essential amino acids, a type of nutrient that the body cannot produce but play a vital role in children's growth and development. Stunting can occur due to a lack of essential amino acids (Wahyudi et al*.,* 2024). The results of the Ninhydrin test from milkfish bone powder are shown in Table 2.

Table *2.* Qualitative protein analysis results of milkfish bone powder using ninhydrin test. *Description*: + = positive (color changes to purple); - = negative (no color change).

No.	Sample	(+/-)
1.	Milkfish bone powder 1	+
2.	Milkfish bone powder 2	+
3.	Milkfish bone powder 3	+

Qualitative analysis of the protein content in milkfish bone powder using the Ninhydrin test in triplicate showed positive results; namely, the color changed to blue-purple. These results indicate the presence of alpha amino acids in milkfish bone powder, which can help fulfill protein nutrition for stunted toddlers (Jeong et al., 2023).

3.2.2 Calcium content

The results of determining the calcium content in milkfish bone powder carried out using the Atomic Absorption Spectroscopy (AAS) method in triplicate showed that 600 mg of milkfish bone powder are shown in Table 3. The average obtained is equivalent to 132.48 mg of calcium in 600 mg of milkfish bone powder. The research results show that the calcium

contents contained in 600 mg of milkfish bones are very high and can help fulfill nutritional requirements for stunted toddlers.

Table 3. Calcium content analysis results of milkfish bone powder using atomic absorption spectroscopy.

No.	Sample	Calcium Content (%)	Average (%)
1.	Milkfish bone powder 1	22.38	
2.	Milkfish bone powder 2	21.77	22.08
3.	Milkfish bone powder 3	22.09	

3.2.3 Protein content

The results of determining protein content in milkfish bone powder carried out using the Kjeldahl method in triplicate showed that 600 mg of milkfish bone powder are shown in Table 4. The average obtained is 44.16 mg in 600 mg of milkfish bone powder. The research results show that the protein content in 600 mg of milkfish bones is lower than that of calcium. High enough temperatures can cause protein denaturation (Setiani et al., 2021). So, the lower protein content can be caused by the protein being denatured during the heating process.

Table 4. Protein content analysis results of milkfish bone powder using kjeldahl method.

No.	Sample	Protein Content (%)	Average (%)
1.	Milkfish bone powder 1	7.28	
2.	Milkfish bone powder 2	7.06	7.36
3.	Milkfish bone powder 3	7.74	

3.3. Capsule evaluation

3.3.1. Capsule disintegration time

Table 5 shows the disintegration time results of the milkfish bone capsule supplements using a disintegration tester. This result meets the requirements: In less than 30 minutes, the entire capsule, except for the capsule shell, has been destroyed (USP, 2023).

Table 5. Disintegration time test results of capsule supplement.

No.	Sample	Disintegration Time
1.	Capsule 1	29 minutes
2.	Capsule 2	29 minutes
3.	Capsule 3	29 minutes
4.	Capsule 4	29 minutes
5.	Capsule 5	29 minutes
6.	Capsule 6	29 minutes

3.3.2. Powder flow properties

The results of testing the flow properties of milkfish bone powder using a flow tester are shown in Table 6. The angle of repose ≤30° indicates excellent flow properties (Andriani et al., 2023).

Table 6. Powder flow properties test results of milkfish bone powder.

Diameter (cm)	Height (cm)	Angle of Repose (°)	Flow Properties
8.9	1.5	$tan^{-1} = \frac{1{,}5\ cm}{0.5(8.9\ cm)}$ $\theta \approx 18.63^{o}$	Excellent

3.3.3. Capsule weight uniformity

The results obtained from all 20 capsules are shown in Table 7. These results show that all 20 capsules were uniform because not a single capsule had a deviation in weight. If weighed one by one, there should be no more than two capsules whose weight each deviates from the average weight specified in column A (5%) and none of the capsules whose weight deviates from the average weight specified in column B (10%). Columns A and B are 5% and 10% because the capsule weighs> 300 mg (USP, 2023).

Table 7. Weight uniformity test result of capsule supplement.

No.	Weight (mg)	No.	Weight (mg)
1.	732	11.	711
2.	732	12.	717
3.	714	13.	751
4.	739	14.	747
5.	735	15.	755
6.	742	16.	756
7.	734	17.	749
8.	735	18.	737
9.	714	19.	747
10.	714	20.	755
Average (mg)	735.8		
Upper limit 5% (mg)	772.59	Upper limit 10% (mg)	809.38
Lower limit 5% (mg)	669.01	Lower limit 10% (mg)	662.22

3.4. Dosage for capsule supplement

Stunting will impact children. Cognitive, motoric, and verbal development will not occur optimally, the immune system will decline, and the risk of obesity and degenerative diseases such as diabetes, stroke, cancer, heart disease, and disability in old age will increase, which will also impact increasing health costs and the potential for death (Simamora et al., 2023).

Low calcium intake could make growth unoptimal. Children with inadequate calcium intake will experience less than optimal growth, including height. Children who are calcium deficient will usually be shorter than children whose calcium needs are appropriately met. Suffering from bone disorders and calcium and vitamin D deficiencies also impact low calcium intake. These deficiencies could cause rachitis. This disease is characterized by soft and brittle bone texture. In addition, the child's growth will be hampered, and muscle pain or weakness may occur. Low calcium intake could increase the risk of osteoporosis in old age. Children whose calcium needs are unmet are more at risk of experiencing bone fractures. The possibility

of experiencing osteoporosis in old age will also increase. Apart from that, calcium deficiency in childhood can also cause osteoporosis in children (Heshmati et al., 2018).

Children's growth does not meet their potential, or stunting in children is caused by a lack of consumption of protein used as a burning agent, so muscles become soft, and hair falls out quickly (USDA, 2015). Proteins also function in an immune protection role. Antibodies are particular and sensitive proteins that can be recognized with foreign objects such as viruses, bacteria, and cells from other organisms. Amino acids are chemical compounds with carboxyl and amino groups and certain side chains. They are categorized as essential and non-essential. Certain serum amino acid levels in stunted children are notably lower than those in non-stunted children, according to several studies. Enough energy intake and high-quality protein are two things that help kids grow linearly (Morris et al., 2022; Endrinikapoulos et al., 2023).

Drugs are rarely administered in their original pure state. They are converted into suitable formulations called dosage forms. Every dosage form is a combination of the drug and other non-drug components. Desirable dosage form properties should be convenient to handle in use and store; stable during storage and use; withstand mechanical shock during transport; flexible in different drug strengths; provide expected therapeutic effect; predictable extent, drug release, onset, intensity, duration of action. In designing supplement dosage, the disintegration and release time of a tablet, capsule, or liquid gel capsule's contents into gastrointestinal fluids is the subject of dosage form performance research. In general, dosage forms with superior performance features have better bioavailability than those with slower disintegration times and easier release of their active ingredients. This procedure is an in vitro method that uses standardized settings and apparatus to measure the rate and amount of phytochemical release into intestinal or gastric fluids. The makeup of capsules, such as gelatin or cellulose, can also affect how well a dose form works (Floyd et al., 2022).

The proportion of food energy likely to be fortified with calcium may be much lower than for some other micronutrients, and the levels of added calcium in foods appear modest. These results imply that because there is no account for expected consumption patterns, models for calculating the maximum acceptable amounts of micronutrients added to meals and supplements may be conservative for calcium. Additionally, it stresses the importance of looking into supplements and fortified food consumption habits nutrient by nutrient (Bourassa et al., 2022).

Due to differences in the amount of calcium and protein needed to fulfill the ideal nutritional needs of stunted toddlers, it is necessary to adjust the dose based on the toddler's age. Based on the literature, the daily calcium requirement for toddlers is 500 mg, but the daily intake of food containing calcium per capita is only 234.46 mg (Valentina et al., 2014). Meanwhile, protein requirements for toddlers are 25-39 mg/day (Fuada & Hidayat, 2015). However, based on research, the protein needs of toddlers are mostly met (Diniyyah & Nindya, 2017). Therefore, calculating capsule dosage requirements for toddlers is more focused on toddlers' needs for calcium (Table 8).

Table 8. Dose requirement data for capsule consumption in toddlers.

No.	Toddler Age	Dosage per Day	Calcium content	Protein Content
1.	1-3 Years	2 × 1 capsule/day	22.08% × 600 mg × 2 capsule = 264.96 mg	7.36% × 600 mg × 2 capsule = 88.32 mg
2.	4-8 Years	3 × 1 capsule/day	22.08% × 600 mg × 3 capsule = 397.44 mg	7.36% × 600 mg × 2 capsule = 132.48 mg

The dosage is determined based on the daily calcium needs of toddlers, which is 700 mg for children aged 1-3 years and 1000 mg for children aged 4-8 years (Fuada and Hidayat, 2015; Valentina et al., 2014). The calculation of the consumption capsule supplement dose uses the percentage of calcium and protein content determined by AAS and Kjeldahl, which is then multiplied by the total weight of the capsule. The capsule consumption frequency is determined to fulfill the calcium requirement.

4. CONCLUSION

The research results show that calcium-protein capsule supplements derived from milkfish bones contain sufficient calcium and protein to help nourish stunted toddlers, thereby reducing the prevalence of stunting in Indonesia. This discovery has the potential to open a breakthrough in launching products for treating stunting in toddlers with the formulation and characteristics of capsule supplements that toddlers can consume.

ACKNOWLEDGMENT

We want to thank Direktorat Jenderal Pendidikan Tinggi, Riset, and dan Teknologi for their help throughout the writing of this article by funding the research. This study was supported by the Faculty of Pharmacy, Universitas Padjadjaran, Indonesia.

CONFLICT OF INTEREST

All authors declared that there was no conflict of interest.

REFERENCES

Andriani, R., Mahmudah, R., Nuralifah, Jannah, S. R. N., Sida, N. A., Hikmah, N., Trinovitasari, N., & Wulandari, W. P. (2023). Formulasi dan Evaluasi Sediaan Granul Antidiabetes Menggunakan Ekstrak Daun Jati (*Tectona grandiss Linn* F.) sebagai Zat Aktif. *Jurnal Mandala Pharmacon Indonesia (JMPI)*, 9(2), 484–491. https://doi.org/10.35311/jmpi.v9i2.410

Azis, M. Y., Putri, T. R., Aprilia, F. R., Ayuliasari, Y., Hartini, O. A. D., & Putra, M. R. (2018). Eksplorasi Kadar Kalsium (Ca) dalam Limbah Cangkang Kulit Telur Bebek dan Burung Puyuh menggunakan Metode Titrasi dan AAS. *al-Kimiya*, 5(2), 74–77. https://doi.org/10.15575/ak.v5i2.3834

Bourassa, M. W., Abrams, S. A., Belizán, J. M., Boy, E., Cormick, G., Quijano, C. D., Gibson, S., Gomes, F., Hofmeyr, G. J., Humphrey, J., Kraemer, K., Lividini, K., Neufeld, L. M., Palacios, C., Shlisky, J., Thankachan, P., Villalpando, S., & Weaver, C. M. (2022). Interventions to Improve Calcium Intake through Foods in Populations with Low Intake. *Annals of the New York Academy of Sciences*, 1511(1), 40–58. https://doi.org/10.1111%2Fnyas.14743

Bu, S. Y., Choi, M. J., Choi, D. S., Jung, Y. M., Jang, I. S., Yang, N., Kim, K., & Park, C. Y. (2022). Perspectives on the Systematic Review for the 2020 Dietary Reference Intakes for Koreans for Calcium. *Nutrition Research and Practice*, 16(1), 89–112. https://doi.org/10.4162/nrp.2022.16.s1.s89

Deran, M. Y. K., Tjendanawangi, A., & Dahoklory, N. (2023). Efektivitas Substitusi Tepung Ikan (*Brevoortia tyrannus*) dengan Tepung Ampas Kelapa (*Cocos nucifera* L.) terhadap Pertumbuhan dan Kelulushidupan Ikan Bandeng (*Chanos chanos*). *Jurnal Vokasi Ilmu-ilmu Perikanan (JVIP)*, 3(2), 147–153. http://dx.doi.org/10.35726/jvip.v3i2.6083

Diniyyah, S. R. & Nindya, T. S. (2017). Asupan Energi, Protein dan Lemak dengan Kejadian Gizi Kurang pada Balita Usia 24-59 Bulan di Desa Suci, Gresik. *Amerta Nutrition*, 1(4), 341-350. https://doi.org/10.20473/amnt.v1i4.2017.341-350

Domili, R. S., & Pertiwi, S. F. (2021). Pemanfaatan Tepung Ampas Kelapa dan Tulang Ikan Sebagai Bahan Baku Pembuatan Cookies untuk Pencegahan Stunting Pada Ibu Hamil. *Jurnal Ilmu Kelautan Kepulauan*, 4(2).

Dwiningrum, R., Pisacha, I. M., & Nursoleha, E. (2023). Analisis Kualitatif dan Kuantitatif Kandungan Protein pada Olahan Bahan Pangan. *Journal Pharmacy Aisyah*, 2(2), 60-67.

Endrinikapoulos, A., Afifah, D. N., Mexitalia, M., Andoyo, R., Hatimah, I., & Nuryanto, N. (2023). Study of the Importance of Protein Needs for Catch-up Growth in Indonesian Stunted Children: A Narrative Review. *SAGE Open Medicine,* 11, 1–9. https://doi.org/10.1177%2F20503121231165562

Esma, G. T., Milloren, S. K. L., Muti, M. B., Nemeno, N. B., & Tibe, A. O. (2023). Efficacy of Milkfish (*Chanos chanos*) Bones as a Liquid Fertilizer on Plant Growth of Lettuce (*Lactuca sativa*). *International Journal of Research Publication and Reviews*, 4(3), 334–344. https://doi.org/10.55248/gengpi.2023.31469

Floyd, Z. E., Ribnicky, D. M., Raskin, I., Hsia, D. S., Rood, J. C., & Gurley, B. J. (2022). Designing a Clinical Study With Dietary Supplements: It is All in the Details. *Frontiers in Nutrition,* 8, 1–7. https://doi.org/10.3389/fnut.2021.779486

Fuada, N. & Hidayat, T. S. (2015). Kecukupan Energi Protein pada Anak-anak (24-59 bulan) di Indonesia. *Jurnal Litbang Provinsi Jawa Tengah*, 13(1), 83–92. https://doi.org/10.36762/jurnaljateng.v13i1.385

Gullapalli, R. P. & Mazzitelli, C. L. (2017). *Gelatin and Non-Gelatin Capsule Dosage Forms*. Amsterdam: Elsevier Inc.

Heshmati, J., Sepidarkish, M., Namazi, N., Shokri, F., Yavari, M., Fazelian, S., Khorshidi, M., & Shidfar, F. (2018). Impact of Dietary Calcium Supplement on Circulating Lipoprotein Concentrations and Atherogenic Indices in Overweight and Obese Individuals: A Systematic Review. *Journal of Dietary Supplements,* 16(3), 357–367. https://doi.org/10.1080/19390211.2018.1440685

Imra., Akhmadi, M. F., Abdiani, I. M., & Irawati, H. (2019). Karakteristik Tepung Tulang Ikan Bandeng (*Chanos chanos*) dari Limbah Industri Baduri Kota Tarakan. *Jurnal Techno-fish*, 3(2), 60-69.

Jeong, S., Jeon, Y., Mun, J., Jeong, S. M., Liang, H., Chung, K., Yi, P. I., An, B. S., & Seo, S. (2023). Ninhydrin Loaded Microcapsules for Detection of Natural Free Amino Acid. *Chemosensors*, 11(49), 1–7. https://doi.org/10.3390/chemosensors11010049

Kuswanti, A., Yuniarsih, N., Anita, Nurulhadi, Z. F., Azzahra, D. F., Yuniar, R. R., & Pandiangan, T. (2023). Literature Review Artikel: Pengaruh Beberapa Jenis Amilum Sebagai Bahan Pengikat terhadap Disintegrasi Tablet Parasetamol. *Jurnal Ilmiah Wahana Pendidikan,* 9(16), 608–615. https://doi.org/10.5281/zenodo.8260417

Lembaga Penerbit Badan Penelitian dan Pengembangan Kesehatan (LPB). (2018). *Laporan Nasional Riskesdas 2018*. Jakarta: Lembaga Penerbit Badan Penelitian dan Pengembangan Kesehatan.

Morris, R., Black, K. A., & Stollar, E. J. (2022). Uncovering Protein Function: From Classification to Complexes. *Essays Biochem,* 66(3), 255–285. https://doi.org/10.1042/EBC20200108

Nugroho, B. A. & Swanjaya, D. (2020). *CNC Modeling for Auto Capsule Filler: Seminar Nasional Inovasi Teknologi* (pp. 115-123). Kediri: Universitas Nusantara PGRI.

Nuurrahmawati, D., Hamim, N., & Hanifah, I. (2023). Hubungan Kualitas Konsumsi Makanan Dengan Kejadian Stunting di Desa Glundengan Kecamatan Wuluhan Kabupaten Jember: Hubungan Kualitas Konsumsi Makanan dengan Kejadian Stunting di Desa Glundengan Kecamatan Wuluhan Kabupaten Jember. *Jurnal Sintesis: Penelitian Sains, Terapan dan Analisisnya*. 4(2), 87-96. https://doi.org/10.56399/jst.v4i2.98

Panjaitan, R. S., Djohansah, V., Septiyani, A., Ardian, K. D., & Asriyanti, L. S. (2023). Qualitative and Quantitative Identification of Carbohydrate and Protein Content in Packaged Chocolate Beverages. *Indonesian Journal of Pharmaceutical Research*, 3(1), 9–19. https://doi.org/10.31869/ijpr.v3i1.4572

Prastika, H. H., Ratnayani, K., Puspawati, N. M., & Laksmiwati, A. A. I. A. M. (2019). Penggunaan Enzim Pepsin untuk Produksi Hidrolisat Protein Kacang Gude (*Cajanus cajan* (L.) Millsp.) yang Aktif Antioksidan. *Cakra Kimia*, 7(2), 180–188.

Purnama, R. C. & Pakerti, A. L. (2022). Analisis Kadar Protein pada Tepung Jagung (*Zea may*s L.) yang Dibeli dengan Merek L di daerah Pasar Semuli Jaya Lampung Utara dengan menggunakan Metode Kjeldahl. *Jurnal Analis Farmasi,* 7(2), 119–129. https://doi.org/10.33024/jaf.v7i2.8186

Puspadina, V., Legowo, D. B., Fitriany, E., Priyoherianto, A., & Damayanti, W. (2021). Effect of Variation of Lubricant Concentration (Magnesium Stearate) on the Physical Quality of Metoclopramid HCl Tablets With Direct Printing Method. *Indonesian Journal of Pharmaceutical Education,* 1(2), 67–75. https://doi.org/10.37311/ijpe.v1i2.10567

Rina, D. E., Samudra, A. G., & Dominica, D. (2023). Formulasi Tablet Ekstrak Alga Coklat (*Sargassum sp*.) dengan Variasi Polivinil Pirolidon Sebagai Bahan Pengikat Metode Granulasi Basah: Formulation of Brown Algae Extract Tablets (*Sargassum sp*.) with Variations Polyvinyl Pyrolidone as Binding Material with Wet Granulation Method. *Medical Sains: Jurnal Ilmiah Kefarmasian*, 8(1), 65–76. https://doi.org/10.37874/ms.v8i1.590

Setiani, B. E., Bintoro, V. P., & Fauzi, R. F. (2021). Pengaruh Penambahan Sari Jeruk Nipis (*Citrus aurantifolia*) sebagai Bahan Penggumpal Alami terhadap Karakteristik Fisik dan Kimia Tahu Kacang Hijau (*Vigna radiata*). *Jurnal Teknologi Pangan dan Hasil Pertanian*, 16(1), 1–16. http://dx.doi.org/10.26623/jtphp.v16i1.4401

Silalahi, K. & Husni, P. (2018). Review: Aplikasi Mikrokristalin Selulosa Dalam Farmasetik. *Farmaka*, 16(1), 380–388. https://doi.org/10.24198/jf.v16i1.17509

Simamora, M., Sipayung, R., Sinaga, J., & Sutrisna, A. A. (2023). Kejadian Stunting dengan Kemampuan Kognitif Anak Usia Sekolah. *Jurnal Online Keperawatan Indonesia*, 6(1), 29–36. https://doi.org/10.51544/keperawatan.v6i1.4304

UNICEF Indonesia. (2021). Towards a Future in Indonesia Without Child Undernutrition. Available from: https://www.unicef.org/indonesia/media/20436/file/Towards%20a%20future%20in%20Indonesia%20without%20child%20undernutrition.pdf [Accessed 13th February 2025].

United States Department of Agriculture (USDA). (2015). *Dietary Guidelines for Americans 2015-2020 8th Edition*. Washington D.C.: United States Department of Agriculture.

United States Pharmacopeia. (2023). *The United States Pharmacopeia, USP 46/The National Formulary, NF 41*. Rockville, Pharmacopeial Convention Inc.

Valentina, V., Palupi, N. S., & Andarwulan, N. (2014). Asupan Kalsium dan Vitamin D pada Anak Indonesia Usia 2-12 Tahun. *Jurnal Teknologi dan Industri Pangan*, 25(1), 83–89. http://dx.doi.org/10.6066/jtip.2014.25.1.83

Wahyudi, D., Nurbaiti, L., & Buanayuda, G. W. (2024). Hubungan Jumlah Asupan Asam Amino Esensial Balita Stunting dan Tidak Stunting di Lokus Stunting Kabupaten Lombok Utara. *Jurnal Medika Hutama*, 5(2), 3879–3894.

Wahyuni, A., Shafira, K., Rizki, M., Lavly, N., Ilham, R.N., Vivia, Y., & Yuniarsih, N. (2023). Review Artikel: Analisis Formulasi dan Evaluasi dalam Penggunaan Variasi Bahan Tambahan pada Lozenges (Tablet Hisap). *Innovative: Journal Of Social Science Research*. 3(2), 9017–9029. https://doi.org/10.31004/innovative.v3i2.1472

Zhao, L. & Lu, W. (2014). Mirror Image Proteins. *Current Opinion in Chemical Biology*, 22, 56–61. https://doi.org/10.1016/j.cbpa.2014.09.019

JPSCR: Journal of Pharmaceutical Science and Clinical Research
Volume 10, Issue 1, Pages 50-59, March 2025
https://doi.org/ 10.20961/jpscr.v10i1.87321

ISSN 2503-331X (Online)

Cost Analysis of Inpatient Heart Failure Treatment in Jogja Hospital Based On INA-CBG's Tariffs In 2023

Ingenida Hadning* and Ahadijatul Kubra

Department of Pharmacy, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Yogyakarta, Indonesia

*Corresponding author: ingenida.hadning@umy.ac.id

Received: May 30, 2024; **Accepted:** March 3, 2025; **Published:** April 28, 2025

Abstract

Heart failure is a catastrophic disease that requires long-term care, resulting in high costs. This study is the first to evaluate the INA-CBG's tariff based on the Minister of Health Regulation Number 3 of 2023 for treating inpatient heart failure. This study aims to determine the difference in the actual costs of inpatient heart failure treatment in Jogja Hospital with INA-CBG's tariff based on Permenkes Number 3 of 2023. This study is an observational study with a cross-sectional research design and retrospective data collection of medical record data and patient cost data in 2023. Data were analyzed using descriptive analysis methods and a one sample t-test. Data were obtained from 104 patients. The results of this study found that the average real cost for the treatment of inpatient heart failure with code I-4-12-I class 1 is IDR10.302.093 ± Rp5.749.499, class 2 is IDR IDR4.184.959 ± Rp681.730, and class 3 is IDR5.123.572 ± Rp2.242.239. The average real cost for the treatment of inpatient heart failure with code I-4-12-II class 1 is IDR9.764.750 ± Rp5.208.499; class 2 is IDR6.734.834 ± Rp3.82.345, and class 3 is IDR8.549.362 ± Rp4.288.906. The average real cost for treating inpatient heart failure with code I-4-12-III class 1 is IDR6.073.828; class 2 is IDR16.885.496, and class 3 is IDR14.326.130±Rp7.565.598. The average real cost was higher than the INA-CBG's tariff, with a significant difference, so the hospital suffered a loss.

Keywords: Cost analysis; Heart failure; INA-CBG's tariff 2023; Real cost

1. INTRODUCTION

According to data obtained by the World Health Organization (WHO) states that in the world, 20 million people die per year due to cardiovascular disorders (Yunita et al., 2020). Based on data obtained (Lilik & Budiono, 2021), the incidence rate of heart disease in Indonesia is increasing from year to year with a prevalence of 1.5% or an estimated 1,017,290 people (Yunita et al., 2020).

One of the causes of heart failure is included in catastrophic disease, apart from being able to cause complications that cause re-hospitalization, also because it requires more serious treatment, which serves to reduce mortality and morbidity. So, treating patients with heart failure is considered the most expensive (Astuti et al., 2021). Factors affecting the cost of care for heart failure are found in long-term treatment patterns and the length of stay (Wulandari et al., 2015).

Since 2008, Indonesia has paid hospitals serving the Jamkesmas program with a prospective payment system, the Diagnosis Related Group System (DRG system), which aims to control costs and maintain service quality. This system continued in 2014 until now through the JKN program under the name Indonesia Case Based Groups (INA-CBG's), where BPJS Health will pay hospitals based on a predetermined amount by the diagnosis of the disease in the patient, along with the actions and drugs that will be given or used with the INA- CBG's system, which is explained in Permenkes Number 3 of 2023 (Kemenkes, 2014; Kemenkes, 2023). In its application of the BPJS Health system, there are still many incidents that show a mismatch between the INA-CBG's tariff and the actual costs, especially in the treatment of catastrophic diseases (diseases that require long-term medical care at a high cost) such as heart failure, stroke, cancer, and also kidney failure (Amalia, 2020).

This study is essential considering the high number of cases and the high cost of treatment for heart failure. This study is the first to evaluate the tariff using INA-CBG's in 2023 and wants to see the hospital's improvements with the new tariff. Previously, a cost analysis study was conducted (Rahman, 2017) at Jogja Hospital, which compared the average actual costs with the INA-CBG's tariff for 2014. The results showed that, based on the difference between direct costs and INA-CBG's rates, Jogja Hospital did not lose money and had established health services by government regulations. After confirming the new INA-CBG tariff in 2023, further research is needed to determine the improvements made by the hospital with the new tariff. This study aimed to determine the difference between the actual costs of inpatient heart failure treatment in Jogja Hospital and the INA-CBG's tariff in 2023. The benefits expected from this research are evaluating hospital services and new tariffs. This study was conducted at Jogja Hospital and compared previous studies that used the INA-CBG's tariff in 2014 with the latest tariff set by the government, namely the INA-CBG's tariff in 2023. Jogja Hospital is one of the government-owned type B regional public hospitals. In addition, Jogja Hospital has set the INA-CBG's tariff in 2023 and has received full accreditation (five stars) from the Hospital Accreditation Commission (KARS).

2. MATERIAL AND METHODS

The study employed an observational design with a cross-sectional approach, approve by the Jogja Hospital Ethics Committee (No. 54/KEPK/RSUD/IX/2023). Data were collected retrospectively by reviewing medical records and treatment costs of heart failure patients from February to September 2023. The sampling method was total sampling, with inclusion and exclusion criteria. Inclusion criteria included heart failure patients admitted to classes 1, 2, and 3 of the JKN program at Jogja Hospital who were diagnosed with mild heart failure, moderate heart failure, and severe heart failure included in INA-CBG's codes I-4-12-I, I-4-12-II, and I-4-12-III, heart failure patients with complete payment data for cost calculation of the exact cost component, and heart failure patients with medical records that could complete patient data. Exclusion criteria were patients who died during the hospitalization period, forced or self-discharged patients, patients who changed treatment classes, and patients who were referred to other hospitals.

The instruments used in this study are cost data, medical record data, patient data recording form, and the INA-CBG's rates based on the Minister of Health Regulation Number 3 of 2023 for heart failure disease. Cost data is the cost of treatment for heart failure patients during inpatient treatment in classes I, II, and III taken from the finance department at Jogja Hospital. The calculated costs are direct medical costs, which include room costs, nursing, laboratory, radiology, drugs and medical equipment costs, doctor visits, support, non-surgical procedures, surgical procedures, and blood services. The data collected from MR were data of heart failure patients in Jogja hospital who underwent hospitalization in class I, II, and III with mild, moderate, and severe severity with INA-CBG's code I-4-12-I, I-4-12-II, I-4-12-III. Patient data recorded included patient characteristics, primary and comorbid diagnoses, and treatment data.

Descriptive statistical analysis was used to describe the average actual cost of treatment for heart failure patients participating in JKN. Descriptive analysis was used to determine the suitability of the average actual cost of treating JKN heart failure patients in the inpatient pharmacy installation of Jogja Hospital with INA-CBG's rates based on the Minister of Health Regulation Number 3 of 2023. The data is appropriate if the average actual cost is less than or equal to the INA-CBG's tariff. To analyze the difference between the average actual cost of treatment for heart failure patients with JKN participants and the INA-CBG's tariff based on the Minister of Health Regulation Number 3 of 2023 at the inpatient pharmacy installation of the Jogja Hospital using the one-sample t-test and Wilcoxon test.

3. RESULTS AND DISCUSSION

3.1. Characteristics of heart failure patients at Jogja Hospital

From February to September 2023, 104 patients with heart failure at Jogja Hospital met the inclusion criteria. The characteristics of heart failure patients at Jogja Hospital are shown in Table 1.

Table 1. Characteristics of patients with heart failure hospitalized at Jogja Hospital in 2023.

Characteristic	Number of Patients (N=104)	Percentage (%)
Sex		
Male	58	55,77
Female	46	44,23
Age		
39-45	6	5,77
46-56	28	26,92
57-70	51	49,04
>70	19	18,27

Based on the results of the study, the incidence of heart failure is more common in male patients. The high incidence of heart failure in men is caused by risk factors such as a history of smoking and an unhealthy lifestyle and the presence of the hormone estrogen in women, which is thought to have an effect in preventing the incidence of cardiovascular disorders by reducing oxidative stress (Astuti Purnamawati et al., 2018).

In addition, in this study, patients who experienced heart failure were most prevalent at the age of> 56 years. The increasing age increases the risk of a person developing heart failure disease due to weakening heart function. With age, the time it takes for fat to accumulate in the arterial wall increases. The accumulation of which continues over time can cause heart disease and stroke (Putri, 2021).

Comorbidities often accompany heart failure, the process of which is the main which is the triggering factor for the primary diagnosis. These comorbidities can be interpreted as conditions outside the primary diagnosis, such as trigger factors and complications that need to be treated to not worsen the condition of heart failure (Sulistiyowatiningsih et al., 2016). Comorbid factors of heart failure patients at Jogja Hospital are shown in Table 2.

Table 2. Distribution of comorbidities among heart failure patients at Jogja Hospital in 2023.

Types of Comorbid	Total (N=342)	Percentage
Impaired Heart Function	119	34,80%
Impaired Kidney Function	38	11,11%
Diabetes Mellitus	38	11,11%
Impaired Pulmonary Function	35	10,23%
Dyslipidemia and Hyperuricemia	24	7,02%
Hypertension	21	6,14%
Infections	20	5,85%
Indigestion	15	4,39%
Anemia	13	3,80%
Skin disorders	7	2,05%
Stroke	4	1,17%
Without comorbidities	4	1,17%
Osteoarthritis	2	0,58%
Liver disorders	1	0,29%
Brain disorders	1	0,29%

This study shows that the most common comorbidities in heart failure patients are heart function disorders, kidney function disorders, and diabetes mellitus. Impaired renal function in heart failure patients can lead to more severe complications compared to those without renal impairment. Patients with both heart failure and impaired renal function often present with additional comorbidities such as hypertension, diabetes, and heart disease, all of which require appropriate management to prevent exacerbation of heart failure symptoms (Sulistiyowatiningsih et al., 2016).

3.2. Treatment cost analysis

This study categorized heart failure patients based on severity and treatment class to determine the average actual cost of each class. The data were grouped according to INA-CBG's code. Table 3 summarizes the average cost components of heart failure treatment. Table 3 shows several components of the highest costs in Jogja Hospital: medicine, room, and electromedical (echocardiography and electrocardiography).

Table 3. Actual cost components of heart failure patients hospitalized at Jogja Hospital in 2023.

Cost Variable (IDR)	Average Cost																	
	Class 1						Class 2						Class 3					
	Mean (IDR)	%	Mean (IDR)	%	Mean (IDR)	%	Mean (IDR)	%	Mean (IDR)	%	Mean (IDR)	%	Mean (IDR)	%	Mean (IDR)	%	Mean (IDR)	%
	I-4-12-I	N (5)	I-4-12-II	N (12)	I-4-12-III	N (1)	I-4-12-I	N (4)	I-4-12-II	N (9)	I-4-12-III	N (1)	I-4-12-I	N (28)	I-4-12-II	N (27)	I-4-12-III	N (17)
Room	2.354.000	22,8	2.193.583	22,4	1.145.000	18,8	661.250	15,8	1.225.000	18,1	2.605.000	15,4	783.107	15,2	1.130.370	13,2	1.980.764	13,8
Nursing	846.200	8,21	840.125	8,60	353.500	5,82	391.625	9,36	713.722	10,6	1.391.000	8,24	461.392	9,01	667.870	7,81	1.248.088	8,71
Laboratory	736.100	7,15	797.625	8,17	576.000	9,48	608.375	14,5	733.111	10,8	1.242.000	7,36	480.410	9,38	885.277	10,3	1.246.264	8,70
Radiology	495.000	4,80	184.583	1,89	1.430.000	23,5	146.250	3,49	195.555	2,90	2.150.000	12,7	175.910	3,43	350.740	4,10	621.764	4,34
Medicine	2.378.953	23,0	2.019.850	20,6	507.025	8,35	605.627	14,4	1.356.256	20,1	4.168.969	24,6	903.398	17,6	1.941.270	22,7	3.486.271	24,3
Visit fee	823.900	8,00	699.125	7,16	448.000	7,38	285.750	6,83	377.055	5,60	1.015.000	6,01	404.316	7,89	625.555	7,32	965.294	6,74
Electromedic	1.517.000	14,7	1.044.291	10,6	650.000	10,7	800.000	19,1	1.007.222	14,9	2.080.000	12,3	933.428	18,2	1.372.277	16,0	2.240.352	15,6
Non-surgical procedure	992.340	9,63	1.317.816	13,5	711.800	11,7	512.582	12,2	848.577	12,6	1.742.800	10,3	740.836	14,4	1.051.832	12,3	1.963.799	13,7
Surgical procedure	158.600	1,54	204.750	2,10	252.500	4,16	173.500	4,15	278.333	4,13	491.000	2,91	266.485	5,20	265.314	3,10	421.764	2,94
Blood service	-	-	463.000	4,74	-	-	-	-	-	-	-	-	9.017	0,18	258.851	3,03	151.764	1,06
Total Cost	**10.302.093**	**100**	**9.764.750**	**100**	**6.073.825**	**100**	**4.184.959**	**100**	**6.734.834**	**100**	**16.885.496**	**100**	**5.123.572**	**100**	**8.549.362**	**100**	**14.326.130**	**100**

Medication costs were the highest, likely due to the complexity of patient conditions, where multiple symptoms and comorbidities require increased medication use. Increased disease severity and a higher number of comorbidities are associated with more extended hospital stays and higher overall medication costs (Giusman & Nurwahyuni, 2022). In heart failure patients at Jogja Hospital, the most widely used drugs are antihypertensive, atrial fibrosis, anti-platelet, antianginal, and hyperlipid.

Table 4 shows five classes of antihypertensive drugs commonly recognized as first-line therapies, namely ACEI / ARB, Beta Blockers, CCBs, and diuretics. The most frequently prescribed at Jogja Hospital were the diuretic furosemide group, used in 102 patients (14.66%). This finding is consistent with research by Etika et al., 2020, which reported that the diuretic and the ACEI groups are the most frequently used medications in hypertension management, due to their established safety profile and efficacy in reducing blood pressure. The use of ACEI is always used as the first line for the treatment of heart failure. Still, it should be noted that this ACEI group can cause worsening of renal function, hyperkalemia, symptomatic hypotension, and angioedema. Therefore, ACEIs are only given to patients with adequate renal function and normal potassium levels (Kemenkes, 2021), as seen in Table 4, where impaired renal function is the second most comorbid type in patients after heart function disorders. Hence, there is more administration of diuretics than in the ACEI group.

Table 4. Treatment patterns among heart failure patients hospitalized at Jogja Hospital in 2023.

Indication	Class	Drugs	Total	Percentage
Antihypertensive	ACEI	Ramipril	34	4,89%
		Catopril	2	0,29%
	ARB	Candesartan	54	7,76%
		Valsartan	10	1,44%
		Irbesartan	6	0,86%
	Beta Blocker	Bisoprolol	59	8,48%
		Carvendilol	14	2,01%
		Propanolol	1	0,14%
	CCB	Amlodipin	27	3,88%
		Nifedipine	11	1,58%
		Diltiazem	1	0,14%
	Diuretics	Furosemid	102	14,66%
		Spironolakton	66	9,48%
		HCT	7	1,01%
Atrial Fibrosis	Digitalis	Digoxin	54	7,76%
Anti-platelet	Inhibitory COX	Aspirin/Aspilet	71	10,20%
	Inhibitory receptor ADP	Clopidogrel	47	6,75%
Anti Anginal	Nitrat	ISDN	52	7,47%
		Nitrogliserin	38	5,46%
Anti Hyperlipid	Statin	Atorvastatin	40	5,75%

The next group most widely used in heart failure patients at Jogja Regional Hospital is the atrial fibrillation drug digoxin group of 54 patients (7.76%). In heart failure patients with

atrial fibrillation, digoxin can be used to slow down the rapid ventricular rate. In addition, digoxin can reduce symptoms and reduce the number of hospitalizations due to worsening symptoms of heart failure (PERKI, 2023).

The treatment that is widely used in heart failure patients is the anti-platelet group, namely aspirin drugs for as many as 71 patients (10.20%). A meta-analysis by the Antithrombotic Trialists' Collaboration showed that aspirin administration could reduce serious vascular events and mortality rates in cardiovascular and cerebrovascular diseases. The response in patients with heart disease in one of the hospitals in Indonesia is also still good, according to the results of research conducted by (A. Yunita et al., 2020), proving that there are no patients who experience aspirin resistance (E. P. Yunita et al., 2015).

Then for the second, namely room costs. The cost of the room, besides being combined with nutritional costs, is influenced by the length of treatment class and the severity of the patient. The longer and higher the class of care and the severity of the patient, the greater the room costs paid (Astuti et al., 2021).

In this study, the electromedical costs (echocardiography and electrocardiography) were also included in the cost of the three most significant components. The total electromedical costs vary depending on how often patients perform electronic examinations such as echocardiography. The more often the patient conducts the electromedical examinations, the greater the treatment costs (Nisa, 2020).

The difference between actual costs and INA-CBG's rates is seen from the average actual costs patients require during hospitalization. Actual costs will be compared with INA-CBG's rates based on class and severity. The results of the difference between actual costs and INA-CBG's rates are listed in Table 5.

It can be seen in Table 3 that patients with code I-4-12-I class 1 and code I-4-12-II class 2 showed that the average actual cost is greater than the INA-CBG's tariff ($p>0.05$), meaning that the average actual cost is greater than the INA-CBG's tariff with a non-significant difference. Patients with code I-4-12-II class 1, code I-4-12-II class 3, and code I-4-12-III class 3 showed that the average actual cost is greater than the INA-CBG's tariff ($p<0.05$), meaning that the average real cost is greater than the INA-CBG's cost rate with a significant difference. Patients with code I-4-12-II class 2 showed that the average actual cost was smaller than the INA-CBG's tariff ($p>0.05$), meaning that the average actual cost was less than the cost rate with a non-significant difference.

The difference between the total real cost and the total INA-CBG's tariff in Table 5 determines whether the hospital experiences a surplus value (profit) or minus (loss). From Table 5, the total real cost of heart failure patients at Jogja Hospital in 2023 amounted to IDR 886.837.128, and the total INA-CBG's cost amounted to IDR 480.479.100. From the difference between the two data, the result is IDR 406.358.028, which means that the financing of heart failure patients in the 2023 period at Jogja Hospital suffered a loss because BPJS Kesehatan could not bear the total costs.

Similar research conducted by (Astuti et al., 2021) stated that the tariff difference was minus IDR 40.158.430 because the real cost of the hospital was more significant than the INA-

CBG's tariff. The real cost of the hospital is influenced by comorbidities that worsen the patient's condition and the length of treatment days—this impacts the increasing number of medical services and support provided.

Another study conducted by One way to overcome the cost difference is to make efficiency so that the tariff and quality of service are balanced. Efficiency can be achieved through drug efficiency, length of treatment, actions, and examinations. Increasing efficiency by implementing clinical pathways as a hospital protocol in implementing or selecting therapy for patients. The application of clinical pathways in heart failure cases is effective in reducing the length of hospitalization and health costs by up to 20%. A clinical pathway is used for cost control and quality control in hospitals. With the clinical path, care and treatment can be appropriately done (Agiwahyuanto et al., 2020).

Previous research by Rahman (2017), which utilized the INA-CBG's tariff based on the Indonesia Minister of Health Regulation Number 59 of 2014, reported a financial surplus. In contrast, using the INA-CBG's tariff established under the Indonesian Minister of Health Regulation Number 3 of 2023, the present study resulted in a financial deficit. The financial loss observed is attributed to a decrease in the government-set INA-CBGs tariff in 2023 compared to the INA-CBGs tariff in 2014. For example, the 2023 INA-CBG's rate for INA-CBG's code I-4-12-I in treatment class 1 is IDR 4,860,200, while the 2014 INA- CBG's rate for the same INA-CBG's code and treatment class is IDR 6,974,900. However, hospitals must also evaluate the components of medicines, rooms, and electromedical costs.

Table 5. Differences between actual costs and INA-CBG's tariff in 2023.

INA-CBG's code	Class	N	Total Cost		Average Cost		Sig. (normality)	P (2-tailed)
			Actual Cost (IDR)	INA-CBG's Tariffs (IDR)	Actual Cost (IDR)	INA-CBG's Tariffs (IDR)		
I-4-12-I	1	5	51.510.465	24.301.000	10.302.093 ±5.749.499	4.860.200	0,624	0,102
	2	4	16.739.836	17.028.800	4.184.959 ±681.730	4.257.200	0,933	0,846
	3	28	143.460.016	102.320.400	5.123.572 ±2.242.239	3.654.300	0,000	0,001
I-4-12-II	1	12	117.177.000	67.610.400	9.764.750 ±5.208.727	5.634.200	0,008	0,005
	2	9	60.613.506	44.416.800	6.734.834 ±3.820.345	4.935.200	0,271	0,219
	3	27	230.832.774	114.380.100	8.549.362 ±4.288.906	4.236.300	0,017	0,000
I-4-12-III	1	1	6.073.825	7.533.200	6.073.825	7.533.200	-	-
	2	1	16.885.496	6.598.700	16.885.496	6.598.700	-	-
	3	17	243.544.210	96.289.700	14.326.130 ±7.565.598	5.664.100	0,048	0,000
Total Cost Difference (+/-)			886.837.128	480.479.100 -406.358.028				

4. CONCLUSION

The results of this study indicated that the average actual cost for patients classified with code I-4-12-I class 1 and code I-4-12-II class 2 was greater than the INA-CBG's tariff, with no significant difference observed. In contrast, the average actual cost for patients with code I-4-12-II class 1, code I-4-12-II class 3, and code I-4-12-III class 3 were also higher than the INA-CBG's tariff, with significant differences. For patients with code I-4-12-II class 2, the average actual cost was smaller than the INA-CBG's tariff, though the difference was not statistically significant. The total actual cost was more significant than the total INA-CBG's tariff, suggesting that BPJS Kesehatan cannot fully cover the actual costs of treating heart failure patients at Jogja Hospital, which would lead to financial losses for the hospital. Therefore, it is recommended that the hospital reassess the 2023 INA-CBG's tariff and evaluate the cost components, including medicines, room charges, and electromedical costs, to better align with actual expenses.

ACKNOWLEDGEMENT

This research is supported by Universitas Muhammadiyah Yogyakarta.

CONFLICT OF INTEREST

All authors declared that there was no conflict of interest.

REFERENCES

Agiwahyuanto, F., Widianawati, E., Ratna Wulan, W., & Basuki Putri, R. (2020). Tarif Rumah Sakit Dengan Tarif Ina-Cbgs Pasien Rawat Inap. *Higeia*, *4*(207), 520–532. https://doi.org/10.15294/higeia/v4i4/37117

Amalia, R. (2020). Analisis Penerapan Indonesia Case Based Groups (INA-CBG's) dalam Pelayanan Badan Penyelenggara Jaminan Sosial (BPJS) Kesehatan di Rumah Sakit Kabupaten Pelalawan. *Pekbis Jurnal*, *12*(2), 106–116.

Astuti, N. D., Irmawati, I., & Apifah, A. (2021). Analisis Tarif Rumah Sakit dan Tarif Ina CBGs Kasus Gagal Jantung Kongestif. *Jurnal Rekam Medis Dan Informasi Kesehatan*, *4*(1), 44–51.

Astuti Purnamawati, D., Arofiati, F., & Relawati, A. (2018). Pengaruh Supportive-Educative System terhadap Kualitas Hidup pada Pasien Gagal Jantung. *Mutiara Medika: Jurnal Kedokteran Dan Kesehatan*, *18*(2). https://doi.org/10.18196/mm.180213

Etika, T., Pristianty, L., & Hidayati, I. R. (2020). Analisis Cost-of-Illness pada Pasien Hipertensi Peserta BPJS Rawat Jalan di Puskesmas Banyuanyar Cost-of-Illness Analysis in Hypertension Outpatients with BPJS (Universal Coverage) in Banyuanyar Primary Healthcare Centre. *Pharmaceutical Journal of Indonesia*, *6*(1), 41–46.

Nisa, B. I. (2020). Faktor-Faktor yang Mempengaruhi Biaya Antara Biaya Riil dan Tarif INA CBGs Pada Pasien Jantung Koroner Rawat Inap JKN di RSUD Tugurejo Semarang Tahun 2019. *Skripsi. Semarang: Fakultas Ilmu Keolahragaan Universitas Negeri Semarang*.

Giusman, R., & Nurwahyuni, A. (2022). Biaya Pengobatan Pasien Rawat Inap Covid-19 Di Rumah Sakit X Tahun 2021. *Jurnal Ekonomi Kesehatan Indonesia*, *7*(2), 96. https://doi.org/10.7454/eki.v7i2.5797

Kemenkes, R. I. (2014). Peraturan Menteri Kesehatan Republik Indonesia Nomor 59 Tahun 2014 tentang Standar Tarif Pelayanan Kesehatan dalam Penyelenggaraan Program

Jaminan Kesehatan. *Menteri Kesehatan RI*, 1-670.

Kemenkes, R. I. (2021). Pedoman Nasional Pelayanan Kedokteran Tata Laksana Gagal Jantung. *Keputusan Menteri Kesehatan Republik Indonesia Nomor Hk.01.07/Menkes/4801/2021*, 1–126.

Kemenkes, R. I. (2023). Peraturan Menteri Kesehatan Republik Indonesia No. 3 Tahun 2023 Tentang Standar Tarif Pelayanan Kesehatan Dalam Penyelenggaraan Program Jaminan Kesehatan. *Menteri Kesehatan RI*, 1-721.

Lilik, N. I. S., & Budiono, I. (2021). Risiko Kematian Pasien Gagal Jantung Kongestif (GJK): Studi Kohort Retrospektif Berbasis Rumah Sakit. *Indonesian Journal of Public Health and Nutrition*, *1*(3), 388–395.

PERKI. (2023). *Pedoman Tatalaksana penyakit gagal jantung* (3rd ed). Perhimpunan Dokter Spesialis Kardiovaskular Indonesia.

Putri, F. O. A. (2021). Gambaran kualitas hidup pada pasien hipertensi yang mengalami komplikasi. *Universitas Muhammadiyah Surakarta*, 1–14. http://eprints.ums.ac.id/91760/

Rahman, Z. A. (2017). *Analisis Biaya Pengobatan Pengobatan Pasien Gagal Jantung Rawat Inap Periode Tahun 2015*. *85*(1), 2071–2079.

Sulistiyowatiningsih, E., Nurul Hidayati, S., & Febrianti, Y. (2016). Kajian Interaksi Obat Pada Pasien Gagal Jantung Dengan Gangguan Fungsi Ginjal Di Instalasi Rawat Inap Rsup Dr. Sardjito Yogyakarta Periode 2009-2013. *Jurnal Ilmiah Farmasi*, *12*(1), 35–43. https://doi.org/10.20885/jif.vol12.iss1.art4

Wulandari, T., Nurmainah, & Robiyanto. (2015). Gambaran Penggunaan Obat Pada Pasien Gagal Jantung Kongestif Rawat Inap Di Rumah Sakit Sultan Syarif Mohamad Alkadrie Pontianak. *Jurnal Farmasi Kalbar*, *3*(1), 1–9.

Yunita, A., Nurcahyati, S., & Utami, S. (2020). Gambaran Tingkat Pengetahuan Pasien Tentang Pencegahan Komplikasi Congestive Heart Failure (Chf). *Jurnal Ners Indonesia*, *11*(1), 98. https://doi.org/10.31258/jni.11.1.98-107

Yunita, E. P., Zulkarnain, B. S., & Aminuddin, M. (2015). Aspirin resistance in coronary artery disease with hypertensive patients. *Indones J Clin Pharm*, *4*(1), 28–38.

JPSCR: Journal of Pharmaceutical Science and Clinical Research
Volume 10, Issue 1, Pages 60-79, March 2025
https://doi.org/10.20961/jpscr.v10i1.92332

ISSN 2503-331X (Online)

Potensi Krim *Solid Lipid Nanoparticle* (SLN) Kombinasi Ekstrak Bunga Gemitir dan Alga Merah untuk Mengatasi Jerawat akibat *Propionibacterium acnes*

The Potency of Solid Lipid Nanoparticle (SLN) Cream Containing Combination of Marigold Flower and Red Algae Extracts against Acne-causing Propionibacterium acnes

Anak Agung Istri Padma Suari[1], Ni Komang Triayu Cita Kartini[1], I Gede Yudistira Perdangga Bandem[1], Ni Kadek Dwi Cahyani Cipta Hantari[1], Ni Putu Eka Leliqia[1,2] dan Ni Putu Ariantari[1,2]*

[1]Program Studi Farmasi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Udayana, Bali, Indonesia
[2]Microbial Natural Products and Biomoleculer Research Group, Universitas Udayana, Bali, Indonesia

*Corresponding author: putu_ariantari@unud.ac.id

Diterima: 19 September 2023; **Disetujui:** 3 Maret 2025; **Dipublikasi:** 10 April 2025

Abstrak

Bunga gemitir (*Tagetes erecta* L.) dan alga merah (*Kappaphycus alvarezii* (Doty) L.M.Liao) merupakan bahan alam yang telah dilaporkan memiliki aktivitas antibakteri dan antioksidan. Penelitian ini bertujuan untuk mengetahui efek antibakteri kombinasi ekstrak etanol bunga gemitir dan alga merah terhadap bakteri penyebab jerawat, *Propionibacterium acnes* ATCC 1223, serta aktivitasnya setelah diformulasi dalam bentuk sediaan krim *solid lipid nanoparticle* (SLN). Serbuk kering bunga gemitir dan alga merah diekstraksi dengan metode maserasi menggunakan pelarut etanol 96%. Terhadap ekstrak yang diperoleh, dilakukan skrining fitokimia. Selanjutnya, masing-masing ekstrak diuji aktivitas antibakterinya terhadap *P. acnes* secara individual dengan metode mikrodilusi. Aktivitas antibakteri kombinasi kedua ekstrak selanjutnya diuji dengan metode mikrodilusi *checkerboard*. Berdasarkan hasil pengujian aktivitas antibakteri, 4 perbandingan kombinasi ekstrak dibuat dalam bentuk sediaan krim SLN yaitu formula F1, F2, F3, dan F4. Sediaan F1–F4 dievaluasi melalui uji organoleptis, homogenitas, pH, daya sebar, dan daya lekat. Aktivitas antibakteri krim SLN diuji dengan metode difusi sumuran. Berdasarkan uji skrining fitokimia yang dilakukan, flavonoid, alkaloid dan fenol terdeteksi pada kedua ekstrak, sedangkan steroid hanya terdeteksi pada ekstrak bunga gemitir. Pada pengujian aktivitas antibakteri ekstrak secara individual, ekstrak bunga gemitir dan alga merah memiliki aktivitas lemah dengan nilai konsentrasi hambat minimum (KHM) sebesar 4 dan 16 mg/mL. Kombinasi ekstrak bunga gemitir dan alga merah menghasilkan aktivitas antibakteri lebih tinggi daripada aktivitas masing-masing ekstrak secara individual terhadap *P. acnes*, dan dikategorikan memiliki efek aditif dengan nilai FKI sebesar 0,5312. Setelah diformulasi dalam bentuk krim SLN, hanya formula F4 yang mengandung 0,1 g ekstrak bunga gemitir dan 0,2 g ekstrak alga merah memberikan aktivitas antibakteri terhadap *P. acnes* dengan diameter zona hambat sebesar 16,76±2,46 mm. Hasil penelitian ini menunjukkan sediaan krim SLN F4 yang mengandung kombinasi ekstrak bunga gemitir dan alga merah potensial untuk diinvestigasi lebih lanjut untuk pengembangan sediaan untuk mengatasi jerawat berbasis bahan alam.

Kata kunci: Alga merah; Bunga gemitir; *Kappaphycus alvarezii* (Doty) L.M.Liao; *Propionibacterium acnes; Tagetes erecta* L.

Abstract

Marigold flowers (Tagetes erecta L.) and red algae (Kappaphycus alvarezii (Doty) L.M.Liao) are natural ingredients reported to have antibacterial and antioxidant activities. This study aimed to determine the antibacterial effect of the combination of ethanol extract from marigold flowers and red algae against acne-causing bacteria, Propionibacterium acnes ATCC 1223, as well as its activity after being formulated into a solid lipid nanoparticle (SLN) cream. Dry marigold flowers and red algae powder were extracted using 96% ethanol. The obtained extract was subjected to phytochemical analysis employing colour reagents. Next, the microdilution method was used to individually test each extract for its antibacterial activity against P. acnes. The antibacterial activity of the combination of these extracts was further tested using the checkerboard microdilution method. Following the antibacterial test, four combination series of marigold flower and red algae were prepared into four SLN cream formulas, i.e., F1, F2, F3, and F4. All formulas were evaluated through organoleptic, homogeneity, pH, spreadability, and adhesiveness tests. Meanwhile, the antibacterial activity of SLN cream was tested using the well diffusion method. Phytochemical analysis indicated flavonoids, alkaloids, and phenols in marigold flower and red algae extracts, while steroid was only detected in marigold flower extract. The antibacterial test of marigold flower and red algae extracts revealed its weak activity with minimum inhibitory concentration (MIC) values of 4 and 16 mg/mL, respectively. Interestingly, the combination of marigold flower and red algae extracts produced higher antibacterial activity than its individual activity against P. acnes, resulting in an additive effect with an FCI value of 0.5312. After being formulated as SLN cream, formula F4 containing 0.1 g marigold flower and 0.2 g red algae extracts gave potent antibacterial activity against P. acnes with a diameter of zone inhibition of 16.76±2.46 mm. The present study indicates the potency of the combination of marigold flower and red algae extracts against acne-causing P. acnes. Further investigation on SLN cream formulation containing the combination of these extracts is promising for developing natural products-based preparations for treating acne.

Keywords: *Marigold flower; Kappaphycus alvarezii (Doty) L.M.Liao; Propionibacterium acnes; Red algae; Tagetes erecta L.*

1. PENDAHULUAN

Kulit merupakan organ penyusun tubuh manusia yang terletak paling luar dan menutupi seluruh permukaan tubuh. Kulit merupakan organ yang pertama kali menerima rangsangan seperti sentuhan, rasa sakit, maupun pengaruh buruk dari luar. Hal tersebut menyebabkan kulit rentan terkena penyakit. Infeksi kulit merupakan penyakit kulit yang umum diderita oleh masyarakat di Indonesia. Salah satu infeksi kulit akibat bakteri yang banyak ditemui adalah jerawat yang diakibatkan oleh infeksi bakteri *Propionibacterium acnes*. Bakteri *P. acnes* merupakan flora normal yang umum ditemukan pada kulit manusia. Namun, terjadinya peningkatan jumlah bakteri pada kulit dapat menyebabkan timbulnya lesi inflamasi pada kulit (Wijaya *et al*., 2024). Prevalensi penderita jerawat di Indonesia pada rentang usia remaja 15-18 tahun sebesar 80-85%, usia di atas 25 tahun sebesar 12%, dan pada rentang usia 35-44 tahun sebesar 3% (Sibero *et al*., 2019). Timbulnya jerawat ini dapat berdampak pada kesehatan mental, emosi, serta rasa percaya diri seseorang (Ayu & Destiwati, 2022). Dalam upaya mengatasi masalah jerawat, tren pengobatan mulai beralih dengan penggunaan bahan alami

(*back to nature*) karena dianggap memiliki efek samping yang lebih ringan dibandingkan dengan pengobatan medis konvensional (Ismiyati & Trilestari, 2014).

Bunga gemitir (*Tagetes erecta* L.) dan alga merah (*Kappaphycus alvarezii* (Doty) L.M.Liao) merupakan bahan alami yang telah dilaporkan di berbagai studi memiliki aktivitas antibakteri dan potensial dikembangkan untuk mengatasi jerawat. Berdasarkan penelitian yang dilakukan oleh Pramitha *et al.* (2018), ekstrak etanol bunga gemitir diketahui mengandung senyawa alkaloid, terpenoid, saponin, fenolik dan flavonoid. Berbagai senyawa alkaloid dilaporkan memiliki aktivitas antibakteri melalui mekanisme penghambatan penyusunan lapisan dinding sel bakteri. Flavonoid menunjukkan aktivitas antibakteri antara lain dengan cara merusak membran sel bakteri melalui pembentukan senyawa kompleks dengan protein ekstraseluler (Amalia *et al.,* 2017). Mekanisme kerja terpenoid sebagai antibakteri yaitu melalui perusakan membran sel bakteri (Wulansari *et al.,* 2020). Fenol memiliki aktivitas antibakteri dengan cara mengganggu proses transpor aktif bakteri (Putri *et al.,* 2014). Saponin bekerja dengan cara menurunkan tegangan permukaan pada dinding sel bakteri, sehingga akan meningkatkan permeabilitas sel dan menyebabkan terjadinya kerusakan pada membran sel bakteri (Rasyid *et al.,* 2018). Selanjutnya, penelitian Cahyaningrum *et al*. (2020) menunjukkan bahwa ekstrak etanol bunga gemitir yang dibuat menjadi sediaan sabun menunjukkan aktivitas antibakteri sedang terhadap bakteri Gram positif *Staphylococcus aureus* dan Gram negatif *Escherichia coli.* Sedangkan, alga merah dilaporkan aktif sebagai antibakteri serta antioksidan (Lumbessy *et al*., 2020). Ekstrak alga merah diketahui memiliki kandungan senyawa bioaktif dari golongan flavonoid, alkaloid, steroid, dan tanin (Marhaeny *et al*., 2024). Selain itu, alga merah jenis ini juga memiliki kandungan karagenan dan senyawa fenol (Saifudin *et al*., 2015). Berdasarkan penelitian oleh Marhaeny *et al*. (2024), diketahui bahwa ekstrak metanol alga merah memiliki aktivitas antibakteri terhadap bakteri Gram positif *S. aureus* dan Gram negatif *E. coli.*

Berdasarkan potensi aktivitas antibakteri dari masing-masing ekstrak bunga gemitir dan alga merah yang telah dilaporkan tersebut, kedua ekstrak ini berpotensi untuk dikombinasikan dalam bentuk sediaan krim dengan formulasi *Solid Lipid Nanoparticle* (SLN), untuk mendapatkan sediaan dengan efek antibakteri lebih baik daripada aktivitas masing-masing ekstrak. Formulasi SLN adalah sebuah pendekatan dalam pengiriman obat yang dapat membantu meningkatkan stabilitas, kontrol pelepasan obat, dan penargetan yang lebih tepat (Paliwal *et al*., 2020). SLN mampu mengatasi beberapa keterbatasan formulasi sediaan topikal tradisional seperti krim dan lotion, yang sering kali kurang efektif dalam mengontrol pelepasan dan penetrasi bahan aktif ke dalam kulit. Penghantaran obat topikal melalui nanopartikel lipid memungkinkan terjadinya pengendapan obat yang tinggi pada area yang ditargetkan secara spesifik, seperti folikel rambut, kelenjar sebaseus, dan kelenjar keringat, sehingga dapat meminimalkan efek samping obat secara sistemik (Ghasemiyeh & Samani, 2020). Formulasi SLN dapat meningkatkan durasi aksi, memudahkan dalam produksi skala besar, serta meningkatkan bioavailabilitas dan biodegradabilitas obat (Gupta *et al*., 2022). Sistem penghantaran SLN dalam beberapa tahun terakhir banyak diaplikasikan dalam penghantaran

senyawa obat atau bahan aktif secara topikal (Aryani *et al*., 2019; Chaturvedi & Sharma, 2023), akan tetapi aplikasinya sejauh ini digunakan untuk formulasi sediaan dengan satu bahan aktif. Oleh karena itu, penelitian ini bertujuan untuk mengevaluasi efek antibakteri kombinasi ekstrak etanol bunga gemitir dan alga merah terhadap bakteri penyebab jerawat, *Propionibacterium acnes* ATCC 1223, serta aktivitasnya setelah diformulasi dalam bentuk sediaan krim SLN. Kombinasi dari ekstrak bunga gemitir dan alga merah pada formulasi krim SLN ini diharapkan memberikan efek antibakteri yang lebih baik untuk bisa mengatasi jerawat yang disebabkan oleh bakteri *P. acnes.*

2. BAHAN DAN METODE

2.1. Alat dan bahan

Alat yang digunakan pada penelitian ini adalah *Laminar Air Flow* (JLabTech®, Jerman), Spektrofotometri UV-VIS (Therm Scientific®, USA), alat-alat gelas (Iwaki®, Jepang), *magnetic stirrer*, seperangkat alat *rotary evaporator* (Heidolph®, Jerman), pH meter (Mediatech®, USA), inkubator (Binder®, Jerman), timbangan analitik (Kern®, Jerman), plat mikrodilusi (*microplate 96 well round bottom* IWAKI®, Jepang), dan autoklaf (Biobase®, China). Pelarut etanol 96%, metanol, dan kloroform, didestilasi ulang sebelum digunakan. Media *Mueller Hinton Agar* (Himedia®, India), media *Mueller Hinton Broth* (Himedia®, India), DMSO (Merck®, Jerman), dan antibiotik klindamisin (Sanbe®, Indonesia) digunakan dalam uji antibakteri. Gliseril monostearat, *Tween* 80, parafin cair, adeps lanae, asam stearat, optiphen, trietanolamin, digunakan sebagai bahan dalam formulasi sediaan.

2.2. Material tanaman

Bunga gemitir (*Tagetes erecta* L.) dibeli di pasar Badung, Bali, pada bulan April 2024. Alga merah didapatkan dari kawasan Pulau Serangan pada bulan Mei 2024. Determinasi sampel bunga gemitir dilakukan di Materia Medica Batu, Malang. Alga merah (*Kappaphycus alvarezii* (Doty) L.M.Liao), nama sebelumnya: *Eucheuma cottonii* Doty) yang digunakan dalam penelitian ini, diidentifikasi dan dideterminasi di Laboratorium Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Udayana. Terhadap material tanaman yang telah dikumpulkan, dilakukan pengolahan pasca panen mulai dari sortasi basah, pencucian bahan, penirisan bahan, proses pelayuan (penyinaran dengan sinar matahari dengan ditutupi kain hitam), dan pengeringan dengan oven pada suhu ± 60ºC. Simplisia kering diubah menjadi serbuk dan diayak hingga diperoleh serbuk kering simplisia.

2.3. Ekstraksi

Serbuk simplisia bunga gemitir ditimbang sebanyak 417 g dan alga merah sebanyak 439 g. Serbuk simplisia kemudian diekstraksi dengan metode maserasi menggunakan etanol 96% dengan perbandingan (1:10) selama 24 jam. Setelah 24 jam, ekstrak disaring sehingga diperoleh ekstrak dan residu. Ekstrak selanjutnya ditampung dan residu diremaserasi dengan etanol 96% dengan volume setengah kali volume maserasi pertama. Kemudian dilakukan pemekatan ekstrak menggunakan alat *vacuum rotary evaporator* untuk memperoleh ekstrak kental.

Rendemen ekstrak kental dihitung berdasarkan perbandingan antara bobot ekstrak dan berat awal simplisia dalam b/b% menggunakan Persamaan 1.

$$\text{Rendemen } (\%\ b/b) = \frac{Bobot\ ekstrak\ (g)}{Bobot\ simplisia(g)} \times 100\%$$

Persamaan 1. Perhitungan rendemen ekstrak kental dalam persen bobot per bobot (% b/b).

2.4. Pengujian susut pengeringan simplisia bunga gemitir dan alga merah

Metode yang digunakan dalam pengujian susut pengeringan ini mengacu pada Farmakope Herbal Indonesia Edisi II (2017). Sebanyak 1 g serbuk simplisia dengan derajat halus nomor 8 ditimbang dalam botol timbang dangkal bertutup yang sebelumnya telah dipanaskan pada suhu 105°C dan ditara. Kemudian, botol timbang dimasukkan ke dalam oven dengan tutup botol yang dibuka dan dikeringkan pada suhu 105°C dengan interval waktu 30 menit hingga diperoleh bobot tetap. Perhitungan susut pengeringan dalam b/b% menggunakan Persamaan 2.

$$\text{Susut pengeringan } (\%\ b/b) = \frac{a-b}{a} \times 100\%$$

Persamaan 2. Perhitungan susut pengeringan dalam persen bobot per bobot (% b/b). Keterangan: a = bobot simplisia sebelum pemanasan (g) dan b = bobot simplisia setelah pemanasan (g).

2.5. Pengujian kadar air ekstrak etanol bunga gemitir

Metode pengujian kadar air ekstrak mengacu pada Farmakope Herbal Indonesia Edisi II (2017). Pengujian kadar air ekstrak etanol bunga gemitir dilakukan dengan metode azeotropis, dengan destilasi toluena. Ekstrak etanol bunga gemitir ditimbang sebanyak 5 g dan dimasukkan ke dalam labu. Kemudian sebanyak 100 mL toluena jenuh air ditambahkan ke dalam labu yang telah berisi ekstrak. Alat destilasi dirangkai dan dilapisi dengan *plastic wrap* pada bagian leher labu. Sebanyak 10 mL toluena jenuh air ditambahkan pada tabung penerima. Labu selanjutnya dipanaskan menggunakan *hot plate* dengan suhu ± 360^{o}C. Volume air dibaca setelah air dan toluena memisah dengan sempurna. Perhitungan kadar air dalam %v/b menggunakan persamaan 3.

$$\text{Kadar air } (\%\ v/b) = \frac{Volume\ air\ (mL)}{Bobot\ ekstrak\ (g)} \times 100\%$$

Persamaan 3. Perhitungan kadar air dalam ekstrak etanol bunga gemitir, dinyatakan dalam persen volume per bobot ekstrak (% v/b).

2.6. Pengujian kadar air ekstrak etanol alga merah

Pengujian kadar air ekstrak etanol alga merah dilakukan dengan metode gravimetri yang mengacu pada Farmakope Herbal Indonesia Edisi II (2017). Sampel ditimbang saksama lebih kurang 10 g, lalu dimasukkan ke dalam wadah yang telah ditara. Kemudian sampel dikeringkan pada suhu 105°C selama 5 jam, lalu ditimbang. Pengeringan dilanjutkan dan dalam selang waktu 1 jam, dilakukan penimbangan kembali sampai perbedaan antara dua penimbangan berturut-turut tidak lebih dari 0,25%. Pengujian dilakukan dalam tiga kali replikasi. Syarat

kadar air ekstrak yang baik adalah tidak lebih dari 10% b/b. Perhitungan kadar air dalam %b/b menggunakan Persamaan 4.

$$\text{Kadar air } (\%\ b/b) = \frac{W0\ (g) - W1\ (g)}{Bobot\ ekstrak\ (g)} \times 100\%$$

Persamaan 4. Perhitungan kadar air dalam ekstrak etanol alga merah. Keterangan: W0 = Bobot cawan + ekstrak sebelum pemanasan dan W1 = Bobot cawan + ekstrak setelah pemanasan.

2.7. Skrining fitokimia ekstrak etanol bunga gemitir dan alga merah

Skrining fitokimia dilakukan menggunakan metode yang diadopsi dari penelitian oleh Putra *et al*. (2023). Beberapa pengujian yang dilakukan pada penelitian ini, yaitu uji flavonoid, alkaloid, terpenoid/steroid, saponin, dan fenol.

2.7.1. Uji flavonoid

Sebanyak 2 mL larutan uji dipipet, lalu dimasukkan ke dalam tabung reaksi. Larutan NaOH 10% ditambahkan sebanyak 2 tetes dan dikocok, hasil positif flavonoid ditandai dengan perubahan warna menjadi kuning, merah atau cokelat.

2.7.2. Uji alkaloid

Sebanyak 2 mL larutan uji dipipet dan dimasukkan ke dalam 2 tabung reaksi yang berbeda. Pada masing-masing tabung ditambahkan 1 mL HCl 2 N. Reagen Mayer sebanyak 2-3 tetes ditambahkan ke dalam tabung reaksi I, hasil positif alkaloid ditandai dengan adanya endapan putih. Reagen Dragendorff sebanyak 2-3 tetes ditambahkan ke dalam tabung reaksi II, hasil positif alkaloid ditunjukkan dengan adanya endapan jingga.

2.7.3. Uji terpenoid dan steroid

Sebanyak 2 mL larutan uji dimasukkan ke dalam tabung reaksi. Asam asetat glasial ditambahkan sebanyak 10 tetes dan asam sulfat pekat sebanyak 2 tetes. Hasil positif steroid ditunjukkan dengan terbentuknya warna biru atau hijau, sedangkan positif terpenoid ditandai dengan warna merah atau ungu.

2.7.4. Uji saponin

Sebanyak 2 mL larutan uji dimasukkan ke dalam tabung reaksi. Aquades ditambahkan ke dalam tabung reaksi. Tabung reaksi dikocok dan diamati busa yang terbentuk. Apabila terbentuk busa stabil tidak kurang dari 10 menit, maka dapat dinyatakan positif mengandung saponin.

2.7.5 Uji fenol

Sebanyak 2 mL larutan uji dimasukkan ke dalam tabung reaksi. Larutan $FeCl_3$ ditambahkan ke dalam tabung reaksi sebanyak 3-4 tetes. Hasil positif fenol ditandai dengan terbentuknya warna hijau, merah, atau hijau kehitaman.

2.8. Pembuatan suspensi bakteri uji

Media MHA dan MHB untuk kultivasi bakteri dan pengujian aktivitas antibakteri disiapkan sesuai prosedur dari produsen media. Bakteri uji yang digunakan adalah *P. acnes* ATCC 1223. Kultur bakteri dilakukan sesuai dengan prosedur pada pedoman *Clinical and Laboratory Standards Institute* (CLSI, 2022). Kultur bakteri uji kemudian diambil menggunakan mikropipet kemudian diinokulasikan ke dalam botol vial steril yang telah berisi media MHB dan diinkubasi selama 18-24 jam pada suhu 37^{o}C. Suspensi bakteri uji diukur absorbansinya hingga diperoleh rentang 0,08-0,13 atau setara dengan absorbansi larutan standar 0,5 McFarland. Setelah itu, dilakukan pengenceran kultur bakteri dengan perbandingan volume 1:20 (1 untuk kultur bakteri dan 20 untuk media MHB), sehingga diperoleh jumlah koloni sebanyak 5×10^{6} CFU/mL.

2.9. Uji aktivitas antibakteri tunggal ekstrak etanol bunga gemitir dan alga merah dengan metode mikrodilusi

Metode mikrodilusi yang digunakan dalam penelitian ini mengacu pada standar yang ditetapkan oleh *Clinical and Laboratory Standards Institute* (CLSI, 2022). Kontrol negatif yang digunakan adalah media MHB, dan klindamisin (0,05 mg/mL) digunakan sebagai kontrol positif. Larutan induk ekstrak etanol bunga gemitir dibuat dengan konsentrasi 16 mg/mL dan ekstrak etanol alga merah dengan konsentrasi 64 mg/mL. Pada setiap sumuran di pelat mikrodilusi, dimasukkan 100 µL media MHB dan larutan ekstrak uji pada sumuran pertama. Selanjutnya, dilakukan pengenceran berseri hingga diperoleh seri konsentrasi ekstrak uji mulai dari 8-15,625$\times10^{-3}$ mg/mL untuk ekstrak etanol bunga gemitir dan seri konsentrasi ekstrak uji mulai dari 32-0,25 mg/mL untuk ekstrak etanol alga merah. Plat mikrodilusi yang telah berisi sampel dan suspensi bakteri uji kemudian diinkubasi pada suhu 35$\pm$2^{o}C selama 18-24 jam. Pengujian aktivitas antibakteri tiap sampel dilakukan sebanyak 3 kali.

2.10. Uji aktivitas antibakteri kombinasi ekstrak etanol bunga gemitir dan alga merah dengan metode mikrodilusi *checkerboard*

Pengujian aktivitas antibakteri kombinasi ekstrak etanol bunga gemitir dan alga merah dilakukan menggunakan metode mikrodilusi *checkerboard.* Larutan induk ekstrak etanol bunga gemitir dibuat dengan konsentrasi 4 kali KHM dan ekstrak etanol alga merah dalam konsentrasi 2 kali KHM. Pengenceran ekstrak etanol bunga gemitir dilakukan di dalam *microplate well* dan pengenceran ekstrak etanol alga merah akan dilakukan di luar *microplate well.* Plat mikrodilusi yang telah berisi sampel dan suspensi bakteri uji diinkubasi pada suhu 35$\pm$2^{o}C selama 18-24 jam (Tayseer *et al*., 2020). Pengujian aktivitas antibakteri tiap sampel dilakukan sebanyak 3 kali. Efek kombinasi ekstrak dievaluasi menggunakan rumus perhitungan Indeks Fraksi Konsentrasi Inhibisi (FKI) berdasarkan Persamaan 5 (Meletiadis *et al.,* 2010).

$$\text{Indeks FKI} = \frac{KHM\ A\ dalam\ Kombinasi}{KHM\ A\ Tunggal} + \frac{KHM\ B\ dalam\ Kombinasi}{KHM\ B\ Tunggal}$$

Persamaan 5. Perhitungan Indeks Fraksi Konsentrasi Inhibisi (FKI). Keterangan: A = Ekstrak etanol bunga gemitir dan B = Ekstrak etanol alga merah.

2.11. Formulasi dan evaluasi sediaan krim *Solid Lipid Nanoparticle* (SLN)

Metode pembuatan dispersi *Solid Lipid Nanoparticle* (SLN) diadopsi dari penelitian oleh Jafar *et al.* (2019) dengan modifikasi pada komposisi ekstrak dan bahan tambahan. Dispersi SLN dibuat dengan basis gliseril monostearat yang dilelehkan. Selanjutnya dibuat campuran lipid dengan melarutkan sejumlah *Tween* 80 dan kombinasi ekstrak etanol bunga gemitir dan alga merah kemudian dipanaskan pada suhu 75ºC. Campuran kemudian disonikasi selama 5 menit. Campuran lipid dimasukkan ke dispersi SLN sambil diaduk menggunakan *magnetic stirrer* selama 30 menit hingga larut dan terbentuk. Variasi konsentrasi ekstrak dibuat dalam F1, F2, F3, dan F4 serta dibuat satu formulasi kontrol (F0) tanpa penambahan ekstrak. Fase minyak (parafin cair, adeps lanae, dan asam stearat, dan propilparaben) dan fase air (trietanolamin dan aquades) dipanaskan di atas *waterbath* pada suhu 70ºC hingga melebur. Dispersi SLN ekstrak kental bunga gemitir dan alga merah dimasukkan ke dalam basis krim lalu digerus hingga homogen. Evaluasi sediaan meliputi uji organoleptis, homogenitas, pH, dan daya sebar (Netto & Jose, 2017).

2.12. Uji aktivitas antibakteri sediaan krim *Solid Lipid Nanoparticle* (SLN) dengan metode difusi sumuran

Pengujian aktivitas antibakteri sediaan krim *Solid Lipid Nanoparticle* (SLN) kombinasi ekstrak etanol bunga gemitir dengan alga merah terhadap bakteri *P. acnes* dilakukan dengan menggunakan metode difusi sumuran. Suspensi bakteri dibuat dengan menggunakan metode sesuai dengan pedoman *Clinical and Laboratory Standards Institute* (CLSI, 2022). Suspensi bakteri kemudian diinokulasikan pada media MHA. Setelah itu dibuat sebanyak 6 lubang pada media untuk diisi dengan sediaan. Setiap formula krim *Solid Lipid Nanoparticle* (SLN), kontrol positif krim klindamisin, dan kontrol negatif (F0) dimasukkan ke dalam sumuran hingga memenuhi lubang sumuran. Cawan Petri kemudian diinkubasi selama 24 jam pada suhu 37ºC. Diameter zona terang (*clear zone*) yang terbentuk di area sekitar lubang diukur dengan menggunakan jangka sorong. Pengujian aktivitas antibakteri tiap sampel dilakukan sebanyak 3 kali.

2.13. Analisis data

Data pengujian aktivitas antibakteri dalam penelitian ini diperoleh dari 3 eksperimen. Hasil pengukuran diameter zona bening pada uji aktivitas antibakteri sediaan krim SLN dengan metode difusi sumuran, ditampilkan sebagai nilai rata-rata (mean)±SD.

3. HASIL DAN PEMBAHASAN

3.1. Ekstraksi

Ekstraksi merupakan suatu tahapan yang bertujuan untuk memisahkan komponen metabolit sekunder dengan campuran komponen lain menggunakan suatu pelarut yang sesuai (Candra *et al.,* 2021). Serbuk simplisia bunga gemitir dan alga merah diekstraksi menggunakan etanol 96% dan dilakukan remaserasi sebanyak dua kali. Hasil ekstraksi disaring kemudian

dipekatkan dengan *vacuum rotary evaporator* pada suhu 30°C. Hasil ekstraksi serbuk simplisia bunga gemitir dan alga merah dapat dilihat pada Tabel 1.

Tabel 1. Hasil ekstraksi serbuk simplisia bunga gemitir dan alga merah.

Sampel	Berat Simplisia (g)	Berat Ekstrak Kental (g)	Rendemen Ekstrak (%)
Bunga Gemitir	100	9,42	9,42
Alga Merah	200	4,84	2,42

Etanol memiliki beberapa keunggulan, seperti relatif tidak toksik, mudah diperoleh, murah, serta dapat digunakan untuk beberapa metode ekstraksi (Hakim & Saputri, 2020). Perhitungan nilai rendemen ekstrak menyatakan jumlah komponen bioaktif yang terkandung di dalam suatu ekstrak kental. Rendemen ekstrak kental bunga gemitir dan alga merah berturut-turut sebanyak 9,42 dan 2,42% b/b. Semakin besar nilai rendemen ekstrak yang diperoleh, maka semakin tinggi pula kandungan senyawa aktif yang terkandung di dalam suatu ekstrak (Senduk *et al*., 2020).

Penelitian oleh Kusmiati *et al.* (2018) melaporkan rendemen ekstraksi sebanyak 20 g serbuk simplisia dari tiga jenis bunga gemitir menggunakan pelarut n-heksana masing-masing sebesar 5,28; 4,40; dan 3,68. Penelitian oleh Aristyanti *et al.* (2017), hasil rendemen ekstraksi bunga dengan pelarut n-heksana, kloroform, dan etil asetat berturut-turut sebesar 9,68%, 14,68%, dan 13,95%. Sementara itu, penelitian Fahrul *et al.* (2021) melaporkan hasil rendemen ekstrak alga merah dengan pelarut etanol 96% d sebesar 3,16% dan pelarut n-heksana sebesar 2,19%. Selanjutnya penelitian oleh Andriani *et al.* (2015), memperoleh nilai rendemen untuk ekstrak n-heksana sebesar 2,50%, petroleum eter sebesar 3,44%, kloroform 1,90%, dan etil asetat sebesar 7,17%. Variasi nilai rendemen disebabkan oleh beberapa faktor seperti ukuran partikel atau derajat halus serbuk simplisia, lama ekstraksi, dan konsentrasi pelarut yang digunakan pada saat ekstraksi (Kusmiati *et al.,* 2018).

3.2. Kadar susut pengeringan simplisia

Penetapan susut pengeringan perlu dilakukan untuk memastikan kualitas mutu simplisia. Penetapan susut pengeringan merupakan salah satu persyaratan yang harus dipenuhi dalam standarisasi tumbuhan yang berkhasiat obat dengan tujuan memberikan batas maksimal (rentang) tentang besarnya senyawa yang hilang pada proses pengeringan. Pemenuhan standar susut pengeringan dengan metode gravimetri menunjukkan bahwa proses pengeringan dilakukan dengan baik dan efisien. Hasil penetapan kadar susut pengeringan simplisia bunga gemitir dan alga merah dapat dilihat pada Tabel 2.

Tabel 2. Hasil penetapan kadar susut pengeringan simplisia bunga gemitir dan alga merah.

Replikasi Pengujian	Simplisia Bunga Gemitir (%b/b)	Simplisia Alga Merah (%b/b)
1	2,00	7,00
2	2,20	7,90
3	2,00	7,20
Rata-rata±SD	2,06 ±0,09	7,36 ±0,38

Hasil susut pengeringan simplisia bunga gemitir yaitu sebesar 2,06% b/b dan simplisia alga merah sebesar 7,36% b/b. Oleh karena itu, hasil pengujian telah sesuai dengan syarat kadar susut pengeringan simplisia menurut Farmakope Herbal Indonesia Edisi II (2017), yaitu tidak lebih dari 10%.

3.3. Kadar Air Ekstrak Kental

Standar kadar air menunjukkan bahwa kandungan air pada ekstrak kental sesuai rentang sehingga tidak mengganggu konsentrasi metabolit lainnya yang ada pada bahan. Bahan tanpa kandungan minyak atsiri seperti alga merah dapat diuji menggunakan metode gravimetri. Apabila terdapat kandungan minyak atsiri pada bahan seperti pada bunga gemitir, pengujian dilakukan dengan metode azeotropis dengan menggunakan pelarut toluen jenuh air. Prinsip dari metode ini adalah pencampuran sampel dengan pelarut yang bersifat *immiscible* (Maryam *et al*., 2020). Hasil penetapan kadar air bunga gemitir dan alga merah dapat dilihat pada Tabel 3. Hasil pengujian kadar air ekstrak telah sesuai dengan syarat berdasarkan Farmakope Herbal Indonesia Edisi II (2017), yaitu tidak lebih dari 10%.

Tabel 3. Hasil penetapan kadar air ekstrak kental bunga gemitir dan alga merah.

Replikasi Pengujian	Ekstrak Bunga Gemitir (% v/b)	Ekstrak Alga Merah (%b/b)
1	6,00	6,20
2	6,00	6,00
3	6,00	6,80
Rata-rata±SD	6,00 ±0	6,35 ±0,47

3.4. Skrining fitokimia

Skrining fitokimia merupakan suatu tahapan yang bertujuan untuk mengidentifikasi golongan metabolit sekunder yang terkandung di dalam suatu ekstrak secara kualitatif. Hasil uji skrining fitokimia ekstrak etanol bunga gemitir dan alga merah (Tabel 4).

Berdasarkan skrining fitokimia yang telah dilakukan, diketahui bahwa ekstrak etanol bunga gemitir mengandung senyawa flavonoid, alkaloid, steroid, dan fenol. Hasil skrining fitokimia ini sesuai dengan penelitian yang telah dilakukan oleh Kusmiati *et al.* (2018), yang melaporkan bahwa serbuk simplisia bunga gemitir memiliki kandungan senyawa dari golongan flavonoid, alkaloid, saponin, steroid/triterpenoid, dan tanin. Selain itu, berdasarkan penelitian yang dilakukan oleh Vijay *et al.* (2013) terhadap serbuk simplisia dan ekstrak metanol bunga gemitir, diperoleh hasil bahwa bunga gemitir mengandung senyawa metabolit berupa alkaloid, flavonoid, steroid/triterpenoid, saponin, tanin, dan fenol. Beberapa senyawa yang telah diisolasi dari bunga gemitir, antara lain lutein, patulitrin, dan rutin (Kusmiati *et al.,* 2018; Moravkar *et al.,* 2023; Rhama & Madhavan, 2011; Santi, 2021).

Hasil skrining fitokimia terhadap ekstrak etanol alga merah menunjukkan ekstrak tersebut mengandung golongan senyawa flavonoid, alkaloid, dan fenol. Hasil ini sejalan dengan penelitian yang dilakukan oleh Kurnia *et al.* (2022) terhadap ekstrak etanol dan fraksi alga merah. Pada penelitian tersebut, ekstrak etanol, fraksi n-heksana dan etil asetat alga merah, dilaporkan mengandung senyawa flavonoid, alkaloid, fenol, dan steroid/triterpenoid,

sedangkan fraksi etanol diketahui hanya mengandung flavonoid, alkaloid, dan fenol. Hingga saat ini, karagenan, alkaloid, fenol, dan flavonoid merupakan metabolit yang sering dilaporkan dari alga merah (Alghazeer *et al.,* 2013; Arianto *et al.,* 2022; Rupert *et al.,* 2022).

Tabel 4. Skrining fitokimia ekstrak etanol bunga gemitir dan alga merah. Keterangan: (+) = Hasil positif/kandungan kimia terdeteksi, (-) = Hasil negatif/kandungan kimia tidak terdeteksi, *Sumber Pustaka = Putra *et al*. (2023).

Golongan Kandungan Kimia	Pereaksi	Hasil Positif Menurut Pustaka*	Hasil Skrining Ekstrak Etanol Bunga Gemitir	Hasil Skrining Ekstrak Etanol Alga Merah
Flavonoid	NaOH 10%	Kuning tua atau jingga	(+)	(+)
Alkaloid	Dragendorff	Endapan jingga	(+)	(+)
	Mayer	Endapan putih	(+)	(+)
Steroid	Liebermann-Burchard	Biru atau hijau	(+)	(-)
Terpenoid	Liebermann-Burchard	Merah atau ungu	(-)	(-)
Saponin	HCl 2 N	Terbentuk busa stabil selama 15 menit	(-)	(-)
Fenol	$FeCl_3$	Merah, hijau, atau hijau kehitaman	(+)	(+)

3.5. Uji aktivitas antibakteri ekstrak etanol bunga gemitir dan alga merah

Pengujian aktivitas antibakteri dengan metode mikrodilusi dilakukan dengan tujuan untuk mengetahui nilai KHM dari ekstrak etanol bunga gemitir dan alga merah terhadap pertumbuhan bakteri *P. acnes* ATCC 1223. Pada penelitian ini, ekstrak etanol bunga gemitir dan alga merah, menunjukkan aktivitas hambatan lemah terhadap bakteri *P. acnes* dengan KHM masing-masing sebesar 4 dan 16 mg/mL, sedangkan kontrol positif klindamisin memberikan nilai KHM sebesar $0{,}7812\times10^{-3}$ mg/mL. Berdasarkan Sartoratto *et al.* (2004), aktivitas antibakteri dinyatakan lemah apabila nilai KHM yang diperoleh pada pengujian > 1,5 mg/mL. Ekstrak etanol bunga gemitir menghasilkan nilai KHM yang lebih kecil jika dibandingkan dengan ekstrak etanol alga merah. Nilai KHM yang lebih kecil menunjukkan bahwa ekstrak etanol bunga gemitir lebih kuat dalam menghambat bakteri *P. acnes* dibandingkan dengan ekstrak etanol alga merah.

Pada penelitian yang dilakukan sebelumnya oleh Verma & Verma (2012), ekstrak etanol bunga gemitir dengan konsentrasi 10 mg/mL dilaporkan memiliki aktivitas antibakteri kuat terhadap bakteri *S. lutea, B. subtilis, E. coli,* dan *B. circulence*, pada pengujian dengan metode difusi. Ekstrak etanol bunga gemitir pada konsentrasi yang sama juga dilaporkan memiliki aktivitas antibakteri kategori sedang terhadap bakteri *S. aureus.* Patulitrin, senyawa flavonoid yang diisolasi dari bunga gemitir dilaporkan aktif terhadap bakteri *Klebsiella pneumoniae* dengan zona hambat sebesar 29,50 mm pada konsentrasi 0,1 mg/mL. Terhadap bakteri *Proteus vulgaris*, patulitrin pada konsentrasi yang sama menunjukkan diameter zona hambat sebesar 28,75 mm. Kemudian, terhadap bakteri *Campylobacter coli, E. coli, Streptococcus mutans,* dan *Streptococcus pyogenes,* patulitrin pada konsentrasi 0,1 mg/mL memberikan efek antibakteri dengan zona hambat sebesar 26,50 mm. Senyawa ini juga aktif terhadap bakteri *Alcaligens*

faecalis, Bacillus cereus dan *Pseudomonas aeruginosa* pada konsentrasi uji yang sama, dengan diameter zona hambat masing-masing sebesar 25,00; 21,50; dan 21,00 mm (Rhama & Madhavan, 2011).

Studi terhadap aktivitas antibakteri dari beberapa spesies alga juga telah banyak dilakukan. Ekstrak metanol alga merah (*E. cottonii*) pada konsentrasi 4% menunjukkan aktivitas antibakteri sedang terhadap bakteri *S. aureus* dan *E. coli* dengan zona hambat masing-masing sebesar 7,85 dan 6,25 mm (Andriani *et al.,* 2015). Ekstrak etanol alga merah *(E. cottonii*) dilaporkan tidak aktif terhadap *P. acnes* pada penelitian Kurnia *et al.* (2022), tetapi menunjukkan aktivitas hambatan terhadap *S. epidermidis* pada konsentrasi 5%. Selain itu, alkaloid dari alga *Cystoseira barbata* dilaporkan aktif terhadap bakteri *Klebsiella* spp. pada konsentrasi 100 mg/mL dengan zona hambat sebesar 35 mm. Ekstrak alkaloid dari alga *Dictyopteris membranacea* pada konsentrasi yang sama juga menunjukkan aksi antibakteri yang sangat kuat terhadap *S. typhi* dengan zona hambat sebesar 35 mm (Alghazeer *et al.*, 2013). Hal ini mengindikasikan kandungan alkaloid kemungkinan berkontribusi dalam aktivitas antibakteri ekstrak alga *C. barbata* dan *D. membranacea* yang dilaporkan sebelumnya, serta aktivitas antibakteri alga merah *K. alvarezii* (nama sebelumnya: *E. cottonii*) yang diuji pada penelitian ini.

3.6. Uji aktivitas antibakteri kombinasi ekstrak etanol bunga gemitir dan alga merah

Hasil uji antibakteri kombinasi ekstrak etanol bunga gemitir dan alga merah terhadap bakteri *P. acnes* ATCC (Tabel 5). Kombinasi ekstrak etanol bunga gemitir dan alga merah menghasilkan nilai KHM kombinasi yang lebih rendah dibandingkan dengan nilai KHM ekstrak secara individual. Dosis yang diperlukan dari masing-masing ekstrak menjadi lebih rendah agar tercapai efek antibakteri yang diinginkan,. Efek antibakteri terhadap bakteri *P. acnes* memerlukan 1/2 KHM ekstrak etanol bunga gemitir dan 1/32 KHM ekstrak etanol alga merah. Efek kombinasi antibakteri kedua ekstrak terhadap bakteri *P. acnes* dilihat dari nilai indeks fraksi konsentrasi inhibitor (FKI). Efek sinergis terjadi apabila nilai FKI kurang atau sama dengan (≤) 0,5; efek aditif ditunjukkan apabila nilai FKI 0,5 hingga kurang atau sama dengan (≤) 1; efek *indifferent* ditunjukkan apabila nilai FKI 1 sampai 4, dan efek antagonis ditunjukkan apabila nilai FKI lebih besar (>) 4 (Costa *et al.,* 2019).

Tabel 5. Hasil efek kombinasi ekstrak etanol bunga gemitir dan alga merah terhadap bakteri *P. acnes* ATCC 1223. Keterangan: Data diperoleh dari 3 kali eksperimen. Indeks FKI dihitung dari nilai KHM tunggal dan KHM kombinasi masing-masing ekstrak.

Sampel Uji	KHM Tunggal	Sampel Uji	KHM Tunggal
Ekstrak Etanol Bunga Gemitir	4	2	0,5312
Ekstrak Etanol Alga Merah	16	0,5	(Efek Aditif)

Berdasarkan perhitungan indeks FKI yang telah dilakukan, diperoleh hasil sebesar 0,5312 yang menunjukkan adanya efek aditif kombinasi kedua ekstrak pada aktivitasnya terhadap bakteri *P. acnes*. Efek aditif merupakan suatu kondisi dimana efek kombinasi ekstrak sama dengan jumlah efek dari masing-masing ekstrak. Hal ini menunjukkan bahwa kedua ekstrak tersebut tidak

saling menghambat atau memperkuat, melainkan bekerja bersama-sama untuk menghasilkan efek antibakteri yang lebih baik (Kurniawidjaja *et al*., 2021). Berdasarkan penelitian ini, kombinasi antara ekstrak etanol bunga gemitir dan alga merah dapat dipertimbangkan untuk menjadi bahan aktif dalam pengembangan sediaan farmasi untuk mengatasi infeksi yang disebabkan oleh bakteri *P. acnes*.

3.7. Formulasi dan evaluasi sediaan krim *Solid Lipid Nanoparticle* (SLN)

Hasil pengujian mikrodilusi kombinasi ekstrak bunga gemitir dan alga merah dijadikan patokan dalam menentukan variasi konsentrasi ekstrak yang diterapkan pada formulasi sediaan krim *Solid Lipid Nanoparticle* (SLN) yaitu formula F1, F2, F3, dan F4 (Tabel 6).

Tabel 6. Formulasi sediaan krim *Solid Lipid Nanoparticle* (SLN) kombinasi ekstrak etanol bunga gemitir dan alga merah.

Bahan	Jumlah Bahan (g)				
	F0	F1	F2	F3	F4
Ekstrak Bunga Gemitir	-	0,1	0,1	0,1	0,1
Ekstrak Alga Merah	-	0,025	0,05	0,1	0,2
Gliseril Monostearat	1	1	1	1	1
Tween 80	3	3	3	3	3
Asam stearat	6	6	6	6	6
Adeps lanae	1	1	1	1	1
Setil alkohol	1	1	1	1	1
Trietanolamin	0,1	0,1	0,1	0,1	0,1
Optiphen	0,05	0,05	0,05	0,05	0,05

Variasi konsentrasi ekstrak berdasarkan aktivitas antibakteri terbaik dari pengujian mikrodilusi *checkerboard*. Formulasi krim Solid Lipid Nanoparticle (SLN) dimulai dengan pembuatan dispersi SLN, ekstrak bunga gemitir dan alga merah diinkorporasikan ke dalam lipid yang didispersikan menggunakan gliseril monostearat dan *Tween* 80. Campuran dispersi jernih kemudian diuji kualitatif melalui pengukuran persentase transmitan menggunakan spektrofotometri UV-Vis pada panjang gelombang 850 nm. Persentase transmitan yang mendekati 100% bahwa ukuran partikel lipid sudah dalam bentuk nanopartikel (Daskar dkk., 2024). Berdasarkan evaluasi fisik sediaan krim Solid Lipid Nanoparticle (SLN) (Tabel 7), formula F3 menunjukkan sediaan krim *Solid Lipid Nanoparticle* (SLN) dengan stabilitas fisik terbaik sedangkan formula F4 memenuhi parameter evaluasi sediaan krim *Solid Lipid Nanoparticle* (SLN) meliputi organoleptis, pH, daya sebar dan daya lekat, namun tekstur sediaan yang dihasilkan kurang rata, sehingga memerlukan optimasi formula lanjutan untuk memperoleh sediaan yang lebih homogen.

3.8. Aktivitas antibakteri sediaan krim *Solid Lipid Nanoparticle* (SLN) kombinasi ekstrak etanol bunga gemitir dan alga merah terhadap Bakteri *P. acnes*

Pengujian aktivitas antibakteri sediaan krim *Solid Lipid Nanoparticle* (SLN) kombinasi ekstrak etanol bunga gemitir dan alga merah dilakukan menggunakan metode difusi sumuran.

Aktivitas antibakteri yang dari sediaan dapat dilihat melalui zona bening yang terbentuk di area sekitar lubang sumuran (Retnaningsih *et al*., 2019). Keunggulan dari metode difusi sumuran adalah kemudahan dalam menghitung diameter zona bening karena sediaan yang diuji tidak hanya beraktivitas di bagian permukaan agar saja, melainkan hingga mencapai bagian dasar agar (Kirtanayasa, 2022). Hasil pengujian aktivitas antibakteri sediaan krim *Solid Lipid Nanoparticle* (SLN) terhadap bakteri *P. acnes* (Tabel 8).

Kemampuan daya hambat antibakteri dapat dibedakan menjadi beberapa kategori, yaitu lemah (≤5 mm), sedang (5-10 mm), kuat (10-20 mm), dan sangat kuat (≥20 mm) (Fiana *et al*., 2020). Data pada Tabel 8 menunjukkan bahwa hanya formula F4 memiliki aktivitas antibakteri yang kuat terhadap *P. acnes* dengan diameter zona bening sebesar 16,76 mm. Aktivitas antibakteri kombinasi ekstrak bunga gemitir dan alga merah atau sediaan yang mengandung kombinasi kedua ekstrak tersebut belum pernah dilaporkan sebelumnya. Akan tetapi, aktivitas antibakteri ekstrak atau sediaan yang mengandung ekstrak tersebut secara individual terhadap *S. aureus* maupun *P. acnes* telah dilaporkan dalam berbagai studi. Pada penelitian yang dilakukan oleh Meetong *et al.* (2023), sediaan gel topikal yang mengandung ekstrak bunga gemitir dengan konsentrasi 0,625% dan 0,125% dilaporkan memiliki aktivitas kuat terhadap bakteri *S. aureus* dengan diameter zona hambat sebesar 15,15 mm. Penelitian oleh Cahyaningrum *et al.* (2023) juga menunjukkan bahwa sediaan krim yang mengandung ekstrak etanol bunga gemitir memiliki mampu menghambat pertumbuhan bakteri *S. aureus* dengan diameter zona hambat terbesar 10,23 mm pada formula krim dengan konsentrasi ekstrak 100% dan diameter zona hambat terkecil sebesar 8,59 mm pada formula krim dengan konsentrasi ekstrak 25%.

Tabel 7. Hasil uji evaluasi sediaan krim *Solid Lipid Nanoparticle* (SLN) kombinasi ekstrak etanol bunga gemitir dan alga merah. Keterangan: *Sumber pustaka = Tungadi *et al*. (2023).

Uji Evaluasi	F0	F1	F2	F3	F4	Syarat Uji Evaluasi*
Organoleptis	Sesuai syarat	Sesuai syarat	Sesuai syarat	Sesuai syarat	Sesuai syarat	Bau tidak tengik dan warna tidak berubah
Homogenitas	Tekstur rata	Tekstur rata	Tekstur rata	Tekstur rata	Tekstur kurang rata	Tekstur rata
pH	6,32	5,92	5,84	5,79	5,55	pH 4,5-6,5
Daya Sebar (cm)	5,6	5,4	5,2	5,2	5	Daya sebar 5-7 cm
Daya Lekat (detik)	1	4	4	6	9	Tidak kurang dari 4 detik

Selanjutnya, pengujian aktivitas antibakteri sediaan gel ekstrak alga merah terhadap bakteri *P. acnes* menunjukkan aktivitas sangat kuat pada formula yang mengandung ekstrak dengan konsentrasi 1%, 3%, dan 5% dengan diameter zona hambat tertinggi sebesar 38,79 mm pada pengujian dengan metode difusi agar (Awaluddin *et al*., 2023). Pada penelitian Sabaani *et al.* (2019), sediaan sabun cair yang mengandung kombinasi ekstrak *Sargassum* sp. dan *Eucheuma* sp. dilaporkan memiliki aktivitas antibakteri sangat kuat pada pengujian antibakteri

terhadap bakteri *P. acnes,* dan diperoleh diameter zona hambat tertinggi sebesar 25,60 mm pada formula yang mengandung 10 mL ekstrak *Sargassum* sp. dan 30 mL ekstrak *Eucheuma* sp. Sementara itu, diameter zona hambat terkecil yang diperoleh adalah 14,00 mm pada formula yang mengandung 20 mL ekstrak *Sargassum* sp. dan 20 mL ekstrak *Eucheuma* sp.

Tabel 8. Hasil uji aktivitas antibakteri sediaan krim *Solid Lipid Nanoparticle* (SLN) Kombinasi ekstrak etanol bunga gemitir dan alga merah terhadap *P. acnes* ATCC 1223. Data diperoleh dari 3 eksperimen; krim klindamisin digunakan sebagai kontrol positif dan formula krim *Solid Lipid Nanoparticle* (SLN) tanpa ekstrak (F0) digunakan sebagai kontrol negatif.

Formulasi Krim *Solid Lipid Nanoparticle* (SLN)	**Diameter Zona Hambat (mm)**			**Rata-Rata Zona Hambat (mm)±SD**
	1	**2**	**3**	
Kontrol positif	30,19	28,36	28,07	28,87±0,93
F0	-	-	-	-
F1	-	-	-	-
F2	-	-	-	-
F3	-	-	-	-
F4	13,42	17,59	19,28	16,76±2,46

Keempat formula sediaan krim *Solid Lipid Nanoparticle* (SLN) yang mengandung kombinasi ekstrak etanol bunga gemitir dan alga merah yang diuji dalam penelitian ini, formula F4 yang mengandung 0,1 g ekstrak etanol bunga gemitir dan 0,2 g ekstrak etanol alga merah menghasilkan aktivitas antibakteri terbaik pada uji terhadap *P. acnes* ATCC 1223 dengan diameter zona hambat sebesar 16,76±2,46 mm. Formula F1–F3 tidak memberikan daya hambat pada bakteri uji. Berdasarkan evaluasi dan stabilitas fisik sediaan, formula F4 menghasilkan krim dengan tekstur kurang rata, tetapi secara keseluruhan hasil evaluasi sediaan berdasarkan pengamatan organoleptis, pH, daya sebar dan daya lekat menunjukkan formula F4 masih memenuhi syarat nilai uji acuan. Penelitian lebih lanjut untuk pengembangan sediaan dalam bentuk *Solid Lipid Nanoparticle* (SLN) yang mengandung kombinasi ekstrak etanol bunga gemitir dan alga merah perlu dilakukan, untuk mendapatkan sediaan dengan aktivitas antibakteri yang baik, menghasilkan sediaan yang homogen dan stabilitas fisik sediaan yang konsisten dalam rentang waktu penyimpanan sediaan.

4. KESIMPULAN

Kombinasi ekstrak bunga gemitir dan alga merah menghasilkan aktivitas antibakteri lebih tinggi daripada aktivitas masing-masing ekstrak secara individual terhadap *P. acnes* ATCC 1223, dan dikategorikan memiliki efek aditif dengan nilai FKI sebesar 0,5312. Setelah diformulasi dalam bentuk sediaan krim SLN, formula F4 yang mengandung 0,1 g ekstrak etanol bunga gemitir dan 0,2 g ekstrak etanol alga merah memberikan aktivitas antibakteri terbaik pada uji terhadap *P. acnes* dengan diameter zona hambat sebesar 16,76±2,46 mm. Hasil penelitian ini menunjukkan potensi penggunaan kombinasi ekstrak etanol bunga gemitir dan alga merah sebagai alternatif sediaan untuk penanganan jerawat yang diakibatkan oleh infeksi *P. acnes*. Penelitian lebih lanjut untuk mendapatkan formula sediaan SLN yang mengandung

kombinasi ekstrak bunga gemitir dan alga merah yang memberikan stabilitas fisik sediaan dan aktivitas antibakteri yang optimum perlu dilakukan.

UCAPAN TERIMAKASIH

Penulis menyampaikan terima kasih kepada Universitas Udayana dan Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi, Republik Indonesia, yang telah memberikan bantuan pendanaan dalam penelitian ini melalui Program PKM-RE Tahun 2024.

DEKLARASI KONFLIK KEPENTINGAN

Semua penulis menyatakan tidak ada konflik kepentingan terhadap naskah ini.

DAFTAR PUSTAKA

Alghazeer, R., Whida, F., Abduelrhman, E., Gammoudi, F., & Naili, M. (2013). In Vitro Antibacterial Activity of Alkaloid Extracts from Green, Red and Brown Macroalgae from Western Coast of Libya. *African Journal of Biotechnology,* 12(51), 7086-7091. https://doi.org/10.5897/AJB2013.13223.

Amalia, A., Sari, I., & Nursanty, R. (2017). Aktivitas Antibakteri Ekstrak Etil Asetat Daun Sembung (*Bluema balsamifera* (L.) DC.) terhadap Pertumbuhan Bakteri *Methicillin Resistant Staphylococcus aureus* (MRSA). *Prosiding Seminar Nasional Biotik 2017*, Aceh, 5(1), 387-391. http://dx.doi.org/10.22373/pbio.v5i1.2160.

Andriani, Z., Fasya, A. G., & Hanapi, A. (2015). Antibacterial Activity of the Red Algae *Eucheuma cottonii* Extract from Tanjung Coast, Sumenep Madura. *ALCHEMY,* 4(2), 93-100. https://doi.org/10.18860/al.v4i2.3197.

Arianto, A., Dalimunthe, A., & Aminah, S. (2022). Evaluation of Ethanol Extract of Red Algae (*Kappahycus alvarezii* Doty): Total Phenolic and Flavonoid Content. *Indonesian Journal of Pharmaceutical and Clinical Research,* 5(2), 30-38. https://doi.org/10.32734/idjpcr.v5i2.8871.

Aristyanti, N. P. P., Wartini, N. M., & Gunam, I. B. W. (2017). Rendemen dan Karakteristik Ekstrak Pewarna Bunga Kenikir (*Tagetes erecta* L.) pada Perlakuan Jenis Pelarut dan Lama Ekstraksi. *Jurnal Rekayasa dan Manajemen Agroindustri,* 5(3), 13-23. https://ojs.unud.ac.id/index.php/jtip/article/view/34075.

Aryani, R., Hidayat, A. F., Darma, G. C. E., & Utami, O. (2019). Effect of Solid Lipid Nanoparticles System on the Stability of Green Tea Leaves (*Camellia sinensis* L. Kuntze) Extract as Sunscreen. *Journal of Physics*, 1375, 1-8. 10.1088/1742-6596/1375/1/012078.

Awaluddin, N., Thayeb, A, M. D. R., Utari, A. U., Awaluddin, A., & Caronge, N. P. (2023). Formulation and Activity Test of Red Algae Extract Gel (*Eucheuma cottonii*) Against *Propionibacterium acnes* Bacteria. *Jurnal Penelitian Pendidikan IPA,* 9(11), 1-8. https://doi.org/10.29303/jppipa.v9i11.5513.

Ayu, D. I. & Destiwati, R. (2022). Komunikasi Intrapersonal Remaja Putri Berjerawat dalam Meningkatkan Kepercayaan Dirinya. *MEDIALOG: Jurnal Ilmu Komunikasi*, 5(1), 259-267. http://dx.doi.org/10.35326/medialog.v5i1.1338.

Cahyaningrum, P. L. & Widyantari, A. A. A. S. S. (2023). Antibacterial Activity of Marigold Flower (*Tagetes erecta* L.) Ethanol Extract Cream against *Staphylococcus aureus*. *Journal of Vocational Health Studies,* 6, 165-172. https://doi.org/10.20473/jvhs.V6.I3.2023.165-172.

Cahyaningrum, P. L., Yuliari, S. A. M., & Mediastari, A. P. A. (2020). Efektivitas Antibakteri Sediaan Sabun Bunga Gemitir (*Tagetes erecta* L.) terhadap *Staphylococcus aureus* dan

Escherichia coli. *The Journal of Muhammadiyah Medical Laboratory Technologist*, 3(2), 11-24. https://doi.org/10.30651/jmlt.v3i2.5374.

Candra, L. M. M., Andayani, Y., & Wirasisya, D. G. (2021). Pengaruh Metode Ekstraksi terhadap Kandungan Fenolik Total dan Flavonoid Total pada Ekstrak Etanol Buncis (*Phaseolus vulgaris* L.). *Jurnal Pijar MIPA*, 16(3), 397-405. https://doi.org/10.29303/jpm.v16i3.2308.

Chaturvedi, P. & Sharma, P. (2024). Formulation and In vitro Evaluation of *Holoptelea integrifolia* Planch. Extract Loaded Solid Lipid Nanoparticles. *Letters in Applied NanoBioScience*, 13(2), 1-10. https://doi.org/10.33263/LIANBS132.088.

CLSI. (2022). *Performance Standards for Antimicrobial Susceptibility Testing, 32nd Edition.* Detroit: Clinical and Laboratory Standards Institute, Wayne, PA.

Costa, R. A., Ortega, D. B., Fulgencio, D. L. A., Costa, F. S., Araujo, T. F., & Barreto, C.C. (2019). Checkerboard Testing Method Indicates Synergic Effect of Pelgipeptins against Multidrug Resistant *Klebsiella pneumoniae*. *Biotechnology Research & Innovation*, 3(1), 187-191. http://dx.doi.org/10.1016/j.biori.2018.12.001.

Daskar, A., Utami, P. I., Astuti, I. Y., & Antoni, F. (2024). Formulasi Dan Karakterisasi Nanopartikel Ekstrak Daun Senggani (*Melastoma malabathricum* L.) pada Berbagai Variasi Komposisi Kitosan dengan Metode Gelasi Ionik. *Jurnal Farmasi Universitas Aisyah Pringsewu*, 3(2), 46-56. https://journal.aisyahuniversity.ac.id/index.php/JFA/article/view/najimis

Depkes RI. (2014). *Farmakope Indonesia. Edisi V*. Jakarta: Departemen Kesehatan Republik Indonesia.

Fahrul, M., Sari, I., & Iriani, D. (2021). Efektivitas Antibakteri Ekstrak Rumput Laut (*Eucheuma cottonii*) dengan Pelarut Berbeda. *Jurnal Agroindustri Halal,* 7(1), 1-8. https://doi.org/10.30997/jah.v7i1.3253.

Fiana, F. M., Kiromah, N. Z. W., & Purwanti, E. (2022). Aktivitas Antibakteri Ekstrak Etanol Daun Sukun (*Artocarpus altilis*) terhadap Bakteri *Staphylococcus aureus* dan *Escherichia coli*. *Jurnal Farmasi Indonesia,* 10-20. https://doi.org/10.23917/pharmacon.v0i0.10108.

Ghasemiyeh, P. & Samani, S. M. (2020). Potential of Nanoparticles as Permeation Enhancers and Targeted Delivery Options for Skin: Advantages and Disadvantages. *Drug Design, Development and Therapy*, 14, 3271-3289. https://doi.org/10.2147/DDDT.S264648.

Gupta, G., Hamawandi, B., Sheward, D. J., Murrell, B., Hanke, L., Mclnerney, G., Blosi, M., Costa, A. L., Toprak, M. S., & Fadeel, B. (2022). Silver Nanoparticles with Excellent Biocompatibility Block Pseudotyped SARS-CoV-2 in the Presence of Lung Surfactant. *Frontiers in Bioengineering and Biotechnology*, 10, 1-16. https://doi.org/10.3389/fbioe.2022.1083232.

Hakim, A. R. & Saputri, R. (2020). Narrative Review: Optimasi Etanol sebagai Pelarut Senyawa Flavonoid dan Fenolik. *Jurnal Surya Medika,* 6(1), 177-180. https://doi.org/10.33084/jsm.v6i1.1641.

Ismiyati, N. & Tri Lestari. (2014). Pengembangan Formulasi Masker Ekstrak Air Daun Alpukat (*Persea americana* Mill) sebagai Antibakteri *Staphylococcus aureus* untuk Pengobatan Jerawat. *Pharmaciana*, 4(1), 1-9. https://doi.org/10.12928/pharmaciana.v4i1.397.

Jafar, G., Agustin, E., & Puryani, D. (2019). Pengembangan Formula *Solid Lipid Nanoparticles* (SLN) Hidrokortison Asetat. *Jurnal Pharmascience,* 6(1), 83-96. https://doi.org/10.20527/jps.v6i1.6080.

Kemenkes RI. (2017). *Farmakope Herbal Indonesia, Edisi II.* Jakarta: Kementerian Kesehatan Republik Indonesia.

Kirtanayasa, I. G. Y. A. (2022). Literatur Review: Aktivitas Antibakteri Beberapa Ekstrak Tanaman terhadap Bakteri Klebsiella Pneumonia. *Gema Agro,* 27(2), 107-111. https://doi.org/10.22225/ga.27.2.5389.107-111.

Kurnia, D., Suhardiman, A., Nurdiansyah, H., & Ghazali, M. (2022). Aktivitas Antibakteri Ekstrak dan Fraksi Makroalga *Eucheuma cottoni* terhadap Bakteri Penyebab Jerawat. *Jurnal Agrotek Ummat,* 9(2), 86-94. https://doi.org/10.31764/jau.v9i2.6161.

Kurniawidjaja, L. M., Lestari, F., Tejamaya, M., & Ramdhan, D. H. (2021). Konsep Dasar Toksikologi Industri. Jakarta: Fakultas Kesehatan Masyarakat Universitas Indonesia.

Kusmiati, Tamat, S. R., & Ilmiarti, T. A. (2015). Isolasi Lutein dari Bunga Kenikir (*Tagetes erecta* L.) dan Identifikasi Menggunakan Fourier Transformed Infra Red dan Kromatografi Cair Spektrometri Massa. *Jurnal Ilmu Kefarmasian Indonesia,* 13(2), 123-130. http://jifi.farmasi.univpancasila.ac.id/index.php/jifi/article/view/64.

Lumbessy, S. Y., Setyowati, D. N., Mukhlis, A., Lestari, D. P., & Azhar, F. (2020). Komposisi Nutrisi dan Kandungan Pigmen Fotosintesis Tiga Spesies Alga Merah (*Rhodophyta sp*.) Hasil Budidaya. *Journal of Marine Research,* 9(4), 431-438. https://doi.org/10.14710/jmr.v9i4.28688.

Marhaeny, A., Pramesti, R., & Suryono, S. (2024). Senyawa Bioaktif *Kappaphycus alvarezii* (Doty) Doty, 1988 Sebagai Antibakteri. *Journal of Marine Research*, 13(1), 121-126. https://doi.org/10.14710/jmr.v13i1.40459.

Maryam, F., Taebe, B., & Toding, D. P. (2020). Pengukuran Parameter Spesifik Dan Non Spesifik Ekstrak Etanol Daun Matoa (*Pometia pinnata* J.R & G.Forst). *Jurnal Mandala Pharmacon Indonesia*, 6(1), 1-12. https://doi.org/10.35311/jmpi.v6i01.39.

Meetong, P., Ananchaiphatthana, P., Asawahame, C., Thanarangsarit, P., & Jutiviboonsuk, A. (2023). Formulation of Topical Gel Containing *Tagetes erecta* L. Floral Extract and Its Antibacterial Activity. *Pharmaceutical Sciences Asia,* 50(2), 157-162. https://doi.org/10.29090/psa.2023.02.22.369.

Meletiadis, J., Pournaras, S., Roilides, E., & Walsh, T. J. (2010). Defining Fractional Inhibitory Concentration Index Cutoffs for Additive Interactions Based on Self-Drug Additive Combinations, Monte Carlo Simulation Analysis, and In Vitro-In Vivo Correlation Data for Antifungal Drug Combinations against *Aspergillus fumigatus*. *Antimicrobial Agents and Chemotherapy,* 54, 602-609. https://doi.org/10.1128/aac.00999-09.

Moravkar, K. K., Laddha, U. D., Patil, M. D., Kale, S. S., Girase, N., Bhairav, B. A., Bhaumik, J., & Chalikwar, S. S. (2023). Extraction of Rutin from *Tagetes erecta* (Marigold) and Preparation of Peroral Nano-Suspension for Effective Antitussive/Expectorant Therapy. *Carbohydrate Polymer Technologies and Applications,* 5, 1-8. https://doi.org/10.1016/j.carpta.2023.100320.

Netto, G., & Jose, J. (2017). Development, Characterization, and Evaluation of Sunscreen Cream Containing Solid Lipid Nanoparticles of Silymarin. *Journal of Cosmetic Dermatology,* 17(6), 1073-1083. https://doi.org/10.1111/jocd.12470.

Paliwal, R., Paliwal, S. R., Kenwat, R., Kurmi, B. D., & Sahu, M. K. (2020). Solid Lipid Nanoparticles: a Review on Recent Perspectives and Patents. *Expert Opinion on Therapeutic Patents*, 30(3), 179-194. https://doi.org/10.1080/13543776.2020.1720649.

Pramitha, D. A. I., Suaniti, N. M., & Sibarani, J. (2018). Aktivitas Antioksidan Bunga Pacar Air Merah (*Impatiens balsamina* L.) dan Bunga Gemitir (*Tagates erecta* L.) dari Limbah Canang. *Chimica et Natura Acta,* 6(1), 8-11. https://doi.org/10.24198/cna.v6.n1.16447.

Putra, I. P. Y. A., Utami, K. S., Hardini, J., Wirasuta, I. M. A. G., Ujam, N. T., & Ariantari, N. P. (2023). Fermentation, Bioactivity and Molecular Identification of Endophytic Fungi Isolated from Mangrove *Ceriops tagal. Biodiversitas*, 24(5), 3091-3098. https://doi.org/10.13057/biodiv/d240565.

Putri, D. D., Nurmagustina, D. E., & Chandra, A. A. (2014). Kandungan Total Fenol dan Aktivitas Antibakteri Kelopak Buah Rosela Merah dan Ungu sebagai Kandidat Feed Additive Alami pada Broiler. *Jurnal Penelitian Pertanian Terapan*, 14(3), 174-180. https://doi.org/10.25181/jppt.v14i3.157.

Rasyid, A., Wahyuningsih, T., & Ardiansyah, A. (2018). Profil Metabolit Sekunder, Aktivitas Antibakteri dan Komposisi Senyawa yang Terkandung dalam Ekstrak Metanol Teripang *Stichopus horrens. Jurnal Ilmu dan Teknologi Kelautan Tropis,* 10(2), 333-340. https://doi.org/10.29244/jitkt.v10i2.19480.

Retnaningsih, A., Primadiamanti, A., & Marisa, I. (2019). Uji Daya Hambat Ekstrak Etanol Biji Pepaya terhadap Bakteri *Escherichia coli* dan *Shigella dysentriae* dengan Metode Difusi Sumuran. *Jurnal Analisis Farmasi,* 4(2), 122-129. https://doi.org/10.33024/jaf.v4i2.2242.

Rhama, S. & Madhavan, S. (2011). Antibacterial Activity of the Flavonoid, Patulitrin Isolated from the Flowers of *Tagetes erecta* L. *International Journal of PharmTech Research,* 3(3), 1407-1409. https://sphinxsai.com/Vol.3No.3/pharm/pdf/PT=30(1407-1409)JS11.pdf.

Rupert, R., Rodrigues, K. F., Thien, V. Y., & Yong, W. T. L. (2022). Carrageenan From *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae): Metabolism, Structure, Production, and Application. *Frontiers*, 13, 1-16. https://doi.org/10.3389/fpls.2022.859635.

Sabaani, N. J., Pnearedondo, M. A. E., & Sepe, M. C. (2019). Antibacterial Activity of Liquid Soap with Combined *Sargassum* sp. and *Eucheuma* sp. Seaweed Extracts. *AACL Bioflux,* 12(5), 1514-1523. https://bioflux.com.ro/docs/2019.1514-1523.pdf.

Saifudin, A., Raharjo, S., & Eso, A. (2015). Uji Aktivitas Antibakteri Ekstrak Metanol Rumput Laut (*Kappaphycus alvarezii*) pada Berbagai Tingkat Konsentrasi terhadap Pertumbuhan Bakteri *Streptococcus mutans*. *Medula*, 3(1), 185-191. https://doi.org/10.46496/medula.v3i1.2541.

Santi, M. D. (2021). Review: Aktivitas Antioksidan Ekstrak Bunga Gemitir (*Tagetes erecta* Linn.). *Jurnal Farmagazine*, 8(1), 25-31. https://doi.org/10.47653/farm.v8i1.534

Sartoratto, A., Machado, A. L. M., Delarmelina, C., Figueira G. M., Marta, C. T. D., Vera, L., & Rehder, G. (2004). Composition and Antimicrobial Activity of Essential Oil from Aromatic Plants Used In Brazil. *Brazilian Journal of Microbiology*, 35(1), 275-280. https://doi.org/10.1590/S1517-83822004000300001.

Senduk, T. W., Montolalu, L. A. D. Y., & Dotulong, V. (2020). Rendemen Ekstrak Air Rebusan Daun Tua Mangrove *Sonneratia alba. Jurnal Perikanan dan Kelautan Tropis,* 11(1), 9-15. https://doi.org/10.35800/jpkt.11.1.2020.28659.

Sibero, H. T., Putra, I. W. A., & Anggraini, D. I. (2019). Tatalaksana Terkini Acne vulgaris. *JK Unila*, 3(2): pp.313-320. https://doi.org/10.23960/jkunila.v3i2.pp313-320.

Tayseer, I., Azzam, H., Al-Karablieh, N., Mayyas, A., & Aburjai, T. (2020). Interaction of Folk Medicinal Plants with Levofloxacin against *Escherichia coli. Journal of Pure and Applied Microciology,* 14(3), 1-7. https://doi.org/10.22207/JPAM.14.3.01.

Tungadi, R., Pakaya, M. S., & Ali, P. D. A. (2023). Formulasi dan Evaluasi Stabilitas Fisik Sediaan Krim Senyawa Astaxanthin. *Indonesian Journal of Pharmaceutical Education*, 3(1), 117-124. https://doi.org/10.37311/ijpe.v3i1.14612.

Verma, P. & Verma, A. (2012). Evaluation of Antibacterial Activity of Different Parts of *Tagetes erecta*. *International Journal of Pharmacy & Life Science,* 3(6), 1766-1768. https://scispace.com/pdf/international-journal-of-pharmacy-life-sciences-evaluation-srfln4rc0l.pdf.

Vijay, K. P., Laxman, B. C., Balasaheb, S. R., Yuvraj, N. R., & Janardhan, P. M. (2013). Pharmacognostic, Physicochemical and Phytochemical Investigation of *Tagetes erecta* Linn Flowers (Asteraceae). *Journal of Biological & Scientific Opinion*, 1(1), 21-24. 10.7897/2321–6328.01124.

Wijaya, S., Kuncahyani, I. D., & Soegianto, L. (2024). Potensi Daun Keji Beling (*Strobilanthus crispus*) sebagai Antibakteri dan Antibiofilm terhadap Bakteri *Cutibacterium acnes*.

Journal of Pharmaceutical Science and Clinical Research, 9(1), 151-163. https://doi.org/10.20961/jpscr.v9i1.74341.

Wulansari, E. D., Lestari, D., & Khoirunissa, M. A. (2020). Kandungan Terpenoid dalam Daun Ara (*Ficus carica* L.) sebagai Agen Antibakteri terhadap Bakteri *Methicillin-Resistant Staphylococcus aureus*. *PHARMACON,* 9(2), 219-225. https://doi.org/10.35799/pha.9.2020.29274.

JPSCR: Journal of Pharmaceutical Science and Clinical Research
Volume 10, Issue 1, Pages 80-89, March 2025
https://doi.org/10.20961/jpscr.v10i1.85091

ISSN 2503-331X (Online)

Pola Resistensi Bakteri Penyebab Ulkus Diabetikum terhadap Antibiotik di Salah Satu Rumah Sakit Swasta di Surakarta

Description of Bacterial Types and Antibiotic Resistance Patterns in Patient with Diabetic Ulcer Infection

Iin Novita Nurhidayati Mahmuda[1], Devi Usdiana Rosyidah[2]*, Flora Ramona Sigit Prakoeswa[3], Yulia Intan Kania[4], Ishmah Nur Faizah[4], Fathiyya Noor Afifah[4] dan Andri Sugeng Prasetyo[4]

[1]Departemen Ilmu Penyakit Dalam, Fakultas Kedokteran, Universitas Muhammadiyah Surakarta, Sukoharjo, Indonesia
[2]Laboratorium Farmakologi, Fakultas Kedokteran, Universitas Muhammadiyah Surakarta, Sukoharjo, Indonesia
[3]Departemen Ilmu Kesehatan Kulit dan Kelamin, Fakultas Kedokteran, Universitas Muhammadiyah Surakarta, Sukoharjo, Indonesia
[4]Program Studi Kedokteran Umum, Fakultas Kedokteran, Universitas Muhammadiyah Surakarta, Sukoharjo, Indonesia

*Corresponding author: dur203@ums.ac.id

Diterima: 29 Februari 2024; **Disetujui:** 14 Mei 2025; **Dipublikasi:** 9 Juni 2025

Abstrak
Infeksi ulkus diabetikum termasuk sebuah komplikasi lanjutan dari diabetes melitus yang masih terus meningkat kasusnya. Apabila belum ditangani secara cepat akan memperlaju tingkat keparahan infeksi. Tujuan penelitian ini untuk mengetahui pola resistensi bakteri penyebab ulkus diabetikum terhadap antibiotik pada pasien ulkus diaetikum. Jenis penelitian menggunakan deskriptif observasional. Sistem kompak VITEK 2 digunakan untuk identifikasi bakteri dan pengujian kerentanan antimikroba sesuai petunjuk. Kemudian, suspensi untuk inokulasi kartu AST GP 71 yang digunakan untuk uji kerentanan obat dilakukan dengan memindahkan 280µL suspensi kultur dari suspensi pertama ke dalam 3mL larutan garam steril untuk memperoleh kekeruhan akhir 10 CFU/mL. Hasil penelitian didapatkan sebanyak 11 jenis bakteri teridentifikasi dari spesimen pus, didapatkan hasil bahwa *Staphylococcus aureus* dan *Eschericia coli* mendominasi bakteri terbanyak.

Kata kunci: Antibiotik; Pola resistensi bakteri; Ulkus diabetikum

Abstract
Diabetic ulcer infection is a further complication of diabetes mellitus, and cases are still increasing. If not treated quickly, it will accelerate the severity of the infection. This study aimed to determine the resistance pattern of bacteria that cause diabetic ulcers to antibiotics in patients with diabetic ulcers. This type of research uses descriptive observational. According to instructions, the VITEK 2 compact system was used for bacterial identification and antimicrobial susceptibility testing. Then, the suspension for inoculation of the AST GP 71 card used for drug susceptibility testing was carried out by transferring 280 µL of the culture suspension from the first suspension into 3 mL of sterile saline solution to obtain a final turbidity of 10 CFU / mL. The study's results obtained 11 types of bacteria identified from pus

specimens; it was found that Staphylococcus aureus and Escherichia coli dominated the most bacteria.

Keywords**:** *Antibiotics; Bacterial resistance patterns; Diabetic ulcers*

1. PENDAHULUAN

Diabetes mellitus (DM) saat ini termasuk suatu ancaman kesehatan tingkat nasional maupun internasional. Pada tahun 2021, *International Diabetic Federation* (IDF) memperkirakan terjadi peningkatan prevalensi diabetes dilihat dari kelompok usia. Prevalensi terendah pada kategori usia 20 hingga 24 tahun (2,2%), dan tertinggi pada kategori 75 hingga 79 tahun (24,7%). Negara Indonesia masuk pada urutan ke- 5 dari 10 negara teratas yang memperoleh pasien diabetes paling tinggi pada dunia, melalui perkiraan jumlah pasien diabetes sebesar 19,5 juta orang serta diperkirakan nanti pada tahun 2045 mencapai 28,6 juta (IDF, 2021).

Diabetes melitus merupakan salah satu penyakit kronis dengan ciri khusus kenaikan kadar gula darah dan dapat menimbulkan risiko komplikasi mikrovaskuler maupun makrovaskuler (Lipsky *et al.,* 2020). Neuropati perifer adalah salah satu komplikasi mikrovaskuler penyakit DM yang menyebabkan hilangnya sensasi tekan dan nyeri pada luka di kaki. Apabila kaki diabetik ini tidak segera diobati dapat meningkatkan risiko terjadinya ulkus diabetikum (Armstrong, 2017).

Ulkus diabetikum merupakan infeksi kronis yang sering ditemukan pada pasien diabetes. Insidensi ulkus diabetikum diperkirakan meningkat hingga 34% di seluruh dunia (McDermott *et al*., 2023). Risiko terkena ulkus diabetikum juga tergolong tinggi di Indonesia, karena banyak pasien diabetes yang tidak terdiagnosis dengan baik (Waspadji, 2014). Ulkus diabetikum ditandai dengan kulit kaku kering, bekas luka, kelainan bentuk dan warna kuku, kaki baal dan kesemutan (PERKENI, 2021). Lokasi ulkus diabetikum tidak hanya pada kaki bisa juga terdapat pada pantat. Infeksi bakteri merupakan penyebab amputasi terbanyak ulkus diabetikum pada kaki (Al-Rubeaan *et al*., 2015).

Resistensi antibiotik adalah suatu keadaan ketika antibiotik digunakan secara tidak rasional. Penelitian sebelumnya di RS Abdul Wahab Sjahrane yang mengungkapkan bahwa antibiotik yang mengalami resistensi didominasi dari Bakteri Gram Negatif. Bakteri Gram Positif yang sering mengalami resistensi adalah *Staphylococcus aureus* dengan hasil resisten terhadap amoksisilin, ampisilin, seftriakson, sefotaksim, dan meropenem, sementara Bakteri Gram Negatif yang sering mengalami resistensi adalah *Pseudomonas aeruginosa* yang resisten terhadap amksisilin, seftriakson, sefotaksim, dan amikasin serta *Acetinobacter baumanii* yang resisten terhadap amoksisilin (Setianingsih *et al*., 2016). Pada penelitian di RSUP Sanglah, didapati jumlah bakteri penyebab infeksi ulkus diabetikum mayoritas merupakan Bakteri Gram Negatif (75%) dengan hasil uji resistensi antibiotik pada kedua bakteri gram menunjukkan resisten tinggi terhadap ampisilin, amikasin, ciprofloxacin, klindamisin, eritromisin, vankomisin, dan benzilpenisilin (Salim *et al*., 2020).

Peningkatan resistensi terhadap antibiotik akan mempengaruhi lama perawatan di rumah sakit, yaitu meningkatnya angka mortalitas dan morbiditas sehingga memungkinkan terjadinya komplikasi selama perawatan (Frieri *et al*., 2017). Oleh karena itu, analisis pola bakteri penyebab ulkus diabetikum sangat penting untuk dilakukan, agar penggunaan antibiotik di masa mendatang dapat lebih bijak dan mengurangi angka kejadian resistensi antibiotik. Berdasarkan paparan di atas, perlu adanya penelitian tentang gambaran jenis bakteri dan pola resistensi antibiotik pada pasien dengan infeksi ulkus diabetikum.

2. METODE

Penelitian ini menggunakan desain penelitian deskriptif observasional dengan metode total sampling design. Penelitian ini dilakukan di RS PKU Muhammadiyah Surakarta pada bulan Oktober hingga November 2023. Populasi yang diambil dalam penelitian ini adalah seluruh pasien ulkus diabetikum yang dirawat inap periode 1 Januari hingga dengan 31 Desember 2022. Data yang digunakan penelitian ini berupa data rekam medis yang meliputi jenis bakteri dan hasil uji sensitivitas antibiotik pada spesimen pus pasien dengan infeksi ulkus diabetikum. Sistem kompak VITEK 2 digunakan untuk identifikasi bakteri dan pengujian kerentanan antimikroba sesuai petunjuk pabrik pembuatnya. Suspensi bakteri untuk inokulasi kartu ID-GP yang digunakan untuk identifikasi otomatis 115 taksa bakteri Gram-positif yang paling signifikan yang tidak membentuk spora disiapkan dengan memindahkan sejumlah koloni kultur murni menggunakan kapas steril atau stik aplikator ke dalam 3 mL larutan garam steril dalam tabung reaksi plastik bening berukuran 12 75 mm dan kekeruhan suspensi disesuaikan dan diukur menggunakan alat pengukur kekeruhan yang disebut DensiChek. Kartu AST-GP71 yang digunakan untuk uji kepekaan obat Bakteri Gram Positif mengandung: penisilin, siprofloksasin, klindamisin, daptomisin, eritromisin, gentamisin, levofloksasin, linezolid, minosiklin, moksifloksasin, nitrofurantoin, oksasilin, quinupristin/dalfopristin, rifampisin, tetrasiklin, tigecycline, trimethoprim/sulfamethoxazole, dan vankomisin.

3. HASIL DAN PEMBAHASAN

3.1. Karakteristik responden penelitian

Karakteristik pasien ulkus diabetikum di salah satu rumah sakit swasta di Surakarta diperoleh data 57 pasien ulkus diabetikum (Tabel 1), terdiri dari 35 orang pria (61,4%) serta 22 orang wanita (38,6%). Usia didominasi berada pada rentang dewasa 28-60 tahun sebanyak 45 pasien (78,94%). Jumlah ulkus tunggal lebih dominan dibandingakan multipel sebanyak 51 orang (89,5%). Lokasi ulkus terbanyak pada kaki kiri sebanyak 25 orang (43,9%). *Outcome* pada pasien ulkus diabetikum didominasi angka hidup sebanyak 49 orang (86,0%). Komorbid paling banyak diderita yaitu hipertensi sebanyak 12 orang (21,1%) diikuti bronkopneumonia sebanyak 8 orang (14,0%), ISK sebanyak 5 orang (8,8%) dan gagal ginjal kronik sebanyak 4 orang (7,0%).

Penelitian serupa dilakukan di Denpasar menyebutkan jumlah laki-laki (46,6%) lebih rendah dibandingkan perempuan (53,4%) (Salim *et al*., 2020). Lokasi ulkus diabetikum pada kaki kanan 21 pasien (36,84%), pada kaki kiri 23 pasien (40,35%), kaki kanan-kiri 7 pasien

(12,28%) dan sisanya 6 pasien (10,52%) pada pantat . Penelitian serupa dilakukan di Banda Aceh, lokasi ulkus didominasi pada kaki kanan sebanyak 26 pasien (45,6%), pada kaki kiri sebanyak 25 pasien (43,9%) dan kaki kanan-kiri sisanya 6 pasien (10,5%) (Abidin *et al*., 2017).

Tabel 1. Presentase karakteristik pasien ulkus diabetikum di salah satu rumah sakit swasta di Surakarta.

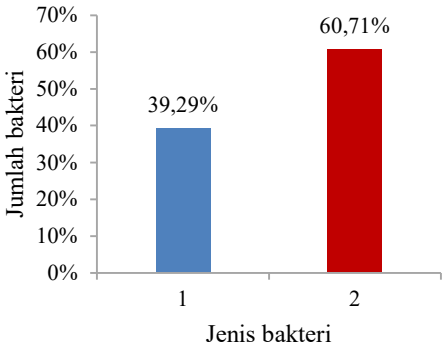
Variabel	Jumlah pasien (n)	Presentase (%)
Usia		
26-60 tahun (Dewasa)	45	78,94
>60 tahun (Lansia)	12	21,05
Jenis Kelamin		
Pria	35	61,4
Wanita	22	38,6
Jumlah ulkus		
Tunggal	51	89,5
Multipel	6	10,5
Lokasi ulkus		
Kaki kanan	22	38,6
Kaki kiri	25	43,9
Kaki kanan-kiri	4	7,0
Pantat	6	10,5
Outcome		
Hidup	49	86,0
Meninggal	8	14,0
Komorbid		
Hipertensi	12	21,1
Gagal ginjal kronik	4	7,0
Ischemic heart disease	1	1,8
Tuberkulosis paru	2	3,5
Bronkopneumonia	8	14,0
Sepsis	3	5,3
Anemia	4	7,0
ISK	5	8,8
Hipertensi + Gagal ginjal kronik	4	7,0
Hipertensi + Sepsis	2	3,5
Gagal ginjal kronik +Sepsis	1	1,8
Anemia + Sepsis	1	1,8
Tidak diketahui	10	17,54

Hasil *outcome* penelitian ini menunjukkan bahwa pasien ulkus diabetikum didominasi oleh pasien hidup sebanyak 49 pasien (86,0%), dengan sebagian besar resisten terhadap dua golongan antibiotik, yaitu Beta laktam dan Makrolida. Sementara itu, dari 8 pasien yang meninggal (14,0%), sebagian besar mengalami resistensi terhadap satu golongan antibiotik beta laktam. Hipertennsi sebagai komorbid tercatat pada 12 pasien (21,1%). Selanjutnya dari sisi usia, mayoritas pasien berada pada rentang usia dewasa 26-60 tahun. Seiring bertambahnya usia elastisitas pembuluh darah dapat menurun, menyebabkan penurunan aliran darah ke

ekstremitas, termasuk kaki. Kondisi ini dapat meningkatkan risiko iskemia atau kurangnya pasokan darah ke jaringan, yang merupakan faktor risiko ulkus diabetikum.

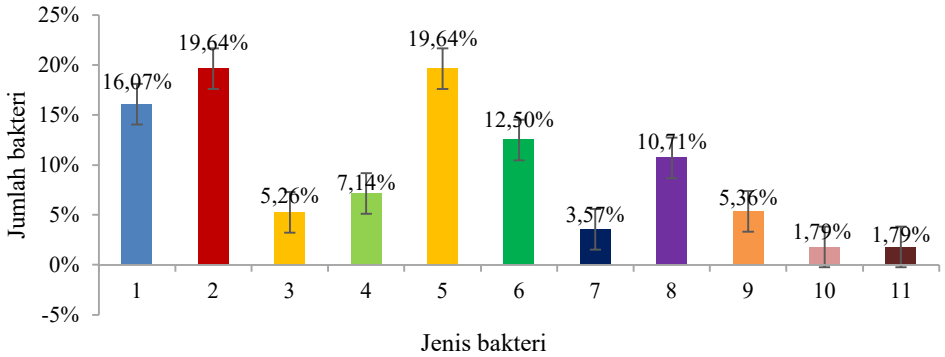
3.2. Spesimen pus

Spesimen pus atau nanah merupakan salah satu jenis spesimen klinis yang sering digunakan untuk mengidentifikasi bakteri penyebab infeksi pada pasien dengan ulkus diabetikum. Pemeriksaan kultur dari spesimen ini memberikan informasi penting mengenai jenis bakteri yang menginfeksi luka, serta menentukan strategi pengobatan yang tepat berdasarkan pola resistensi antibiotik.



Gambar 1. Jenis bakteri spesimen pus pasien ulkus diabetikum di salah satu rumah sakit swasata di Surakarta. Keterangan: jenis Bakteri Gram Positif (1) dan jenis Bakteri Gram Negatif (2).

Berdasarkan hasil penelitian yang diperoleh dari 56 pasien ulkus diabetikum, ditemukan bahwa mayoritas isolat bakteri berasal dari golongan Bakteri Gram Negatif sebanyak 60,17%, sedangkan Bakteri Gram Positif sebesar 39,29%. Presentase ini menunjukkan dominasi Bakteri Gram Negatif sebagai penyebab infeksi pada ulkus diabetikum, yang umumnya memiliki tingkat resistensi antibiotik lebih tinggi dibandingkan Bakteri Gram Positif (Gambar 1).

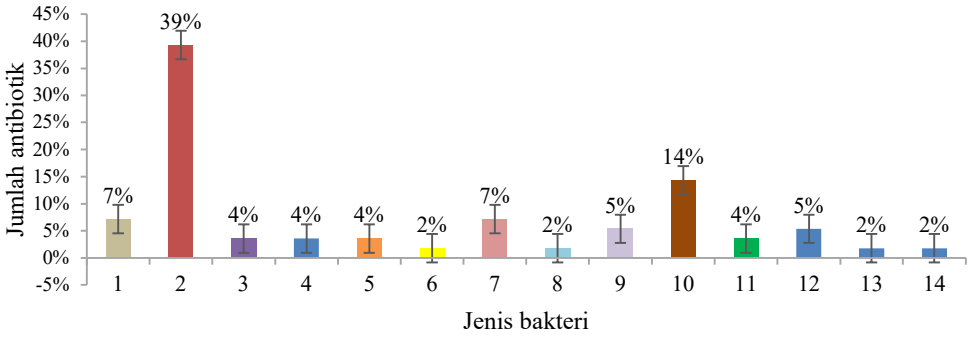


Gambar 2. Hasil kultur bakteri spesimen pus pasien ulkus diabetikum di salah satu rumah sakit swasata di Surakarta. Keterangan: *Streptococcus sp* (1), *Staphylococcus aureus* (2), *Staphylococcus haemolyticus* (3), *Klebsiella pneumonia* (4), *Escherichia coli* (5), *Pseudomonas aeruginosa* (6), *Pseudomonas fluorescens* (7), *Proteus mirabilis* (8), *Enterobacter cloacae* (9), dan *Providencia stuartii* (10).

Identifikasi jenis bakteri pada spesimen pus pasien ulkus diabetikum bertujuan untuk mengetahui patogen dominan yang menyebabkan terjadinya infeksi serta memberikan dasar pemilihan antibiotik yang rasional. Hasil identifikasi jenis bakteri menunjukkan bahwa bakteri yang paling dominan adalah *Escherichia coli* dan *Staphylococcus aureus* sebesar 19,64%, diikuti oleh *Streptococcus sp* sebanyak 16,07%, serta *Pseudomonas aeruginosa* dan *Proteus mirabilis* masing-masing sebesar 12,05% dan 10,71%. Bakteri lain seperti *Staphylococcus haemolyticus*, *Klebsiella pneumonia, Pseudomonas fluorescens, Enterobacter cloacae,* dan *Providencia stuartii* menunjukkan persentase lebih kecil (Gambar 2). Penelitian lain di RSUD. Dr. Moewardi menunjukkan bahwa Bakteri Gram Negatif didominasi oleh *Eschericia coli* sebanyak 130 pasien, sedangkan Bakteri Gram Positif terbanyak *Staphylococcus aureus* sebanyak 56 pasien (Sub Instalasi Laboratorium Mikrobiologi Klinik RSUD Dr. Moewardi, 2022).

3.3. Penggunaan antibiotik

Berdasarkan data rekam medis penggunaan antibiotik pada pasien rawat inap ulkus diabetikum (Gambar 3), penggunaan antibiotik tunggal yang palingumum pada pasien ulkus diabetikum adalah ceftriaxone (golongan sefalosporin) sebanyak 22 pasien (39%). Terapi kombinasi yang paling sering digunakan adalah ceftriaxone (golongan sefalosporin) dan metronidazole (golongan nitronidazole) sebanyak 8 pasien (14%).



Gambar 3. Data rekam medis penggunaan antibiotik pada pasien rawat inap ulkus diabetikum di salah satu rumah sakit swasata di Surakarta. Keterangan: cefotaxime (1), ceftriaxone (2), ceftazidim (3), cefuroxime (4), cefradoksil (5), amoxicillin (6), sulbactam (7), clindamycin (8), ciprofloxacin (9), ceftriaxone + metronidazole (10), cefuroxime + metronidazole (11), clindamycin + metronidazole (12), cefixime + metronidazole (13), meropenem + metronidazole (14).

Penelitian sebelumnya juga menunjukkan bahwa ceftriaxone merupakan antibiotik tunggal yang paling sering digunakan pada kasus serupa. Ceftriaxone termasuk dalam golongan antibiotik beta laktam dan mempunyai aktivitas bakterisidal, efektif terhadap Bakteri Gram Positif dan Bakteri Gram Negatif (Wright *et al*., 2023). Tujuan pemberian antibiotik kombinasi adalah memperlambat risiko resistensi bakteri dan mendapatkan efek sinergis (Kementerian Kesehatan RI, 2021). Pemberian antibiotik kombinasi pada penelitian ini mayoritas menggunakan kombinasi metronidazol dan ceftriaxone sebanyak 8 pasien (14,0%). Kombinasi

antara metronidazol dan ceftriaxone efektif dalam membunuh bakteri penyebab infeksi ulkus diabetikum (Zubair *et al*., 2011).

3.4. Pola resistensi Bakteri Gram Positif penyebab infeksi ulkus diabetikum

Pola resistensi antibiotik pada Bakteri Gram Positif di salah satu rumah sakit swasta di Surakarta (Tabel 2) menunjukkan bakteri *Staphylococcus aureus* merupakan bakteri terbanyak pertama yang resisten terhadap semua golongan antibiotik beta laktam, kuinolon, tetrasiklin, makrolida, aminoglikosida, sulfonamid, dan glikopeptida. *Streptococcus sp* menjadi bakteri terbanyak kedua dan sebagian besar resisten terhadap 3 golongan antibiotik kuinolon, tetrasiklin, dan aminoglikosida. *Staphylococcus haemolyticus* menjadi bakteri terbanyak ketiga sebagian besar resisten terhadap 5 golongan antibiotik kuinolon, tetrasiklin, makrolida, aminoglikosida, sulfonamid, dan glikopeptida. Penelitian lain di RSUD Dr. Moewardi menyebutkan *Staphylococcus aureus* terbukti sebagian besar mengalami resisten terhadap 2 golongan antibiotik Beta laktam dan Tetrasiklin (Sub Instalasi Laboratorium Mikrobiologi Klinik RSUD Dr. Moewardi, 2022).

Tabel 2. Pola resistensi Bakteri Gram Positif penyebab infeksi ulkus diabetikum terhadap golongan antibiotik. Keterangan antibiotik: AMX (amoxycillin), AMP (ampicilin), CFR (cefradoxil), CZO (cefazolin), CTX (cefotaxime), FOX (cefoxitin), CAZ (ceftazidime), CRO (ceftriaxone), CIP (ciprofloxacin), MFX (moxifloxacin HCL), LVX (levofloxacin), DOX (doxycyclin), TCY (tetracyclin), CLI (clindamycin), ERY (erythromycin), GEN (gentamycin), SXT (cotrimoksazol), VAN (vankomycin). Kriteria: >90% = Sensitif > 90% (antibiotik direkomendasikan pada pasien), >90% = Intermediet 70-90% (antibiotik masih bisa direkomendasikan pada pasien sesuai arahan dokter), >90% = Resisten < 70% (antibiotik tidak direkomendasikan pada pasien), ☐ = Antibiotik tidak diuji.

Golongan Antibiotik	Antibiotik	Bakteri Gram Positif		
		Streptococcus sp	*Staphylococcus aureus*	*Staphylococcus haemolyticus*
Beta Laktam	AMX	100%	50%	
	AMP	88,9%	50%	
	CFR	75%	70%	
	CZO	75%	66,7%	
	CTX	77,8%	45,5%	
	FOX	75%	60%	
	CAZ	75%	40%	
	CRO	75%	55,6%	100%
Kuinolon	CIP	57,1%	45,5%	66,7%
	MFX	66,7%	36,4%	100%
	LVX	66,7%	27,3%	33,3%
Tetrasiklin	DOX	25%	50%	
	TCY	33%	27,3%	66,7%
Makrolida	CLI	75%	81,8%	66,7%
	ERY	77,8%	63,6%	66,7%
Aminoglikosida	GEN	37,5%	50%	0%
Sulfonamid	SXT	87,5%	54,5%	66,7%
Glikopeptida	VAN	87,5%	70%	33,3%

3.5. Pola resistensi Bakteri Gram Negatif penyebab infeksi ulkus diabetikum

Pola resistensi antibiotik pada Bakteri Gram Negatif di salah satu rumah sakit swasta di Surakarta (Tabel 3) menunjukkan bakteri *Eschericia coli* merupakan bakteri terbanyak pertama yang menunjukkan sebagian besar resisten terhadap 7 golongan antibiotik beta laktam, aminoglikosida, polipeptida, tetrasiklin, sulfonamid, kuinolon, dan kloramfenikol. *Pseudomonas aeruginosa* menjadi bakteri terbanyak kedua yang resisten terhadap semua golongan antibiotik beta laktam, aminoglikosida, karbapenem, polipeptida, tetrasiklin, sulfonamid, kuinolon, dan kloramfenikol. *Proteus mirabilis* menjadi bakteri terbanyak ketiga yang sebagian besar resisten terhadap 7 golongan antibiotik beta laktam, aminoglikosida, polipeptida, tetrasiklin, sulfonamid, dan kuinolon.

Tabel 3. Pola resistensi bakteri gram negative penyebab infeksi ulkus diabetikum terhadap golongan antibiotik. Keterangan antibiotik: CIP (ciprofloxacin), FEP (cefepime), CFM (cefixime), CTX (cefotaxime), CAZ (ceftazidime), CRO (ceftriaxone), AMC (amoxycillin/ clavulanat), AMP (ampicilin), AMK (amikasin), GEN (gentamisin), KAN (kanamisin), MEM (meropenem), IPM (imipenem), CHL (chloramphenicol), SAM (sulbactam), COL (colistin sulfat), FOS (fosfomycin), TCY (tetracyclin), SXT (cotrimoksazol), TZP (tazobactam), CZO (cefazolin). Kriteria: >90% = Sensitif > 90% (antibiotik direkomendasikan pada pasien), >90% = Intermediet 70-90% (antibiotik masih bisa direkomendasikan pada pasien sesuai arahan dokter), >90% = Resisten < 70% (antibiotik tidak direkomendasikan pada pasien), ☐ = Antibiotik tidak diuji. Keterangan Bakteri Gram Negatif: ECO (*Eschericia coli*), KP (*Klebsiella pneumonia sp*), PA (*Pseudomonas aeruginosa*), PF (*Pseudomonas fluorescens*), PM (*Proteus mirabilis*), ECL (*Enterobacter cloacae*), PS (*Providencia stuartii*), CF (*Citrobacter freundii*).

Golongan	Antibiotik	Bakteri Gram Negatif							
		ECO	KP	PA	PF	PM	ECL	PS	CF
Beta Laktam	FEP	60%	33,3%	20%	0%	33,3%	33,3%	100%	100%
	CFM	16,7%	0%	20%	0%	33,3%	33,3%	100%	100%
	CTX	16,7%	0%	25%	0%	40%	33,3%	0%	
	CAZ	45,5%	66,7%	28,6%	0%	50%	33,3%	100%	100%
	CRO	30%	66,7%	0%	0%	50%	33,3%	100%	100%
	AMC	83,3%	0%	0%	0%	40%	0%	0%	
	AMP	10%	0%	0%	0%	16,7%	0%	0%	0%
	CFR	14,3%	0%	0%	0%	20%	0%	0%	
	CZO	25%	100%	0%		60%			0%
	SAM	50%	0%	0%	50%	40%	33,3%	100%	0%
Aminoglikosida	AMK	62,5%	100%	33,3%	0%	50%	50%	0%	100%
	GEN	9,1%	66,7%	50%	0%	40%	50%	0%	100%
	KAN	50%	0%	0%	0%	40%	50%	0%	100%
	FOS	83,3%	100%	0%	50%	100%	100%	0%	
	TZP	90,9%	50%	42,9%	50%	100%	100%	100%	100%
Karbapenem	MEM	90,9%	100%	28,6%	50%	100%	100%	100%	100%
	IPM	100%	100%	0%	50%	100%	100%	100%	
Polipeptida	COL	20%	100%	50%	100%	20%	50%		
Tetrasiklin	TCY	16,7%	0%	0%	0%	20%	33,3%	0%	
Sulfonamid	SXT	63,6%	0%	0%	0%	16,7%	33,3%	0%	0%
Kuinolon	CIP	18,2%	33,3%	28,6%	0%	33,3%	100%	100%	0%
Kloramfenikol	CHL	50%	100%	0%	0%	40%	0%	0%	

Klebsiella pneumonia sp menjadi bakteri terbanyak keempat yang sebagian besar resisten terhadap 5 golongan antibiotik beta laktam, aminoglikosida, tetrasiklin, sulfonamid, dan kuinolon. *Enterobacter cloacae* menjadi bakteri gram kelima yang sebagian besar resisten

terhadap 6 golongan antibiotik beta laktam, aminoglikosida, polipeptida, tetrasiklin, sulfonamid, dan kuinolon. *Providencia stuartii* menjadi bakteri gram keenam yang sebagian besar resisten terhadap 5 golongan antibiotik beta laktam, aminoglikosida, tetrasiklin, sulfonamid, dan kloamfenikol. *Citrobacter freundii* sebagian besar intermediet dan sisanya mengalami resisten terhadap 3 golongan antibiotik beta laktam, sulfonamid dan kuinolon.

Pada Bakteri Gram Negatif terbanyak *Eschericia coli* sebagian besar resisten terhadap 7 golongan antibiotik misalnya, beta laktam, aminoglikosida, polipeptida, tetrasiklin, sulfonamid, kuinolon, dan kloramfenikol. Penelitian serupa di RSUD Dr. Moewardi menyebutkan *Eschericia coli* terbukti sebagian besar resisten terhadap 3 golongan antibiotik beta laktam, kuinolon, dan aminoglikosida (Sub Instalasi Laboratorium Mikrobiologi Klinik RSUD Dr. Moewardi, 2022). Penelitian lain menyebutkan *Methicillin resistant staphylococcus aureus* yang ditemukan pada 4 kasus, seluruhnya resisten terhadap benzylpenicillin, ciprofloxacin, levofloxacin, dan moxifloxacin (Salim *et al.,* 2020).

Keterbatasan penelitian ini adalah pengambilan sampel tidak mempertimbangkan waktu pemberian antibiotik, sebaiknya sampel diambil sebelum pemberian antibiotik. Selain itu, pengambilan sampel pada penelitian ini tidak mempertimbangkan faktor-faktor yang dapat mempengaruhi pola resistensi, seperti riwayat perawatan sebelumnya. Pola resistensi antibiotik yang merupakan hasil dari penelitian ini tidak dapat diterapkan di rumah sakit dengan tingkat pelayanan maupun daerah yang berbeda. Pada penelitian ini, beberapa antibiotik belum dilakukan uji sensitivitas antibiotik, sehingga belum diketahui pasti apakah antibiotik tersebut dapat diberikan kepada pasien ulkus diabetikum.

4. KESIMPULAN

Jumlah Bakteri Gram Negatif lebih dominan sebagai penyebab infeksi ulkus diabetikum dibandingkan Bakteri Gram Positif di salah satu rumah sakit swasta di Surakarta. Jenis Bakteri Gram Positif penyebab infeksi ulkus diabetikum terbanyak adalah *Staphylococcus aureus* sebanyak 11 pasien, sedangkan Bakteri Gram Negatif terbanyak adalah *Eschericia coli* sebanyak 11 pasien. Pola resistensi antibiotik tertinggi pada Bakteri Gram Positif ditunjukkan oleh *Staphylococcus aureus*, yang resisten terhadap seluruh golongan antibiotik beta laktam, kuinolon, tetrasiklin, makrolida, aminoglikosida, sulfonamid, dan glikopeptida. Sementara itu, *Eschericia coli* menunjukkan pola resistensi antibiotik Bakteri Gram Negatif tertinggi terhadap golongan antibiotik beta laktam, aminoglikosida, polipeptida, tetrasiklin, sulfonamid, kuinolon, dan kloramfenikol.

UCAPAN TERIMAKASIH

Penulis mengucapkan terima kasih kepada pihak rumah sakit yang telah memberikan izin pengambilan data penelitian.

DEKLARASI KONFLIK KEPENTINGAN

Semua penulis menyatakan tidak ada konflik kepentingan terhadap naskah ini.

DAFTAR PUSTAKA

Abidin, Z., Meuraxa, R., Aceh, B., Fitria, E., Nur, A., Marissa, N., & Ramadhan, N. (2017). *Karakteristik Ulkus Diabetikum pada Penderita Diabetes Mellitus di RSUD dr . Zainal Abidin dan RSUD Meuraxa Banda Aceh*. 153–160.

Al-Rubeaan, K., Al Derwish, M., Ouizi, S., Youssef, A. M., Subhani, S. N., Ibrahim, H. M., & Alamri, B. N. (2015). Diabetic foot complications and their risk factors from a large retrospective cohort study. *PLoS ONE*, *10*(5), 1–17. https://doi.org/10.1371/journal.pone.0124446.

Armstrong, D. G., Boulton, A. J. M., & Bus, S. A. (2017). Diabetic Foot Ulcers and Their Recurrence. *New England Journal of Medicine*, *376*(24), 2367–2375. https://doi.org/10.1056/nejmra1615439

Frieri, M., Kumar, K., & Boutin, A. (2017). Antibiotic resistance. *Journal of Infection and Public Health*, *10*(4), 369–378. https://doi.org/10.1016/j.jiph.2016.08.007.

IDF. (2021). International Diabetes Federation. In *Diabetes Research and Clinical Practice* (Vol. 102, Issue 2). https://doi.org/10.1016/j.diabres.2013.10.013.

Kementerian Kesehatan Republik Indonesia. (2021). Pedoman Penggunaan Antibiotik. *Pedoman Penggunaan Antibiotik*, 1–97.

Lipsky, B. A., Senneville, É., Abbas, Z. G., Aragón-Sánchez, J., Diggle, M., Embil, J. M., Kono, S., Lavery, L. A., Malone, M., van Asten, S. A., Urbančič-Rovan, V., & Peters, E. J. G. (2020). Guidelines Diabetic foot infection. *Diabetes/Metabolism Research and Reviews*, *36*(S1), 1–24. https://doi.org/10.1002/dmrr.3280.

McDermott, K., Fang, M., Boulton, A. J. M., Selvin, E., & Hicks, C. W. (2023). Etiology, Epidemiology, and Disparities in the Burden of Diabetic Foot Ulcers. *Diabetes Care*, *46*(1), 209–211. https://doi.org/10.2337/dci22-004.

PERKENI. (2021). Pedoman Pengelolaan dan Pencegahan Diabetes Melitus Tipe 2 Dewasa di Indonesia 2021. *Global Initiative for Asthma*, 46. www.ginasthma.org.

Salim, S. E., Sukrama, I. D. M., Fatmawati, N. N. D., & Hendrayana, M. A. (2020). Pola Bakteri Pada Pasien Kaki Diabetik Dan Resistensinya Terhadap Antibiotik Di Rumah Sakit Umum Pusat Sanglah Periode 1 Januari 2017 – 28 Februari 2018. *Jmu*, *9*(10), 98–104.

Setianingsih, Y., Fadraersada, J., Ibrahim, A., & Ramadhan, A. M. (2016). Pola Resistensi Bakteri terhadap Antibiotik pada Pasien Diabetic Foot di RSUD Abdul Wahab Sjahranie Samarinda Periode Agustus-Oktober 2016. Proceeding of Mulawarman Pharmaceuticals Conferences, 4, 407–414. https://doi.org/10.25026/mpc.v4i1.212

Sub Instalasi Laboratorium Mikrobiologi Klinik RSUD Dr. Moewardi. (2022). *Pola Kepekaan Kuman RSUD Dr. Moewardi Periode Januari – Desember 2022*.

Zubair, M., Malik, A., & Ahmad, J. (2011). Clinico-microbiological study and antimicrobial drug resistance profile of diabetic foot infections in North India. *Foot*, *21*(1), 6–14. https://doi.org/10.1016/j.foot.2010.10.003.

Waspadji S. (2014). Komplikasi kronik diabetes: Mekanisme terjadinya, diagnosis, dan strategi pengelolaan. Dalam setiati S, Alwi I, Sudoyo AW, Simadibrata M, Setiyohadi B, Syam AF. Buku ajar ilmu penyakit dalam. Edisi ke 6. Jakarta: Pusat Penerbitan Ilmu Penyakit Dalam FKUI, pp 2359-66.

Wright, A., Wood, S., De Silva, J., & Bell, J. S. (2023). Systemic Antimicrobial Therapy for Diabetic Foot Infections: An Overview of Systematic Reviews. Antibiotics, 12(6), 1–16. https://doi.org/10.3390/antibiotics12061041.

JPSCR: Journal of Pharmaceutical Science and Clinical Research
Volume 10, Issue 1, Pages 90-99, March 2025
https://doi.org/10.20961/jpscr.v10i1.94947

ISSN 2503-331X (Online)

Antibacterial Activity of Zerumbone from Extract of *Zingiber zerumbet* (L.) Roscoe ex Smith Rhizomes

Soerya Dewi Marliyana*, Desi Suci Handayani, Nida Nafisah Hayati and Risna Ramadhani

Program Studi Kimia, Universitas Sebelas Maret, Surakarta, Indonesia

*Corresponding author: msoerya@staff.uns.ac.id

Received: November 8, 2024; **Accepted:** May 9, 2025; **Published:** June 15, 2025

Abstract

The rhizome of *Zingiber zerumbet*, known as Lempuyang Gajah, is commonly used in the community for medicinal purposes due to its diverse biological activities, particularly its antibacterial compounds. Among these compounds, zerumbone is one of the major secondary metabolites found in *Z. zerumbet* rhizomes. This research focused on the isolation of zerumbone and the exploration of its antibacterial properties. The isolation process involved the use of the maceration method with acetone as the solvent, while various chromatographic techniques such as Liquid Vacuum Chromatography, Column Chromatography, and Thin Layer Chromatography were employed for fractionation and purification of zerumbone. The identification of zerumbone was accomplished through GC-MS data analysis. The antibacterial activity of zerumbone was tested against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa* using the agar diffusion method. This compound exhibited moderate antibacterial activity against *P. aeruginosa* with an inhibition zone of 9.42 mm. The rhizome of *Z. zerumbet* can serve as an alternative source of zerumbone for further studies in the field of medicine.

Keywords: Antibacterial activity; Isolation; Zerumbone; *Zingiber zerumbet*

1. INTRODUCTION

Indonesia is a country that has a wealth of biodiversity. This diversity is an unlimited source of organic compounds. It is widely known that plants in the *Zingiberaceae* family mostly contain chemical compounds that can be used as medicinal plants in traditional medicine (Chavan et al., 2018; Haque et al., 2019). The Zingiberaceae family contains the genus Zingiber which has more than one type of species. One of the species of the genus *Zingiber* is *Zingiber zerumbet*, known as lempuyang gajah. *Z. zerumbet* rhizome has been widely used in traditional medicine in inflammation, diarrhea, stomach cramps, headache, toothache, asthma, carbuncles, cough, mouth ulcers, ear inflammation, gastritis, arthritis, fever, flatulence, allergies, poisoning, antioxidant and bacterial infections (Koga et al., 2016; Jantan et al., 2019; Akhtar et al., 2019; Ahmadabadi et al., 2019; Ramzan et al., 2022; Assiry et al., 2023).

Zerumbone is a major compound contained in several plants of the *Zingiberaceae* family, including *Z. spectabile*, *Z. roseum*, *Z. aromaticum*, *Z. zerumbet*, *Z. americans*, etc. According to some studies, zerumbone is mostly found in *Z. zerumbet* plants compared to other

Zingiber species. The zerumbone content in essential oil of *Z. zerumbet* is 90.62% (Mulyani, 2010), while in *Z. spectabile* it is 30% (Sadhu et al., 2007), and *Z. roseum* is 6.5% (Al-Amin et al., 2019). Researcher successfully identified the major constituents contained in essential oil from pentane extract of *Z. zerumbet* rhizomes including zerumbone (48.13%), α-humulene (17.23%), humulene epoxide I (7.88%), and humulene epoxide II (5.74%) (Yu et al., 2008).

Infectious diseases are still a health problem in both developing and developed countries. Patients with infectious diseases caused by bacteria are generally given therapy in the form of antibiotics. Inaccurate use of antibiotics can cause resistance in bacteria. This shows that a new antibacterial compound is needed that can overcome the resistance problem. Biological antibacterial agents can be obtained from fungi, bacteria, bacteriocins and plants (Prastiyanto et al., 2020). Zerumbone is one of the major compounds found in *Z. zerumbet* which is a terpenoid compound with a cyclic structure containing one oxygen atom, which gives it biological activity properties such as antibacterial, antifungal, and anti-inflammatory. In previous studies, zerumbone has been successfully isolated from the essential oil of *Z. zerumbet* rhizomes and is known to have better antibacterial activity when compared to its essential oil against *S. aureus* bacteria (MTCC 96) with an inhibition zone of 13 ± 2 mm (Padalia et al., 2018). Zerumbone from the essential oil of *Z. zerumbet* rhizomes also has potential as an antibacterial against *S. mutans* (Silva et al., 2018). Isolation of zerumbone is mostly obtained from essential oils which require methods that are not simple, so as another alternative, zerumbone isolation is carried out from *Z. zerumbet* extract. Isolation of zerumbone from *Z. zerumbet* rhizome extract is still limited and has not been widely reported. Therefore, it is necessary to isolate zerumbone from acetone extract of *Z. zerumbet* rhizome and test its antibacterial activity against gram-positive and gram-negative bacteria. Furthermore, *Z. zerumbet* can be one of the plant sources for obtaining zerumbone and can be used as a source of potential medicinal compounds.

2. MATERIAL AND METHODS

2.1. Materials

The materials *Z. zerumbet* rhizome from Pasar Gedhe, Surakarta. Technical solvents such as acetone, ethyl acetate, n-hexane. Silica gel 60 (0.040-0.063 mm, Merck, Germany) and TLC 60 PF254 (0.25 mm, Merck, Germany) were utilized for chromatography. Cerium (IV) (Merck, Germany) and H_2SO_4 (Merck, Germany) were used as spotting reagents. Dimethyl Sulfoxide (DMSO) (Merck, Germany), NaCl 0.9% (Merck, Germany), blank disks (6 mm), amoxicillin, and imipenem antibiotic disks (6 mm) were utilized in the antibacterial activity test. Four bacterial strains were obtained from the microbiology laboratory of the Faculty of Medicine, UNS, namely *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 11229, and *Pseudomonas aeruginosa* ATCC 27853. The research utilized a GCMS-QP2010S Shimadzu instrument, vacuum liquid chromatography (VLC), column chromatography, a rotary evaporator (RE-2010), and a UV254 lamp.

2.2. Isolation and identification of Zerumbone

The isolation of zerumbone from *Z. zerumbet* rhizomes, as described in research (Al-Amin et al., 2019), was conducted with slight modifications. A total of 1.75 kg of dried powdered rhizomes was extracted with acetone at room temperature, utilizing 4 L of solvent for 24 hours, with this process repeated three times. After filtering the extracting solvent, the filtrate was concentrated under reduced pressure at 40 °C using a rotary evaporator, yielding 125.73 g of crude acetone extract, corresponding to an 7.18% yield based on the dried powder. A 15 g portion of the acetone extract was subjected to fractionation through vacuum liquid chromatography (VLC) with a solvent mixture of n-hexane and ethyl acetate in various ratios (9.5:0.5; 9:1; 8:2; 7:3; 6:4; and 5:5), which yielded 17 fractions. TLC analysis guided the selection of fractions for purification. TLC plates were visualized under a UV lamp (λ254) and then treated with the spotting reagent $Ce(SO_4)_2$.

Based on the TLC analysis, 3.23 g of fraction E (FE) was purified using a mixture of n-hexane and ethyl acetate in a 9.7:0.3 ratio (250 mL), yielding 23 fractions. Fractions FE10-23 (58 mg) displayed a single spot as a white crystal. These fractions were then analyzed for structural identification using GC-MS spectroscopy.

2.3. Determination of antibacterial activity of Zerumbone

The determination of antibacterial activity followed the protocol outlined in reference (Clinical and Laboratory Standards Institute, 2018) with slight modifications. Antibacterial activity was assessed using the disc paper diffusion method. Specifically, paper discs (6 mm) were impregnated with a 20 µL mixture of pure isolate and negative control (DMSO) with varying concentrations of 16, 32, 64, 128, 256, 512 and 1024 (µg/mL). Bacterial suspension was prepared by diluting one inoculating loop of bacterial culture in 4 mL of sterile NaCl (0.9%), adjusting turbidity to the standard 0.5 McFarland standard. A single inoculating loop of bacteria was evenly spread in a zigzag pattern on Mueller-Hinton Agar (MHA) plates using aseptic techniques. Samples and reference antibiotics (Amoxicillin 20 µg/disc and Imipenem 20 µg/disc) along with a control (20 µL DMSO/disc), were evenly placed on the MHA plates and incubated for 24 hours at 35±2 °C. The inhibition of bacterial growth was determined by measuring the diameter of the inhibition zone.

3. RESULTS AND DISCUSSION

3.1. Isolation of Zerumbone

Maceration from *Z. zerumbet* rhizome powder produced a concentrated extract as a thick blackish brown extract. The *Z. zerumbet* rhizome extract (15 g) fractionation using VLC obtained 17 fractions (F_A-F_Q). Fraction E (F_E) forms white slightly light brown crystal-like solids which are thought to contain impure F_E compounds, so further purification is needed using column chromatography. The TLC profile of VLC results is shown in Figure 1.

Fraction F_E (3.23 g) from VLC was tested using TLC (Figure 2) to determine its separation pattern. The eluents used for the elution process with TLC were n-hexane: ethyl acetate with a ratio of (9: 1); (9.9: 0.1); (9.8: 0.2); (9.7: 0.3); (9.6: 0.4); and (9.5: 0.5).

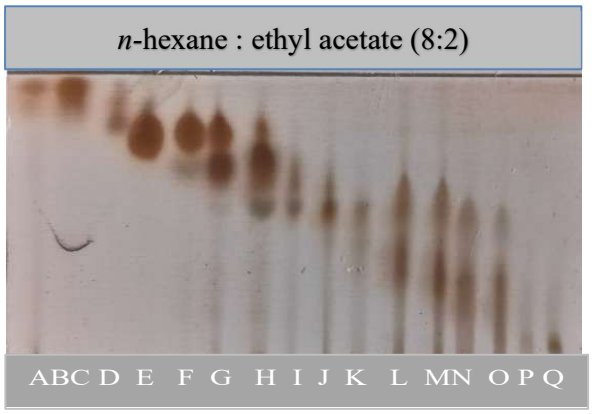


Figure 1. TLC profile of 17 fractions with n-hexane: ethyl acetate (8: 2) eluent.

Based on the TLC profile obtained in Figure 2, it can be seen that there is one elongated spot in each concentration variation. However, in this study, solvent variation was used in the ratio of 9.7: 0.3 because it was seen that the comparison produced spot stains that were lower than the other comparisons. This is done to maximize the process of separating compounds when column chromatography is performed. The results of column chromatography obtained 23 fractions which were then tested for purity using TLC. Fraction E_{1-6} is a non-polar compound because it elutes first. These compounds may be oils, fats, triterpenes, and other non-polar compounds (Ritna et al., 2016). Based on the TLC analysis, fraction E_{10-23} showed one spot on all three eluent variations and was predicted to be the target zerumbone which was further identified by GCMS. The yield of zerumbone that was successfully isolated was 1.53 g of the column chromatography results. The schematic diagram illustrating the isolation and purification process of zerumbone from the acetone extract are presented in Figure 3.

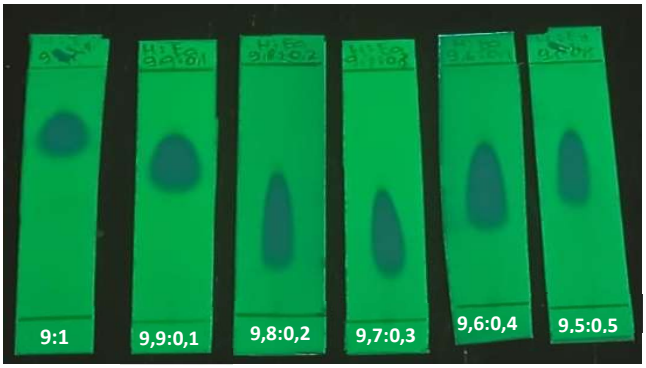


Figure 2. TLC profile of F_E with variation of n-hexane: ethyl acetate eluent ratio

The structure of fraction E_{10-23} was identified using GC-MS analysis. The GC chromatogram of isolate E_{10-23} displayed a single peak (Figure 4), indicating the presence of a single major compound. Based on the chromatogram data, it showed that the E_{10-23} isolate is pure with a retention time of 30.789 minutes and an abundance of 99.76%. The results of mass

spectra analysis of compounds detected by GC-MS contained in isolate E_{10-23} were compared with the mass spectra of standard compounds from WILEY 229.LIB.

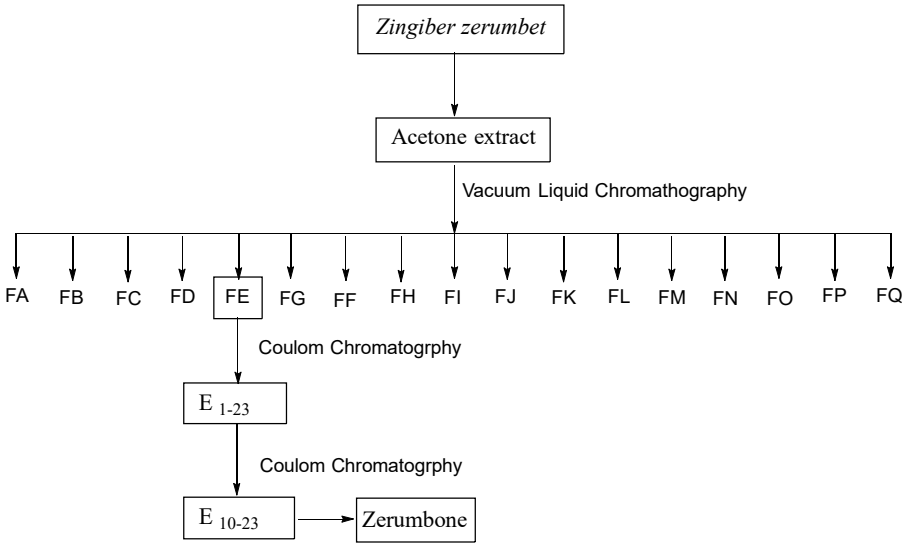


Figure 3. The schematic diagram illustrating the isolation and purification of Zerumbone from the acetone extract.

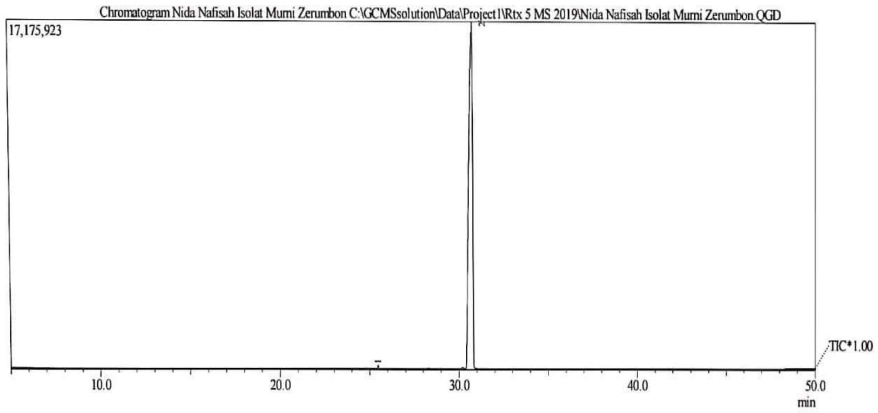


Figure 4. The GC chromatogram of compound isolate E_{10-23}.

The mass spectra of isolate E_{10-23} are shown in Figure 5a, while the mass spectra of standard compounds from the library are shown in Figure 5b. Seen in Figure 5 (a, b), the fragmentation pattern of the mass spectra of isolate E_{10-23} is almost similar to the fragmentation pattern in the mass spectra of zerumbone ($C_{15}H_{22}O$) with Similarity index (SI) 89%. Therefore, it is possible that isolate E_{10-23} is zerumbone. GC-MS data is in accordance with research conducted by Diastuti et al. (2022), which states that the GC-MS data of zerumbone obtained has a retention time of 28.146 minutes, an abundance of 96.78%, and a molecular mass of M+ 218 m/z. The mass spectrum of isolate E_{10-23} gives a peak of molecular ion M+218 which is the molecular

weight of $C_{15}H_{22}O$. Isolate $E_{10\text{-}23}$ has a base peak mass spectrum of 107 m/z. The fragmentation of compound isolate $E_{10\text{-}23}$ is shown in Figure 6.

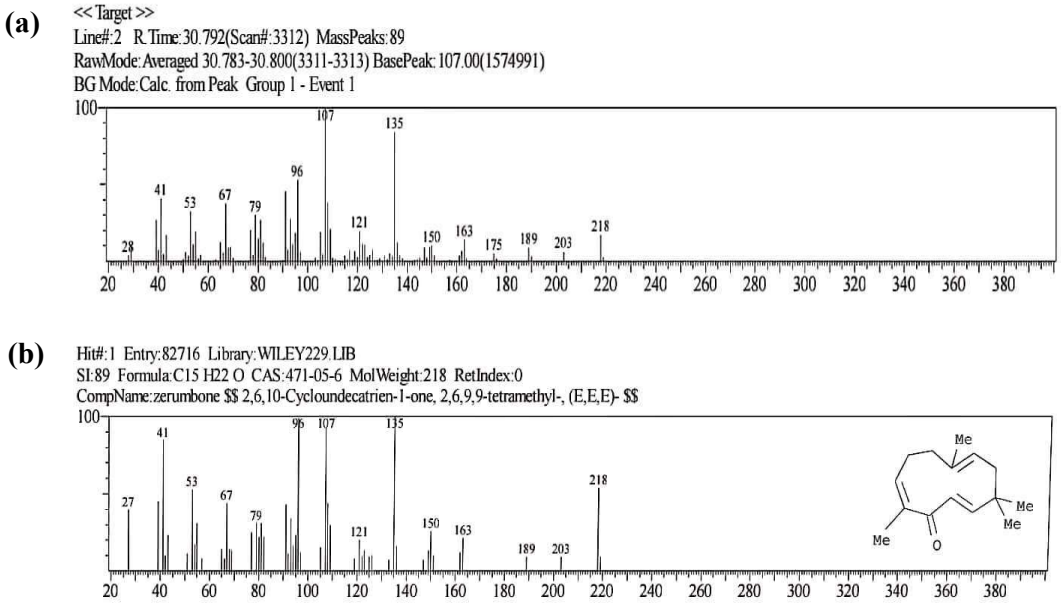


Figure 5. (a). Mass spectra of compound isolate $E_{10\text{-}23}$, (b). Mass spectra of zerumbone.

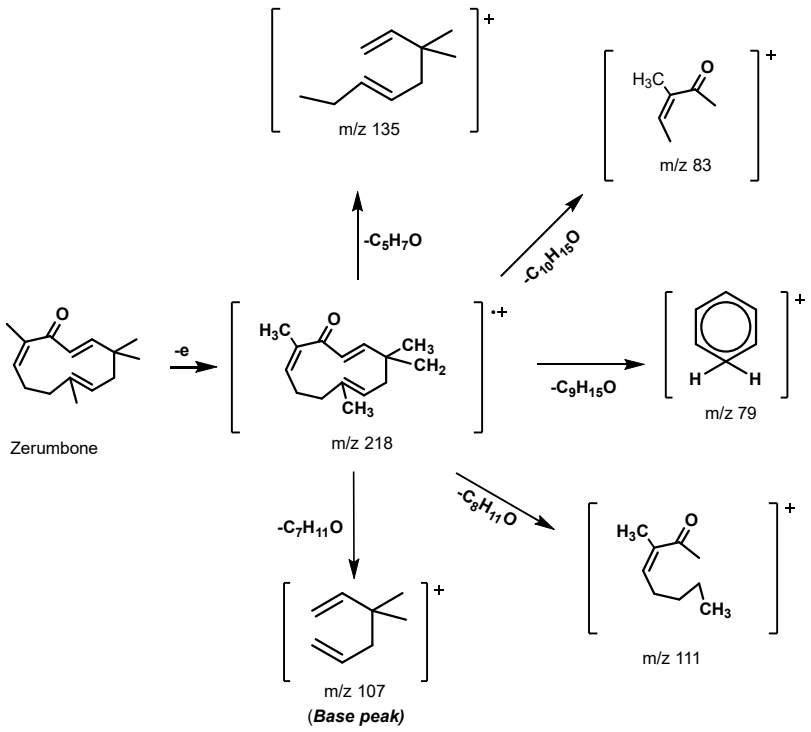


Figure 6. Zerumbone fragmentation pattern.

The fragmentation of Zerumbone results in the release of C_5H_7O, producing the $[C_{10}H_{15}]^+$ with an m/z value of 135. The release of $C_7H_{11}O$ generates the $[C_8H_{11}]^+$ with an m/z value of

107. Additionally, the release of $C_9H_{15}O$ results in the $[C_6H_7]^+$ with an m/z value of 79. The $[C_9H_{16}O]^+$ with an m/z value of 111 is formed from the release of $C_8H_{11}O$. Lastly, the release of $C_{10}H_{15}O$ produces the $[C_6H_{10}O]^+$ with an m/z value of 83.

3.2. Antibacterial in vitro testing

In the antibacterial activity test, the value of concentration is linearly proportional to the diameter of inhibition. This is in accordance with research conducted by researchers (Trisia et al., 2018), which states that one of the factors that can affect the activity of antimicrobial materials, namely the concentration of antimicrobial materials. The inhibition produced by antimicrobial materials will be higher if the concentration is also high. This can be proven through the measurement of the inhibition diameter of *P. aeruginosa* bacteria. The results of the antibacterial activity test and measurement of the inhibition zone diameter of zerumbone can be seen in Table 1 and Figure 7.

Table 1. Results of Zerumbone inhibition zone diameter measurement against *S. aureus, S. epidermidis, P. aeruginosa and E. coli* bacteria. *Description*: Disk diameter 6 mm, inoculum volume 20 µL. Negative control: DMSO.

Sample	Consentration (ppm)	Inhibition Zone Diameter (mm)			
		S. epidermidis	***S. aureus***	***E. coli***	***P. aeruginosa***
Zerumbone	16	0	0	0	7.92
	32	0	0	0	8.33
	64	0	0	0	8.42
	128	0	0	0	8.53
	256	0	0	0	8.78
	512	0	0	0	8.97
	1024	0	0	0	9.42
Amoxylline (+)		31.34	37.74	21.82	-
Imipenem (+)		-	-	-	26.66
DMSO (-)		0	0	0	0

Based on Table 1, zerumbone showed an inhibition zone of *P. aeruginosa* of 9.42 mm but showed no activity against *S. epidermidis*, *S. aureus* and *E. coli* bacteria. Al-Amin et al. (2019) reported that zerumbone had an inhibition zone of 11 mm against *P. aeruginosa*. Meanwhile, *S. aureus* and *E. coli* showed inhibition zones of 12 mm and 11 mm, respectively. The antibacterial test results revealed that zerumbone exhibits varying levels of antibacterial activity against different bacterial species, indicating that its effectiveness depends on the nature and characteristics of each bacterium. Each bacterial species has a unique level of tolerance to specific substances or compounds. According to Syarifah et al., (2018) stated that differences in the sensitivity of bacteria to antibacterial compounds can be influenced by the structure of the bacterial cell wall. Gram-negative bacteria only contain a small layer of peptidoglycan and do not contain teichoic acid, so the walls of Gram-negative bacteria are more susceptible to physical disturbances, such as antibiotics or other antibacterial agents. The mechanism of terpenoids as antibacterials is to react with the lipid fraction of the bacterial plasma membrane which results in changes in membrane permeability, if accumulated continuously it can result

in lysis of intracellular material due to the formation of cavities in the lipid bilayer (Kapitan et al., 2017).

The positive control inhibition diameter value is used as a comparison to determine the antibacterial activity of zerumbone. Classification of bacterial growth inhibition values according to Mahmudah & Atun (2017), grouped into four groups: weak response (diameter ≤5 mm), moderate (diameter 5-10 mm), strong (diameter 10-20 mm), and very strong (diameter ≥20 mm). Based on the inhibition value, it can be seen that zerumbone has moderate inhibition against *P. aeruginosa* bacteria.

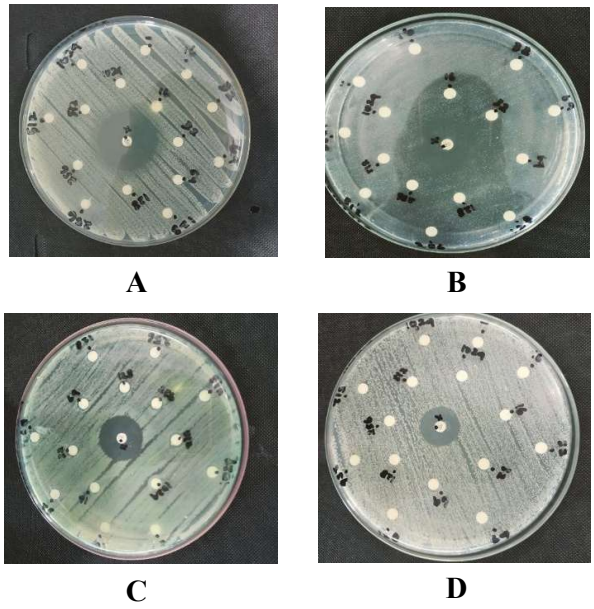


Figure 7. The antibacterial activity of zerumbone against the bacteria. *Description*: (A) *S. aureus*, (B) *S. epidermidi*s, (C) *P. aeruginosa*, and (D) *E. coli*.

4. CONCLUSIONS

Zerumbone was successfully isolated from the acetone extract of *Z. zerumbet* rhizome, resulting in a yield of 1.529 g (51% of the initial 3 g chromatography mass). This compound exhibited moderate antibacterial activity against *P. aeruginosa* forming a 9.42 mm inhibition zone, but it showed no antibacterial effect against *S. aureus*, *S. epidermidis*, or *E. coli*. The rhizome of *Z. zerumbet* could serve as an alternative source of zerumbone for further medical research.

ACKNOWLEDGMENTS

This research was funded by Sebelas Maret University through a group research grant with a contract number 194.2/UN27.22/PT.01.03/2024.

DECLARATION OF CONFLICT OF INTEREST

All authors declare that there is no conflict of interest in this manuscript

DAFTAR PUSTAKA

Ahmadabadi, H. K., Vaez-Mahdavi, M. R., Kamalinejad, M., Shariatpanahi, S. S., Ghazanfari, T., & Jafari, F. (2019). Pharmacological and Biochemical Properties of *Zingiber zerumbet* (L.) Roscoe ex Sm. and Its Therapeutic Efficacy on Osteoarthritis of Knee. *Journal of Family Medicine and Primary Care*, *8*(12), 3798. https://doi.org/10.4103/JFMPC.JFMPC_594_19

Akhtar, N. M. Y., Jantan, I., Arshad, L., & Haque, M. A. (2019). Standardized ethanol extract, essential oil and zerumbone of *Zingiber zerumbet* rhizome suppress phagocytic activity of human neutrophils. *BMC Complementary and Alternative Medicine*, *19*(1), 331. https://doi.org/10.1186/S12906-019-2748-5/TABLES/4

Al-Amin, M., Siddiqui, M. A., Ruma, S. A., Eltayeb, N. M., Nurun Nahar Sultana, G., Salhimi, S. M., & Faiz Hossain, C. (2019). Antimicrobial activity of the crude extract, fractions and isolation of zerumbone from the rhizomes of *Zingiber roseum*. *J Res Pharm*, *23*(3), 559–566. https://doi.org/10.12991/jrp.2019.163

Assiry, A. A., Ahmed, N., Almuaddi, A., Saif, A., Alshahrani, M. A., Mohamed, R. N., & Karobari, M. I. (2023). The antioxidant activity, preliminary phytochemical screening of *Zingiber zerumbet* and antimicrobial efficacy against selective endodontic bacteria. *Food Science & Nutrition*, *11*(8), 4853–4860. https://doi.org/10.1002/FSN3.3462

Chavan, J. J., Kshirsagar, P. R., Pai, S. R., & Pawar, N. V. (2018). Micropropagation, metabolite profiling, antioxidant activities and chromatographic determination of bioactive molecules across in vitro conditions and subsequent field cultivation stages of 'Shampoo Ginger' (*Zingiber zerumbet* L. Roscoe ex Sm). *Biocatalysis and Agricultural Biotechnology*, *16*, 79–89. https://doi.org/10.1016/J.BCAB.2018.07.015

Clinical and Laboratory Standards Institute. (2018). *Performance Standards for Antimicrobial Susceptibility Testing*. 28th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.

Diastuti, H., Asnani, A., Delsy, E. V. Y., Pamukasari, R., & Indriani, S. (2022). Toxicity and Antimicrobial Activity of Zerumbon from *Zingiber zerumbet* Rhizome. *Molekul*, *17*(3), 328–334. https://doi.org/10.20884/1.JM.2022.17.3.5808

Haque, M. A., Jantan, I., Harikrishnan, H., & Ghazalee, S. (2019). Standardized extract of *Zingiber zerumbet* suppresses LPS-induced pro-inflammatory responses through NF-κB, MAPK and PI3K-Akt signaling pathways in U937 macrophages. *Phytomedicine : International Journal of Phytotherapy and Phytopharmacology*, *54*, 195–205. https://doi.org/10.1016/J.PHYMED.2018.09.183

Jantan, I., Haque, M. A., Ilangkovan, M., & Arshad, L. (2019). Zerumbone from *Zingiber zerumbet* inhibits innate and adaptive immune responses in Balb/C mice. *International Immunopharmacology*, *73*, 552–559. https://doi.org/10.1016/J.INTIMP.2019.05.035

Kapitan, O. B., Ambarsari, L., dan Falah, S. (2017). In Vitro Antibakteri Ekstrak Etanol Puni (*Zingiber zerumbet*) Asal Pulau Timor. *Savana cendana*, 2(02), 29-32.

Koga, A. Y., Beltrame, F. L., & Pereira, A. V. (2016). Several aspects of *Zingiber zerumbet*: a review. *Revista Brasileira de Farmacognosia*, *26*(3), 385–391. https://doi.org/10.1016/J.BJP.2016.01.006

Mahmudah, F. L., & Atun, S. (2017). Uji Aktivitas Antibakteri Dari Ekstrak Etanol Temu Kunci (*Boesenbergia pandurata* Roxb) TERHADAP BAKTERI *Streptococcus mutans*. *Jurnal Penelitian Saintek*, *22*(1), 59. https://doi.org/10.21831/JPS.V22I1.15380

Mulyani, S. (2010). Komponen dan anti-bakteri dari fraksi kristal minyak Z. zerumbet. *Majalah Farmasi Indonesia,* 21(3), 178–184.

Padalia, R. C., Verma, R. S., Chauhan, A., Singh, V. R., Goswami, P., Singh, S., Verma, S. K., Luqman, S., Chanotiya, C. S., & Darokar, M. P. (2018). *Zingiber zerumbet* (L.) *Roscoe* ex Sm. from northern India: Potential source of zerumbone rich essential oil for antiproliferative and antibacterial applications. *Industrial Crops and Products*, *112*, 749–

754. https://doi.org/10.1016/J.INDCROP.2018.01.006

Prastiyanto, M. E., Wardoyo, F. A., Wilson, W., & Darmawati, S. (2020). Antibacterial Activity of Various Extracts of *Averrhoa bilimbi* against Multidrug Resistant Bacteria. *Biosaintifika*, *12*(2), 163–168. https://doi.org/10.15294/BIOSAINTIFIKA.V12I2.23600

Ramzan, M., Karobari, M. I., Heboyan, A., Mohamed, R. N., Mustafa, M., Basheer, S. N., Desai, V., Batool, S., Ahmed, N., & Zeshan, B. (2022). Synthesis of Silver Nanoparticles from Extracts of Wild Ginger (*Zingiber zerumbet*) with Antibacterial Activity against Selective Multidrug Resistant Oral Bacteria. *Molecules (Basel, Switzerland)*, *27*(6). https://doi.org/10.3390/MOLECULES27062007

Ritna, A., Anam, S., & Khumaidi, A. (2016). Identifikasi Senyawa Flavonoid Pada Fraksi Etil Asetat Benalu Batu (*Begonia sp.*) ASAL KABUPATEN MOROWALI UTARA. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal)*, *2*(2), 83–89. https://doi.org/10.22487/J24428744.2016.V2.I2.5957

Sadhu, S. K., Khatun, A., Ohtsuki, T., & Ishibashi, M. (2007). First isolation of sesquiterpenes and flavonoids from *Zingiber spectabile* and identification of zerumbone as the major cell growth inhibitory component. *Natural Product Research*, *21*(14), 1242–1247. https://doi.org/10.1080/14786410701371421

Syarifah, R., Fakhrurrazi, A. Harris, A. Sutriana, Erina, dan Winaruddin. (2018). Uji Daya Hambat Ekstrak Biji Buah Pada (*Myristica fragrans Houtt*) terhadap Pertumbuhan Bakteri *Escherichia coli*. *Jurnal Mahasiswa Veteriner*, 2(3), 361-372.

Silva TM, Pinheiro CP, Orlandi PP, Pinheiro CC, Pontes GS. Zerumbone from *Zingiber zerumbet* (L.) *Smith*: a potential prophylactic and therapeutic agent against the cariogenic bacterium *Streptococcus mutans*. *BMC Complementary and Alternative Medicine*. 2018;18:301:1-9. https://doi.org/10.1186/s12906-018-2360-0

Trisia, A., Philyria, R., & Toemon, A. N. (2018). Uji Aktivitas Antibakteri Ekstrak Etanol Daun Kalanduyung (*Guazuma ulmifolia* Lam.) terhadap Pertumbuhan *Staphylococcus aureus* dengan Metode Difusi Cakram (Kirby-Bauer). *Anterior Jurnal*, *17*(2), 136–143. https://doi.org/10.33084/ANTERIOR.V17I2.12

Yu, F., Harada, H., Yamasaki, K., Okamoto, S., Hirase, S., Tanaka, Y., Misawa, N., & Utsumi, R. (2008). Isolation and functional characterization of a b-eudesmol synthase, a new sesquiterpene synthase from *Zingiber zerumbet* Smith. FEBS Letter, 582(5), 565-572. https://doi.org/10.1016/j.febslet.2008.01.020

JPSCR: Journal of Pharmaceutical Science and Clinical Research
Volume 10, Issue 1, Pages 100-113, March 2025
https://doi.org/10.20961/jpscr.v10i1.98721

ISSN 2503-331X (Online)

Aktivitas Penghambatan Dipeptidyl peptidase-4 (DPP-4) dan Skrining Fitokimia Ekstrak Buah Api-api (*Avicennia marina*)

Inhibition Activity of Dipeptidyl Peptidase-4 (DPP-4) and Phytochemical Screening Extract of White Mangrove Fruit

Dwi Hadi Setya Palupi[1*], Sri Haryanti[2], Masitoh Suryaning Prahasiwi[1] dan Retno Sari Utomo[2]

[1]Program Studi S1 Farmasi, STIFAR Yayasan Pharmasi Semarang, Semarang, Indonesia
[2]Program Magister Farmasi, STIFAR Yayasan Pharmasi Semarang, Semarang, Indonesia

*Corresponding author: dwi.palupi@ymail.com

Diterima: 23 Januari 2025; **Disetujui:** 26 Mei 2025; **Dipublikasi:** 22 Juni 2025

Abstrak

Pengobatan farmakologis untuk diabetes didasarkan pada peningkatan ketersediaan insulin dan perbaikan sensitivitas insulin. Saat ini, terapi berbasis glukagon-like peptide 1 (GLP-1) bertujuan untuk mengendalikan glukosa melalui penghambatan enzim DPP-4. Api-api (*Avicennia marina)* merupakan *mangrove* yang banyak terdapat di Indonesia, namun pemanfaatannya sebagai obat oleh masyarakat masih terbatas. Penelitian ini bertujuan untuk mengidentifikasi kandungan metabolit sekunder yang terdapat pada ekstrak maupun fraksi buah api-api dan menentukan aktivitas penghambatannya terhadap enzim dipeptidyl peptidase-4 (DPP-4). Ekstraksi dilakukan dengan cara maserasi dalam etanol 96%, dilanjutkan fraksinasi dengan metode cair-cair untuk mendapatkan fraksi n-heksan, etil asetat, dan air. Skrining fitokimia dilakukan dengan uji warna dan kromatografi lapis tipis (KLT). Aktivitas penghambatan enzim DPP-IV dilakukan menggunakan metode fluoresensi dengan substrat fluorogenik Gly-Pro-aminomethyl-coumarin (AMC). Rendemen hasil ekstraksi buah api-api (*Avicennia marina*) sebesar 48,06% sedangkan fraksinasi dari ekstrak menghasilkan fraksi air dengan rendemen terbesar (31,54%). Hasil skrining fitokimia dan KLT memperlihatkan ekstrak etanol dan fraksi etil asetat buah api-api mengandung flavonoid, triterpenoid/steroid, tanin, dan saponin. Fraksi air terdeteksi mengandung flavonoid, tanin, dan terpenoid, sedangkan fraksi n-heksan hanya mengandung flavonoid dan triterpenoid. Aktivitas penghambatan DPP-4 fraksi n-heksan, fraksi etil asetat, dan fraksi air dalam nilai IC_{50} berturut-turut yaitu 5,19; 1,36; dan 2,51 mg/mL. Fraksi etil asetat buah api-api putih memiliki aktivitas penghambatan DPP-4 terbaik, sehingga dapat menjadi sumber antidiabetik alami.

Kata kunci: *Avicennia marina*; Diabetes melitus; Inhibitor DPP-4; Metabolit sekunder; Skrining fitokimia

Abstract

The goal of pharmacological treatment for diabetes is to improve insulin sensitivity and increase insulin availability. By blocking the DPP-4 enzyme, glucagon-like peptide 1 (GLP-1)-based therapy currently seeks to regulate blood sugar. Although white mangrove (Avicennia marina) is a common mangrove plant in Indonesia, people still use it sparingly, especially for medicinal purposes. This study aims to identify the secondary metabolite content in the white

mangrove fruit extracts and fractions and determine their dipeptidyl peptidase-4 (DPP-4) inhibitory activity. Extraction was performed by maceration in 96% ethanol, followed by fractionation using the liquid-liquid method to obtain n-hexane, ethyl acetate, and water fractions. Phytochemical screening was conducted using color tests and thin-layer chromatography (TLC). The DPP-IV enzyme inhibition activity was performed using the fluorescence method with the fluorogenic substrate Gly-Pro-aminomethyl-coumarin (AMC). The yield of the extraction of white mangrove fruit (Avicennia marina) was 48.06%, while the fractionation of the extract produced an aqueous fraction with the highest yield (31.54%). The results of phytochemical screening and TLC show that the ethanol extract and ethyl acetate fraction of the white mangrove fruit contain flavonoids, triterpenoids/steroids, tannins, and saponins. The aqueous fraction was detected to contain flavonoids, tannins, and terpenoids, while the n-hexane fraction only contained flavonoids and triterpenoids. The DPP-4 inhibition activity of the n-hexane fraction, ethyl acetate fraction, and water fraction with IC50 values of 5.19; 1.36; and 2.51 mg/mL, respectively. The ethyl acetate fraction of the white mangrove fruit has the best DPP-4 inhibitory activity, making it a potential source of natural antidiabetic agents.

***Keywords*:** *Avicennia marina*; Diabetes mellitus; DPP-4 inhibitors; Phytochemical screening; Secondary metabolites

1. PENDAHULUAN

Diabetes melitus (DM) merupakan penyakit metabolisme kronis yang ditandai dengan hiperglikemia karena produksi insulin yang tidak mencukupi atau resistensi insulin (Yan *et al*., 2021). Penanganan DM harus dilakukan secara maksimal mengingat penyakit ini memiliki komplikasi yang serius apabila tidak ditangani dengan baik. Komplikasi seperti neuropati, nefropati, retinopati, penyakit kardiovaskuler, stroke, sindrom kaki diabetik bahkan kematian bisa saja terjadi jika kadar gula darah tidak terkontrol. Oleh karena itu menemukan pengobatan yang tepat dapat membantu mengatasi masalah ini.

Saat ini sudah banyak obat antidiabetes sintetik yang beredar di pasaran dimana penggunaan obat anti diabetes bertujuan untuk mengendalikan kadar gula darah dan mencegah komplikasi penyakit ini. Namun, sebagian besar obat-obatan tersebut memiliki efek samping yang mungkin muncul akibat penggunaan jangka panjang antara lain masalah pencernaan, hipoglikemia, serta dampak ke organ-organ vital seperti hati, dan ginjal. Efek samping membuat kepatuhan penderita diabetes terhadap pengobatannya menjadi terganggu (Adiputra, 2023). Oleh karena itu, penelitian tentang pengobatan alternatif DM dari sumber alami, terutama tanaman, telah meningkat pesat dalam beberapa tahun terakhir.

Dipeptidyl peptidase-4 (DPP-4) merupakan salah satu target terapi baru dalam penatalaksanaan DM tipe 2. Enzim DPP-4 berperan dengan cepat menonaktifkan hormon incretin; glucagon like peptide-1 (GLP-1) dan glukosa dependen insulinotropic polypeptide (GIP) yang berfungsi sebagai stimulator prandial penting sekresi insulin dan pengatur glukosa darah (Deacon, 2020). Penghambatan terhadap DPP-4 meningkatkan kontrol glikemik pada pasien DM tipe 2. Keunggulan dari obat kelompok penghambat DPP-4 antara lain memiliki profil keamanan yang baik, ditandai risiko rendah terjadinya hipoglikemia, serta efektivitas dalam menurunkan kadar hemoglobin glikosilasi (HbA1c), termasuk pada pasien dengan

gangguan ginjal kronis (Marín-Peñalver *et al.*, 2016). Namun, harga yang relatif tinggi membatasi penggunaannya sehingga seringkali tidak menjadi pilihan utama dalam terapi. Sebagai alternatif, diperlukan pencarian kandidat golongan penghambat DPP-4 yang berasal dari sumber alam sangat potensial untuk dikembangkan.

Salah satu bahan alam yang berpotensi untuk digunakan dan dikembangkan sebagai obat adalah tanaman api-api putih (*Avicennia marina*). Secara empiris, masyarakat menggunakan daun dan buah *A. marina* untuk mengobati beberapa jenis penyakit seperti hepatitis, cacar, dan rematik. Penelusuran terhadap senyawa bioaktif *A. marina* telah dilakukan beberapa peneliti dan diketahui bahwa terdapat polifenol, flavonoid, steroid, terpenoid, alkaloid, glikosida jantung, saponin, tanin, dan antrakuinon dalam daun serta buah (Vasanthakumar *et al*., 2019; Fernandes & Noor'an, 2019; Rozirwan *et al*., 2022). Senyawa-senyawa bioaktif tersebut dianggap bertanggung jawab atas aktivitas farmakologi seperti antibakteri, antijamur, antivirus, antiplasmodial, antitumor, antiulkus, antioksidan, dan antikanker (Sohaib *et al*., 2022; Yassien *et al*., 2021; Andriani *et al*., 2021).

Ekstrak etanol daun *A. marina* juga telah terbukti sebagai agen antidiabetes pada model mencit diabetes yang diinduksi oleh streptozotocin (Okla *et.al*, 2019). Aktivitas antidiabetes ini dikaitkan dengan mekanismenya sebagai antioksidan (Singh, 2021). Kandungan senyawa bioaktif dalam buah *A. marina* yang memiliki kemiripan dengan daun, memungkinkan juga adanya potensi antidiabetes. Aktivitas antidiabetes fraksi buah api-api (*Avicennia marina*) melalui penghambatan DPP-4 sejauh ini belum diketahui dan secara in vitro ditetapkan melalui penelitian ini.

2. BAHAN DAN METODE

2.1. Bahan

Bahan yang digunakan dalam penelitian ini adalah buah api-api putih, etanol 96%, n-heksan, etil asetat, aquadest, asam sulfanilat (Merck, Jerman), HCl (Merck, Jerman), NaNO2 (Merck, Jerman), NaOH (Univar, USA), asam asetat, H2SO4 (Merck, Jerman), serbuk Mg (Merck, Jerman), amil alkohol, reagen mayer, reagen dragendorff, reagen buchardat, FeCl3 (Merck, Jerman), NaCl 10%, Gelatin 1%, HCl 2N, asam asetat anhidrat (Univar, USA), metanol, lempeng silica gel 60 F_{254}, asam formiat (Merck, Jerman), toluene (Merck, Jerman), kloroform (Merck, Jerman), asam asetat glacial (Merck, Jerman), anisaldehid asam sulfat, kit DPP-4 *screening assay* (Elabscience®), DMSO (Merck, Jerman).

2.2. Metode

2.2.1. Pengambilan dan pengolahan sampel

Sampel penelitian berupa buah tanaman api-api putih (*Avicennia marina*) yang diambil dari daerah Kaliwungu, Kabupaten Kendal. Tumbuhan terlebih dahulu dilakukan determinasi di Laboratorium Biologi Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang. Sampel berupa buah api-api putih (*Avicennia marina*) dikeringkan kemudian dihaluskan.

2.2.2. Ekstraksi

Ekstraksi dilakukan dengan metode remaserasi. Serbuk buah api-api putih sebanyak 500 gram direndam dalam etanol 96% dengan perbandingan 1:10 (b/v) selama 3 × 24 jam, dengan penggantian pelarut setiap 24 jam. Filtrat hasil maserasi dikumpulkan, disaring, kemudian diuapkan menggunakan *rotary evaporator* dilanjutkan dengan *waterbath* hingga diperoleh ekstrak dengan konsistensi yang sesuai (ekstrak kental).

2.2.3. Fraksinasi

Fraksinasi ekstrak dilakukan dengan metode cair-cair bertingkat menggunakan pelarut dengan kepolaran berbeda, yaitu n-heksana, etil asetat, dan air. Sebanyak 10 gram ekstrak etanol ditambahkan dengan aquadest 50 ml dengan perbandingan 1:5 (b/v), diaduk kemudian ditambahkan dengan pelarut n-heksana (1:1, b/v), dikocok perlahan menggunakan corong pisah hingga terbentuk dua lapisan yaitu lapisan air dan lapisan n-heksan. Selanjutnya lapisan n-heksan dipisahkan untuk dipekatkan sehingga diperoleh fraksi n-heksana (FNH). Fase air kemudian difraksinasi dengan etil asetat (1:1, b/v) dan dikocok perlahan menggunakan corong pisah. Setelah terjadi pemisahan lapisan etil asetat dipisahkan dan dipekatkan sehingga diperoleh fraksi etil asetat (FEA) sedangkan lapisan air dikentalkan dengan *waterbath* sehingga diperoleh fraksi air (FA).

2.2.4. Penapisan fitokimia

Penapisan fitokimia dilakukan dengan uji warna dan KLT untuk metabolit sekunder flavonoid, alkaloid, tanin, saponin, dan triterpenoid/steroid.

2.2.4.1. Uji flavonoid

Ekstrak atau fraksi teruji sebanyak 1 mL ditambahkan 3 mL etanol 70%, dan dikocok, selanjutnya dipanaskan dalam penangas air dan disaring. Filtrat hasil penyaringan ditambahkan serbuk Mg sebanyak 0,1 gram serta 2 tetes HCl pekat dan amil alkohol. Uji positif flavonoid ditandai dengan adanya warna merah, kuning hingga jingga pada lapisan amil alkohol (Supomo *et.al.*, 2019)

2.2.4.2. Uji alkaloid

Ekstrak atau fraksi sebanyak 0,5 gram ditambahkan 1 ml asam klorida 2 N dan 9 ml air suling, kemudian dipanaskan di atas penangas air selama 2 menit. Sampel didinginkan dan disaring dan filtratnya digunakan untuk pengujian alkaloida. Selanjutnya, ke dalam 3 tabung reaksi masing-masing dimasukkan 0,5 ml filtrat. Pada setiap tabung ditambahkan dua tetes pereaksi meliputi pereaksi *mayer, dragendorff, dan bouchardat*. Hasil positif senyawa alkaloid pada pereaksi *mayer* adalah terbentuknya endapan putih hingga kekuningan. Pada pereaksi *dragendorf*, senyawa alkaloid positif dengan terbentuknya endapan merah bata. Sementara itu, untuk uji dengan peraksi *bauchardat* positif ditandai terbentuknya endapan coklat (Fernandes dan Noor'an, 2019).

2.2.4.3. Uji tanin

Ekstrak atau fraksi teruji ditimbang sebanyak 0,5 gram, larutkan dalam aquadest hingga tidak berwarna, selanjutnya ambil filtrat sebanyak 2 mL dan ditetesi pereaksi $FeCl_3$ sebanyak 1-2 tetes. Hasil positif ditandai dengan warna hijau, biru dan hitam (Widiawati dan Asih, 2024).

2.2.4.4. Uji saponin

Ekstrak dan fraksi buah api-api putih ditambahkan 10 mL air panas kemudian didinginkan dan dikocok hingga muncul buih. Larutan didiamkan selama 2 menit, kemudian diteteskan HCl 2 N. Hasil positif ditunjukkan dengan terbentuknya buih stabil selama 10 menit (Fernandes dan Noor'an, 2019).

2.2.4.5. Uji triterpenoid/ steroid

Ekstrak dan fraksi buah api-api putih ditambahkan dengan asam asetat anhidrid sebanyak 10 tetes dan H_2SO_4 pekat sebanyak 2 tetes. Larutan dikocok perlahan dan dibiarkan selama beberapa menit. Jika menghasilkan warna hijau dan biru maka mengandung steroid, sedangkan jika menghasilkan warna merah dan ungu maka mengandung triterpenoid (Fernandes dan Noor'an, 2019).

2.2.4.6. Uji kromatografi lapis tipis

Pengujian KLT meggunakan fase diam Silika G60 F_{254}. Plat KLT dibuat dengan panjang 10 cm dan lebar 3 cm. Ekstrak dan fraksi buah api-api putih dilarutkan dengan pelarutnya ditotolkan pada plat, dielusi dengan fase gerak yang disesuaikan senyawa teruji.

2.2.5. Uji aktivitas penghambatan enzim Dipeptidyl peptidase-4 (DPP-4)

Kit skrining inhibitor DPP-4 (Elabscience) digunakan untuk mengevaluasi aktivitas penghambatan DPP-4 dari sampel uji berdasarkan metode uji fluoresensi. Sampel (10 µL) dengan konsentrasi berbeda dalam DMSO masing-masing dipipet ke dalam *96-microwell plate* diikuti dengan penambahan buffer uji yang diencerkan (30 µL), larutan enzim DPP-4 rekombinan tikus yang diencerkan (10 µL), dan substrat fluorogenic Gly-Pro-aminomethyl-coumarin (AMC) yang diencerkan (50 µL). Pada sumur kontrol negatif dan positif, sampel masing-masing diganti dengan pelarut dan standar sitagliptin. *Microwell plate* dikocok dan diinkubasi selama 30 menit pada suhu 37°C. Setelah inkubasi, fluoresensi gugus AMC bebas yang dihasilkan dari reaksi diamati pada rentang panjang gelombang eksitasi 350–360 nm dan rentang panjang gelombang emisi 450–465 nm menggunakan *microplate reader*. Persentase penghambatan dihitung menggunakan Persamaan 1.

$$\text{Persentase inhibisi (\%)} = \left[\left(\text{OD}_{kontrol} - \text{OD}_{sampel}\right)/\left(\text{OD}_{kontrol}\right)\right] \times 100$$

Persamaan 1. Rumus perhitungan persentase penghambatan enzim inhibitor Dipeptidyl peptidase-4 (DPP-4). Keterangan: $OD_{kontrol}$ adalah absorbansi kontrol negatif dan OD_{sampel} adalah absorbansi sampel.

3. HASIL DAN PEMBAHASAN

3.1. Determinasi tanaman

Hasil determinasi yang dilakukan di Laboratorium Biologi Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang menunjukkan bahwa tanaman yang akan digunakan adalah benar Api-api (*Avicennia marina*) berdasarkan surat keterangan identifikasi No 051/EL-AFM/VIII/2024.

3.2. Ekstraksi dan fraksinasi buah api-api

Proses ekstraksi buah api-api dilakukan melalui metode maserasi menggunakan pelarut etanol 96%. Etanol dipilih sebagai pelarut dengan pertimbangan keamanan karena tidak berpotensi toksik (Azis *et al.,* 2014). Metode maserasi dipilih dengan pertimbangan untuk menghindari kerusakan terutama untuk senyawa-senyawa yang *thermolabile* khususnya senyawa golongan flavonoid yang dalam hal ini diduga memiliki potensi dalam aktivitas antidiabetes. Pengulangan maserasi juga dilakukan untuk memaksimalkan penarikan senyawa bioaktif dari serbuk simplisia. Maserat yang diperoleh dilakukan penguapan pelarut secara vakum menggunakan *rotary evaporator* hingga diperoleh ekstrak kental.

Ekstrak kental buah api-api difraksinasi dengan metode cair-cair. Fraksinasi bertujuan untuk memisahkan senyawa berdasarkan tingkat kepolaran yang berbeda. Pelarut yang digunakan adalah n-heksan (non polar), etil asetat (semi polar), dan air (polar). Persentase rendamen ekstrak dan fraksi yang diperoleh dapat dilihat pada Tabel 1. Berdasarkan hasil rendemen fraksi, kemungkinan senyawa bioaktif yang terkandung dalam buah api-api putih cenderung bersifat polar. Hal ini disimpulkan dari besarnya rendemen untuk fraksi air. Namun penelitian oleh Fernandes & Noor'an (2019) justru menemukan hasil berbeda, dimana senyawa bioaktif dengan persentase terbesar dalam pemeriksaan kuantitatif menggunakan GC-MS adalah γ-sitosterol. Senyawa γ-sitosterol merupakan fitosterol dengan sifat semi polar yang berdasar studi *in silico* sangat berpotensi sebagai antidiabetes (Balamurugan *et al*., 2015).

Tabel 1. Rendemen ekstrak dan fraksi buah api-api (*Avicennia marina*)

Sampel	Rendemen (%)
Ekstrak etanol	48,06
Fraksi n-Heksan	0,55
Fraksi etil asetat	10,71
Fraksi air	31,54

3.3. Skrining fitokimia ekstrak dan fraksi buah api-api

Skrining fitokimia ekstrak etanol dan fraksi *A. marina* dilakukan melalui uji warna dan dikonfirmasi dengan melakukan kromatografi lapis tipis (KLT). Pengujian meliputi uji keberadaan flavonoid, alkaloid, tanin, saponin, dan terpenoid atau steroid.

3.3.1. Uji warna

Skrining fitokimia dengan uji warna sebagai pengetahuan awal secara kualitatif mengenai kandungan senyawa bioaktif pada buah api-api putih. Hasil menunjukkan bahwa ekstrak dan

fraksi etil asetat mengandung senyawa flavonoid, tanin, saponin dan terpenoid (Tabel 2). Fraksi air terdeteksi mengandung flavonoid, tanin, dan terpenoid, sedangkan untuk fraksi n-heksan hanya terdapat flavonoid dan triterpenoid.

Penelitian terdahulu, menunjukkan hasil yang berbeda dengan tidak terdeteksinya tannin dan saponin pada ekstrak etanol buah api-api putih yang diperoleh dari Hutan Penelitian dan Pendidikan Muara Kaeli. Hal itu kemungkinan karena perbedaan tempat tumbuh. Selain itu, hasil ekstraksi senyawa fitokimia juga dapat dipengaruhi oleh faktor yang bersumber dari sampel maupun dari proses ekstraksi. Bagian tanaman, asal tanaman, ukuran partikel, cara pengeringan dan kadar air merupakan beberapa faktor dari sampel. Faktor ekstraksi meliputi jenis pelarut, cara ekstraksi, komposisi pelarut, suhu dan durasi ekstraksi (Shaikh & Patil, 2020).

Hasil rendemen dari fraksinasi menggunakan n-heksana paling kecil karena jika dilihat dari tingkat kepolaran termasuk pelarut non polar. Oleh karena itu n-heksan hanya mengekstrak senyawa dengan kepolaran yang rendah. Secara umum, flavonoid lebih larut dalam pelarut polar, tetapi beberapa flavonoid glikosida seperti isoflavon, flavanon, flavon, dan flavonol memiliki afinitas terhadap pelarut non-polar (Rodríguez *et al*., 2020). Terpenoid dapat terekstrak dengan pelarut non-polar atau polar (Mierza *et al.*, 2023) dan ini terbukti bahwa baik pada fraksi n-heksan, etil asetat maupun fraksi air terdeteksi keberadaan terpenoid.

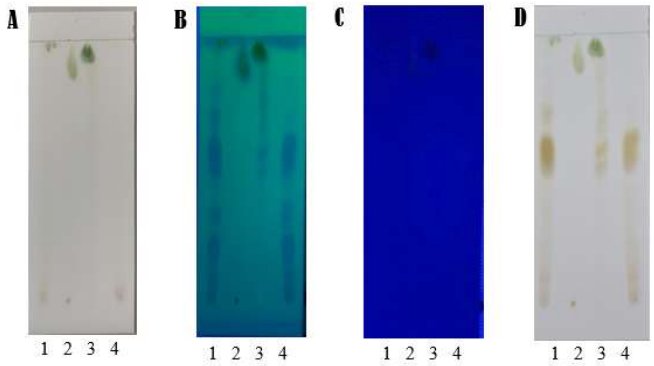
Tabel 2. Hasil uji kualitatif warna ekstrak dan fraksi buah api-api (*Avicennia marina*). Keterangan: (+) terdeteksi senyawa dan (-) tidak terdeteksi senyawa.

Kandungan Senyawa	Ekstrak Etanol 96%	Fraksi n-Heksan	Fraksi Etil Asetat	Fraksi Air
Flavonoid	(+)	(+)	(+)	(+)
Alkaloid	(-)	(-)	(-)	(-)
Tanin	(+)	(-)	(+)	(+)
Saponin	(+)	(-)	(+)	(-)
Terpenoid	(+)	(+)	(+)	(+)

3.3.2. Kromatografi Lapis Tipis (KLT)

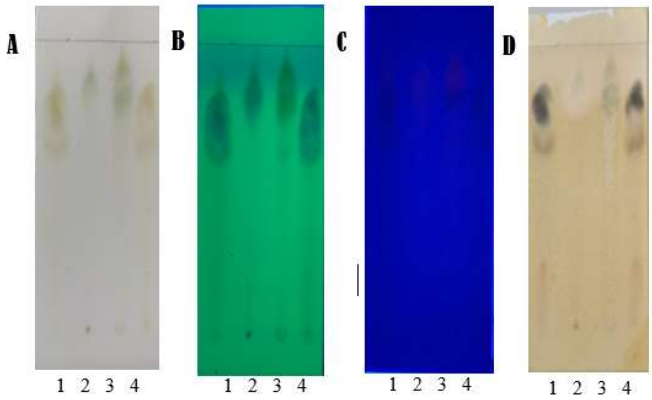
Pengujian KLT dilakukan untuk mengonfirmasi hasil uji warna dengan pemisahan senyawa berdasarkan perbedaan kepolaran. Metode KLT merupakan metode pemisahan senyawa dengan menggunakan 2 fase berbeda yaitu fase diam dan fase gerak. Silika gel G60 F_{254} adalah fase diam yang digunakan sedangkan fase gerak merupakan campuran dari 2 atau lebih larutan dengan tingkat kepolaran berbeda. Fase gerak berbeda disesuaikan dengan senyawa yang sedang dipisahkan.

Pada pengujian keberadaan flavonoid, hasil pengamatan memperlihatkan adanya noda berwarna kuning kehijauan setelah diuapi dengan ammonia. Uap ammonia yang bereaksi dengan gugus hidroksi fenolik pada flavonoid akan membentuk garam dan senyawa kinoid yang berwarna kuning kehijauan. Noda tersebut merupakan tanda positif untuk keberadaan flavonoid. Noda kuning kehijauan terlihat pada ekstrak maupun ketiga fraksi (Gambar 1). Flavonoid merupakan golongan fenol dengan sifat kepolaran yang bervariasi mulai dari polar (glikosida flavonoid), semi polar (aglikon flavonoid), dan non polar (isoflavon). Sifat kepolaran itulah yang menjelaskan terdeteksinya flavonoid baik pada ekstrak maupun ketiga fraksi.



Gambar 1. Profil KLT ekstrak dan fraksi buah api-api putih pada fase diam silika gel GF_{254} dan fase gerak etil asetat : asam formiat : air (8:1:1). Keterangan: sinar tampak (A), sinar UV 254 nm (B), UV 366 nm (C), sinar tampak dengan uap ammonia (D), ekstrak etanol 96% (1), Fraksi n-heksan (2), Fraksi etil asetat (3), Fraksi air (4).

Uji KLT untuk senyawa alkaloid dilakukan dengan fase gerak etil asetat, metanol, dan air dengan perbandingan 9:2:2 dan penampakan noda setelah disemprot dengan pereaksi *dragendorf.* Pengamatan lempeng di bawah lampu UV 254 maupun 366 nm tidak memperlihatkan keberadaan alkaloid (tidak berfluoresensi biru). Hasil KLT ini menegaskan hasil uji warna (dengan pereaksi *dragendorff, meyer, maupun bouchardat*) yaitu bahwa pada ekstrak dan fraksi buah api-api tidak terkandung alkaloid.

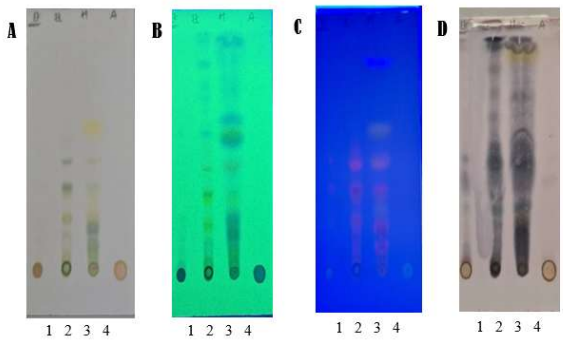


Gambar 2. Profil KLT ekstrak dan fraksi buah api-api putih pada fase diam silika gel GF_{254} dan fase gerak metanol dan etil asetat (7:3). Keterangan: sinar tampak (A), sinar UV 254 nm (B), UV 366 nm (C), sinar tampak setelah disemprot FeCl3 (D), Ekstrak etanol 96% (1), Fraksi n-heksan (2), Fraksi etil asetat (3), Fraksi air (4).

Kandungan tanin dalam ekstrak dan fraksi buah api-api putih ditegaskan keberadaannya dengan fase gerak metanol dan etil asetat (7:3). Setelah proses elusi serta penyemprotan dengan penampak bercak $FeCl_3$ terdapat noda bercak berwarna hitam pada sinar tampak seperti terlihat pada Gambar 2. Ion Fe^{3+} dari $FeCl_3$ akan bereaksi dengan tanin membentuk kompleks kalium trisianoferik Feri (III) yang berwarna hijau-hitam (Putria *et al.,* 2022). Hasil menunjukkan

bahwa ekstrak etanol dan ketiga fraksi mengandung tanin. Gugus OH pada tanin membuat tanin bersifat polar yang memungkinkan tanin dapat larut dalam pelarut polar (air) maupun semi polar (etil asetat). Tanin juga dimungkinkan larut pada pelarut non polar sebagai senyawa tanin terhidrolisis (Iriany *et al*., 2021), sehingga pada hasil KLT fraksi n-heksan terdeteksi adanya senyawa ini.

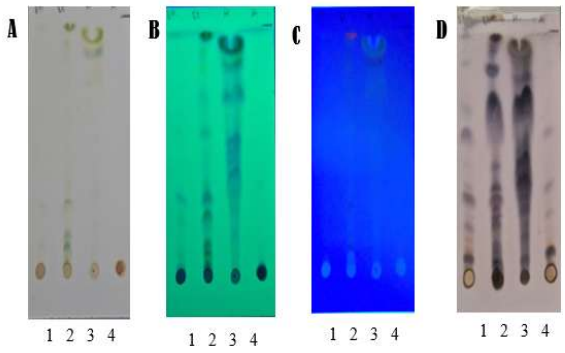
Uji KLT untuk senyawa saponin dalam ekstrak dan fraksi dilakukan menggunakan fase gerak kloroform: asam asetat glasial: metanol: air (64:32:12:8). Penyemprotan dengan penampak bercak anisaldehid-asam sulfat memunculkan noda bercak berwarna biru keunguan pada sinar tampak. Anisaldehid mendestruksi gugus fungsional tertentu pada struktur saponin yang ditandai dengan bercak warna biru keunguan (Dewi *et al.*, 2018). Saponin adalah suatu glikosida yang memiliki dua tipe struktur kimia dasar dari suatu aglikon (sapogenin). Secara umum saponin akan larut dalam pelarut polar maupun semi polar, dan dalam bentuk terhidrolisis (aglikon) akan terlarut dalam pelarut non polar. Hasil KLT menunjukkan bahwa saponin terdeteksi pada fraksi n-heksan dan fraksi etil asetat (Gambar 3).



Gambar 3. Profil KLT ekstrak dan fraksi buah api-api putih pada fase diam silika gel GF_{254} dan fase gerak kloroform : asam asetat glacial : methanol : air (64:32:12:8). Keterangan: sinar tampak (A), sinar UV 254 nm (B), UV 366 nm (C), sinar tampak setelah disemprot anisaldehid-asam sulfat (D). Ekstrak etanol 96% (1), Fraksi n-heksan (2), Fraksi etil asetat (3), Fraksi air (4).

Modifikasi fase gerak untuk pengembangan ekstrak pada pengujian triterpenoid/ steroid telah dilakukan dan diperoleh kombinasi n-heksan : etil asetat (7:3) sebagai fase gerak yang paling optimal dalam memisahkan senyawa. Noda warna biru jika diamati di bawah sinar UV 366, dan berwarna merah ungu-ungu (pada sinar tampak) setelah disemprot pereaksi anisaldehid menandakan hasil positif untuk triterpenoid/ steroid (Khairani & Indriani, 2024). Noda warna ungu merupakan hasil reaksi kondensasi atau oksidasi antara anisaldehid dengan gugus fungsional terpenoid (Hasil positif mengandung terpenoid ditunjukkan pada ekstrak dan ketiga fraksi seperti dapat dilihat pada Gambar 4. Ini mempertegas hasil uji warna yang juga memperlihatkan adanya kandungan senyawa triterpenoid/steroid. Terpenoid merupakan unit isoprene (C_5H_8) yang cenderung bersifat non polar maupun semi polar. Namun, beberapa terpenoid juga bersifat polar ketika terikat gula sebagai glikosida (Jiang *et al.*, 2016). Hal-hal tersebut yang menjelaskan terdeteksinya terpenoid pada ekstrak dan fraksi buah api-api.

Api-api (*Avicennia marina*), sebagai salah satu spesies dari genus *Avicennia*, telah banyak didokumentasikan mengandung berbagai metabolit unik dari beragam kelas kimia. Metabolit-metabolit ini diduga berperan dalam aktivitas farmakologisnya. Golongan flavonoid dan terpenoid merupakan senyawa yang paling sering ditemukan dalam tanaman dari genus *Avicennia* (Thatoi *et al*.,2016). Pemeriksaan menggunakan GC-MS memperlihatkan bahwa persentase γ-sitosterol, ditemukan paling besar pada ekstrak etanol buah api-api putih (Fernandez & Noor'an, 2019). Studi *docking* (*in silico*) menunjukkan bahwa γ-sitosterol menjadi kandidat potensial untuk dikembangkan menjadi agen hipolipidemik yang efektif. Selain itu, review terkait aktivitas senyawa bioaktif tanaman telah mencatat bahwa triterpenoid/steroid juga terbukti berpotensi sebagai antihiperglikemia melalui studi *in vitro* maupun *in silico* (Shaikh, 2021), salah satunya melalui mekanisme penghambatan enzim *Dipeptidyl peptidase*-4 (DPP-4).



Gambar 4. Profil KLT ekstrak dan fraksi buah api-api putih pada fase diam silika gel GF_{254} dan fase gerak n-heksan : etil asetat (7:3). Keterangan: sinar tampak (A), sinar UV 254 nm (B), UV 366 nm (C), sinar tampak setelah disemprot anisaldehid-asam sulfat (D). Ekstrak etanol 96% (1), Fraksi n-heksan (2), Fraksi etil asetat (3), Fraksi air (4).

3.2.3. Uji aktivitas penghambatan enzim DPP-4

Efek penghambatan enzim DPP-4 oleh masing-masing fraksi buah api-api dianalisis berdasarkan nilai IC_{50}, dengan sitagliptin sebagai kontrol positif. Hasil pengujian ditampilkan pada Tabel 3. Fraksi etil asetat menunjukkan kekuatan penghambatan tertinggi terhadap enzim DPP-4, sedangkan potensi penghambatan terendah dapat diamati pada fraksi n-heksana. Berdasarkan nilai IC_{50}, fraksi etil asetat menunjukkan aktivitas penghambatan tertinggi terhadap enzim DPP-4, diikuti oleh fraksi air, dan yang terendah adalah fraksi n-heksan. Hasil skrining fitokimia dalam fraksi etil asetat menunjukkan keberadaan flavonoid, steroid, tanin, dan saponin. Senyawa-senyawa bioaktif tersebut diduga bertanggungjawab memberikan aktivitas penghambatan DPP-4.

Hasil dalam penelitian ini sesuai dengan penelitian yang telah ada bahwa senyawa semi polar memiliki potensi lebih besar dalam aktivitas penghambatan DPP4 (Quek *et al*., 2021; Purnomo, 2015). Senyawa-senyawa steroid dalam tanaman (fitosterol) seperti stigmasterol, β maupun γ-sitosterol adalah golongan senyawa yang lebih banyak terekstraksi dalam pelarut

semi polar. Berdasar studi *in silico* diketahui fitosterol dapat mengikat protein target DPP4. Aktivitas penghambatan DPP-4 fitosterol dinyatakan kuat karena mempunyai energi bebas pengikatan rendah, meskipun sejauh ini secara *in vitro* pendapat tersebut tidak terbukti (Gupta *et al*., 2020).

Tabel 3. Aktivitas penghambatan DPP-4 fraksi-fraksi buah api-api (*A. Marina*) .

Sampel teruji	IC_{50} (mg/mL)
Fraksi n-heksan	5,19
Fraksi etil asetat	1,36
Fraksi air	2,51
Sitagliptin	0,07

Golongan senyawa lain yang juga dianggap bertanggungjawab memberikan aktivitas penghambatan DPP-4 adalah flavonoid (Yaribeygi *et al*., 2018). Sebagai golongan senyawa yang cukup besar dalam tanaman, terdapat bermacam flavonoid dengan tingkat kepolaran yang berbeda-beda. Jenis pelarut semi polar memungkinkan banyak senyawa flavonoid akan terekstraksi di dalamnya. Aktivitas penghambatan flavonoid terhadap enzim DPP-4 melalui mekanisme pengikatan secara langsung terhadap situs aktif enzim, menghambat pembelahan inkretin (GLP-1) sehingga mengakibatkan peningkatan sekresi insulin dan menurunkan kadar glukosa darah (Fan *et al*., 2013; Pan *et al.,* 2022).

Penelitian ini telah mengeksplorasi potensi antidiabetik melalui penghambatan aktivitas DPP-4 fraksi-fraksi dari ekstrak etanol buah api-api. Penghambatan aktivitas DPP-4 akan meningkatkan waktu paruh hormon incretin, yang berkontribusi terhadap pengendalian kadar glukosa dalam darah untuk DM tipe 2 dengan merangsang pelepasan insulin, penghambatan glukagon, proliferasi sel β, menurunkan laju pengosongan lambung, dan menginduksi rasa kenyang. DPP-4 terlibat dalam inaktivasi GLP-1 sebagai peptida insulinotropik yang kuat (Rameshrad, 2020). Penghambatan aktivitas DPP-4 dapat menjadi metode yang efektif untuk pengobatan DM tipe 2 dengan meningkatkan sekresi insulin. Namun pengujian terhadap ekstrak dan fraksi buah api-api ini masih sebatas *in vitro*, sehingga perlu dilakukan pengujian *in vivo.* Hal itu terkait dengan adanya pengaruh farmakodinamika dan farmakokinetika suatu senyawa bioaktif di dalam tubuh terhadap efek farmakologi yang dihasilkan. Penelitian lanjutan diperlukan untuk menguji efektivitas fraksi etil asetat dari buah api-api putih sebagai agen antidiabetik melalui uji *in vivo*.

4. KESIMPULAN

Ekstraksi buah api-api (*Avicennia marina*) menggunakan pelarut etanol 96% menghasilkan rendemen sebesar 48,06% dan fraksinasi terhadap ekstrak menunjukkan rendemen tertinggi pada fraksi air sebesar 31,54%. Uji fitokimia menunjukkan bahwa ekstrak etanol dan fraksi etil asetat buah api-api mengandung flavonoid, triterpenoid/steroid, tanin, dan saponin. Fraksi air teridentifikasi mengandung flavonoid, tanin, dan terpenoid, sementara fraksi n-heksan hanya terdapat flavonoid dan triterpenoid. Di antara ketiga fraksi, fraksi etil asetat

menunjukkan aktivitas penghambatan DPP-4 tertinggi dengan nilai IC_{50} sebesar 1,36 mg/mL sehingga berpotensi dikembangkan sebagai kandidat penghambat inhibitor DPP-4 alami untuk terapi diabetes melitus tipe 2.

UCAPAN TERIMAKASIH

Terima kasih kepada Direktorat Riset, Teknologi, dan Pengabdian Masyarakat yang telah memberikan dana hibah penelitian yang memungkinkan tim peneliti untuk dapat melakukan penelitian ini.

DEKLARASI KONFLIK KEPENTINGAN

Semua penulis menyatakan tidak ada konflik kepentingan terhadap naskah ini.

DAFTAR PUSTAKA

Adiputra, R. (2023) Efek Samping Penggunaan Obat Anti Diabetes Jangka Panjang : Sebuah Meta Analisis. *J. Kesehat. Tambusai*, vol. 4, no. 3, pp. 3951–3959

Andriani, S., Widiastuti, E. L., Nurcahyani, N., Rosa, E., and Busman, H. (2021) "Cytotoxic activity of methanol extraction of Avicennia marina and taurin in the HeLa cancer cells," *J. Phys. Conf. Ser.*, vol. **1751**, no. 1, https://doi.org/10.1088/1742-6596/1751/1/012045

Balamurugan, R., Stalin, A., Aravinthan, A. (2015) γ-sitosterol a potent hypolipidemic agent: In silico docking analysis. *Med Chem Res* **24**: 124–130. https://doi.org/10.1007/s00044-014-1075-0

Deacon, C.F. (2020) Dipeptidyl peptidase 4 inhibitors in the treatment of type 2 diabetes mellitus. *Nat Rev Endocrinol* **16**, 642–653. https://doi.org/10.1038/s41574-020-0399-8

Dewi, N. L. A., Adnyani, L. P. S., Pratama, R. B. R., Yanti, N. N. D., Manibuy, J. I., Warditiani N. K. (2018) Pemisahan, Isolasi, dan Identifikasi Senyawa Saponin dari Herba Pegagan (Centella asiatica L. Urban). *Jurnal Farmasi Udayana*, Vol 7, No 2: 68-76

Fan, J., Johnson, M. H., Lila, M. A., Yousef, G., & de Mejia, E. G. (2013). Berry and Citrus Phenolic Compounds Inhibit Dipeptidyl Peptidase IV: Implications in Diabetes Management. *Evidence-based complementary and alternative medicine : eCAM*, *2013*, 479505. https://doi.org/10.1155/2013/479505

Fernandes, A. dan Noor'an, R.F. 2019. Uji Fitokimia Dan Gc-Ms Buah Api-Api (Avicennia marina (FORSSK.) VIERH). *Jurnal Insan Farmasi Indonesia*, **2**: 261–270. DOI: 10.36387/jifi.v2i2.321

Gupta, A., Narkowicz, C. K., Al-Aubaidy, H. A., Jelinek, H. F., Nichols, D. S., Burgess, J. R., & Jacobson, G. A. (2020). Phytosterol supplements do not inhibit dipeptidyl peptidase-4. *Diabetes & metabolic syndrome*, *14*(5), 1475–1478. https://doi.org/10.1016/j.dsx.2020.07.019

Iriany, Angkasa, H., Namira, C.A. (2021) Ekstraksi Tanin dari Buah Balakka (Phyllanthus emblica L.) dengan Bantuan Microwave: Pengaruh Daya Microwave, Perbandingan Massa Kering Terhadap Jumlah Pelarut Etil Asetat. *Jurnal Teknik Kimia USU*, Vol. 10, No. 1: 8-12

Jiang, Z., Kempinski, C., & Chappell, J. (2016). Extraction and Analysis of Terpenes/Terpenoids. *Current protocols in plant biology*, *1*, 345–358. https://doi.org/10.1002/cppb.20024

Khairani, A.C., Indriani, N. (2024) Identifikasi Kandungan Senyawa Fitokimia Minyak Buah Merah (Pandanus conoideus Lam.) *Jurnal Kolaboratif Sains*, Volume 7 No. 12, 4599–4603. DOI: 10.56338/jks.v7i12.6335

Mierza, V., Antolin, S., Ichsani, A., Dwi, N., Sridevi, A., Dwi, S. (2023) Isolasi dan Identifikasi Senyawa Terpenoid. *Jurnal Surya Medika (JSM),* Vol 9 No 2: Page 134–141. DOI: 10.33084/jsm.v9i2.5681

Marín-Peñalver, J. J., Martín-Timón, I., Sevillano-Collantes, C., & Del Cañizo-Gómez, F. J. (2016). Update on the treatment of type 2 diabetes mellitus. *World journal of diabetes*, *7*(17), 354–395. https://doi.org/10.4239/wjd.v7.i17.354

Okla, M. K., Alamri, S. A., Alatar, A. A., Hegazy, A. K., Al-Ghamdi, A. A., Ajarem, J. S., Faisal, M., Abdel-Salam, E. M., Ali, H. M., Salem, M. Z. M., & Abdel-Maksoud, M. A. (2019). Antioxidant, Hypoglycemic, and Neurobehavioral Effects of a Leaf Extract of *Avicennia marina* on Autoimmune Diabetic Mice. *Evidence-based complementary and alternative medicine : eCAM*, *2019*, 1263260. https://doi.org/10.1155/2019/1263260

Pan, J., Zhang, Q., Zhang, C., Yang, W., Liu, H., Lv, Z., Liu, J., & Jiao, Z. (2022). Inhibition of Dipeptidyl Peptidase-4 by Flavonoids: Structure-Activity Relationship, Kinetics and Interaction Mechanism. *Frontiers in nutrition*, *9*, 892426. https://doi.org/10.3389/fnut.2022.892426

Purnomo, Y., Soeatmadji, D. W., Sumitro, S. B., Widodo, M. A. (2015) Anti-diabetic potential of Urena lobata leaf extract through inhibition of dipeptidyl peptidase IV activity *Asian Pac J Trop Biomed* Volume 5, Issue 8, 645-649. https://doi.org/10.1016/j.apjtb.2015.05.014

Putria, D. K., Salsabila, I., Darmawan, S. A. N., Eka Wulan G Pratiwi, E. W. G., Nihan, Y. A. (2022) Identifikasi Tanin pada Tumbuh-tumbuhan di Indonesia. *PharmaCine*, Vol. 3 No.1: 11-24. https://doi.org/10.35706/pc.v3i1.7238

Quek, A., Kassim, N. K., Lim, P. C., Tan, D. C., Mohammad Latif, M. A., Ismail, A., Shaari, K., & Awang, K. (2021). α-Amylase and dipeptidyl peptidase-4 (DPP-4) inhibitory effects of *Melicope latifolia* bark extracts and identification of bioactive constituents using *in vitro* and *in silico* approaches. *Pharmaceutical biology*, *59*(1), 964–973. https://doi.org/10.1080/13880209.2021.1948065

Rameshrad, M., Razavi, B. M., Lalau, J. D., De Broe, M. E., & Hosseinzadeh, H. (2020). An overview of glucagon-like peptide-1 receptor agonists for the treatment of metabolic syndrome: A drug repositioning. *Iranian journal of basic medical sciences*, *23*(5), 556–568. https://doi.org/10.22038/ijbms.2020.41638.9832

Rodríguez De Luna, S. L., Ramírez-Garza, R. E., & Serna Saldívar, S. O. (2020). Environmentally Friendly Methods for Flavonoid Extraction from Plant Material: Impact of Their Operating Conditions on Yield and Antioxidant Properties. *TheScientificWorldJournal*, *2020*, 6792069. https://doi.org/10.1155/2020/6792069

Rozirwan, Nugroho, R.Y., Hendri, M.I., Fauziyah, Putri, W.A., & Agussalim, A. (2022). Phytochemical profile and toxicity of extracts from the leaf of Avicennia marina (Forssk.) Vierh. collected in mangrove areas affected by port activities. *South African Journal of Botany*. Vol 150, 903-919. https://doi.org/10.1016/j.sajb.2022.08.037

Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603–608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834.

Shaikh, S., Lee, E. J., Ahmad, K., Ahmad, S. S., Lim, J. H., & Choi, I. (2021). A Comprehensive Review and Perspective on Natural Sources as Dipeptidyl Peptidase-4 Inhibitors for Management of Diabetes. *Pharmaceuticals (Basel, Switzerland)*, *14*(6), 591. https://doi.org/10.3390/ph14060591

Singh, A. K., Yadav, D., Sharma, N., & Jin, J. O. (2021). Dipeptidyl Peptidase (DPP)-IV Inhibitors with Antioxidant Potential Isolated from Natural Sources: A Novel Approach

for the Management of Diabetes. *Pharmaceuticals (Basel, Switzerland)*, *14*(6), 586. https://doi.org/10.3390/ph14060586

Sohaib, M., Al-Barakah, F. N. I., Migdadi, H. M., & Husain, F. M. (2022). Comparative study among *Avicennia marina*, *Phragmites australis,* and *Moringa oleifera* based ethanolic-extracts for their antimicrobial, antioxidant, and cytotoxic activities. *Saudi journal of biological sciences*, *29*(1), 111–122. https://doi.org/10.1016/j.sjbs.2021.08.062

Supomo, Warnida, H., Sahid (2019). Perbandingan Metode Ekstraksi Ekstrak Umbi Bawang Rambut (Allium Chinense G.Don.) Menggunakan Pelarut Etanol 70% Terhadap Rendemen Dan Skrining Fitokimia. *Jurnal Riset Kefarmasian Indonesia,* 1(1):30-40. DOI: 10.33759/jrki.v1i1.15

Thatoi, H., Samantaray, D., & Das, S. K. (2016). The genus Avicennia, a pioneer group of dominant mangrove plant species with potential medicinal values: a review. *Frontiers in Life Science*, 9(4), 267–291. https://doi.org/10.1080/21553769.2016.1235619

Vasanthakumar, S., Dineshkumar, G., & Jayaseelan, K. (2019). Phytochemical Screening, GC-MS Analysis and Antibacterial Evaluation of Ethanolic Leaves Extract of Avicennia marina. *Journal of Drug Delivery and Therapeutics*. DOI: 10.22270/jddt.v9i4-A.3431

Widiawati, Asih, E. N. A. (2024). Potensi skrining fitokimia dan aktivitas antioksidan ekstrak daun Avicennia marina dan Avicennia alba dari Selat Madura. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 27(5), 393-406. http://dx.doi.org/10.17844/jphpi.v27i5.52421

Yan, L. S., Cheng, B. C., Zhang, S. F., Luo, G., Zhang, C., Wang, Q. G., Fu, X. Q., Wang, Y. W., & Zhang, Y. (2021). Tibetan Medicine for Diabetes Mellitus: Overview of Pharmacological Perspectives. *Frontiers in pharmacology*, *12*, 748500. https://doi.org/10.3389/fphar.2021.748500

Yassien, E. E., Hamed, M. M., Abdelmohsen, U. R., Hassan, H. M., & Gazwi, H. S. S. (2021). In vitro antioxidant, antibacterial, and antihyperlipidemic potential of ethanolic Avicennia marina leaves extract supported by metabolic profiling. *Environmental science and pollution research international*, *28*(21), 27207–27217. https://doi.org/10.1007/s11356-021-12496-7

Yaribeygi, H., Atkin, S. L., & Sahebkar, A. (2019). Natural compounds with DPP-4 inhibitory effects: Implications for the treatment of diabetes. *Journal of cellular biochemistry*, *120*(7), 10909–10913. https://doi.org/10.1002/jcb.28467

JPSCR: Journal of Pharmaceutical Science and Clinical Research
Volume 10, Issue 1, Pages 114-129, March 2025
https://doi.org/10.20961/jpscr.v10i1.100041

ISSN 2503-331X (Online)

Physical and SPF Value Stability Studies Avocado Oil Nano-emulgel with Carbopol 980 as A Gel Base

Ayu Shabrina[1]*, Diva Ma'arif Pangestu[2], Siska Amaliyah[2], Malinda Prihantini[1], Junvidya Heroweti[3] and Listyana Dewi Prastiwi[4]

[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Wahid Hasyim, Semarang, Indonesia
[2]Faculty of Pharmacy, Universitas Wahid Hasyim, Semarang, Indonesia
[3]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Wahid Hasyim, Semarang, Indonesia
[4]Faculty of Pharmacy, Universitas Muhammadiyah Kalimantan Timur, Samarinda, Indonesia

*Corresponding author: shabrina@unwahas.ac.id

Received: March 2, 2025; **Accepted:** May 27, 2025; **Published:** June 22, 2025

Abstract
Avocado oil (AVO) is a natural source of many unsaturated fatty acids that can help protect the skin from harmful UV radiation. The high content of unsaturated fatty acids in avocado oil can affect product stability. One way to overcome the stability of avocado oil for topical preparation is by formulating it into a gel base using Carbopol 980. The purpose of this study was to determine the physical stability and SPF value of avocado oil nano-emulgel (NE) with variations of Carbopol 980 as a gel base for 28 days of storage. AVONE was made with 5% AVO and Carbopol 980 base variation of 0.5% (NE1), 1.0% (NE2;), and 1.5% (NE3.) AVONE was stored in a climatic chamber at 30° ± 2°C with RH 65% ± 5%. The samples were tested for physical and SPF values before storage on days 7, 14, 21, and 28. The data obtained were analyzed statistically using one-way Anova. The AVONE on organoleptic parameters were stable; the resulting colour is broken-white with a distinctive oil aroma and thick and homogeneous texture. The pH value ranged from 6.21 ± 0.02 - 7.21 ± 0.02, with all formulas stable during storage. The viscosity value ranged from 13.28±0.23 - 47.22±0.89 dPa.s; the viscosity of NE3 was stable during storage. Adhesion and spreadability showed good ability to adhere to the skin. The SPF values ranged from 8.95±0.43 – 22.41±0.21, and NE3 was stable during storage. Avocado oil nano-emulgel with Carbopol 980 concentration of 1.5% was stable during storage.

Keywords: Avocado oil; Carbopol; Emulgel; Nano-emulgel; Stability

1. INTRODUCTION

Avocado oil is a natural source of many unsaturated fatty acids such as oleic, linoleic, and palmitic acids, which can help protect the skin from harmful UV radiation (Suradnyana et al., 2023). Avocado oil contains unsaturated fatty acids totalling 85.72%(Li et al., 2019). The higher content of unsaturated fatty acids in avocado oil make it easy to oxidize and lead to be spoiled oil (Hermanto et al., 2010).

Avocado oil can increase collagen synthesis, reduce the number of inflammatory cells, accelerate coagulation, and accelerate epithelial regeneration (Ebad Sichani et al., 2021). Avocado oil is a rich source of oleic acid (unsaturated fatty acid), which can increase collagen synthesis. However, the content of unsaturated fatty acids in avocado oil can cause oxidation reactions due to its poor stability(A. P. De Oliveira et al., 2013). Avocado oil has a comedogenic rating of 3 when used directly on the skin(Ghani et al., 2021). Avocado oil may clog pores in some skin types, potentially causing acne or skin issues in sensitive individuals. One way to overcome the instability of avocado oil is to formulate it into nano-emulgel (Eid et al., 2013; Scomoroscenco et al., 2021)

Previous research has formulated avocado oil in several dosage forms, such as nanoemulsion (NS), emulgel and nano-emulgel (NE) with Carbopol 940 (Dewi et al., 2024; Eid et al., 2013; Shabrina, 2024). Avocado oil NS showed good stability and globule size. The avocado oil SPF value and MED (Minimum Erythemal Dose) of outcomes are inferior to NS, potentially due to the gel base's effect. Consequently, it is essential to investigate the physical stability and SPF value of avocado oil formulated as a nano-emulgel with carbopol 980 to ensure product stability and efficacy. Nano-emulgel using Carbopol 980 offers advantages over cream formulations, including enhanced stability of the emulsion system, which is augmented by the increased viscosity of the aqueous phase due to the presence of a gelling agent. This configuration facilitates the delivery of both hydrophilic and hydrophobic compounds, as the nano-emulgel comprises a biphasic oil and water system (Priani et al., 2020). Another advantage of the nano-emulgel form is the presence of an oil phase component in the emulsion system as a suitable carrier for hydrophobic active substances such as avocado oil, which is difficult if formulated into a form that contains a lot of water, such as a gel(Hardenia et al., 2014). Nano-emulgel preparations are also known to adhere better than cream preparations, thereby increasing comfort and effectiveness of use (Sreevidya, 2019). Several polymer bases, including Carbopol 980, can be utilized to produce nano-emulgel.

Carbopol 980 is utilized as a basis owing to its advantages, including the transparent physical appearance of the nano-emulgel, compatibility, stability, and suitable viscosity (Nabillah et al., 2022). Carbopol 980 is recognized for providing a stable formulation at room temperature and during accelerated storage conditions (Shelke & Kulkarni, 2019). Carbopol is a base due to its excellent stability, absorption, spreadability, and non-sticky properties, making it suitable for sunscreen formulations (Ikhtiyarini & Sari, 2022). Carbopol 980, used as a gelling agent with a concentration of 0.5%, produces an ideal preparation. Carbopol 980 in nano-emulgel preparations produces stable preparations for 3 months at room temperature and accelerated storage conditions (Shelke & Kulkarni, 2019). However, despite these known advantages, there is a lack of research specifically evaluating how variations in Carbopol 980 concentration affect the physical stability and SPF value of nano-emulates incorporating avocado oil as the active component. It is necessary to investigate the impact of different base concentrations on the overall performance of the formulation. Based on the above background, it is necessary to conduct research related to the physical and SPF value stability of avocado oil

nanoemulsion in the form of nano-emulgel preparations with variations in Carbopol 980 base. This base type prevents phase separation and ensures uniform dispersion of UV-filtering actives critical for consistent SPF performance.

2. MATERIAL AND METHODS

2.1. Materials

The material used in this study was avocado oil (cosmetic grade) obtained from PT Daarjeling Aroma, Bandung, with a certificate of analysis. The carrier materials were Tween 80, Span 80, Sorbitol, Paraffin Liquid, TEA, methyl paraben, propyl paraben, and Carbopol 980 obtained from PT Multi Kimia Raya Semarang with cosmetic grade. DMSO and distilled water were obtained from PT Multi Kimia Raya Semarang with analytical grade.

The tools used in this study are a set of climatic chambers (Taisite HWS-70BX®), a viscometer (Rheosys Merlin II®) with type of cone and plate, a set of adhesion test equipment, a set of spreadability test equipment, and UV-Vis spectrophotometer (Shimadzu UV-1800®).

2.2. Methods

The preparations of nano-emulgel began with nanoemulsion production (Table 1). Tween 80 and PEG 400 were heated at 35°C in a different beaker glass. Tween 80 and PEG 400 were mixed using a magnetic stirrer at 700 rpm. The avocado oil was then added dropwise into the mixture, stirred at 1200 rpm until homogenous, and showed a clear and transparent nanoemulsion. The product was tested for particle size, polydispersity index and zeta potential to ensure that the preparation falls into nanoemulsion category.

Table 1. Formula of avocado oil nanoemulsion with carbopol 980 variations.

Materials	Concentration (%b/v)		
	NE1	NE2	NE3
Avocado oil		7.5	
Tween 80		30	
PEG 400		40	
Carbopol 980	0.50	1.00	1.50
Tween 80	12.50	12.50	12.50
Span 80	2.50	2.50	2.50
Sorbitol	1.00	1.00	1.00
Paraffin Liquid	1.25	1.25	1.25
Methyl parabene	0.18	0.18	0.18
Propyl paraben	0.02	0.02	0.02
Triethanolamine	0.80	0.80	0.80
Distilled water		Up to 100	

The preparation of nano-emulgel begins with weighing each ingredient according to the formula in Table 1. The gel phase (Carbopol 980) was made by dispersing the polymer base (Carbopol 980) into hot water at 80°C and stirring in a mortar until a thick gel mass was formed. The emulsion phase was then made consisting of an aqueous phase (methylparaben, and tween 80) and an oil phase (propylparaben, liquid paraffin, and span 80), each phase of which was

first homogenized in a waterbath that had been adjusted to a temperature of 70°C. The oil phase was added to the water phase in a glass beaker. The mixture was stirred using a magnetic stirrer set at 40°C and a speed of 500 rpm; then distilled water and sorbitol were added and stirred until homogeneous, after which avocado oil was added little by little with stirring until homogeneous. The homogeneous emulsion phase is mixed with the gel base (Carbopol 980) that has been formed. Each formula was produced for three replications.

Avocado oil nano-emulgel was stored in a climatic chamber at 30° ± 2°C with RH 65% ± 5%. Samples were tested before storage and on days 7, 14, 21, and 28 (Nabillah et al., 2022)(FDA, 2013). The parameters tested were:

2.2.1. Organoleptical test

Organoleptic testing is done by direct observation using the five senses, including colour, odour and consistency of the nano-emulgel made (Depkes RI, 1995).

2.2.2. Homogeneity test

A nano-emulgel preparation of 0.1 g was applied to a transparent glass plate and observed for homogeneity. The test preparation must show a homogeneous arrangement, indicated by the absence of coarse grains on the glass object (Voigt, 1984).

2.2.3. pH test

The pH measurement was carried out using a pH meter at room temperature. Before use, the pH meter electrode is washed, rinsed with distilled water, and dried. The tool is calibrated using a standard buffer solution of pH four and pH 7 (BPOM RI, 1995). The pH test is also carried out to determine if the pH of the nano-emulgel preparation meets the requirements according to SNI 16-3499-1996. A good pH for the skin is 4.5-8 (Chandra & Rahman, 2022).

2.2.4. Viscosity test

The viscosity test was conducted with a Rheosys Merlin II-type cone and plate viscometer. A sample of 0.5 g was put into the plate, and then the cone was positioned to start the measurement. Viscosity and flow curves are generated automatically with the Rheosys Micra application (Nabillah et al., 2022).

2.2.5. Adhesion test

The adhesion test is carried out by weighing the preparation as much as 0.25 grams placed on a glass object that has been determined in the area than another glass object is placed above. The glass object was then mounted on the test device, given a load of 1 kg for 5 minutes, and then released with a load weighing 80 grams. The time is recorded until the two glass objects are released (Allen, 1998).

2.2.6. Spreadability test

A total of 0.5 grams of nano-emulgel preparation was placed in the centre of a transparent glass covered with graph paper underneath, then covered with another transparent glass on top,

given a load (50 g, 100 g, 150 g, 200g and 250g) and allowed to stand for 1 minute, measuring the diameter of the nano-emulgel spread area released (Voigt, 1984).

2.2.7. SPF value test

Determination of SPF value was done using a UV-Vis spectrophotometer by weighing 1 gram of avocado oil nano-emulgel, then put into a 10 ml volumetric flask and dissolved with DMSO as much as 5 ml. The solution was vortexed until completely dissolved. The solution that has been obtained is measured with a UV-Vis spectrophotometer at a wavelength of 290-320 nm using DMSO as a blank; then the absorbance value is recorded at every 5 nm interval. The recorded absorbance value were used to calculated the Sun Protection Factor (SPF) using the Mansur method (Mansur et al., 1986):

$$SPF = CF \times \Sigma_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Equation 1. Calculation of Sun Protection Faactor (SPF) using the Mansur method *(Mansur et al.,* 1986). *Description*: CF = correction factor, Abs (λ) = absorption of sunscreen product, Σ_{290}^{320}EE (λ) = erythemal effect spectrum, I (λ) = intensity of light spectrum.

2.3. Data analysis

Organoleptic and homogeneity test data were described descriptively, while pH, viscosity, adhesion, spreadability, and SPF values were analyzed statistically using one-way Anova to detect any physical changes in all formulas during 28 days of storage. Nano-emulgel is concluded as a stable product if no significant changes or significance values were > 0.05.

3. RESULTS AND DISCUSSION

The result of the nanoemulsion of avocado oil can be seen in Figure 1 and Table 2. The product was categorized as a nanoemulsion according to its particle size.

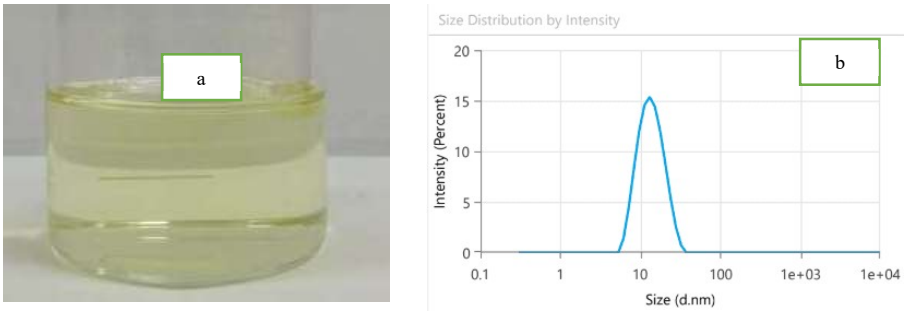


Figure 1. The result of the organoleptic test (a) and particle size (b) of avocado oil nanoemulsion.

Based on the result above, it can be concluded that the preparation was categorized as a nanoemulsion. The result of the particle size was below 100 nm, and the polydispersity index was below 0.5, showing that the nanoemulsion had a homogenous globule size (Widyastuti & Saryanti, 2023). The zeta potential was below 30 mV, indicating that the nanoemulsion had a good stability(Mardiyanto et al., 2024). Tween 80 with PEG 400 can create transparent and

stable nanoemulsions close to a micellar system(Artanti et al., 2021). Tween 80 is a hydrophilic surfactant that effectively decreases surface tension in nanoemulsion formulations (Suryani et al., 2020).

Table 2. The results of the physical test of avocado oil nanoemulsion and nano-emulgel. *Description*: NE1 = nano-emulgel with 0,50% of Carbopol 980, NE2 = nano-emulgel with 1,00% of Carbopol 980, NE3 = nano-emulgel with 1,50% of Carbopol 980. Data displayed n = 3 ±SD. *Significantly different with nanoemulsion.

Parameters	Result			
	Nanoemulsion	Nano-emulgel		
		NE1	NE2	NE3
Physical appearance	Clear and transparent liquid	Broken white with soft texture		
Particle size (nm)	12.74 ± 0.24	320.72 ± 1.22*	323.00 ± 1.87*	322.00 ± 0.92*
Polydispersity index	0.1415 ± 0.02	0.4318 ± 0.04*	0.4325 ± 0.07*	0.4320 ± 0.05*
Zeta potential (mV)	22.5 ± 1.15	-28.1 ± 1.08*	-29.3 ± 0.81*	-28.7 ± 1.02*

The physical test results of NE1, NE2 and NE3 were significantly different with nanoemulsion ($p = 0.001$), attributed to the aggregation of globules with the bases and other solvents in the emulgel. Carbopol could experience entrapment within the nanoemulsion system, increasing particle size relative to the nanoemulsion(Abdullah et al., 2023). Particle aggregation within the system can affect various parameters, including the polydispersity index and zeta potential(Al-Awady et al., 2017). The PDI data indicated that the nanoemulsion was monodisperse. This monodisperse system was also demonstrated in nano-emulgel. Prior studies indicated that nanoemulsions incorporated into hydrogels yield a polydispersity index of less than 0.5 and maintain stability for up to 90 days of storage(Alhasso et al., 2023). The zeta potential in all nanoemulgel formulations exhibited negative values, influenced by the carboxylate group of carbopol. Carboxylic groups can engage in electrostatic interactions when avocado oil droplets are integrated into the base, resulting in a stable colloidal structure(Alhasso et al., 2023). This result was in line with Shabrina et al.(2024) that nanoemulsion dispersed in carbopol as a gel base might experience changes such as zeta potential and globule size.

The results of the organoleptic test of avocado oil nano-emulgel preparations for NE1, NE2 and NE3 can be seen in Figure 2.

Figure 2. The result of the organoleptic test of avocado oil nano-emulgel with Carbopol 980 variations (NE1: 0,50%; NE2: 1,00%; NE3: 1,50%)

The results of organoleptic testing of avocado oil NE did not show any colour changes during 28 days of storage. The texture of the preparation in NE1 was slightly thick, while NE2 and NE3 had a thicker texture. The difference in texture of the three formulas was due to the different concentrations of Carbopol 980 base used in each formula. According to research by Nabillah et al. (2022), the greater the concentration of gelling agent used, the thicker the preparation's texture will be. After 28 days of storage, the three formulas became thinner in texture. This is because gel preparations might experience a syneresis. This event was caused by the release of water from the preparation, where the preparation shrinks so that it tends to squeeze water out of the preparation (Kuncari et al., 2014).

The homogeneity test results of avocado oil nano-emulgel preparations for NE1, NE2 and NE3 can be seen in Figure 3.

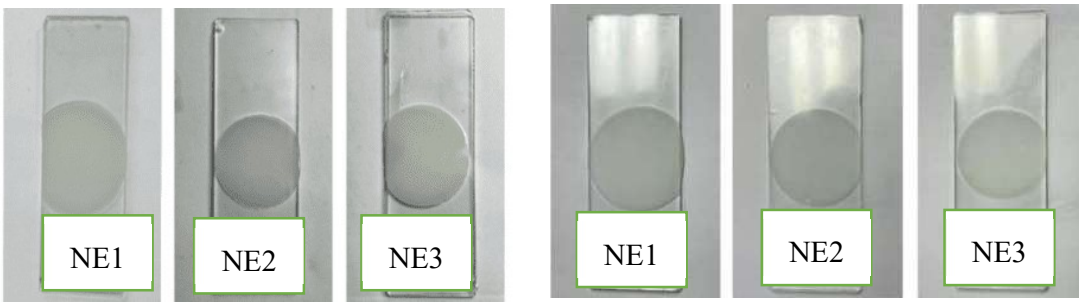


Figure 3. The result of Avocado oil nano-emulgel homogeneity test results before storage (a) and after the storage (b) with Carbopol 980 variations (NE1: 0,50%; NE2: 1,00%; NE3: 1,50%).

As shown in the picture above, NE1, NE2 and NE3 avocado oil nano-emulgel obtained homogeneous results for 28 days of storage. All formulas showed the absence of coarse grains or unmixed particles. The preparation exhibited inhomogeneity, as evidenced by coarse grains on the glass object (Voigt, 1984). The results of the homogeneity test obtained are in line with the research conducted by Nabillah et al. (2022), showing that the different concentrations of Carbopol 980 do not affect the homogeneity of the preparation.

The pH test results of avocado oil nano-emulgel preparations for NE1, NE2 and NE3 can be seen in Table 3.

The pH test results show that NE1 has a higher pH than NE2 and NE3. This indicates that increasing the concentration of Carbopol 980 can affect the pH of the nano-emulgel preparation. The greater the concentration of Carbopol 980 used, the lower the pH will be. avocado oil nano-emulgel pH in NE1, NE2 and NE3 obtained results between 6.21 ± 0.02 - 7.21 ± 0.02. The pH value obtained is based on the range of pH requirements (SNI) for topical preparations, namely 4.5-8.

Based on the statistical analysis, the results did not show any significant difference in pH ($p>0.05$) in the three formulas, which means that there is no significant difference in the pH

value of avocado oil nano-emulgel preparations during the storage cycle, so it can be said that all formula is stable for 28 days of storage.

Table 3. The result of pH stability of avocado oil nano-emulgel with Carbopol 980 as a gel base. Description*: NE1 = nano-emulgel with 0,50% of Carbopol 980, NE2 = nano-emulgel with 1,00% of Carbopol 980, NE3 = nano-emulgel with 1,50% of Carbopol 980, Data displayed n = 3 ±SD.*

Day	pH		
	NE1	NE2	NE3
Before storage	7.21±0.02	6.87±0.01	6.21±0.02
7	7.20±0.02	6.89±0.01	6.24±0.02
14	7.21±0.02	6.87±0.01	6.24±0.04
21	7.20±0.02	6.86±0.03	6.23±0.02
28	7.21±0.02	6.86±0.03	6.22±0.01
p value	0.543	0.671	0.774

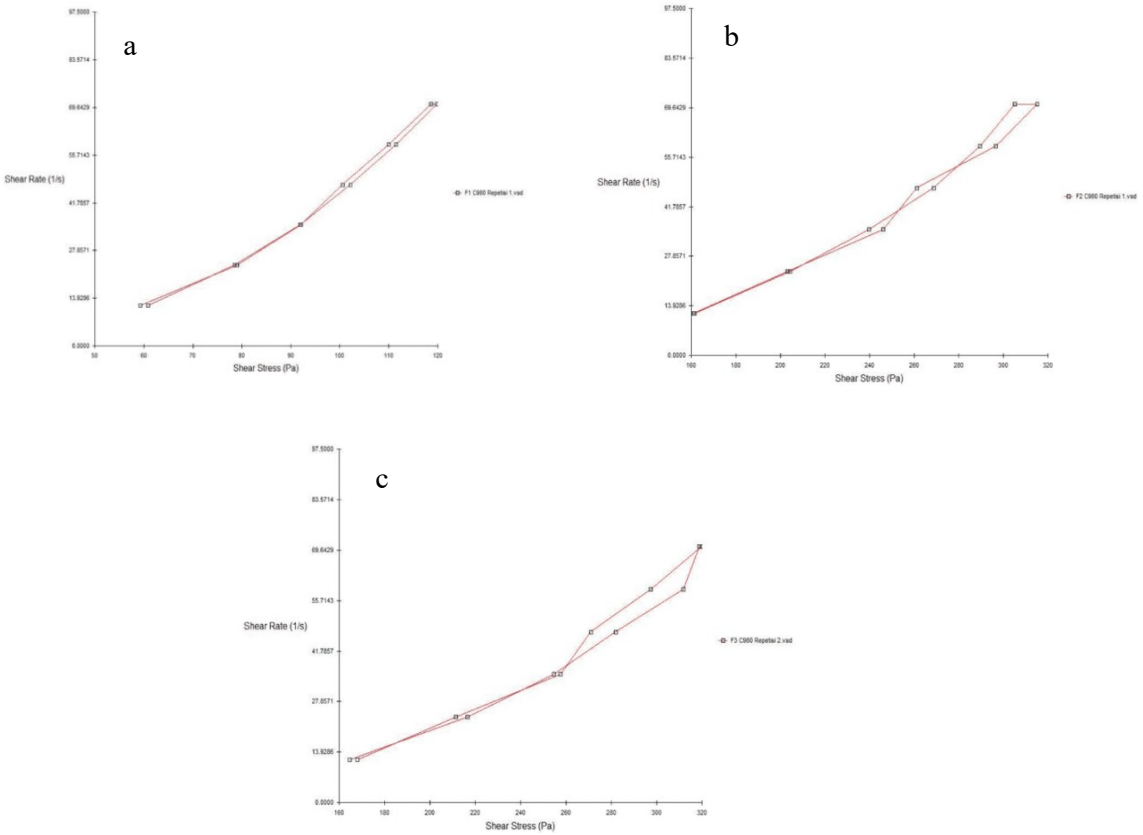
The viscosity test results of avocado oil nano-emulgel preparations for NE1, NE2 and NE3 (Table 4). The viscosity results showed changes during 28 days of storage in NE1, NE2 and NE3, but the changes in the three formulas are still within the range that meets the requirements for good nano-emulgel viscosity values, namely 3-300 dPa.s (Yuliandri et al., 2021). Based on the results of the viscosity values obtained showed that NE3 has a higher viscosity value than NE1 and NE2, and this is in line with previous research conducted by Nabillah et al. (2022), which shows that the higher concentration of Carbopol 980, the viscosity value of the preparation obtained increases. The increasing viscosity indicates a thicker preparation (Suhesti et al., 2022).

Table 4. The result of the viscosity test of avocado oil nano-emulgel with carbopol 980 as a gel base. Description*: NE1 = nano-emulgel with 0,50% of Carbopol 980, NE2 = nano-emulgel with 1,00% of Carbopol 980, NE3 = nano-emulgel with 1,50% of Carbopol 980, Data displayed n = 3 ±SD.*

Day	Viscosity (dPa.s)		
	NE1	NE2	NE3
Before storage	16.88±0.08	43.16±0.07	45.33±0.66
7	15.61±0.22	40.65±0.25	46.42±0.91
14	15.48±0.15	39.32±0.22	46.38±0.82
21	14.31±0.25	38.35±0.41	47.30±0.78
28	13.28±0.23	37.45±0.28	47.22±0.89
p value	0.001	0.024	0.512

Based on the statistical analysis, the viscosity value of NE3 did not show any significant difference ($p>0.05$), meaning there was no significant difference in the viscosity value of avocado oil nano-emulgel preparations during storage. In NE1 and NE2, there were significant changes ($p<0.05$) in the viscosity values of avocado oil nano-emulgel preparations during storage. Both NE1 and NE2 experienced a decrease in viscosity during storage. According to

research by Rismawati et al. (2020), this decrease in viscosity can occur due to the longer temperature and storage time. This is because the nano-emulgel experiences syneresis. Syneresis occurs due to the matrix structure or gel fibres that persist in solidifying, ultimately leading to the expulsion of water and enabling the liquid to migrate to the surface (Rao et al.,



2023; Zalivskaya et al., 2021). This might be caused by the container system (Rao et al., 2023). The rheogram of avocado oil NE can be seen in Figure 4 and Figure 5.

Figure 4. Rheogram of avocado oil nano-emulgel with carbopol 980 as a gel base before storage of (a) NE1 (0,50%), (b) NE2 (1,00%), and (c) NE3 (1,50%).

The rheogram results show that nano-emulgel NE1, NE2 and NE3 rheology were categorized as pseudoplastic. Pseudoplastic is characterized by a slightly curved upward graph shape (Patricia & Yuliani, 2015). Pseudoplastic flow is characterized by a flow curve that intersects the origin (0,0), in contrast to plastic flow, indicating that pseudoplastic flow lacks a yield value. The viscosity of pseudoplastic substances decreases with increasing rate of shear. This occurs in long-chain molecules such as polymers. The pseudoplastic system is also called a dilute shear system because the viscosity decreases by increasing the shear stress (Kuncari et al., 2014). The rheology profile of all formulas did not show any changes. The rheology of Carbopol 980 is not affected by temperature and storage duration but by the cross-linker used during formulation (Islam et al., 2004). This type of rheology has advantages, such as increasing

viscosity after application when the shear is removed, helping the product stay in place and not drip off.

The pseudoplastic nature contributes to a non-dripping, non-sticky texture at rest and a smooth, pleasant feel during application—essential for user satisfaction with topical products like sunscreens. Pseudoplastic gels decrease viscosity under shear stress, such as during application, making them easier to spread on the skin or other surfaces. This behaviour ensures good adherence and uniform coverage, crucial for topical formulations (Jo et al., 2021). Gel with pseudoplastic rheology can withstand mechanical stresses during processing, such as pumping or extrusion, and application without breaking down or separating, contributing to long-term stability (Dantas et al., 2016).

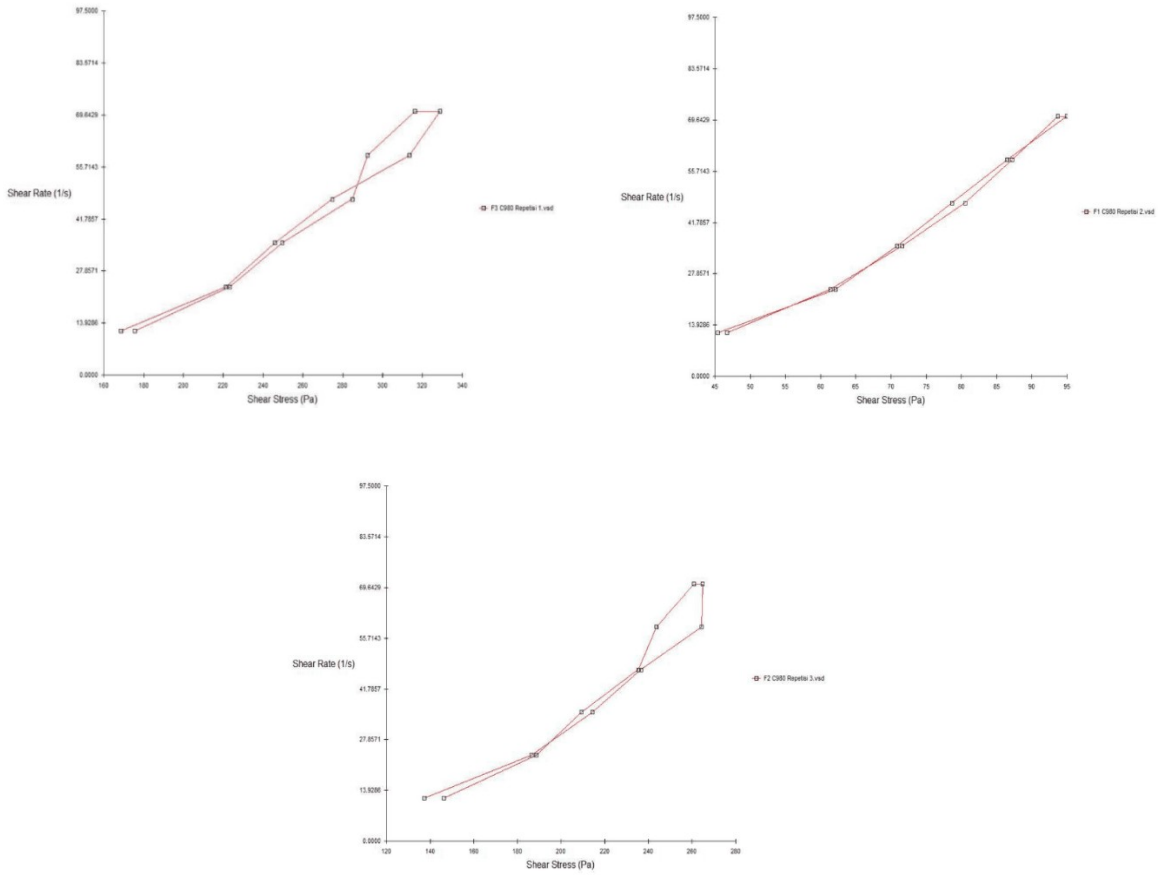


Figure 5. Rheogram of avocado oil nano-emulgel with carbopol 980 as a gel base after storage of (a) NE1 (0,50%), (b) NE2 (1,00%), and (c) NE3 (1,50%).

The results of the avocado oil nano-emulgel preparation adhesion test for NE1, NE2 and NE3 (Table 5). The results of the avocado oil nano-emulgel adhesion test showed that NE3 had a longer adhesion time than NE1 and NE2, but the adhesion of all formulas still met the requirements for good topical preparation adhesion, which is not less than 4 seconds. The results of the avocado oil nano-emulgel adhesion test show that the higher the concentration of

Carbopol 980, the more viscosity of the resulting preparation will increase so that the adhesion time will be longer (Numberi, 2020).

Based on the results of statistical analysis, the adhesion value of avocado oil nano-emulgel preparations shows significant results ($p>0.05$) in NE3, which means that there is no significant difference in the adhesion value of avocado oil nano-emulgel preparations during the storage cycle, so it can be said that NE3 is stable for 28 days of storage. In NE1 and NE2, the significance results ($p<0.05$) mean that there are significant differences in the adhesion value of avocado oil nano-emulgel preparations during the storage cycle, so it can be said that NE1 and NE2 are not stable during 28 days of storage. The adhesion value started changing on day 21. The adhesion values of NE1 and NE2 have decreased during storage due to a reduction in viscosity. However, the adhesion values remain adequate for effective topical preparation, not exceeding 4 seconds (Numberi, 2020).

Table 5. The result of the adhesion test of avocado oil nano-emulgel with carbopol 980 as a gel base. Description*: NE1 = nano-emulgel with 0,50% of Carbopol 980, NE2 = nano-emulgel with 1,00% of Carbopol 980, NE3 = nano-emulgel with 1,50% of Carbopol 980, Data displayed n = 3 ±SD.*

Day	Adhesion (minute)		
	NE1	NE2	NE3
Before storage	5.43±0.07	15.20±0.08	30.34±0.07
7	5.40±0.11	15.32±0.14	30.27±0.14
14	5.81±0.09	15.34±0.09	30.25±0.08
21	4.76±0.03	14.74±0.06	30.27±0.15
28	4.22±0.18	14.36±0.26	30.31±0.20
p value	0.000	0.000	0.541

The results of the spreadability test of avocado oil nano-emulgel preparations for NE1, NE2 and NE3 can be seen in Table 6. The spreadability of the skin preparation is crucial to evaluate as it pertains to the user's ease of application. The broader the active material, the more effectively it will be dispersed (Numberi, 2020). The avocado oil nano-emulgel spreadability test results showed that NE1 can spread more widely than NE2 and NE3. The results showed that the higher the concentration of the gelling agent, the smaller the spreadability value obtained. The results are due to the interconnection between spreadability and viscosity. As the viscosity of the preparation increases, its spreadability decreases; conversely, a decrease in viscosity results in enhanced spreadability.

Based on the results of statistical analysis, the value of the spreadability of avocado oil nano-emulgel shows significant results ($p>0.05$) in NE3, which means that there was no significant difference in the value of the spreadability of avocado oil nano-emulgel preparations during the storage cycle, so it can be said that NE3 is stable for 28 days of storage. Whereas in NE1 and NE2, the significant results ($p<0.05$) mean a significant difference in the value of the spreadability of avocado oil nano-emulgel preparations during the storage cycle. The

spreadability values of NE1 and NE2 increased during storage, influenced by viscosity and reduced adhesion. However, spreadability should not be considered absolute data, as no literature provides an exact ideal value (Numberi, 2020).

Table 6. The result of the spreadability test of avocado oil nano-emulgel with Carbopol 980 as a gel base. *Description*: NE1 = nano-emulgel with 0,50% of Carbopol 980, NE2 = nano-emulgel with 1,00% of Carbopol 980, NE3 = nano-emulgel with 1,50% of Carbopol 980, Data displayed n = 3 ±SD.

Day	Spreadability (cm)		
	NE1	NE2	NE3
Before storage	5.14±0.10	3.72±0.02	3.54±0.02
7	5.19±0.10	3.75±0.04	3.57±0.05
14	5.17±0.13	3.85±0.02	3.57±0.05
21	5.45±0.02	4.00±0.02	3.53±0.02
28	5.38±0.02	3.97±0.01	3.61±0.03
p value	0.025	0.012	0.146

The results of the SPF value test for avocado oil nano-emulgel preparations for NE1, NE2 and NE3 can be seen in Table 7. The results of the SPF value showed that all of the formulas were categorized as having maximum protection. This aligns with previous research conducted by Shabrina et al. (2024) on nanoemulsion and nanoemulgel preparations using 5% avocado oil, which resulted in the maximum protection category(Shabrina, 2024). The SPF values measured over a 28-day storage period in NE1 and NE2 exhibited fluctuations, while NE3 remained stable. Several factors cause SPF value, including the type of sunscreen solvent, concentration and combination of sunscreens, type of emulsion, and the interaction and effect of other formulation components, including esters, emollients, and emulsifiers, which are some of the elements that affect the estimated SPF value. Increased or decreased absorption of UV radiation may be due to some of these variables(Mbanga et al., 2014).

Table 7. The result of the SPF value of avocado oil nano-emulgel with carbopol 980 as a gel base. *Description*: NE1 = nano-emulgel with 0,50% of Carbopol 980, NE2 = nano-emulgel with 1,00% of Carbopol 980, NE3 = nano-emulgel with 1,50% of Carbopol 980, Data displayed n = 3 ±SD.

Day	SPF Value		
	NE1	NE2	NE3
Before storage	9.02±0.81	11.93±0.38	22.38±0.24
7	8.95±0.43	12.01±0.36	22.41±0.21
14	9.65±0.09	11.96±0.08	22.34±0.32
21	9.71±0.27	12.01±0.23	22.42±0.27
28	9.80±0.18	11.98±0.02	22.37±0.23
p value	0.468	0.255	0.107

Based on the results of statistical analysis, the SPF value of avocado oil nano-emulgel preparations showed significant results ($p>0.05$) in all formulas, which means that there is no

significant difference in the SPF value of avocado oil nano-emulgel preparations during the storage cycle.

Nano-emulgel with Carbopol 980 base obtained physically stable results. The results obtained by nano-emulgel are by the previously determined TPP (target product profile) (R. S. Oliveira et al., 2022). Carbopol 980 was the best at modifying the rheological properties and viscosity of nanoscale emulsions designed for topical applications(Kumara et al., 2015). Carbopol 980 demonstrated favourable test outcomes, indicating its potential as an effective carrier for topical formulations (Md et al., 2020). This research is in line with the previous result that using 1.5% w/v Carbopol 980 as a gelling agent resulted in rheologically stable results and was acceptable for topical application (Shukla et al., 2019; Verma et al., 2023). Increasing the concentration of Carbopol 980 as a gelling agent can improve the physical properties of the preparation (Kelessidis et al., 2011). Carbopol 980, as a gelling agent with a concentration of 1.5%, has good stability and did not show significant physical/chemical changes in nano-emulgel preparations (Thombre et al., 2022)(Wani et al., 2015).

4. CONCLUSION

Based on the result above, it can be concluded that avocado oil in nano-emulgel preparation with 1,5% of Carbopol 980 was stable during 28 days of storage at 30±2°C and RH of 65±5%.

ACKNOWLEDGEMENT

The author would like to thank Yayasan Wahid Hasyim, Semarang, for funding this research through DIPA Unwahas 2023 in the Interdisciplinary Research scheme.

CONFLICT OF INTEREST

All authors declared that there was no conflict of interest.

REFERENCES

Abdullah, N. I. W., Samsudin, M. N., & Othman, S. F. C. (2023). Assessing the Stability of Peppermint Oil Encapsulated in Hydrogel Beads. *Journal of Tropical Life Science*, *13*(1), 51–60. https://doi.org/10.11594/jtls.13.01.06

Al-Awady, M. J., Fauchet, A., Greenway, G. M., & Paunov, V. N. (2017). Enhanced antimicrobial effect of berberine in nanogel carriers with cationic surface functionality. *J. Mater. Chem. B*, *5*(38), 7885–7897. https://doi.org/10.1039/C7TB02262J

Alhasso, B., Ghori, M. U., & Conway, B. R. (2023). Development of a Nanoemulgel for the Topical Application of Mupirocin effectively releases Mupirocin. *Pharmaceutics*, *15*, 1–26.

Allen, L. V. (1998). *The Art and Terminology of Pharmaceutical Compounding*. American Pharmaceutical Association, Washington DC.

Artanti, A. N., Prihapsara, F., Mas'ud, N., & Ermawati, D. E. (2021). Optimization of the Proportion of Surfactant, Co-Surfactant, and Candlenut Oil for Self-Nanoemulsifying Drug Delivery System (SNEDDS) of Secang Heartwood (Caesalpinia sappan L.) Methanolic Extract. *ALCHEMY Jurnal Penelitian Kimia*, *17*(1), 124. https://doi.org/10.20961/alchemy.17.1.39054.124-131

Chandra, D., & Rahman. (2022). Uji Fisikokiimia Sediaan Emulgel Ekstrak Etanol Goji Berry

(Lycium barbarum L.). *Medfarm: Jurnal Farmasi Dan Kesehatan*, *11*(2), 219–228.

Dantas, M. G. B., Reis, S. A. G. B., Damasceno, C. M. D., Rolim, L. A., Rolim-Neto, P. J., Carvalho, F. O., Quintans-Junior, L. J., & Da Silva Almeida, J. R. G. (2016). Development and Evaluation of Stability of a Gel Formulation Containing the Monoterpene Borneol. *Scientific World Journal*, *2016*, 10–13. https://doi.org/10.1155/2016/7394685

De Oliveira, A. P., Franco, E. D. S., Rodrigues Barreto, R., Cordeiro, D. P., De Melo, R. G., De Aquino, C. M. F., E Silva, A. A. R., De Medeiros, P. L., Da Silva, T. G., Góes, A. J. D. S., & Maia, M. B. D. S. (2013). Effect of semisolid formulation of persea americana mill (Avocado) oil on wound healing in rats. *Evidence-Based Complementary and Alternative Medicine*, *2013*. https://doi.org/10.1155/2013/472382

Depkes RI. (1995). *Farmakope Indonesia keempat* (Edisi IV). Departemen Kesehatan Republik Indonesia, Jakarta.

Dewi, R. H. T. M., Sholihah, N., Nofitasari, R., Adhityasmara, D., & Shabrina, A. (2024). The Potential of Avocado Oil for Topical Use: A Narrative Review. *Jurnal Ilmu Farmasi Dan Farmasi Klinik*, *21*(1), 106. https://doi.org/10.31942/jiffk.v21i1.11218

Ebad Sichani, M. R., Farid, M., & Khorasgani, E. M. (2021). Histomorphological Examination of Skin Wound Healing Under the Effect of Avocado Oil in Wistar Rats. *Acta Veterinaria Eurasia*, *47*(3), 121–128. https://doi.org/10.5152/actavet.2021.20096

Eid, A. M., Elmarzugi, N. A., & El-Enshasy, H. A. (2013). Development of Avocado Oil Nanoemulsion Hydrogel Using Sucrose Ester Stearate. *Journal of Applied Pharmaceutical Science*, *3*(12), 145–147. https://doi.org/10.7324/JAPS.2013.31226

FDA. (2013). ASEAN guideline on stability study of drug product. *Asean Guideline On Stability Study Of Drug Product (R1) Revision*, *May*, 1–40.

Ghani, H., Rahman, R. K., Liu, K., & Cubelli, S. (2021). An Investigation of Makeup Ingredients and Their Effects on Acne Cosmetica with Dermatologic Practice Recommendations. *SKIN: Journal of Cutaneous Medicine*, *5*(5), 474–481. https://doi.org/10.25251/skin.5.5.4

Hardenia, A., Jayronia, S., & Jain, S. (2014). Emulgel : An Emergent Tool in Topical Drug Delivery. *International Journal of Pharmaceutical Sciences and Research*, *5*(5), 1653–1660. https://doi.org/10.13040/IJPSR.0975-8232.5(5).1653-60

Hermanto, S., Muawanah, A., & Wardhani, P. (2010). Analisis Tingkat Kerusakan Lemak Nabati dan Lemak Hewani Akibat Proses Pemanasan. *Jurnal Kimia VALENSI*, *1*(6), 262–268. https://doi.org/10.15408/jkv.v1i6.237

Ikhtiyarini, T. A., & Sari, A. K. (2022). Efektivitas Penggunaan Basis Gel pada Sediaan Emulgel Effectiveness of Basic Use for Emulgel Preparations. *Camellia*, *1*(1), 19–25.

Islam, M. T., Rodríguez-Hornedo, N., Ciotti, S., & Ackermann, C. (2004). Rheological characterization of topical carbomer gels neutralized to different pH. *Pharmaceutical research*, *21*(7), 1192–1199. https://doi.org/10.1023/b:pham.0000033006.11619.07

Jo, M., Chang, M. J., Goh, K. K. T., Ban, C., & Choi, Y. J. (2021). Emulsion-filled gels' gels' rheology, microstructure, and storage stability are stabilized solely by maize starch modified with octenyl succinylation and pregelatinization. *Foods*, *10*(4). https://doi.org/10.3390/foods10040837

Kelessidis, V. C., Poulakakis, E., & Chatzistamou, V. (2011). Use Carbopol 980 and carboxymethyl cellulose polymers as rheology modifiers of sodium-bentonite water dispersions. *Applied Clay Science*, *54*(1), 63–69. https://doi.org/10.1016/j.clay.2011.07.013

Kumara, P., Prakash, N. K. S., Lokesh, & Manral, D. K. (2015). Viscoelastic Properties and Rheological Characterization of Carbomers. *Int. J. Latest Res. Eng. Technol., 1*(6), 2454–5031.

Kuncari, E. S., Iskandarsyah, & Praptiwi. (2014). Evaluasi, Uji stabilitas fisik dan sineresis sediaan gel yang mengandung minoksidil,apigenin dan perasan herba seledri (Apium graveolens L.) Evaluation , Physical stability test and syneresis of gel containing minoxidil, apigenin and celery (Apium graveo. *Bul. Penelit. Kesehat*, *42*(4), 213–222.

Li, Y., Liu, Y., Deng, D., Liang, J., Chen, W., Chen, X., & Li, J. (2019). Study on Extracting Avocado Oil from Avocado Pulp by Aqueous Extraction. *IOP Conference Series: Earth and Environmental Science*, *330*(4). https://doi.org/10.1088/1755-1315/330/4/042027

Mansur JS, Breder MNR, Mansur MCA, A. R. (1986). *Mansur JS, Breder MNR, Mansur MCA, Azulay RD.1986. Determination of Sun Protection Factor for Spectrophotometry. An Bras Dermatol Rio De Janeiro, 61, 121- 4*.

Mardiyanto, Mohadi, R., Fithri, N. A., & Kurniawan, G. (2024). Optimization of Nanoemulsion Formula Containing Erythromycin with VCO and Varying Concentrations of Tween-80 and PEG-400. *Science and Technology Indonesia*, *9*(3), 697–709. https://doi.org/10.26554/sti.2024.9.3.697-709

Mbanga, L., Mpiana, P. T., Bokolo, K., Mvingu, K., Ngoy, B., Muwara, K., & Mulenga, M. (2014). Sun Protection Factor (SPF) Determination of Cosmetic Formulations Made in Kinshasa (DR Congo) by in-vitro Method Using UV-VIS Spektrophotometer. *Journal of Physical and Chemical Sciences*, *2*(1), 1–5.

Md, S., Alhakamy, N. A., Aldawsari, H. M., Kotta, S., Ahmad, J., Akhter, S., Alam, M. S., Khan, M. A., Awan, Z., & Sivakumar, P. M. (2020). Improved analgesic and anti-inflammatory effect of diclofenac sodium by topical nanoemulgel: Formulation development—in vitro and in vivo studies. *Journal of Chemistry*, *2020*. https://doi.org/10.1155/2020/4071818

Nabillah, S., Noval, N., & Hidayah, N. (2022). FORMULASI DAN EVALUASI NANO HIDROGEL EKSTRAK DAUN SERUNAI (Chromolaena odorata L.) DENGAN VARIASI KONSENTRASI POLIMER CARBOPOL 980. *Jurnal Ilmiah Ibnu Sina (JIIS): Ilmu Farmasi Dan Kesehatan*, *7*(2), 340–349. https://doi.org/10.36387/jiis.v7i2.995

Numberi, A. M. (2020). Uji Stabilitas Fisik Sediaan Masker Gel dari Ekstrak Alga Merah (Poryphyra sp). *Majalah Farmasetika*, *5*(1), 1–17. https://doi.org/10.24198/mfarmasetika.v5i1.24066

Oliveira, R. S., da Silva, D. F., Mota, S., Garrido, J., Garrido, E. M., Lobo, J. M. S., & Almeida, I. F. (2022). Design of an Emulgel for Psoriasis Focused on Patient Preferences. *Applied Sciences (Switzerland)*, *12*(7). https://doi.org/10.3390/app12073260

Patricia, P., & Yuliani, S. H. (2015). Pembuatan Dan Uji Aktivitas Sediaan Unguenta Scarless Wound Dengan Ekstrak Binahong Dan Zat Aktif Aspirin. *Jurnal Farmasi Sains Dan Komunitas*, *12*(2), 54–60.

Priani, S. E., Abdilla, S. A., & Suparnan, A. (2020). Pengembangan Sediaan Mikroemulsi Gel Antijerawat Mengandung Minyak Kulit Batang Kayu Manis (Cinnamomum burmanni Nees ex Bl). *Jurnal Ilmiah Farmasi Farmasyifa*, *3*(1), 9–17. https://doi.org/10.29313/jiff.v3i1.5464

Rao, M. R., Deshpande, S., & Deshpande, P. (2023). Dapsone-Loaded Mixed Micellar Gel for Treatment OF Acne Vulgaris. *AAPS PharmSciTech*, *24*(5), 109. https://doi.org/10.1208/s12249-023-02564-1

Rismawati, D., Aji, N., & Herdiana, I. (2020). Pengaruh Butylated Hydroxyanisole terhadap Stabilitas dan Karakteristik Emulgel Kombinasi Ekstrak Jahe Merah dan Minyak Peppermint. *Jurnal Ilmiah Kefarmasian*, *1*(2), 35–41.

Scomoroscenco, C., Teodorescu, M., Raducan, A., Stan, M., Voicu, S. N., Trica, B., Ninciuleanu, C. M., Nistor, C. L., Mihaescu, C. I., Petcu, C., & Cinteza, L. O. (2021). Novel gel microemulsion as topical drug delivery system for curcumin in

dermatocosmetics. *Pharmaceutics*, *13*(4). https://doi.org/10.3390/pharmaceutics13040505

Shabrina, A. (2024). Physical Stability and Effectivity Comparison of Nanoemulsion and Nano-emulgel of Avocado Oil with Carbopol 940 Variation. *Indonesian Journal of Pharmaceutical Science and Technology*, *6*(1), 8–17.

Shabrina, A., Firdausi, Z. M., Poerba, A. T., Anisa, D., & Heroweti, J. (2024). Sunscreen effectivity and physical characterization of avocado oil in nanoemulsion using isopropyl myristate variations. *Pharmaciana*, *14*(1), 69–79. https://doi.org/10.12928/pharmaciana.v14i1.28214

Shabrina, A., Heroweti, J., Yumni, G. G., Poerba, A. T., & Diana, A. (2024). Physical Stability and Effectivity Comparison of Nanoemulsion and Nano- emulgel of Avocado Oil with Carbopol 940 Variation. *Indonesia Journal of Pharmaceutical Sciences and Technology*, *6*(1), 6–17.

Shelke, O., & Kulkarni, A. (2019). Formulation, development and evaluation of nifedipine emulgel for treatment of anal fissures using polymeric emulsifiers. *Indian Journal of Pharmaceutical Education and Research*, *53*(2), s74–s81. https://doi.org/10.5530/ijper.53.2s.51

Shukla, K. V., Sharma, A., & Yadav, M. (2019). Formulation Development and Evaluation of Ethosomal Gel of Acyclovir for the Treatment of Herpes Zoster. *Journal of Drug Delivery and Therapeutics*, *9*(4-A), 664–668. http://jddtonline.info/index.php/jddt/article/view/3548

Sreevidya, V. S. (2019). An Overview on Ebola. *Global Dermatology*, *2*(1), 92–97. https://doi.org/10.15761/god.1000122

Suhesti, T. S., Rohman, M. M. H., & Sunarto, S. (2022). Formulation of Gel Hand Sanitizer of Nagasari Leaf Extract (Mesua ferrea L.). *Indonesian Journal of Pharmaceutical Science and Technology*, *1*(1), 31. https://doi.org/10.24198/ijpst.v1i1.36465

Suradnyana, I. G. M., Juliadi, D., & Suena, N. M. D. S. (2023). Formulasi serta Uji Aktivitas Antioksidan dan Tabir Surya Krim Ekstrak Aseton Biji Buah Alpukat. *Jurnal Ilmiah Medicamento*, *9*(1), 42–51. https://doi.org/10.36733/medicamento.v9i1.5504

Suryani, Sahumena, M. H., Mabilla, S. Y., Ningsih, S. R., Adjeng, A. N. T., Aswan, M., Ruslin, Yamin, & Nisa, M. (2020). Preparation and Evaluation of Physical Characteristics of Vitamin E Nanoemulsion Using Virgin Coconut Oil (VCO) and Olive Oil as Oil Phase with Variation Concentration of Tween 80 Surfactant. *Research Journal of Pharmacy and Technology*, *13*(7), 3232–3236. https://doi.org/10.5958/0974-360X.2020.00572.7

Thombre, N. A., Niphade, P. S., D. Ahire, E., & J. Kshirsagar, S. (2022). Formulation Development and Evaluation of Microemulsion Based Lornoxicam Gel. *Biosciences Biotechnology Research Asia*, *19*(1), 69–80. https://doi.org/10.13005/bbra/2968

Verma, P., Dutt, R., & Anwar, S. (2023). Formulation and development of novel probiotic loaded gel for the treatment of caffeine induced anxiety. *Journal of Applied Pharmaceutical Science*, *13*(6), 121–127. https://doi.org/10.7324/JAPS.2023.100861

Voigt, R. (1984). *Buku Ajar Teknologi Farmasi*. diterjemahkan oleh Soewandi, S. N., UGM Press, Yogyakarta.

Wani, R. R., Patil, M. P., Dhurjad, P., Chaudhari, C. A., & Kshirsagar, S. J. (2015). Microemulsion Based Gel: A Novel Approach In Delivery of Hydrophobic Drug. *International Journal for Pharmaceutical Research Scholars*, *4*(2), 671–677.

Widyastuti, A. I., & Saryanti, D. (2023). Formulasi dan Evaluasi Sediaan Nanoemulsi Ekstrak Umbi Bawang Putih (Allium sativum L.). *Jurnal Sains Dan Kesehatan*, *5*(2), 178–185. https://doi.org/10.25026/jsk.v5i2.1677

Yuliandri, M., Saádah, H., & Warnida, H. (2021). Pengaruh Konsentrasi Carbopol 940 Sebagai Gelling Agent Terhadap Sifat Fisik Dan Stabilitas Gel Hand. *Library.Usd.Ac.Id*, 117–

124. http://www.library.usd.ac.id/Data PDF/F. Farmasi/Farmasi/108114120_full.pdf

Zalivskaya, A., Fadeeva, D., Shestopalova, N., Avtina, N., Radyukova, V., & Ivanova, V. (2021). Comparative characteristics of gel bases for semisolid dosage forms. *BIO Web of Conferences*, *40*, 03008. https://doi.org/10.1051/bioconf/20214003008